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INSTRUMENTAL COLOUR, TEXTURE AND SENSORY PROPERTIES OF PLJESKAVICA (TRADITIONAL SERBIAN MEAT PRODUCT) AFFECTED BY ADDITION OF FRESHLY EXTRACTED *Allium ursinum* L.

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ABSTRACT

Pljeskavica is a traditional Serbian burger-type meat product that is also very popular across the Balkan countries. The use of natural supplements containing various bioactive compounds, such as wild garlic (*Allium ursinum* L.) extracts, has gained more importance in the last several decades and has promising results for the prevention of the processes leading to detoriation. The present study aimed to evaluate the influence of *Allium ursinum* L. freshly squeezed extract (FSAULE) on colour, texture and sensory attributes of raw and grilled pljeskavica during cold (10 days) and freeze storage (90 days). Control (CON) and three experimental treatments containing different concentrations of FSAULE were prepared – AUL1 (1.32 mL/kg), AUL2 (4.40 mL/kg) and AUL3 (8.79 mL/kg). Redness and yellowness of raw meat and backfat particles were not altered after the addition of different amounts of FSAULE throughout cold and freeze storage. Meat and backfat particles of raw AUL3 were significantly darker compared to CON and other AULs. The results of texture profile analysis indicate that the addition of different amounts of FSAULE did not alter instrumental texture properties. According to the check-all-that-apply analysis of sensory properties, AUL3 was mostly characterized by affirmative attributes – pleasant odour, taste, saltiness, softness, juiciness and ideal texture. The affirmative attributes that were not present in AULs, but were present in CON, were detected by a small number of consumers – from 2.8% (pleasant colour) to 15.0% (pleasant odour). Most consumers found that intense garlic odour (85.0%) and intense garlic-like taste (83.9%) were not present in either CON or AULs.

Keywords: pljeskavica; pattie; wild garlic extract; cold storage, instrumental colour, instrumental texture, CATA sensory analysis

INTRODUCTION

One of the quintessential representatives of Serbian cuisine, embodying the rich tradition of meat-based delicacies, is pljeskavica (pronounced [pʎêskaʋitsa]), a classical patty/hamburger-type meat product. Alongside another renowned minced meat product, ćevapi (pronounced [tɕeʋǎːpi]), pljeskavica stands as a most popular meat product in Serbia and the Western Balkan region (**Stajić & Kalušević, 2021**). Pljeskavica is made from ground meat (pork, beef, mutton or a blend of those) and fatty tissue, with the addition of salt, and is shaped like a burger. When grilled, it acquires a smoky brown exterior, while preserving a succulent interior. It can also be stuffed with ingredients such as cheese, ham and onion. The name pljeskavica derives from the Serbian word "pljesakati", which means to clap. It denotes the technique used to shape and flatten the meat for grilling and give it a destinctive thin round shape (**Tomasevic *et al.*, 2018**), similar to the English word "patty".
Burger and patty-type meat products have attained immense popularity and represent a hallmark of the fast-food industry (**Stajić & Kalušević, 2021**). A multinational fast food company, for instance, has reported selling > 75 burgers per second, equating to approximately100 billion annually worldwide (**Spencer *et al.*, 2005**), while in the US alone consumers indulge in 5 billion burgers per year (**Prayson *et al.*, 2008**). The dynamic rhythm of modern life imposes short-term meal preparation as an imperative. In response, affordable, convenient, and sensory-pleasing options, such as burgers and patties, have garnered widespread popularity in supermarkets, fast-food chains, and restaurants across the globe. These patty/burgers, made from beef, pork, mutton, chicken and other meats can be shallow pan-fried, smoked, flame-broiled, or grilled before consumption. Typically, the fat and protein contents of such meat products are within 6%–14% and 12%–18%, respectively, with considerable variability attributed to the specific ingredients used and their respective proportions. Smaller-sized burgers have also found their place as snack bites or nuggets (**Bhattacharya, 2023**).
Burgers are prepared from processed meat which contains proteins of high biological value, essential fatty acids, vitamins and minerals (**Angiolillo *et al.*, 2015**). However, the consumption of these products, especially in excessive quantities, carries certain risk factors, notably the high level of saturated fat, cholesterol and sodium. Such imbalances have been linked to heightened mortality rates and an increased susceptibility to several non-communicable chronic diseases (**Pereira & Vicente, 2013**). Modern consumers are looking for healthier and more convenient meat products, which are easy to prepare. Furthermore, they express a distinct preference for products prepared without synthetic chemical additives, while retaining an appealing appearance and flavor. The use of food additives remains undisputed because it provides superiority in terms of technological properties. However, there is continuous public concern about the risks associated with their consumption, such as allergic reactions, inflammatory responses, carcinogenicity and behavioural disorders, such as hyperactivity (**Zheng & Wang, 2001; Abedini *et al.*, 2023**). Consequently, food technologists face a challenging task, as they endeavour to innovate and create novel, healthier or functional products that meet the evolving consumer demand (**Varela & Fiszman, 2013; Munekata *et al.*, 2020; Wang *et al.*, 2022**).
Established processing procedures (such as grinding, salting and cooking) used in burger production contribute to the increasing formation of reactive oxygen species, which makes them very susceptible to oxidation. The oxidation of lipids and proteins is the primary factor responsible for the progressive decline in the quality and shelf life of patties during storage. This oxidative degradation leads to the loss of flavour, texture, and nutrients, significantly affecting the acceptance of meat products and shortening their shelf-life (**Ergezer & Serdaroğlu, 2018; Gómez *et al.*, 2020**). Additionally, burgers are prone to spoilage mainly due to the action of enzymatic systems of microorganisms, during both the processing stage and storage. The use of natural supplements containing various bioactive compounds sourced from materials such as plants, fruits, vegetables, oilseeds, spices, thyme, honey, bee pollen, and grains, offers a prospective approach to mitigate the aforementioned spoilage processes (**Lu *et al.*, 2018; Sedlacek-Bassani *et al.*, 2020; Kurćubić *et al.*, 2023**). On the other hand, plant extracts contain colourants (e.g. polyphenolic compounds) which can alter colour properties and by extension sensory quality of meat products (**Shah et al., 2014**). For example, wild garlic extracts (Allium ursinum L.) contain chlorophylls and carotenoids (**Voća et al., 2022**) which can alter typical red/pink colour of minced meat products. Also, since different reactions occur during heat treatment, addition

of plant extract can lead to colour changes of grilled products. Odour and taste can also be altered, but not necessarily bad since the same compounds from plant extracts are present in species used in meat products, such as allicin which is present in both wild garlic and garlic. Therefore, research is needed to establish the necessary concentrations of plant extracts to satisfy both good shelf-life and sensory quality requirements.

The utilazation of plant essential oils and extracts as natural ingredients with antioxidant properties has garnered significant interest and is evident in an increasing number of research (**Aliakbarlu & Khalili Sadaghiani, 2015; Śmiecińska *et al.*, 2022; Kurćubić et al., 2023**). These plant-derived extracts exhibit strong free radical scavenging capability and antibacterial activity due to the presence of phenolic acids, stilbenes and flavonoids (**Aquilani *et al.*, 2018**). Many of these plant extracts have GRAS status (Generally Recognized as Safe) – the addition of these phytochemicals or substances to food is considered safe by experts, and their use is exempt from the usual Federal Food Safety Act (**Roberts & Haighton, 2016**). *Allium ursinum* L. (wild gallic, ramson), marked as Medicinal Plant of the Year by the Association for the Protection and Research on European Medicinal plants in 1992 (**Sharma *et al.*, 2017**), contains, in high amounts, several compounds (e. g. polyphenols, flavonoids) that possess antimicrobial and antioxidant properties (**Liu *et al.*, 2017; Tomšik *et al.*, 2017**).

In previous research conducted by **Kurćubić *et al.*** (**2023**) and **Kurćubić *et al.*** (**2022**) chemical characterization and antibacterial activity (minimum inhibitory concentrations and minimum bactericidal concentration values) of freshly squeezed *Allium ursinum* L. extract (FSAULE) were examined, as well as microbiological and technological properties and proximate composition of burgers with added FSAULE.

This research, which is follow-up on mentioned research **Kurćubić *et al.***, (**2023**) and **Kurćubić *et al.*** (**2022**), aimed to investigate the effects of freshly squeezed *Allium ursinum* L. extract on instrumental colour, texture, sensory properties and their changes during cold and freeze storage of pljeskavica prepared from a mixture of beef and pork.

MATERIAL AND METHODS

Pljeskavica preparation

The pljeskavica treatments and FSAULE were prepared as described by **Kurćubić *et al.*** (**2023**). The leaves of *Allium ursinum* L. were collected in Central Serbia in March 2020, in the lower part of Mt Ovčar, a protected area of exceptional features (The Ovčar-Kablar Gorge, latitude 43°54′02.8“, longitude 20°11′54.7″ and 391 m above sea), at its pre-flowering stage. Fresh plant leaves were hand selected for freshly squeezed extract preparation. This approach is justified because there is a risk that due to exposure to high temperatures, oxygen, and light (to which they are sensitive), certain bioactive compounds may degrade in the WGE. The species was identified, and the voucher specimen was deposited in the Herbarium of the Institute of Botany and Botanical Garden "Jevremovac," University of Belgrade–number 17817 BEOU. Freshly squeezed wild garlic extract (FSAULE) was prepared by cold-pressing chopped leaves in a manual squeezer (garlic press BL-3455, Blaumann, Budapest, Hungary). Squeezing was repeated several times to obtain approximately 300 mL of representative extract. Four treatments of pljeskavica were prepared – control and three modified treatments. The basic composition of products was the same for all treatments: 50% pork shoulder, 20% beef shoulder, 15% fatty beef trimmings with 30% fat, 10% water, 2% table salt, 0.5% white pepper, 0.8% sweet red ground pepper, and 1% "Pergeta" cooking supplement with vegetables (Meat & Trade, Novi Sad, Serbia). Garlic powder (0.2%, Meat & Trade, Novi Sad, Serbia) was added to the control (CON), while in the modified treatments FSAULE was added in different amounts: 1.32 mL FSAULE/kg (15 mg allicin/kg (15 ppm) – AUL1), 4.40 mL FSAULE/kg (50 mg allicin/kg (50 ppm) – AUL2) and 8.79 mL FSAULE/kg (100 mg allicin/kg (100 ppm) – AUL3). According to the literature data (Ankri and Mirelman 1999; Patent Application Publication Pub. No.: US 2007/0160725 A1, 2007, United States;) the minimum LD_{50} allicin concentration against food borne pathogens (e.g. *Escherichia coli* and *Staphyloccocus aureus*) is 15 µg/mL (15 ppm). The amounts of FSAULE added were calculated according to the concentration of allicin in FSAULE (11.375 mg/ml) which was determined in previous research by **Kurćubić *et al.*** (**2023**). Pljeskavicas were grilled in a combi oven (RATIONAL AG, Mod. SCC WE 101, Landsberg am Lech, Germany) at a programmed temperature of 285 °C, for 9 minutes. Combi ovens allow the thermal processing of foods of different flavours (e.g. fish and pork) in the same chamber, without them affecting each other, and it enables the achievement of uniformity of the heat treatment.

The whole process was repeated three times (three independent batches), and samples (pljeskavicas) were stored at +4 °C (cold storage) for 10 days and at −20 °C (freeze storage) for 90 days. Four pljeskavicas from each treatment were used on days 1, 5, 10 and 90 to examine the colour and texture properties.

Instrumental colour determination

Colour was measured using the Computer vision system (CVS). Photographs (in raw image format) were taken from each pljeskavica (raw and cooked) with the equipment and under conditions as described by **Tomasevic *et al.*** (**2019**). Seven readings (5 × 5 pixels measuring area) were taken from each pljeskavica's photograph, separately of meat parts and fat parts, using a Photoshop Average Color Sampler Tool. Average values of these seven readings were calculated and used as one iteration for statistical analysis. C^* (chroma) and h (hue angle) were calculated using the standard equations:

$$C^* = [(a^*)^2 + (b^*)^2]^{1/2} \text{ and } h = \arctan b^*/a^*$$

Browning index was calculated according to **Bozkurt & Bayram** (**2006**):

$$BI = \frac{[100 \times (x - 0.31)]}{0.17}$$

$$x = \frac{(a^* + 1.75 \times L^*)}{(5.645 \times L^* + a^* - 3.012 \times b^*)}$$

Total colour difference (ΔE^*) of treatments with FSAULE, relative to CON, was determined using the standard equation:

$$\Delta E^* = [(L^*_{AUL} - L^*_{CON})^2 + (a^*_{AUL} - a^*_{CON})^2 + (b^*_{AUL} - b^*_{CON})^2]^{1/2}$$

AUL – Pljeksavica with FSAULE; CON – control

Instrumental texture analysis

After grilling, pljeksavicas were cooled to room temperature and 3 cylindrical samples (16 mm in radius and original height ≈ 14±1 mm) were taken from each pljeskavica for texture profile analysis, which was performed using the universal texture analyzer (TA.XT Plus; Stable Micro System, Ltd., Godalming, UK). Samples were compressed twice to 50% of their original height (the height of each sample was measured immediately before compression) with a compression aluminium platen of 25 mm (P/25) and a 5 kg load cell. Pre-test speed was 60 mm/min, test speed was 60 mm/min, and post-test speed was 300 mm/min. Hardness, adhesiveness, springiness, cohesiveness and chewiness were evaluated and obtained using the Exponent software (Stable Micro Systems, Godalming, United Kingdom).

Sensory analysis

Check-all-that-apply (CATA) method was used to examine the influence of different amounts of *Allium ursinum* L. extracts on the sensory profile of pljeskavica. Before analysis, all CATA questions were defined (**Jorge *et al.*, 2015**) in a panel discussion by five professors from the Department of Animal Source Food Technology, Faculty of Agriculture, University of Belgrade and the Department of Food Technology, Faculty of Agronomy, University of Kragujevac. The specific sensory characteristics of this type of product and AU extracts were considered when CATA questions were defined. The panel defined the following terms for describing colour (pleasant colour, pale colour, atypical colour), odour (pleasant odour, atypical odour, garlic odour, intense garlic odour), taste (tasty, salty, atypical taste, taste like garlic, intense taste like garlic) and texture (soft, juicy, ideal texture). The attributes were presented to the consumers at random.

Sixty consumers (aged 19–62, 61.67% male, 38.33% female) participated in the CATA survey. They were selected for consuming pljeskavicas/burgers at least once a week. All consumers were selected among students and staff members of the Faculty of Agronomy, University of Kragujevac and were not informed about recipe formulations. After heat treatment, pljeskavicas were cut into six pieces, randomly coded with three-digit numbers and also randomly served (one per consumer) on white plastic plates under daily light. Consumers were asked to complete a CATA questionnaire and to rate overall acceptance or liking (on a 0–10 scale: 0–extremely unacceptable, 10–extremely acceptable) of every treatment. They used water at room temperature to cleanse their palate between samples. The sensory analysis was performed in a single testing session. All participant have given consent to participate in sensory analyis.

Statistical analysis

The results were subjected to two-way ANOVA to evaluate the effect of FSAULE addition, storage time, and its interaction. Software Statistica 12.5 (StatSoft, Inc., Tulsa, OK, USA) was used for data analysis and the results were presented as a mean ± standard deviation (SD). Differences between means were determined using Tukey's HSD test at the significance level $p < 0.05$. The results of CATA survey were analysed using XLSTAT-Sensory/CATA data analysis (2020) as following procedure described by **Stajić *et al.*** (**2020**). The analysis included data obtained from the survey of four products (three treatments with FSALE (AULs) and control (CON) considered as ideal product) by sixty consumers on fourteen attributes, which are recorded in binary format – 0: attribute absent (No) and 1: attribute present (Yes). Moreover, data about the overall acceptance were also included. Two groups of results were presented. First, based on a correspondence analysis, a biplot projection was presented which allows product positioning, including CON (ideal products). The second group of results is related to the penalty analysis and provides data as to whether the observed attributes were absent (No) or present (Yes) in AULs and at the same time absent or present in CON, and how this influenced the overall acceptance scores. To interpret the results, the observed attributes were divided into three groups: affirmative sensory attributes (pleasant colour, pleasant odour, tasty, soft, juicy, ideal texture and salty) for which we presented the frequency (%) of the absence in AULs but presence in CON (No/Yes), and presence in both AULs and CON (Yes/Yes); negative sensory attributes (pale colour, atypical colour, atypical odour and atypical taste) for which we presented the frequency (%) of presence in AULs, but absence in CON (Yes/No), and absence in both AULs and CON (No/No); garlic-like sensations – garlic odour, intense garlic odour, garlic taste and intense garlic taste, for which we presented the frequency (%) presence in AULs, but absence in CON (Yes/No), and of absence in both AULs and CON (No/No).

RESULTS AND DISCUSSION

Instrumental colour determination

The results obtained by instrumental colour measurements were presented in Tables 1–4. The addition of different amounts of FSAULE did not alter *a** and *b** values of raw meat and raw backfat particles – there were no significant differences ($p > 0.05$) between treatments after the addition of different amounts of FSAULE throughout cold storage (Table 1; except in *a** of raw backfat on day 5) and freeze storage (Table 2). Cold storage significantly affected ($p < 0.05$) colour properties (except on *b** values of backfat) in all treatments – all pljeskavicas became darker, less red and less yellow. However, relations within treatments were similar to those on day 1, suggesting that amounts of these changes were similar in all treatments. The lightness of raw meat and raw backfat particles was altered only in pljeskavica with the highest amount of FSAULE – raw meat particles were darker ($p < 0.05$) in AUL3 compared to all other treatments, while backfat particles were darker compared to CON on day 1 and lighter ($p < 0.05$) in all AUL treatments on day 10.

Table 1 Colour properties of raw pljeskavica as affected of FSAULE content and cold storage

		CON	AUL1	AUL2	AUL3
raw meat					
*L**	day1	63.68±1.86^{bC}	64.89±0.50^{bC}	63.50±2.14^{bC}	58.82±1.04^{aC}
	day5	53.93±3.58^{bB}	52.46±1.55^{abB}	52.82±1.33^{abB}	46.04±1.11^{aB}
	day10	44.71±2.39^{bA}	44.50±3.44^{abA}	46.68±0.68^{bA}	39.75±0.58^{aA}
*a**	day1	35.96±2.71^{aC}	34.14±1.95^{aC}	36.32±2.22^{aC}	38.21±1.50^{aC}
	day5	29.82±0.91^{aB}	28.89±2.23^{aB}	28.54±2.22^{aB}	25.18±2.50^{aB}
	day10	19.14±1.45^{aA}	19.21±2.43^{aA}	20.36±1.07^{aA}	19.25±0.84^{aA}
*b**	day1	14.07±1.37^{aC}	13.18±1.25^{aB}	14.21±1.08^{aB}	15.25±0.39^{aB}
	day5	10.71±0.81^{aB}	10.93±1.35^{aB}	11.71±1.49^{aB}	10.54±2.03^{aA}
	day10	6.07±1.68^{aA}	7.25±1.24^{aA}	6.43±0.91^{aA}	7.71±0.31^{aA}
raw backfat					
*L**	day1	82.61±0.91^{bC}	81.46±0.39^{abB}	80.32±0.94^{abA}	78.93±1.28^{aA}
	day5	77.79±0.92^{aB}	77.82±0.21^{aA}	78.46±0.61^{aA}	77.21±1.66^{aA}
	day10	73.29±0.65^{aA}	76.86±1.08^{bA}	77.79±1.43^{bA}	76.96±1.83^{bA}
*a**	day1	10.75±0.36^{aB}	12.11±1.02^{aB}	12.07±0.92^{aC}	12.79±0.74^{aC}
	day5	12.29±1.06^{bB}	10.11±0.61^{abB}	8.29±1.56^{aB}	8.21±2.41^{aB}
	day10	7.46±1.46^{aA}	5.75±0.51^{A}	4.86±1.16^{A}	4.93±0.88^{A}
*b**	day1	2.00±1.15^{aA}	2.93±0.54^{aA}	2.54±0.77^{aA}	3.25±0.41^{aA}
	day5	3.93±0.58^{aA}	4.00±0.53^{aA}	2.96±0.97^{aA}	3.61±0.78^{aA}
	day10	3.89±1.08^{aA}	3.64±0.89^{aA}	4.11±1.11^{aA}	5.32±1.07^{aA}

$^{a–b}$ Values (mean±SD) in the same row with different superscripts are significantly different ($p < 0.05$) considering different amounts addition of FSAULE;
$^{A–C}$ Uppercase letters are used for comparing the samples considering the effect of storage. Values in the same column for the same property, with different superscripts are significantly different ($p < 0.05$) considering storage time.

Table 2 Colour properties of raw and grilled pljeskavica as affected of FSAULE content and freeze storage

			CON	AUL1	AUL2	AUL3
raw meat	*L**	day1	63.68±1.86^{bB}	64.89±0.50^{bB}	63.50±2.14^{bB}	58.82±1.04^{aB}
		day90	59.96±1.23^{bA}	61.07±0.94^{bA}	59.57±1.48^{bA}	55.25±1.42^{aA}
	*a**	day1	35.96±2.71^{aA}	34.14±1.95^{aA}	36.32±2.22^{aA}	38.21±1.50^{aA}
		day90	33.68±2.68^{aA}	32.00±1.82^{aA}	34.04±2.05^{aA}	35.75±1.31^{aA}
	*b**	day1	14.07±1.37^{aA}	13.18±1.25^{aA}	14.21±1.08^{aA}	15.25±0.39^{aA}
		day90	13.11±1.43^{aA}	12.21±1.25^{aA}	13.07±0.82^{aA}	14.18±0.38^{aA}
raw backfat	*L**	day1	82.61±0.91^{bA}	81.46±0.39^{abA}	80.32±0.94^{abA}	78.93±1.28^{aA}
		day90	80.61±0.91^{bA}	79.46±0.39^{bA}	78.64±0.78^{abA}	77.04±1.15^{aA}
	*a**	day1	10.75±0.36^{aA}	12.11±1.02^{aA}	12.07±0.92^{aA}	12.79±0.74^{aA}
		day90	10.89±1.03^{aA}	11.14±1.37^{aA}	11.75±0.92^{aA}	12.46±2.67^{aA}
	*b**	day1	2.00±1.15^{aA}	2.93±0.54^{aA}	2.54±0.77^{aA}	3.25±0.41^{aA}
		day90	2.39±1.13^{aA}	2.79±0.55^{aA}	2.46±1.58^{aA}	3.79±1.12^{aA}
grilled meat	*L**	day1	44.39±0.68^{bB}	46.29±0.95^{bB}	44.86±0.42^{bB}	37.21±1.07^{aB}
		day90	41.82±0.82^{bA}	43.61±1.15^{bA}	42.32±0.73^{bA}	34.86±1.08^{aA}
	*a**	day1	18.75±0.99^{aA}	17.64±1.08^{aA}	17.82±1.02^{aA}	19.00±0.39^{aA}
		day90	17.79±1.01^{aA}	16.75±1.22^{aA}	16.93±1.19^{aA}	18.11±0.18^{aA}
	*b**	day1	22.43±1.38^{aA}	20.57±1.85^{aA}	18.14±2.12^{aA}	18.29±3.35^{aA}
		day90	21.14±1.15^{aA}	19.32±1.80^{aA}	16.93±1.96^{aA}	17.14±3.17^{aA}
	BI	day1	101.20±6.38^{aA}	86.91±6.21^{aA}	81.22±7.51^{aA}	109.74±21.91^{aA}
		day90	101.48±6.39^{abA}	86.98±6.58^{abA}	80.80±7.37^{aS}	109.69±21.24^{bA}
grilled backfat	*L**	day1	59.61±1.53^{aA}	61.11±1.00^{aA}	61.25±1.19^{aA}	59.54±0.54^{aA}
		day90	58.79±1.95^{aA}	60.21±1.18^{aA}	60.43±1.08^{aA}	58.50±0.58^{aA}
	*a**	day1	12.79±1.10^{aA}	11.11±0.56^{aA}	11.75±1.26^{aA}	12.07±0.59^{aA}
		day90	12.46±1.11^{aA}	10.89±0.65^{aA}	11.61±1.21^{aA}	11.79±0.54^{aA}
	*b**	day1	12.36±1.39^{bA}	7.93±1.50^{aA}	7.43±0.88^{aA}	8.89±1.16^{aA}
		day90	12.04±1.65^{bA}	7.75±1.73^{aA}	7.25±0.88^{aA}	8.50±1.24^{aA}

$^{a–b}$ Values (mean±SD) in the same row with different superscripts are significantly different ($p < 0.05$) considering different amounts addition of FSAULE
$^{A–C}$ Uppercase letters are used for comparing the samples considering the effect of storage. Values in the same column for the same property, with different superscripts are significantly different ($p < 0.05$) considering storage time.

The L^* and a^* values of the pork muscle highly correlate to the total hem pigment content and myoglobin oxidation – MetMb content (**Lindahl *et al.*, 2001**). In this research, all pljeskavicas were prepared with the same amount of the same types of meat, therefore the total hem pigment content is irrelevant. In that case, lower L^* values (darker meat) and higher a^* values (more red meat) are correlated to the higher MetMb contents (**Lindahl *et al.*, 2001**). Since there are no significant differences within all treatments regarding a^* values (and b^* values as well) throughout the cold storage, the significant differences between AUL3 and all other treatments regarding L^* values could not be associated with myoglobin fractions content caused by prooxidative or antioxidative properties of the FSAULE towards myoglobin. The significantly lower L^* values in the treatment with the highest content of the FSAULE were probably caused by the colorants present in the FSAULE (e.g. chlorophylls and carotenoids (**Voća *et al.*, 2022**)). The instrumental colour properties (L^*, a^* and b^* values) of fresh ground pork could be affected by the type of plant extracts because they contain potent colourants such as polyphenolic compounds (**Shah *et al.*, 2014**).

Filipčev *et al.* (2023) using wild garlic (WG) aqueous extract in different amounts (low, middle, high), in pasta preparation, reported significantly lower L^* values and higher a^* values in all non-cooked pasta, compared to control, and significantly higher b^* values in pasta with high amount of WG extract. After cooking, significantly lower L^* values and higher a^* values were found in pasta with middle and high WG extract amounts, while significantly higher b^* values (compared to control) were found in all pasta. When powdered WG and encapsulated WG were used, the differences (compared to control) were more pronounced – significantly lower L^* values and higher a^* and b^* values in both non-cooked and cooked pasta. These results, together with the results of our research, indicate that the addition of wild garlic into foods can induce colour changes depending on the food matrix, amount of WG added and the manner of WG preparation (e.g. extract type, powder, encapsulated) because it contains natural pigments that gave colour to raw food and undergo different reactions (e.g. Maillard reaction) during heat treatment.

Lighter backfat could be associated with the antioxidative properties of the FSAULE, which was demonstrated to some extent in our previous research (**Kurćubić *et al.*, 2023**). Moreover, research indicates that garlic added to meat products in different forms (fresh, powder or oil) inhibits lipid oxidation (**Nieto *et al.*, 2013**). However, **Mancini *et al.* (2020)** indicate the limited antioxidant capacity of the garlic powder added to rabbit meat burgers, proposing research with the use of different garlic products or concentrations.

After 90 days of freeze storage (Table 2), significantly lower L^* values of meat particles have been obtained in raw and grilled pljeskavicas. However, relations within treatments did not change compared to day 1 (before freezing), as was the case regarding all other observed colour properties. Contrary to cold storage, a^* and b^* values remained stable during freeze storage.

After grilling cold stored products, the colour properties of meat particles (Table 3) displayed the properties of raw pljeskavica – AUL3 was the darkest ($p < 0.05$) compared to others, while a^* and b^* values did not differ within treatments. After grilling, backfat particles did not differ compared to control in L^* and a^* values, while b^* values were significantly lower in all AUL treatments. Colour property changes of cold stored pljeskavicas after grilling were sporadic compared to the ones grilled on 1st day of storage. However, relations within grilled treatments, regarding meat and backfat colour properties, were the same as on 1st day, except in b^* values of backfat. Backfat particles of grilled pljeskavicas on 1st day with FSAULE had significantly ($p < 0.05$) lower b^* values. However, after grilling, b^* values of backfat particles were lower on days 5 and 10 compared to 1st day, not significantly only in AUL3, which led to different relations between treatments on day 10. Freeze storage did not alter the relations between treatments regarding the colour properties of meat and backfat of grilled pljeskavicas.

Table 3 Colour properties of grilled pljeskavica as affected of FSAULE content and cold storage of raw samples

		CON	AUL1	AUL2	AUL3
grilled meat					
L^*	day1	44.39 ± 0.68^{bA}	46.29 ± 0.95^{bA}	44.86 ± 0.42^{bA}	37.21 ± 1.07^{aA}
	day5	47.25 ± 0.84^{bA}	44.71 ± 1.18^{bA}	43.61 ± 2.09^{bA}	39.46 ± 1.11^{aA}
	day10	47.07 ± 3.62^{bA}	44.50 ± 1.27^{bA}	47.00 ± 1.06^{bA}	38.61 ± 2.25^{aA}
a^*	day1	18.75 ± 0.99^{aA}	17.64 ± 1.08^{aA}	17.82 ± 1.02^{aA}	19.00 ± 0.39^{aA}
	day5	17.25 ± 0.18^{aA}	17.04 ± 0.61^{aA}	17.04 ± 0.63^{aA}	17.11 ± 0.90^{aA}
	day10	16.96 ± 1.22^{abA}	16.93 ± 2.13^{abA}	15.57 ± 0.80^{aA}	19.04 ± 0.44^{bA}
b^*	day1	22.43 ± 1.38^{aA}	20.57 ± 1.85^{aA}	18.14 ± 2.12^{aA}	18.29 ± 3.35^{aA}
	day5	17.79 ± 0.97^{aA}	16.43 ± 1.70^{aA}	18.29 ± 2.16^{aA}	15.21 ± 1.31^{aA}
	day10	19.54 ± 0.98^{aA}	15.25 ± 3.40^{aA}	17.29 ± 4.07^{aA}	15.54 ± 0.67^{aA}
BI	day1	101.20 ± 6.38^{aB}	86.91 ± 6.21^{aA}	81.22 ± 7.51^{aA}	109.74 ± 3.75^{aB}
	day5	73.54 ± 4.84^{aA}	73.63 ± 7.36^{aA}	83.79 ± 12.74^{aA}	81.06 ± 8.53^{aA}
	day10	80.57 ± 11.10^{aAB}	69.07 ± 7.75^{aA}	70.91 ± 11.76^{aA}	87.53 ± 9.01^{aAB}
grilled backfat					
L^*	day1	59.61 ± 1.53^{aA}	61.11 ± 1.00^{aA}	61.25 ± 1.19^{aA}	59.54 ± 0.54^{aA}
	day5	63.93 ± 2.76^{aAB}	64.07 ± 1.48^{a}A	64.18 ± 1.29^{aAB}	62.39 ± 2.94^{a}A
	day10	65.39 ± 2.38^{aB}	65.75 ± 2.54^{a}A	67.71 ± 2.84^{aB}	62.46 ± 3.06^{a}A
a^*	day1	12.79 ± 1.10^{aB}	11.11 ± 0.56^{aA}	11.75 ± 1.26^{aB}	12.07 ± 0.59^{aB}
	day5	8.71 ± 1.87^{aA}	8.64 ± 1.27^{aA}	8.14 ± 0.59^{aA}	9.21 ± 0.92^{aA}
	day10	8.64 ± 0.54^{abA}	9.07 ± 1.06^{abA}	7.07 ± 0.88^{aA}	10.39 ± 1.17^{bAB}
b^*	day1	12.36 ± 1.39^{bB}	7.93 ± 1.50^{aB}	7.43 ± 0.88^{aB}	8.89 ± 1.16^{aA}
	day5	4.71 ± 1.71^{aA}	5.11 ± 1.31^{aAB}	4.71 ± 0.74^{aAB}	6.11 ± 0.69^{aA}
	day10	4.07 ± 0.34^{aA}	4.39 ± 1.46^{aB}	4.04 ± 1.30^{aA}	7.82 ± 1.40^{bA}

$^{a–b}$ Values (mean±SD) in the same row with different superscripts are significantly different ($p < 0.05$) considering different amounts addition of FSAULE.

$^{A–B}$ Uppercase letters are used for comparing the samples considering the effect of storage. Values in the same column for the same property, with different superscripts are significantly different ($p < 0.05$) considering storage time.

During heat treatment of meat products without added nitrite, browning occurs as a result of denatured MetMb formation (**Parthasarathy & Bryan, 2012**) and Maillard reaction on the surface (between sugars (added and/or from species (e.g. red paprika) and meat proteins) (**Feiner, 2006**). Browning index (BI) could represent a quantification tool for browning reactions. Regarding this research, after grilling there are no significant differences between all treatments. However, these results occur due to the high values of standard deviations indicating a wide range of result intervals. This could be the result of beef and pork meat used in pljeskavica preparations, indicating that most likely, the BI could not be used as a colour indicator in burger-type meat products that contain mixed meats (e.g. beef and pork) after heat treatment, because these meats undergo different changes during grilling.

The total colour difference (ΔE*) could provide additional data to gain more complete insight into the color difference between two products. ΔE* was calculated by comparing it to the CON colour properties of 1st day – CON1, on both, raw and grilled samples (Table 4).

On 1st day, immediately after preparation (ΔE*1), ΔE* values of raw meat particles of AUL1 and AUL2 were within the 2.8–3.1 range, while twice higher values were calculated in AUL3. However, after 5 and 10 days of cold storage, ΔE* values of CON were similar to AUL1 and AUL2 indicating similar colour changes, while ΔE* values of AUL3 were somewhat higher. ΔE* values of raw backfat particles in AUL treatments were within the 2.6–4.6 range and increased during cold storage. However, similar to the case of meat particles, changes in colour properties of CON treatment after 5 and 10 days of cold storage were similar to AUL treatments, though somewhat higher. After freeze storage, when compared to CON1, three treatments (CON, AUL1 and AUL2) had similar ΔE* values. In contrast, AUL3 had twice higher values regarding meat and backfat particles, indicating more intense colour changes when the highest amount of FSAULE was added.

Regarding grilled pljeskavicas, on day 1 (compare to CON1) progressively higher ΔE* values were observed with increase of FSAULE amounts. Samples of all treatments (included CON) grilled during cold storage had higher ΔE* values (compare to CON1), the highest observed in AUL3 mostly affected by the lowest L^* values (Table 3) indicating that FSAULE compounds in mutual reactions or in reactions with meat compounds during grilling cause more darker products` surface. The opposite was observed in ΔE* values of grilled backfat particles – ΔE* values were the lowest in AUL3. After freeze storage, ΔE* values of grilled meat particles show progressively higher values, while similar values of backfat particles were observed within modified treatments.

ΔE* values after freeze storage (ΔE* 1–90 – day 1 vs. day 90) was similar in all treatments indicating that freeze storage did not alter colour properties.

Ramírez-Navas and Rodríguez De Stouvenel (2012) reported that ΔE* values lower than 2.7 probably are not noticeable to the human eye. On the other side, **Altmann *et al*.** (**2022**) reported that colour differences of raw pork (loin) were clearly discernible to consumers when ΔE* value were ≥ 1. In the study of characteristics of ćevapi (another Serbian renowned minced meat product) **Djekic *et al.*** (**2023**) pointed that ΔE* values higher than 10 were considered significant visible differences while within 2–10 range there was a perceptible difference. Regarding our research, ΔE* values on 1st day of both raw and grilled meat and backfat particles were within 3–9 range indicating perceptible difference.

Table 4 Values of total colour differences (ΔE*)⁺ of raw and grilled pljeskavica

		CON	AUL1	AUL2	AUL3
raw meat	ΔE*1	/	3.08±1.66	2.76±0.88	6.04±3.63
	ΔE*5	12.88±1.63	14.28±1.98	14.08±1.27	20.68±1.62
	ΔE*10	27.05±2.06	27.06±2.53	25.12±1.15	30.02±0.87
	ΔE*90	4.48±0.64	5.61±1.51	5.28±1.14	9.06±2.77
grilled meat	ΔE*1	/	3.62±0.78	4.87±3.12	8.59±1.24
	ΔE*5	5.85±1.06	6.73±2.53	5.16±0.61	9.11±2.40
	ΔE*10	5.29±1.56	8.08±1.47	7.98±1.41	9.13±2.51
	ΔE*90	3.04±0.47	4.34±1.02	6.61±2.76	11.11±1.33
raw backfat	ΔE*1	/	2.58±1.30	2.85±1.42	4.61±1.86
	ΔE*5	5.59±0.81	5.38±0.54	5.32±0.54	6.90±0.64
	ΔE*10	10.33±0.64	8.03±1.17	8.16±0.47	9.13±0.94
	ΔE*90	2.26±0.28	3.71±1.19	4.28±0.81	4.77±1.83
grilled backfat	ΔE*1	/	5.19±3.49	5.47±1.01	3.91±1.94
	ΔE*5	9.91±5.36	9.56±4.31	10.12±2.10	8.35±3.77
	ΔE*10	11.10±3.71	10.92±1.02	13.00±5.03	6.23±4.97
	ΔE*90	1.27±0.54	5.43±3.39	5.45±1.17	4.53±1.56
ΔE* 1–90 raw	meat	4.48±0.64	4.49±0.65	5.76±1.92	4.47±0.56
	fat	2.26±0.28	2.31±0.22	1.61±0.41	2.87±0.68
ΔE* 1–90 grilled	meat	3.04±0.47	3.09±0.35	2.97±0.36	2.78±0.37
	fat	2.26±0.28	2.31±0.22	1.61±0.41	2.87±0.68

⁺ values were calculated comparing L^*, a^* and b^* values of all treatments during cold and after freeze storage with L^*, a^* and, b^* values of CON on 1st day.

Instrumental texture

Research studies indicate that compounds in garlic (garlic essential oil) have a visible influence on meat proteins, inducing the formation of protein cross-links and therefore increasing product toughness (**Nieto *et al.*, 2013**). However, the results of the texture profile analysis (Table 5) from this research indicate that the addition of different amounts of FSAULE did not alter instrumental texture properties. Similar results were also reported in the research that used different plant extracts in burger/patty formulations (**Bellucci *et al.*, 2021**). During cold storage, CON did not significantly differ ($p > 0.05$) compared to AUL treatments regarding all observed instrumental texture properties in all-time sections (except AUL2 hardness and cohesiveness on day 5). Additionally, during cold storage, no clear patterns of changes in instrumental texture properties were observed. Similar observations were made after freeze storage, with the difference regarding springiness and cohesiveness – after 90 days of freeze storage AUL2 and AUL3 had significantly higher springiness and cohesiveness compared to CON.

Table 5 Results of instrumental texture analysis of grilled pljeskavicas

		CON	AUL1	AUL2	AUL3
cold storage					
Hardness (N)	day1	16.48±3.89^{abA}	18.92±1.82^{bAB}	14.75±2.43^{aA}	15.95±1.75^{abA}
	day5	21.07±1.96^{bB}	18.03±2.25^{abA}	16.31±2.57^{aA}	19.38±2.49^{bB}
	day10	18.29±2.24^{abAB}	20.95±1.37^{bB}	15.84±1.92^{aA}	17.82±1.98^{aAB}
Springiness	day1	0.85±0.02^{aA}	0.84±0.03^{aA}	0.86±0.02^{aA}	0.85±0.02^{aA}
	day5	0.86±0.02^{aA}	0.88±0.02^{aB}	0.87±0.03^{aA}	0.85±0.01^{aA}
	day10	0.89±0.03^{aA}	0.87±0.02^{aAB}	0.89±0.03^{aA}	0.89±0.04^{aB}
Cohesiveness	day1	0.71±0.02^{abA}	0.69±0.05^{aA}	0.75±0.04^{bA}	0.72±0.04^{abAB}
	day5	0.70±0.04^{aA}	0.75±0.02^{abB}	0.76±0.04^{bA}	0.71±0.02^{abA}
	day10	0.74±0.04^{aA}	0.73±0.02a^{AB}	0.78±0.05^{aA}	0.76±0.05^{aB}
Chewiness (N)	day1	10.02±2.20^{aA}	11.01±1.28^{aA}	9.54±1.84^{aA}	9.72±1.30^{aA}
	day5	12.79±1.49^{aB}	11.95±1.60^{aAB}	10.77±1.80^{aA}	11.69±1.29^{aAB}
	day10	11.92±1.15^{abAB}	13.40±0.74^{bB}	10.93±1.08^{aA}	12.14±1.95^{abB}
freeze storage					
Hardness (N)	day1	16.48±3.89^{abA}	18.92±1.82^{bA}	14.75±2.43^{aA}	15.95±1.75^{abA}
	day90	20.77±2.25^{aB}	21.55±1.93^{aA}	20.47±1.70^{aB}	19.52±2.51^{aB}
Springiness	day1	0.85±0.02^{aA}	0.84±0.03^{aA}	0.86±0.02^{aA}	0.85±0.02^{aA}
	day90	0.85±0.04^{aA}	0.88±0.02^{abA}	0.90±0.03^{bB}	0.89±0.03^{bB}
Cohesiveness	day1	0.71±0.02^{abA}	0.69±0.05^{aA}	0.75±0.04^{bA}	0.72±0.04^{abA}
	day90	0.69±0.05^{aA}	0.73±0.04^{abA}	0.75±0.03^{bA}	0.75±0.04^{bA}
Chewiness (N)	day1	10.02±2.20^{aA}	11.01±1.28^{aA}	9.54±1.84^{aA}	9.72±1.30^{aA}
	day90	12.30±1.51^{aA}	13.75±1.52^{aB}	13.89±1.81^{aB}	13.27±2.83^{aB}

$^{a–b}$ Values (mean±SD) in the same row with different superscripts are significantly different ($p < 0.05$) considering different amounts addition of FSAULE

$^{A–B}$ Uppercase letters are used for comparing the samples considering the effect of storage. Values in the same column for the same property, with different superscripts are significantly different ($p < 0.05$) considering storage time.

Sensory analysis

The results of CATA analysis, presented in Figures 1 and 2, indicate that all products were similar in terms of their sensory attributes. The correspondence analysis, presented in Figure 1, explained 93.88% of the total variations. Among all treatments, AUL3 was characterised mainly by ideal texture, pleasant odour, tasty, soft, juicy, salty, pleasant colour and atypical odour, attributes which can be marked as affirmative (except atypical odour). On the other side, compared to other treatments, AUL1 was mainly characterised by garlic odour and taste like garlic, two of four garlic-like sensations, attributes which can show whether an increase of FSAULE amount led to increased perception of garlic-like sensations. All treatments were the least characterised by atypical colour and intense taste like garlic.

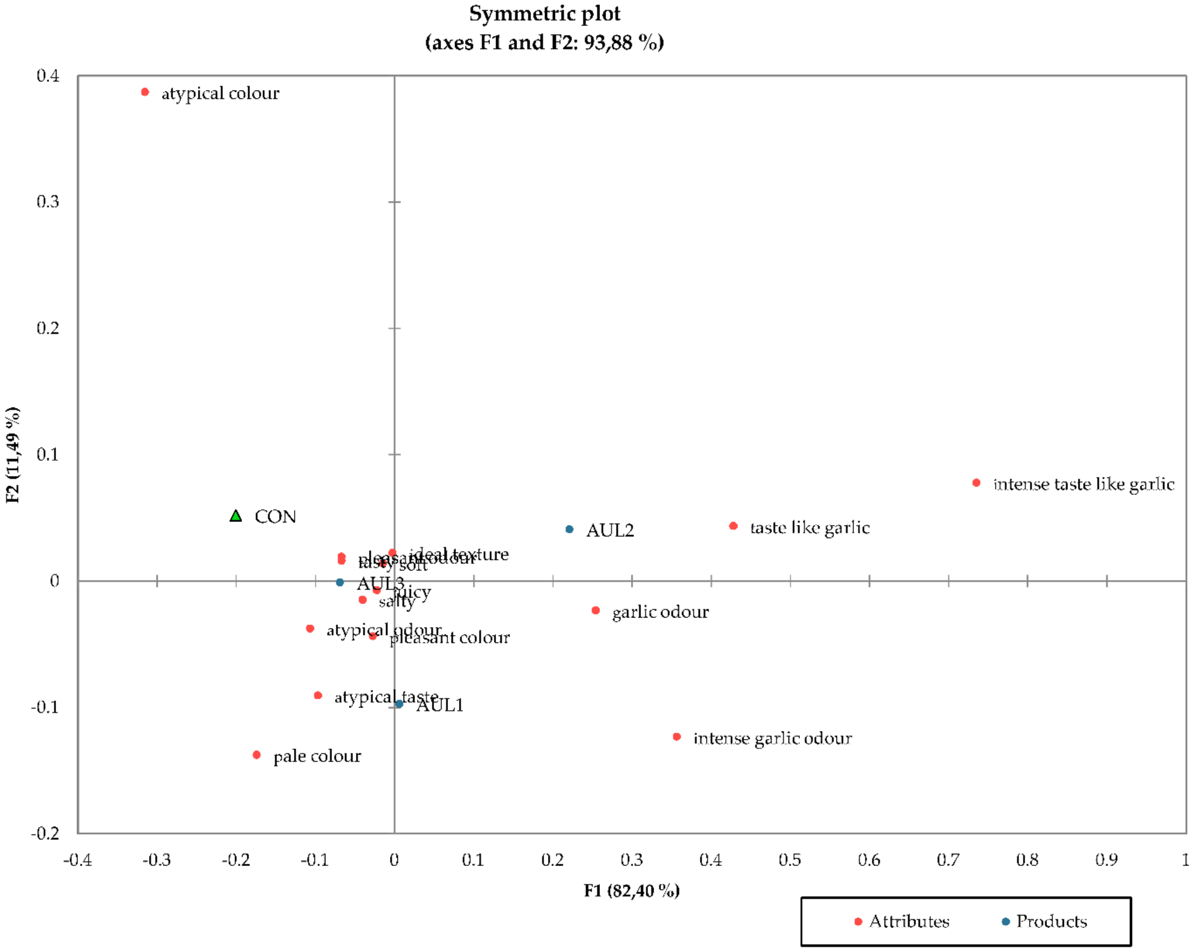

Figure 1 Biplot projection of the pljeskavicas and the sensory attributes in the first and second dimensions of correspondence analysis performed on data obtained from CATA questions

Figures 2–4 shows the frequencies (%) of absence (No) and presence (Yes) of sensory attributes in pljeskavicas with FSAULE (AULs) and, at the same time, absence or presence in control. All sensory attributes were divided into three groups: affirmative attributes (pleasant colour, pleasant odour, tasty, soft, juicy, ideal texture and salty – Fig. 2), negative attributes (pale colour, atypical colour, atypical odour and atypical taste – Fig. 3) and garlic-like sensations (garlic odour, intense garlic odour, taste like garlic and intense taste like garlic – Fig. 4). Regarding affirmative attributes, more than a half of consumers marked that all these attributes were present in both AULs and CON (Yes/Yes). Moreover, from 70 to 84% consumers marked that pleasant colour, odour and taste were present in both AULs and CON. This indicates that the addition of different amounts of FSAULE probably did not alter the colour, odour and taste. On the other side, every 7–10th consumer found that pleasant odour, tasty, soft, juicy and ideal texture were not present in AULs, but were present in CON. In that case, mean liking scores dropped by 0.94, 1.68, 0.90, 0.79 and 1.47, respectively. However, this could not be attributed to the FSAULE addition because 7.2–17.2% of consumers also marked that stated attributes were present in AULs and not in CON.

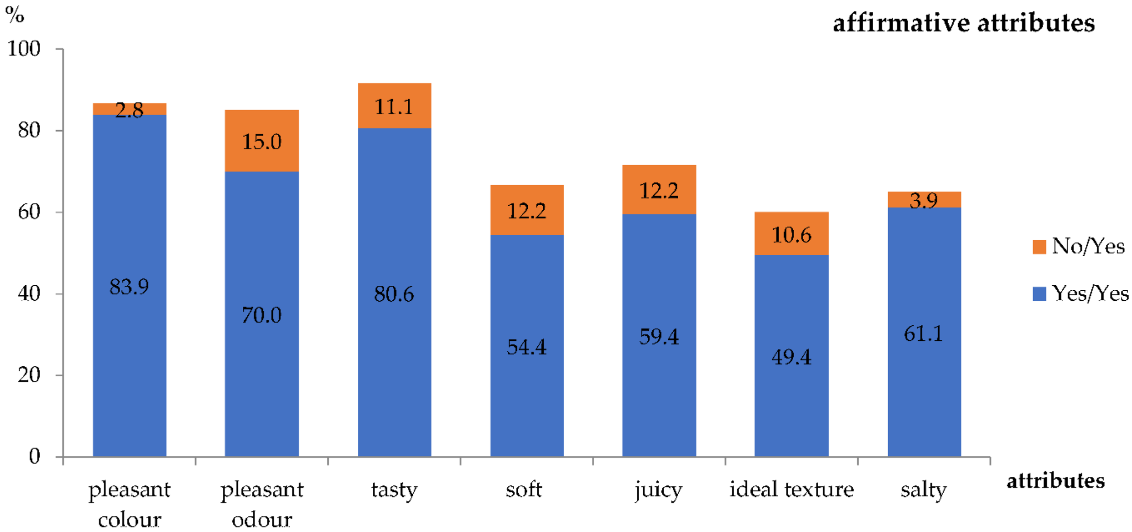

Figure 2 Frequencies (%) of absence (No) and presence (Yes) of affirmative sensory attributes in pljeskavicas with FSAULE (AULs) and at the same time absence or presence in CON – AULs/CON.

The results of affirmative attributes are complemented by the negative attributes results (Fig. 3) – only 3.3–13.9% marked that these attributes were present in AULs, but absent in CON. Contrary to this, at least 2/3 of consumers marked that these attributes were not present in either AULs or CON. This also indicates that the majority of consumers did not negatively perceive changes in colour (which were instrumentally detected) and did not notice or negatively perceive changes in odour and taste. When consumers marked that negative attributes were present in AULs but not present in CON, mean liking scores were lower from 0.36 (pale colour) to 1.36 (atypical taste), while mean liking scores were higher by 0.79 in the case of atypical colour.

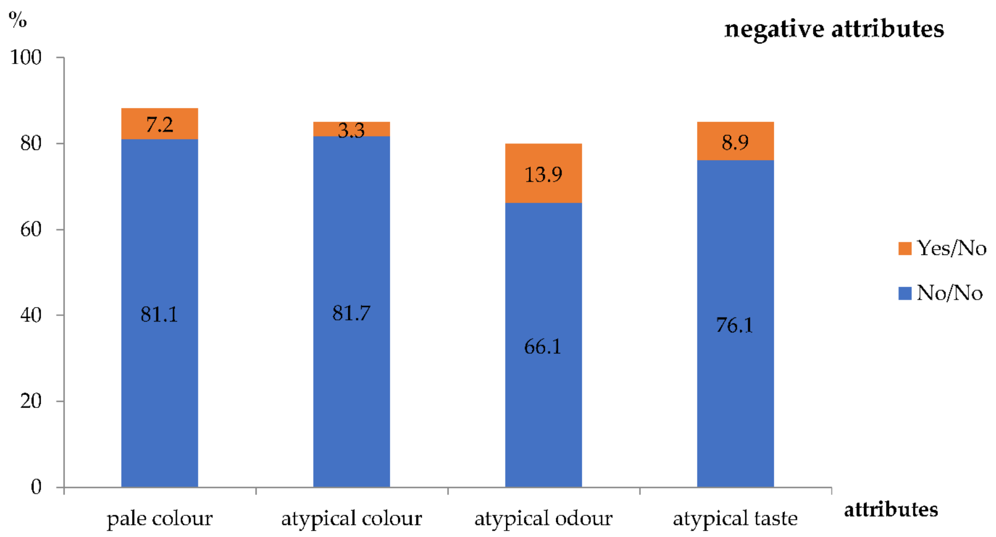

Figure 3 Frequencies (%) of absence (No) and presence (Yes) of negative sensory attributes in pljeskavicas with FSAULE (AULs) and at the same time absence or presence in CON – AULs/CON.

Most of the consumers did not mark the presence of intense garlic sensations (intense garlic odour and intense garlic taste) in both AULs and CON (No/No – Fig. 4). Moreover, when they marked the presence of intense garlic odour and intense taste like garlic in AULs, but not in CON (Yes/No), the mean liking scores dropped only by 0.24 and 0.21, respectively. On the other hand, the No/No option regarding garlic odour and taste like garlic was recorded by 74.4 and 63.3% of consumers, respectively. Only regarding taste like garlic, a noticeable number of consumers (26.7%) mark it as present in AULs but not in CON, however, without influence on acceptance – the mean liking score was higher by 0.03 compared to the No/No liking score. Therefore, when consumers noticed atypical odour and atypical taste in AULs but not in CON (Yes/No – Fig. 3) and the mean liking scores were lower by 0.62 and 1.36 respectively, this was not a result of the addition of FSAULE.

The research of **Śmiecińska *et al.*** (**2022**) reported that rabbit meat burgers with ramson powder (0.35 g/kg) received significantly higher marks in terms of appearance, hardness, juiciness and overall acceptability compared to control and treatment with the same amount of garlic powder. Moreover, in terms of aroma-intensity, flavour-intensity, off-odours and off-flavour, there were no differences compared to treatments with garlic powder and a mixture of garlic+ramsons powder, which is very similar to the results of this research. The research of **Śmiecińska *et al.*** (**2022**) complements this and our previous research **Kurćubić *et al.*** (**2023**), confirming that ramsons can be used in burger-type meat products as a garlic replacement.

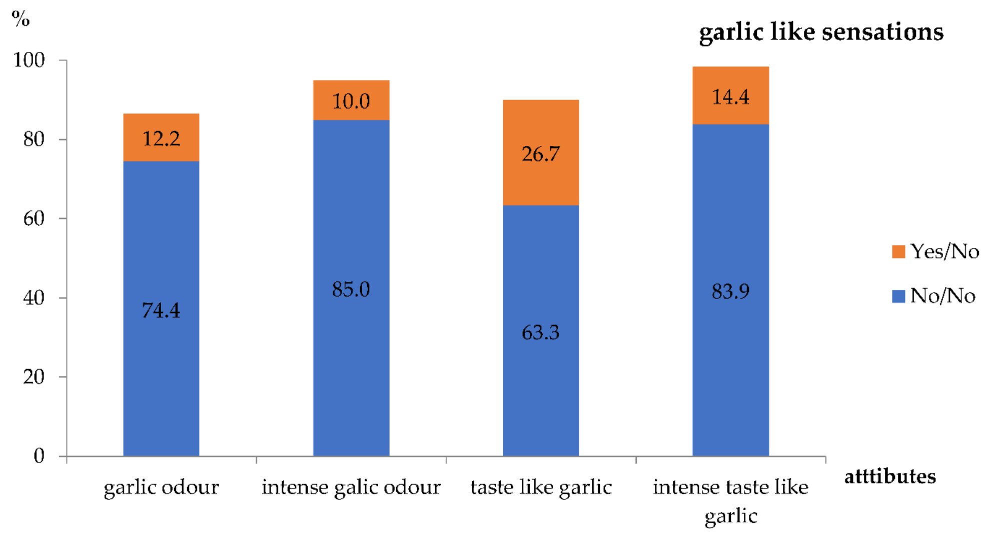

Figure 4 Frequencies (%) of absence (No) and presence (Yes) of garlic-like sensations in pljeskavicas with FSAULE (AULs) and at the same time absence or presence in CON – AULs/CON.

CONCLUSIONS

The utilization of natural supplements containing various bioactive compounds, such as wild garlic (*Allium ursinum* L.) extracts, has gained increasing attention in the last several decades and exhibits promising results in the prevention of the spoilage-related processes. However, it is important to consider both the technological and sensory properties of such products. In particular, the use of freshly squeezed *Allium ursinum* L. extract in higher amounts in burger-type meat products could result in darker (both raw and grilled) products, likely attributed to the presence of natural colourants within the FSAULE. Nevertheless, this change in colouration did not lead to negative perceptions among consumers, as most participants indicated that all positive sensory attributes, including an appealing color, remained in both control and FSAULE-treated samples.

Based on the results obtained in this research, the application of freshly squeezed *Allium ursinum* L. extract demostrates excellent potential in burger-type products. Additionally, further research could be focused on exploring the utilization of higher extract concentrations and alternative extraction techniques, such as supercritical extraction, which may yield extracts with enhanced beneficial properties.

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