

Effect of Storage Time and Packaging Conditions on Microbiological Stability of Veggie Burgers

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This study investigates the impact of storage time and packaging conditions on the microbiological stability of veggie burgers. Two types of veggie burgers, one based on millet porridge and the other on chickpea and broccoli, were prepared with and without the addition of 0.1% rosemary essential oil. The burgers were packaged using three different methods: vacuum packaging (VAC), modified atmosphere packaging (MAP), and packaging under an unchanged atmosphere (ATM). Microbiological analyses were conducted over a 24-day storage period at 2 ± 0.5 °C to evaluate the total viable count (TVC), Enterobacteriaceae, and microscopic filamentous fungi. Results indicated that the addition of rosemary oil significantly improved the microbiological stability of the burgers across all packaging methods. VAC and MAP packaging methods were more effective in reducing microbial growth compared to ATM. Texture analysis showed no significant differences between burgers with and without rosemary oil. The organoleptic evaluation revealed that while the addition of rosemary oil enhanced microbiological stability, it negatively impacted the taste and aroma, making the burgers less desirable to consumers. Further research and formulation adjustments are recommended to optimize the sensory attributes while maintaining microbiological safety.

Keywords: veggie burgers, microbiological stability, storage time, packaging conditions, rosemary essential oil

1 Introduction

In following the prevailing food trends, consumers take into account many factors that influence their purchasing choices. These include religious, cultural, environmental, financial, psychological, but also health considerations. Sometimes, this knowledge comes from unverified sources of information, which can hinder the introduction of new, correct dietary principles. Following certain types of diets for health reasons is associated with the desire to lose weight, as well as problems related to intolerances such as lactose or gluten. Many consumers still do not realize that the need for elimination diets should only apply to those affected by food intolerances and not be a dietary trend. In healthy individuals, an improperly composed diet that excludes the above products can lead to nutritional deficiencies (Bielawska et al., 2019, Gadzała & Lesiów 2019).

The development of new, innovative, health-promoting products and new food production and storage methods

is currently the biggest challenge for manufacturers. Instead of chemical preservatives, their alternatives are being tested, which are additives of natural compounds that prevent food spoilage and protect against pathogenic microorganisms (Bondi et al., 2017).

1.1 Essential Oils in Food

Essential oils are obtained from raw plant materials used in the therapeutic, food, pharmaceutical, and cosmetic industries. The plant species from which essential oils can be extracted are usually considered to be all those plants that contain more than 0.01% essential oil in the dry weight of the raw material. In individual plant species, the same organs produce the oil (Nurzyńska-Wierdak, 2015). Essential oils are defined as volatile mixtures of organic substances derived from plant secretions or parts. Essential oils are often obtained by steam distillation, but can also be extracted by extrusion, adsorption and organic solvents (Adaszyńska & Swarczewicz, 2012).

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The addition of essential oils to foods to prevent the spread of pathogens that adversely affect the shelf life of those foods at all stages of food processing is gaining popularity. Essential oils' antioxidant, anti-inflammatory and antimicrobial properties have been studied in depth in the literature. However, when it comes to the mechanism of action of essential oils in inhibiting food spoilage, there are still many inaccuracies and gaps in understanding this process (Osemwegie et al., 2024).

1.2 Convenience Food

Convenience foods are those suitable for consumption straight out of the package or require a short heat treatment before consumption. It is food that is appropriately packaged, portioned, preserved, and constitutes all or part of a meal. It is very attractive to consumers in terms of speed and ease of preparation. We can distinguish between ready-to-eat foods: ready to process, ready to cook, ready to heat, ready to eat and ready to serve (Adamczyk 2010; Makała, 2011; Scholliers, 2015; Chen et al., 2020; Temgire et al., 2021).

Food spoilage is synonymous with food deterioration. The first signs of deterioration can be seen as soon as the food is produced. Packaging is used to reduce the rate of negative changes in the food storage process. The food industry constantly strives to extend the freshness of food. This objective is the main driving force behind the use of ever new packaging and food packaging methods (Pluta et al., 2013).

Packaging is undoubtedly one of the most popular, widely used methods to prolong freshness, shelf life and guarantee products' microbiological safety (Michalska-Požoga & Rydzkowski, 2013).

2 Material and Methods

2.1 Raw Materials for the Burger

The following raw materials and products were used to make the burgers. Buckwheat, carrots, white onions, red onions, wheat flour, breadcrumbs, sesame seeds, sunflower seeds, rapeseed oil, ginger root, soy sauce, parsley, broccoli, chickpeas, eggs, yeast flakes, semolina, garlic, mustard, cream cheese, white salt, black pepper, cardamom, nutmeg, hot pepper, sweet pepper, garlic, herbs de Provence. These products were purchased from a local shop (N 50.018769, E 22.019356 Rzeszów, Poland).

2.2 Essential Oil

EO prepared from *Rosmarinus officinalis* L. was purchased from Slovak company Hanus (Nitra, Slovakia) and was

extracted by steam distillation of dried fruits. It was stored in the dark at 4 °C throughout the analyses.

2.3 Burger Production and Packaging Technology

To make the burgers with a millet porridge base, the first step was to cook the millet porridge according to the instructions on the packet. Carrots, ginger, chopped onions and parsley were grated. All the ingredients were mixed using a MESKO – AGD model KU2 – 4E mixer. The resulting mass was weighed and divided into two equal parts. To one part, 0.1% of the oil was added to the weight of the ingredients and constituted the test sample.

To prepare the broccoli burgers, the broccoli was cooked, cooled and chopped. Meanwhile, the chickpeas were drained and then blended with oil. The cheese was grated, the onion was chopped, the garlic was pressed, and the remaining ingredients were added and mixed in a "Mesko-AGD" planetary mixer (Skarżysko-Kamienna, Poland). The prepared mass was weighed and divided into two equal parts. To one of them 0.1% rosemary oil was added in relation to the weight of the ingredients – this constituted the test sample, while the other part of the mass – with no oil added – constituted the control sample.

The second part of the mixture without the essential oil was used as a control. Burgers weighing approximately 50 g (before baking) were formed from the prepared mass and placed on a baking tray lined with baking paper. The burgers were baked at 200 °C for 30 minutes in a Sveba Dahlen oven (Fristad, Sweden). After this time, the burgers were cooled and packed.

The burgers were packaged individually under an unchanged atmosphere (ATM); the packaging material was PE string bags measuring 10 × 10 cm, 40 µm thick. Vacuum packing (VAC) of the burgers was carried out using a VBN-18SB vacuum packer from RM Gastro, the packaging material was PA/PE foil pouches 10 × 14 cm, 80 µm thick. BIOGON C30/MAPAX 3 gas was used for modified atmosphere gas (MAP) packaging of the burgers, with 70% nitrogen and 30% carbon dioxide, the packaging material was 10 × 14 cm PA/PE plastic bags, 80 µm thick.

The following variants of veggie burgers were made as part of the study:

- KK – burger based on millet groats without the addition of essential oil (control sample).
- KO – burger based on millet groats with 0.1% addition of rosemary oil (research sample).
- BK – burger based on chickpeas and broccoli seeds without the addition of essential oil (control sample).

- BO – burger based on chickpea seeds and broccoli with 0.1% rosemary essential oil added (research sample).

2.4 Microbiological Analysis of Burgers

The burgers obtained in the study were subjected to microbiological analysis. Packaged by three different methods, the burger samples, the control samples, as well as the samples with essential oil added, were stored in a cold room at 2 ± 0.5 °C. The first samples were taken on the day the burgers were made on the laboratory scale and marked as 'day 0'. Samples were taken at three-day intervals until the 24th day after the burgers were made and packaged, for surface culture on three types of microbiological media to check their microbiological stability.

Six burgers of each type were allocated for microbiological evaluation, from which 10 replicates were randomly sampled. The material for microbiological tests was secured in 100 mL containers. Samples weighing 5.00 ± 0.1 g were cut from the burgers and transferred to sterile disposable containers. A total of 45 ml of saline (0.9% NaCl) was added to the sample containers and mixed in a BagMixer® 400S homogenizer (stomacher) (Interscience, Saint Nom la Brétèche, France). Serial dilutions from 10^{-1} to 10^{-5} were prepared from the solutions. To determine the total viable count (TVC), the samples were cultured in Trypticasein Soy Lab-Agar medium (TSA, Biocorp, Paris, France) according to (ISO 11133:2014). The samples were incubated for 48 h at 37 °C under aerobic conditions. For the isolation of Enterobacteriaceae, Violet Red Bile Glucose Agar (VRBL, Biocorp, Paris, France) was used according to ISO (ISO 9308-1:2014). The inoculated plates were incubated at 37 °C for 24 h. To determine the number of microscopic filamentous fungi, Malt Extract Agar (MEA, Sigma-Aldrich, St. Louis, MO, USA) was used according to (ISO 16212:2017). The plates were incubated at 25 °C for 48 h. Microbiological tests were performed in triplicate for each sample and each medium. After incubation, the bacterial colonies of the plates were counted, with plate counts ranging from 15 to 150 colonies for Enterobacteriaceae and 30 to 300 colonies for TVC (ISO 4833-1:2013).

2.5 Testing Burger Texture Parameters

The texture parameters of burgers based on millet groats were tested using a TPA test (double compression test) using an EZ-Test EZ-LX Universal Testing Machine (Shimadzu, Japan) with a maximum nominal force of 1,000 N. The determination was carried out using a probe with a diameter of 25 mm. The crosshead travel was 50 mm.min⁻¹, to a compression of 50% of the sample height. The determination resulted in the following

parameters: hardness, brittleness, gumminess and chewiness, and elasticity. Measurements were made in three independent repetitions.

2.6 Organoleptic Evaluation of Burgers

The burgers without added essential oil were subjected to organoleptic evaluation, which constituted the control sample, and the burgers with added rosemary oil at a concentration of 0.1% constituted the test sample. The organoleptic evaluation of the burgers was carried out using a 5-point scale by a group of 12 people. It was assumed that the award of 5 points indicated a burger of very good quality, while the award of 1 point indicated unacceptable quality. Weighting factors were applied. In both burger variants, product characteristics such as colour, taste, aroma, texture, and overall appearance were assessed. Respondents were given samples of the burgers, both the control sample and the research sample, and were asked to score them from 1 to 5 according to the organoleptic evaluation sheets (Table 1). The scores obtained were added up and an average was drawn for both burger variants.

2.7 Statistical Analysis

Statistical analysis was performed using Statistica version v.13.1 (Stat-Soft), performing univariate and multivariate analysis of variance (ANOVA) and a post-hoc test (Fisher's NIR) for each microbiological study parameter described, with a significance level of $p \leq 0.05$.

3 Results and Discussion

3.1 Evaluation of the Total Viable Count

Table 2 shows the results of the microbiological analysis of TVC in burgers with and without rosemary oil added, packaged using different packaging methods. On the first day, immediately after heat treatment, the burger samples with and without the addition of rosemary oil on a millet groats base were not packaged with VAC and MAP. The first day was a control sample. However, for statistical purposes, the values from the first day were applied to all packaging variants.

The TVC of the KK burgers immediately after heat treatment was $2.26 \log \text{CFU.g}^{-1}$ for the variant without added essential oil, while the KO variant (with added oil) reduced the total microbial content of the test product, as it was $1.81 \log \text{CFU.g}^{-1}$, and this difference was statistically significant ($p \leq 0.05$).

Throughout the entire storage period of the KK and KO ATM burgers, an increase in total microbial counts was observed in the majority of the samples of the burgers on a millet-based basis with and without essential oil (KO

Table 1 Organoleptic evaluation sheet using the 5-punt method for the burger without added essential oil (own elaboration)

No.	Quality differentiator	Weighting factor	5 pt	4 pt	3 pt	2 pt	1 pt
1.	colour	0.15	– the color is very inviting, brown.	– the color is inviting, brown.	– colour uninviting, visible burning.	– color inadequate and uninviting, visible burning.	– color unacceptable, uninviting, visible burning.
2.	taste	0.25	– taste adequate, desirable, perceptible vegetable flavor.	– flavor suitable, less desirable, perceptible vegetable flavor.	– taste adequate, without foreign components.	– taste inadequate, perceptible foreign aftertaste.	– taste strongly inappropriate, perceptible foreign components.
3.	fragrance	0.2	– aroma desirable, perceptible pleasant vegetable aroma.	– flavor aroma, less desirable, perceptible vegetable smell.	– odor neutral, without foreign components.	– smell inadequate, noticeable foreign odor.	– odor strongly inappropriate, perceptible foreign odors.
4.	texture	0.2	– soft, tender, perceptible pieces of the ingredients used.	– tender, perceptible chunks of the ingredients used, gently falling apart.	– decaying, noticeable chunks of ingredients used, dry.	– very decaying, dry, hard.	– very decaying, very dry, very hard.
5.	general appearance	0.2	– shape suitable, characteristic of burgers, visible pieces of ingredients used, inviting appearance.	– shape characteristic of burgers, visible pieces of ingredients used, adequate appearance.	– slightly deformed shape, visible oversized pieces of ingredients, adequate appearance.	– clear deformation of shape, uninviting appearance.	– strongly distorted shape, unsuitable appearance.

Table 2 Microbiological analysis of the total number of microorganisms

Storage day	Variant	ATM	VAC	MAP	Factor I	Variant	ATM	VAC	MAP	Factor I	Variant	ATM	VAC	MAP	Factor I
1	KK	2.26 ^b ±0.12	2.26 ^b ±0.12	2.26 ^b ±0.12	–	BK	2.31 ^b ±0.05	2.31 ^{ab} ±0.05	2.31 ^b ±0.05	–		2.31 ^b ±0.05	2.31 ^{ab} ±0.05	2.31 ^b ±0.05	–
	KO	1.81 ^a ±0.03	1.81 ^a ±0.03	1.81 ^a ±0.03	–	BO	1.85 ^a ±0.15	1.85 ^a ±0.15	1.85 ^a ±0.15	–		1.85 ^a ±0.15	1.85 ^a ±0.15	1.85 ^a ±0.15	–
3	KK	2.69 ^{cA} ±0.09	2.39 ^{bA} ±0.09	2.24 ^{bA} ±0.24	0.26	BK	2.61 ^{bA} ±0.06	2.54 ^{b,cA} ±0.01	2.51 ^{bA} ±0.03	0.29		2.59 ^{bA} ±0.06	2.55 ^{b,cA} ±0.03	2.44 ^{bA} ±0.12	0.47
	KO	2.30 ^{bA} ±0.00	2.15 ^{a,bA} ±0.15	2.15 ^{a,bA} ±0.15	0.65	BO	2.59 ^{bA} ±0.06	2.15 ^{a,bA} ±0.15	2.55 ^{b,cA} ±0.03	0.47		3.63 ^{dB} ±0.05	3.08 ^{d,eA} ±0.12	3.04 ^{cA} ±0.04	≤0.05
6	KK	4.02 ^{fA} ±0.02	3.69 ^{d,eA} ±0.09	3.30 ^{c,dA} ±0.30	0.14	BK	3.17 ^{cA} ±0.26	3.15 ^{cA} ±0.15	3.15 ^{cA} ±0.15	0.40		4.01 ^{eA} ±0.11	3.89 ^{dA} ±0.19	3.89 ^{dA} ±0.19	0.85
	KO	3.39 ^{dA} ±0.09	3.30 ^{cA} ±0.30	3.15 ^{cA} ±0.15	0.72	BO	4.01 ^{eA} ±0.11	3.65 ^{d,eA} ±0.05	3.65 ^{d,eA} ±0.05	0.79		4.71 ^{fgA} ±0.09	4.63 ^{h,i,j,kA} ±0.04	4.49 ^{e,f,gA} ±0.01	0.15
9	KK	3.98 ^{fC} ±0.02	3.81 ^{eB} ±0.03	3.63 ^{dA} ±0.03	≤0.05	BK	3.94 ^{gA} ±0.24	3.47 ^{c,dA} ±0.01	3.47 ^{c,dA} ±0.01	≤0.05		4.55 ^{fgB} ±0.01	4.43 ^{h,iB} ±0.06	4.20 ^{d,eA} ±0.05	≤0.05
	KO	3.65 ^{eB} ±0.05	3.40 ^{c,dA} ±0.02	3.47 ^{c,dA} ±0.01	≤0.05	BO	4.55 ^{fgB} ±0.01	4.15 ^{eA} ±0.03	4.15 ^{eA} ±0.03	≤0.05		4.94 ^{h,i,jA} ±0.34	4.93 ^{j,k,lA} ±0.15	4.90 ^{g,h,iA} ±0.05	0.99
12	KK	4.58 ^{hB} ±0.02	4.42 ^{gA} ±0.02	4.36 ^{eA} ±0.02	≤0.05	BK	4.71 ^{fgA} ±0.09	4.36 ^{eA} ±0.02	4.36 ^{eA} ±0.02	≤0.05		4.71 ^{fgA} ±0.09	4.63 ^{h,i,j,kA} ±0.04	4.49 ^{e,f,gA} ±0.01	0.15
	KO	4.23 ^{gB} ±0.03	4.20 ^{fA,B} ±0.03	4.15 ^{eA} ±0.03	≤0.05	BO	4.71 ^{fgA} ±0.09	4.15 ^{eA} ±0.03	4.15 ^{eA} ±0.03	≤0.05		4.94 ^{h,i,jA} ±0.34	4.93 ^{j,k,lA} ±0.15	4.90 ^{g,h,iA} ±0.05	0.99
15	KK	4.80 ^{iA} ±0.02	4.69 ^{g,h,iA} ±0.01	4.54 ^{e,fA} ±0.06	0.23	BK	4.80 ^{iA} ±0.02	4.54 ^{e,fA} ±0.06	4.54 ^{e,fA} ±0.06	0.23		4.94 ^{h,i,jA} ±0.34	4.93 ^{j,k,lA} ±0.15	4.90 ^{g,h,iA} ±0.05	0.13
	KO	4.28 ^{gA} ±0.02	4.23 ^{fA} ±0.05	4.18 ^{eA} ±0.00	0.16	BO	4.80 ^{iA} ±0.02	4.18 ^{eA} ±0.00	4.18 ^{eA} ±0.00	0.16		4.94 ^{h,i,jA} ±0.34	4.93 ^{j,k,lA} ±0.15	4.90 ^{g,h,iA} ±0.05	0.13
18	KK	4.98 ^{iA} ±0.02	4.69 ^{g,h,iA} ±0.09	4.30 ^{eA} ±0.30	0.72	BK	5.26 ^{kB} ±0.08	4.30 ^{eA} ±0.30	4.30 ^{eA} ±0.30	0.72		5.26 ^{kB} ±0.08	5.09 ^{k,l,m,A,B} ±0.05	4.98 ^{h,iA} ±0.02	0.09
	KO	4.39 ^{gA} ±0.09	4.30 ^{fA} ±0.30	4.15 ^{eA} ±0.15	0.72	BO	4.87 ^{g,h,iA} ±0.03	4.15 ^{eA} ±0.15	4.15 ^{eA} ±0.15	0.72		4.87 ^{g,h,iA} ±0.03	4.24 ^{g,h,iA} ±0.24	4.45 ^{e,fA} ±0.15	0.15
21	KK	4.97 ^{iB} ±0.01	4.82 ^{h,iA} ±0.01	4.80 ^{fgA} ±0.02	≤0.05	BK	5.48 ^{kA} ±0.04	4.80 ^{fgA} ±0.02	4.80 ^{fgA} ±0.02	≤0.05		5.48 ^{kA} ±0.04	5.32 ^{l,mA} ±0.06	5.24 ^{iA} ±0.06	0.11
	KO	4.69 ^{h,iB} ±0.01	4.50 ^{fg,hA} ±0.01	4.46 ^{e,fA} ±0.01	≤0.05	BO	5.24 ^{j,k,lB} ±0.06	4.46 ^{e,fA} ±0.01	4.46 ^{e,fA} ±0.01	≤0.05		5.24 ^{j,k,lB} ±0.06	4.45 ^{h,i,jA} ±0.15	4.74 ^{g,h,iA} ±0.04	≤0.05
24	KK	5.45 ^{kB} ±0.02	5.35 ^{jA,B} ±0.05	5.26 ^{hA} ±0.00	≤0.05	BK	5.59 ^{kA} ±0.04	5.26 ^{hA} ±0.00	5.26 ^{hA} ±0.00	≤0.05		5.59 ^{kA} ±0.04	5.46 ^{mA} ±0.01	4.82 ^{g,h,iA} ±0.52	0.30
	KO	5.10 ^{jA} ±0.10	4.93 ^{iA} ±0.15	4.96 ^{g,hA} ±0.18	0.71	BO	5.40 ^{kA} ±0.04	4.96 ^{g,hA} ±0.18	4.96 ^{g,hA} ±0.18	0.71		5.40 ^{kA} ±0.04	4.90 ^{j,k,lA} ±0.30	4.77 ^{g,hA} ±0.07	0.17
Multivariate analysis of variance (ANOVA)															
Factor 1		≤0.05	≤0.05	≤0.05			≤0.05	≤0.05	≤0.05			≤0.05	≤0.05	≤0.05	
Factor 2		≤0.05	≤0.05	≤0.05			≤0.05	≤0.05	≤0.05			≤0.05	≤0.05	≤0.05	
Factor 1 × Factor 2		0.05	0.97	0.92			0.59	0.16	0.50			0.59	0.16	0.50	

Parameters marked with the same lowercase letter in a column are not statistically significantly different at a significance level of $p \leq 0.05$. Parameters marked with the same uppercase letter in a row are not statistically significantly different at a significance level of $p \leq 0.05$. Factor 1 – day of storage; Factor 2 – variant; Factor 1 – packaging method. KK – millet goats-based burger without essential oil, BO – millet goats-based burger with essential oil added, BK – broccoli-based burger without essential oil, BO – broccoli-based burger with essential oil added

and KK). For burgers without essential oil, no statistically significant differences were found between days 6 and 9 of storage and days 18 and 21 of storage. However, for burgers with essential oil, no significant statistical differences were found between 12, 15 and 18 days of storage. Total microbial counts for ATM-packed products without essential oil ranged from 2.26 to 5.45 log CFU.g⁻¹ throughout the storage period, while those with essential oil ranged from 1.81 to 5.10 log CFU.g⁻¹. For each of the time periods studied, the number of colony-forming units in the samples packaged under unchanged atmosphere (ATM) was statistically significantly different between the burger based on millet groats without (KK) and that with essential oil (KO).

The total microbial count for the burger in the variant without the addition of essential oil immediately after heat treatment was 2.31 log CFU.g⁻¹, while the addition of oil to the product reduced the total microbial count and the value was 1.85 log CFU.g⁻¹, and the difference between the tested variants was statistically significant ($p > 0.05$).

Throughout the storage period of the BK and BO burgers using the ATM packaging method, an increase in the total microbial count was observed for both burgers without and with the addition of essential oil (BK and BO). For burgers without added rosemary oil, no statistically significant differences were found between 1 and 3 days of storage, 12 and 15, 15 and 18, as well as 18, 21, and 24 days of storage. In contrast, no significant statistical differences were found for burgers with essential oil added between 12 and 15, 12 and 18, 18 and 21, and 21 and 24 days of storage. The total microbial count for burgers packaged by the ATM method without essential oil ranged from 2.31 to 5.59 log CFU.g⁻¹ throughout the storage period, while for burgers with added oil it ranged from 1.85 to 5.40 log CFU.g⁻¹. On days 3, 9, 12, 21 and 24 of storage using the ATM method, no statistically significant differences in the number of colony-forming units were observed between the burger based on chickpea and broccoli seeds (BK) and the one with added essential oil (BO).

The total microbial count, for VAC-packed KK products (without essential oil), ranged from 2.26 to 5.35 log CFU.g⁻¹ throughout the storage period, while for KO products (with rosemary oil) it ranged from 1.81 to 4.93 log CFU.g⁻¹. In the majority of cases, an increase in total microbial counts was observed in the samples of the burgers based on millet groats with and without essential oil (KO and KK). For burgers without added rosemary oil, no significant statistical differences were found between storage days 1 and 3, storage days 6 and 9, storage days 12, 15 and 18, and storage days 15, 18 and 21. In contrast,

for burgers with rosemary oil added, no statistically significant differences were found between 1 and 3 days of storage, 6 and 9 days of storage, and between 12, 15, 18 and 21 days of storage. In 6 of the 8 time periods (1, 6, 9, 15, 18 and 24 days of storage), the number of colony-forming units in the vacuum-packed samples (VAC), was statistically significantly different between the burgers based on millet groats without (KK) and those with the addition of essential oil (KO).

In the VAC packaging method, an increase in the total microbial count was observed throughout the storage period for the BK and BO burger samples based on chickpea and broccoli seeds, while for the burger samples with added oil, a significant decrease in the total microbial count was also observed between days 15 and 18, but the count increased again on subsequent days.

BK burgers without added essential oil showed a statistically significant increase in total microbial count between days 3, 6, 9 and 12 of storage. In contrast, for the BO burger (with the addition of essential oil), statistically significant differences were shown between 1 and 3, 6, 9, 12 days of storage and also between 15 and 18 days of storage.

The total microbial counts for the VAC-packed BK burgers ranged from 2.31 to 5.46 log CFU.g⁻¹ throughout the storage period, while for the BO burgers, they ranged from 1.85 to 4.90 log CFU.g⁻¹.

Throughout the storage period of the MAP-packaged burgers, an increase in total microbial counts was observed in the majority of the burger samples based on the millet groats with and without essential oil (KO and KK). For the burgers without rosemary oil, no statistically significant differences were found between days 1 and 3 of storage, days 6 and 9 of storage and between days 12, 15 and 18 of storage. In contrast, for burgers with rosemary oil, no statistically significant differences were found between 1 and 3 days of storage, 6 and 9 days of storage, and between 12, 15, 18 and 21 days of storage. Total microbial counts for MAP-packed products without essential oil ranged from 2.24 to 5.26 log CFU.g⁻¹ throughout the storage period, while those with rosemary oil ranged from 1.81 to 4.96 log CFU.g⁻¹. In 1 of the 8 time periods (1 day of storage), the number of colony-forming units in the samples packaged in modified atmosphere gas (MAP) was statistically significantly different between the burger based on millet groats without (KK) and that with essential oil (KO).

The results obtained in this study are reflected in scientific results available in databases. Thakur et al. (2019), in a study on the shelf life of chicken patties, used three levels of oregano essential oil to reduce microbial

growth on the product and found lower levels of bacteria than the control (without essential oil). The results of a study by Ahmed et al. (2022) are also promising, as they examined the effect of polyphenolic compounds present in cloves on the microbiological properties, lipid stability and sensory attributes of beef burgers during cold storage at 4 °C, for a period of 21 days and tested at 7-day intervals. The authors of this study observed a lower number of TVCs compared to control samples. In a study by Vergara et al. (2021), which investigated the effect of different forms of oregano on the quality of millet groats burgers vacuum-packed and stored in a cold store for 14 days, higher values of total microbial count were obtained in the samples analysed than in the vegetarian burgers. In millet groats burgers without added rosemary oil (control sample), the total microbial count, ranged from 4.76 log CFU.g⁻¹, on the day of packaging, to 7.45 log CFU.g⁻¹, on day 14 of storage. In millet groats burgers with 0.08% oregano oil, the values ranged from 5.33 log CFU.g⁻¹ to 7.71 log CFU.g⁻¹. In a study by Assanti et al. (2021), the microbiological stability of chicken meat-based burgers with a combination of chitosan dip, vacuum-packed and stored for 12 days, was tested. Vacuum-packed chicken burgers without the addition of chitosan dip reached a total microbial count of 7 log CFU.g⁻¹ on the 12th day of storage, and this value was higher than that of the burgers tested in their own work with and without the addition of rosemary oil. The value of the total microbial count increased with increasing storage time, similarly to the vegetarian burgers. In the work of Vergara et al. (2021), the effect of different forms of rosemary on the quality of millet groats burgers vacuum-packed and stored under refrigeration

for 14 days was studied. For the control sample (without added rosemary), results ranging from 5.11 log CFU.g⁻¹ to 6.98 log CFU.g⁻¹ were obtained, on the day of packaging and on day 14 of storage. In the work of Kryževičūtė et al. (2017), the effect of raspberry pomace extract on the quality and shelf life of beef burgers packed in a modified gas atmosphere (80% O₂ and 20% CO₂) and stored under refrigeration for 26 days was investigated. Values of total microbial counts ranged from about 5 log CFU.g⁻¹ on day 1 of storage to about 7 log CFU.g⁻¹ on day 26 of storage.

3.2 Evaluation of Total Enterobacteriaceae and Total Number of Microscopic Filamentous Fungi

Enterobacteriaceae and of microscopic filamentous fungi were not detected for 24 days of storage of KK, KO, BK, BO burgers.

3.3 Testing of Texture Parameters

When the texture of the individual parameters was tested, there were no significant statistical differences between burgers with rosemary oil added compared to burgers without.

Table 3 shows the results of the texture analysis of the burgers based on millet groats with and without added rosemary oil.

The hardness value for the burgers based on millet groats (KK) averaged 11.4 N. The value for the rosemary oil (KO) millet groats-based burgers was higher, averaging 12.42 N. The firmness of the burgers on the basis of millet groats with added rosemary oil was 8.79% higher

Table 3 Results of texture analysis of burgers based on millet groats

Parameters	KK	KO	The difference between KK i KO [%]
Hardness [N]	11.42 ±0.56	12.42 ±1.60	8.79
Springiness	0.37 ±0.37	0.26 ±0.26	28.70
Brittleness [N]	1.34 ±0.63	1.41 ±0.89	5.31
Rubberiness [N]	1.49 ±0.14	1.73 ±0.30	15.89
Chewiness [N]	0.55 ±0.56	0.48 ±0.53	13.78
Resilience	0.040 ±0.004	0.043 ±0.007	5.39
Parameters	BK	BO	The difference between BK i BO [%]
Hardness [N]	25.00 ±4.17	28.58 ±4.49	14.32
Springiness	0.265 ±0.05	0.268 ±0.08	1.13
Brittleness [N]	-	-	-
Rubberiness [N]	7.90 ±2.55	9.47 ±2.37	19.87
Chewiness [N]	2.13 ±0.97	2.63 ±1.29	23.47
Resilience	0.09 ±0.02	0.11 ±0.01	22.22

KK – millet groats-based burger without essential oil, KO – millet groats-based burger with essential oil added, BK – broccoli-based burger without essential oil, BO – broccoli-based burger with essential oil added

than for the burger without added oil. The springiness of the KK burgers was 28.70% higher than that of the KO burger. It was 0.37 for the burger without added rosemary oil, while it was 0.26 for the burger with added rosemary oil. The crispness of the KK burger was 1.34 N, while that of the KO burger was 1.41 N. This value was 5.31% higher for burgers with rosemary oil than for those without. The value of the 'gumminess' parameter was also 15.89% higher for burgers with essential oil than for burgers without such an addition. This was 1.49 N for the KK burgers and 1.73 N for the KO burgers. The chewiness was higher with the KK burgers at 0.55 N and 0.48 N for the KO burgers. The difference was 13.78%. The parameter resilience was 5.39% higher for the KO burgers. The value of the KK parameter was 0.040, while it was 0.043 for the KO burgers.

The results of the texture analysis of the broccoli and chickpea seed-based burgers are shown in Table 3. The hardness of the BK burger was 25.00 N, while the hardness for the BO burger was equal to 28.58 N. The hardness of the burger with oil was 14% higher than that of the burger from the control trial. The springiness of the BK burger was 0.265 ± 0.05 N, while the springiness of the BO burger was equal to 0.268 ± 0.08 N. The difference in results was negligible, as it was only 1%. The tenderness of the BO burger was 9.47 N and was 19% higher than the rubberiness in the BK control sample (7.90 N). For the gumminess parameter, the BO burger (2.63 N) scored 23% higher than the BK burger (2.13 N). The resilience parameter for the BO burger was 0.11 and was 11% higher than for the BK, where it was 0.09 N.

The hardness of the burgers in the meat variant, depending on the measurement method used, are very divergent. In the work of Hanula et al. (2022), the firmness of the grilled beef burger was 75.82 ± 5.07 N, in contrast, in the study of Cegiełka (2012), this parameter for the chicken meat burger was 8.2 ± 0.41 N. In another study, the firmness of a grilled beef burger was 31.62 N by Szpicer et al. (2020). According to a study by Godschalk-Broers et al. (2022), which compared the properties of chicken patties and beef burgers and their vegetarian substitutes available commercially, the hardness of beef burgers

was 210.04 N. Also, the plant-based burger analogues examined by Godschalk-Broers et al. (2022), mostly soy- or other plant-based, had much higher hardness values, ranging from 69.81 N to 395.42 N. However, such large differences are due to the different texture testing methodology and the difference in the accessories used for testing. In the study by Mabrouki et al. (2023), vegetable pasties based on pea protein with sunflower oil, rapeseed oil, coconut fat, fibre and water were tested. The hardness of the samples so made varied from 1.56 N to 4.93 N.

In the study by Petrat-Melin and Dam (2023), the Springiness of the beef burger was 0.76 ± 0.01 N, while for the beef hybrid burger with extruded pea starch, it was equal to 0.77 ± 0.01 N, it follows that the tested veggie burgers in the variant with and without oil had a lower Springiness compared to the beef burger and the hybrid burger. In the study by Szpicer et al. (2020), the Springiness of the grilled beef burger was 0.30. This was comparable to the values obtained in a texture study of veggie burgers. According to a study by Spitzer et al. (2020), the Rubberiness of the grilled beef burger was 5.50 N. This means that the grilled beef has a higher Rubberiness than the veggie burgers studied in their own work. The chewiness of the grilled beef burger in the study by Szpicer et al. (2020) was 1.62 N and was higher than the chewiness results obtained in their own work. In the study by Rai and Garai (2023), the resilience parameter of the ragi flour and oyster mushroom product tested was 0.062, a value that was slightly higher than that obtained in the study of texture parameters of veggie burgers.

3.4 Organoleptic Evaluation of Burgers

The burgers produced were subjected to an organoleptic evaluation in terms of the following quality characteristics: overall appearance, colour, texture, aroma and flavour, as well as the average score with weighting factors (Table 1). The evaluation was carried out by a team of 12 people with proven sensory sensitivity.

The results of the organoleptic evaluation are shown in Table 4 and Figure 1. The evaluation was carried

Table 4 Results of organoleptic evaluation of burgers

Variant	Colour	Flavour	Fragrance	Texture	General appearance	Average score
KK	4.83 ± 0.39	4.58 ± 0.67	4.75 ± 0.62	4.75 ± 0.45	4.83 ± 0.39	4.74 ± 0.41
KO	4.83 ± 0.39	3.50 ± 1.09	4.25 ± 0.96	4.67 ± 0.49	4.67 ± 0.65	4.32 ± 0.64
BK	3.86 ± 0.14	4.76 ± 0.29	5.00 ± 0.00	4.80 ± 0.09	4.30 ± 0.61	4.59 ± 0.34
BO	3.93 ± 0.12	3.24 ± 0.75	4.25 ± 0.39	4.60 ± 0.39	4.10 ± 0.39	3.99 ± 0.51

KK – millet groats-based burger without essential oil, KO – millet groats-based burger with essential oil added, BK – broccoli-based burger without essential oil, BO – broccoli-based burger with essential oil added

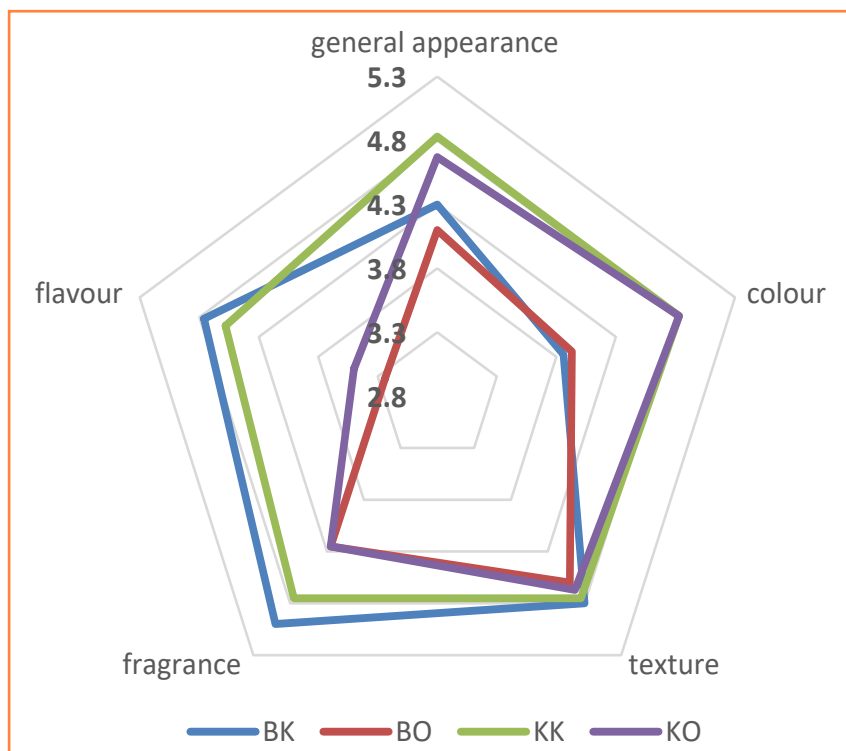


Figure 1 Results of organoleptic evaluation of burgers with and without essential oil added
 KK – millet groats-based burger without essential oil, KO – millet groats-based burger with essential oil added, BK – broccoli-based burger without essential oil, BO – broccoli-based burger with essential oil added

Considering the average score of the burgers on the basis of millet groats, it can be concluded that the KK burger received higher scores (4.74 points). At the same time, it should be emphasized that the KO burger, which received lower scores (4.32 points), was not characterized by negative features, but was only less desirable than the KK burger. Due to this ingredient, the taste and smell of the KO burger were rated lower. Respondents rated the other characteristics at a similar level.

The BK burger was characterized by better taste – a score of 4.76 points – while the BO burger received a score of 3.24 points. Also, for aroma, the BK burger scored higher (5 points) than the BO burger (4.25 points). The BK burger had a better texture, it was rated at 4.80 points, while the BO burger received a score of 4.60 points. The colour of the products was rated at a very similar level. For the BK burger, it was 3.86 points, while for the BO burger, it was 3.93 points. Correspondents also rated the overall appearance of the BK burger better, which received a score of 4.30 points, while the BO burger oil received 4.10 points. The organoleptic evaluation of the burgers showed that the BK burger of essential oil was characterized by better quality parameters, its average score in relation to the weighting factors was 4.59, while the BO burger was rated at 3.99. The biggest differences between the produced burgers were shown in the parameters of smell and taste. It was clearly declared that the aroma and flavour of the essential oil was too strong. Other parameters such as colour, texture, and overall appearance were evaluated similarly, with slight differences in the numerical values.

out in a laboratory designed for this type of analysis. The test was carried out at room temperature. The evaluation team consisted of 12 people with proven sensory sensitivity. Respondents received coded samples on white porcelain plates. According to the evaluators, the KK burger (mean score of 4.74 points) received a higher overall score than the KO burger (mean score of 4.32 points). The colour of the millet groats-based burgers, both without and with the addition of rosemary oil, was rated the same by the respondents (4.83 points), the addition of rosemary oil, did not affect the colour of the millet groats-based burgers in any way. The taste of the KK burgers was rated much higher by respondents (4.58 points) than the KO burgers (3.50 points). The lower rating of the burgers with rosemary oil was due to the overly pungent, intense flavour of the burger with this additive.

Respondents felt that it significantly affected the acceptability of this type of burger on a millet porridge base. The smell of the burgers on KK, was rated higher (4.75 points) than the KO burgers (4.25 points). This was related to the intense smell of the added rosemary oil used in the burgers. Respondents did not find the smell repulsive or negative, but rather less appealing due to its over-proneness. The texture of the KK (4.75 points) and KO (4.67 points) burgers were rated at a similar level. Based on the ratings given by the respondents, it can be concluded that the addition of rosemary oil did not significantly affect the texture of the burgers. The overall appearance of the KK burgers (4.83 points) was rated slightly higher than that of the KO burgers (4.67 points). It can be concluded from this that rosemary oil did not significantly affect the overall appearance of the burgers.

4 Conclusions

1. The use of vacuum packaging and modified gas atmosphere packaging reduced the total number of microorganisms, compared to normal, unaltered atmosphere packaging, in the veggie burgers during refrigerated storage for 24 days.
2. The addition of 0.1% rosemary oil improved the microbiological stability of the produced burgers in each of the packaging methods tested.
3. Texture testing showed no differences for individual parameters between the burger with rosemary oil addition and the burger without oil addition.
4. The burger without added essential oil showed better scores in the organoleptic evaluation test, especially for the taste and aroma parameter, which was found to be too intense and noticeable in the sample with oil.
5. The burger with rosemary oil requires further research and modification of the composition. In spite of its poorer ratings in the consumer survey, it has a predisposition and potential to be produced on an industrial scale especially due to its better results in microbiological tests, which will allow an extended shelf life.

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