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HYGIENIC QUALITY OF CHICKPEA SPREADS IN RELATION TO THE USED INGREDIENTS AND STORAGE CONDITIONS

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ABSTRACT

The aim of research was to verify variability of selected parameters of hygienic quality of chickpea spreads in relation to the used ingredients and storage conditions. Four basic groups of chickpea spreads (A, B, C, D) contained the same ingredients (boiled chickpea, salt, garlic). The decisive factor for assessing their hygienic quality was the addition of two different vegetable oils and lemon juice. The chickpea spreads were analyzed fresh, then after 24 and 48 hours of storage at a temperature of 4 and 8 °C. Using the plate dilution method was detected the presence of the total viable count (TVC), coliform bacteria, *Bacillus cereus*, microscopic filamentous fungi (MMF) and yeast. The following parameters were determined within the physic-chemical analyses: pH, water activity and salt content. The data were processed to the analysis of variance (ANOVA) in the general linear models (GLM), Scheffe's test and Pearson correlation coefficients (r_{xy}). The presence of MFF and yeast in samples of chickpea spreads was minimal. A higher amount of yeast was detected in the spread with sunflower oil (B - on average of $4.9.10^2$ CFU/g). The least amounts of coliform bacteria were found in the group of chickpea spreads with olive oil (<4.10^1 CFU/g). The presence of the bacterium *B. cereus* was not proven (<1.10^1 CFU/g). On the contrary, in chickpea spreads there was a higher representation of TVC (from 3.08 to 4.52 log CFU/g). High water activity (0.95 to 0.99) together with pH range (6.16 to 6.45), chickpea spreads are an ideal medium for the growth of various microorganisms. The average values of NaCl in fresh chickpea spreads were from 0.624 g/100 g (D) to 0.783 g/100 g (B). Most correlations between individual indicators in chickpea spreads were not statistically significant ($P>0.05$). A statistically highly significant difference between the two variables - pH and a_w ($P<0.01$) was detected in sample A8b. The results showed that the chickpea spread with olive oil and lemon juice had the best properties in terms of safety. However, spreads represent an ideal environment for the growth of various microorganisms.

Keywords: chickpea spread, hygienic quality, ingredients, storage, analysis

INTRODUCTION

Chickpea (*Cicer arietinum L.*) is an old-world legume (edible plant seeds) and is traditionally incorporated into many culinary recipes for its nutty flavor and versatile, sensory use in food **(Wallace *et al.*, 2016)**. It is one of the most consumed legumes in the world. Chickpea has a high content of protein, fiber, polyunsaturated fatty acids, vitamins, minerals, and polyphenols. This legume is considered a reliable source of energy and an excellent option for consumers looking for healthy food **(Martínez-Preciado *et al.*, 2020)**. They contain 18-30% protein, 4.7-8.2% fat and 44% carbohydrates, vitamins, and minerals. Essential amino acids are sufficiently represented in chickpea seeds, even approaching the values of products of animal origin **(Zetochová, 2021)**. Most legumes, such as beans, chickpeas, etc. it is highly versatile and adaptable, and should continue to be considered an important food by cooks and consumers. As consumers seek healthier diets or health through their diets, the culinary status of beans and other legumes is expected to continue to rise **(Amin and Borchgrevink, 2022)**. Legume-based products and ingredients offer opportunities for new products aimed at retail consumers and food service customers, by improving processing technologies, resulting in new products such as bean pasta, bean-based burgers, as well as "ground meat" that are now commercially available on the global market **(Jackson *et al.* 2022)**. Legume spreads are an innovative product and an alternative to traditional animal spreads or pâtés. They contain all the nutritional components of legumes: high-quality proteins, complex carbohydrates, fiber, minerals, vitamins, and antioxidants. The concept of commercially available legume spreads is new as non-dairy and reduced fat/calorie spreads are becoming popular with health-conscious people **(Kirse-Ozolina *et al.*, 2016a)**. Due to their nutritional composition, spreads can be a source of contaminating bacteria. The quality of each of the raw materials used, which are intended for the preparation of spreads, is important for the final quality of the spread. Contamination can occur during cultivation, especially from irrigation water, during collection, processing, storage and during transport. An important risk factor is spoilage by insects or rodents or any cross-contamination during handling **(Barker, 2018)**. The storage quality of legumes decreases exponentially with an increase in equilibrium relative humidity and temperature. In addition to microbial deterioration, specific quality changes attributed to storage are associated with taste (must, sour/bitter), color change (browning, darkening) and hard-to-cook defects (HTC – reduced water binding, longer cooking time). Under adverse storage conditions, storage mistakes occur, leading to significant loss of grain quality and economic loss. The overall final quality of legumes is linked to the control of critical physical and chemical properties and biochemical processes during production and post-harvest handling and storage **(Dugesar *et al.*, 2021)**. If the spreads were not prepared in hygienic conditions, bacteria may be present in them. A principal factor of contamination is the quality of the ingredients and the equipment used during the preparation of the spreads. Spreads are included in menus and eaten with various foods. The microbial risk is so high and can cause health problems of various nature **(Ahmed and Uddin, 2020)**. If the food has a sufficiently high acidity (pH<4.6) or a sufficiently low water activity (a_w<0.85), it can be intended for storage, the conditions of which are determined by the manufacturer **(Boyer *et al.*, 2020)**. The main problem with the production of spreads or dips is their limited shelf life, as they are prone to spoilage and quality degradation. Refrigeration slows the growth of microorganisms but does not stop it. Therefore, it is important to prevent microbial contamination in food during preparation. Prevention includes: buying quality food and other ingredients needed to make spreads; observance of technological procedures, as well as personal and operational hygiene focused on the cleanliness of work aids; use of proven sanitation practices; setting and maintaining the required temperature during the storage of spreads and comparable products (4 °C) **(Klug *et al.*, 2018)**.

The purpose of this study was to analyze and evaluate selected indicators of the hygienic quality of chickpea spreads in relation to the used ingredients and storage conditions.

MATERIAL AND METHODS

Material

In accordance with the above, the aim of the research was to analyze the hygienic quality of chickpea spreads stored in different model situations in relation to the presence or absence of lemon juice and the type of edible oil (extra virgin olive or sunflower oil). For microbiological and physic-chemical analysis, four basic groups of chickpea spreads (A, B, C, D) were prepared, which contained the same ingredients (boiled chickpea, salt, garlic), and differed in the oil and lemon juice content. Sample preparation: dried chickpea was soaked in water for 15 hours. Subsequently, it was washed with water and boiled for 1 hour. The cooked chickpea was mixed and divided into four bowls (samples A, B, C, D) in the same amount, 250 g. According to the type of sample, the following ingredients were added to the mixed chickpea: 100 ml of sunflower or extra virgin olive oil, 2 g of salt, 2 g of pressed garlic and 1 ml of lemon juice. To create a fine and spreadable structure of the spreads, all the ingredients were mixed very well with a hand mixer. The base sample in each combination was divided into 5 equal parts of 50 g each. The chickpea spreads prepared in this way were stored in bowls with a lid for 24 or 48 hours at two different temperatures (4 and 8 °C). All sample combinations are shown in Table 1.

Under the Ministry of Health of the Slovak Republic **Decree No. 125/2017**, spreads (own production) belong to semi-finished products and can be stored for a maximum of 24 hours at a temperature of 0 to 4 °C. In terms of health safety, it is not possible under the mentioned decree to store creams, foams, spreads, dressing (own production) at higher temperatures or freeze them before heat treatment (-18 °C). These requirements have also been applied in our analyses and have been supplemented by additional storage conditions resulting from the experience of Slovak households.

Table 1 Labelling of chickpea spreads samples

Sample	
A	Chickpea spread with extra virgin olive oil
A4a	Chickpea spread with extra virgin olive oil after 24 hours of storage at 4 °C
A4b	Chickpea spread with extra virgin olive oil after 48 hours of storage at 4 °C
A8a	Chickpea spread with extra virgin olive oil after 24 hours of storage at 8 °C
A8b	Chickpea spread with extra virgin olive oil after 48 hours of storage at 8 °C
B	Chickpea spread with sunflower oil
B4a	Chickpea spread with sunflower oil after 24 hours of storage at 4 °C
B4b	Chickpea spread with sunflower oil after 48 hours of storage at 4 °C
B8a	Chickpea spread with sunflower oil after 24 hours of storage at 8 °C
B8b	Chickpea spread with sunflower oil after 48 hours of storage at 8 °C
C	Chickpea spread with extra virgin olive oil and fresh lemon juice
C4a	Chickpea spread with extra virgin olive oil and fresh lemon juice after 24 hours of storage at 4 °C
C4b	Chickpea spread with extra virgin olive oil and fresh lemon juice after 48 hours of storage at 4 °C
C8a	Chickpea spread with extra virgin olive oil and fresh lemon juice after 24 hours of storage at 8 °C
C8b	Chickpea spread with extra virgin olive oil and fresh lemon juice after 48 hours of storage at 8 °C
D	Chickpea spread with sunflower oil and fresh lemon juice
D4a	Chickpea spread with sunflower oil and fresh lemon juice after 24 hours of storage at 4 °C
D4b	Chickpea spread with sunflower oil and fresh lemon juice after 48 hours of storage at 4 °C
D8a	Chickpea spread with sunflower oil and fresh lemon juice after 24 hours of storage at 8 °C
D8b	Chickpea spread with sunflower oil and fresh lemon juice after 48 hours of storage at 8 °C

Methods

The samples were analyzed fresh, then after 24 and 48 hours of storage at temperatures of 4 and 8 °C (Table 1). **Microbiological parameters of hygienic quality:** coliform bacteria (CB), total viable count (TVC), microscopic filamentous fungi (MFF), *Bacillus cereus*. Plate dilution method was used to determine mentioned microorganisms and the samples were prepared by smear or embedding. The microbiological characteristic is given in the Table 2.

Table 2 Characteristic of microbiological analysis

Microorganism	Dilution	Volume	Culture medium	Cultivation	Slovak technical norm/ISO
TVC	10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}	1 ml embedding	PCA agar	30±1 °C, 72±3 hours	ISO STN 4833
Coliforms	10^{-1}, 10^{-2}, 10^{-3}	1 ml embedding	VRBL agar	30±1 °C, 24 hours	STN EN ISO 4832
Bacillus cereus	10^{-1}, 10^{-2}	0,1 ml smear	MYP agar	30 ±1 °C, 20 hours	STN EN ISO 7932
Yeast and MFF	10^{-1}, 10^{-2}	0.1 ml smear	DRBC agar	25±1 °C, 5 days	ISO 7954

- Determination of total viable count of microorganisms – PCA agar (Plate Count Agar). This agar is a non-selective growth medium and is commonly used to monitor total bacterial growth in the sample. Components of PCA agar: agar, glucose, yeast extract, enzymatic casein hydrolysate.
- Determination of coliforms – VRBL agar (Violet Red Bile Lactose). The agar is determined for the reproduction and isolation of growth-demanding bacteria. Components of VRBL agar: agar, lactose, crystal violet, sodium chloride, yeast extract, neutral red, meat peptone, bile salts.
- Determination of bacteria *Bacillus cereus* – MYP agar (Plate Count Agar). This agar is used to determine the number of *Bacillus* in food samples. Components of MYP agar: agar, D-mannitol, sodium chloride, meat peptone, meat extract, phenol red.
- Determination of microscopic filamentous fungi – DRBC agar (Dichloran Rose-Bengal Chloramphenicol agar). This agar is used as a selective medium to isolate and determine the count of yeasts and filamentous fungi (MFF) found in food with water activity (a_w) greater than 0.95. Components of DRBC agar: agar, dextrose, magnesium sulphate, dichlorane, dihydrophosphate potassium, meat peptone Bengal red.

The culture mediums were prepared by weighing the corresponding amount of dehydrated nutrient soil and subsequently mixing in distilled water. Culture medium (except the VRBL agar) was then sterilized in an autoclave at the temperature of 121 °C, pressure of 120 kPa, for 15 minutes.

Physic-chemical parameters of hygienic quality: pH, water activity (a_w) and salt (only in fresh samples). Measurements of individual parameters were repeated three times in each sample.

- Determination of pH – pH meter (Testo).
- Determination of water activity – FA-st lab device (GBX Scientific ltd).
- Determination of salt content – M926 Chloride Analyzer. The devise measures chloride ions in a water solution. A sample volume of 0.5 ml is required for the measurement and the result is shown in mg/l Cl^- or mg % (mg/100ml) NaCl.

While determinations of water activity and pH were made directly by measuring on the devices, the salt content was recalculated according to the below formulas.

Calculation of salt content in %: x = NaCl in %

$x = A^{x} 0.0084$ A = value from the device in mg/l

Statistical evaluation

For statistical evaluation of the results was used the SAS program package, version 8.2. The program allows the analysis of several variables at once. For the SAS software, the data were sorted in order at beginning, when the SAS file and the sorting procedure were created from it. The sorted data were used in the SAS software for mathematical-statistical calculations of the indicators of the descriptive characteristics by groups according to the values of the quantities and the statistical evidence of the difference between the groups of chickpea spreads.

The data were subjected to the analysis of variance (ANOVA) in the general linear models (GLM), *t*-test, Scheffe´s test and Pearson correlation coefficients (r_{xy}). Statistical comparison of differences between individual groups of chickpea spreads was made using Scheffe´s test. For the results, the p value of the respective achieved statistical significance was evaluated at the selected level of significance α=0.05. The linear relationship between the two variables was evaluated according to the Pearson correlation coefficient (r_{xy}). The result of the correlation relationship (r) between two variables was statistically evaluated at a significance level of α=0.05, α=0.01, and α=0.001 **(Cohen, 1988)**.

RESULTS AND DISCUSSION

In accordance with the set goals, 4 types of chickpea spreads were kept under different conditions and then microbiologically and physic-chemically analyzed.

Microbiological parameters of chickpea spread hygienic quality

During the preparation process, chickpea spread is susceptible to bacterial cross-contamination from ingredients, utensils, and the environment. As part of our research, apart from cooking the chickpea, the spread was not heat-treated during its preparation and no chemical preservative was added to it. These conditions limit its shelf life, which can also be affected by improper handling during serving. The total viable count of microorganisms is one of the key indicators in the field of hygiene management. Although in **Ordinance No. 06267/2006-SL** of the Ministry of Agriculture of the Slovak Republic and the Ministry of Health of the Slovak Republic, there are no defined limits for the occurrence of TVC in spreads, foams, and creams, to detect potential microbiological contamination of spreads, it was performed such determinations.

As is shown in Figure 1, the average values of TVC ranged from 3.08±0.17 log CFU/g in a sample of chickpea spread with olive oil analyzed after 24 hours of storage at 4 °C (A4a), up to a maximum value 4.52±0.18 log CFU/g in the olive oil sample that was analyzed after 48 hours at 8 °C (A8b). In general, chickpea spreads with fresh lemon juice contained slightly lower TVC than those without lemon juice. Overall, it can be assessed that the TVC of all samples analyzed after 24 hours of storage at 4 °C decreased compared to fresh samples. Subsequently, with increased storage temperature or storage time, TVC gradually increased (24 h, 8 °C → 48 h, 4 °C → 48 h, 8 °C).

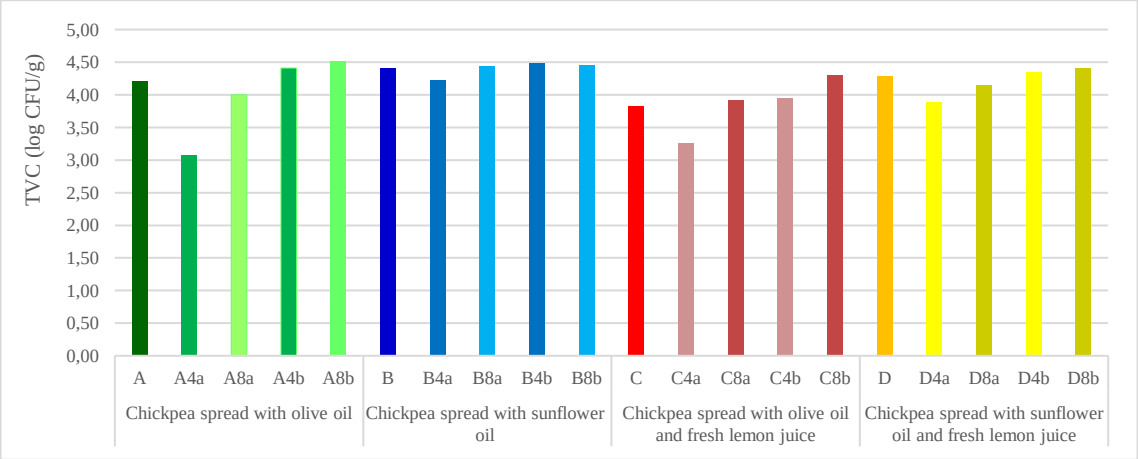

Figure 1 The presence of TVC in individual combinations of chickpea spreads

The initial microbial quality of chickpea spreads is directly influenced by its ingredients, production technology, storage conditions and preparation/consumption environment. Due to the internal factors, high water activity, high protein, and carbohydrate content together with the medium pH range, chickpea spread represents an ideal medium for the growth of various microorganisms, especially if it is stored in unsuitable conditions **(Al-Qadiri *et al.*, 2021)**. Hummus is a popular food in many European countries, the United States, but also in Asia, thanks to its excellent nutritional profile. However, parallel to the growing consumption of hummus, several outbreaks of infection caused by *Listeria monocytogenes*, *Salmonella spp. and Escherichia coli*. The main ingredient of hummus, cooked chickpea, is usually exposed to sufficient heat so that harmful vegetative pathogens are eliminated. However, inadequate hygienic procedures, poor microbial quality of water and packaging materials can cause the multiplication of undesirable microorganisms and subsequently cause disease **(Tayyarcan *et al.*, 2022)**. The shelf life of hummus products, whether with or without the use of tahini paste, is quite limited (1-7 days in a refrigerated environment). This is due to the current absence of chemical preservatives and additives capable of modifying chemical and microbiological profiles to extend shelf life **(Haddad et al., 2021)**.

As part of a deeper assessment of the analyses, the results were supplemented with statistical analysis using the Scheffe test. Table 3 shows that up to 65.79% of the individual combinations of chickpea spreads had statistically significant differences (P<0.05).

Table 3 Statistical evaluation of differences in TVC between different conditions of chickpea spreads storage and processing

F test	**131,71^{+++}**																		
Indicator	**A4a**	**A8a**	**A4b**	**A8b**	**B**	**B4a**	**B8a**	**B4b**	**B8b**	**C**	**C4a**	**C8a**	**C4b**	**C8b**	**D**	**D4a**	**D8a**	**D4b**	**D8b**
A	+	-	+	+	+	-	+	+	+	+	+	+	+	-	-	+	-	-	+
A4a		+	+	+	+	+	+	+	+	-	-	+	+	+	+	-	+	+	+
A8a			+	+	+	+	+	+	+	-	+	-	-	+	+	-	-	+	+
A4b				-	-	+	-	-	-	+	+	+	+	-	+	+	+	-	-
A8b					+	+	-	-	-	+	+	+	+	+	+	+	+	+	-
B						+	-	-	-	+	+	+	+	-	-	+	+	-	-
B4a							+	+	+	+	+	+	+	-	-	+	-	-	+
B8a								-	-	+	+	+	+	-	+	+	+	-	-
B4b									-	+	+	+	+	+	+	+	+	+	-
B8b										+	+	+	+	+	+	+	+	-	-
C											-	-	-	+	+	-	-	+	+
C4a												-	+	+	+	-	+	+	+
C8a													-	+	+	-	-	+	+
C4b														+	+	-	-	+	+
C8b															-	+	-	-	-
D																+	-	-	+
D4a																	-	+	+
D8a																		+	+
D4b																			-

+: Statistically significant difference according to Scheffe's test (P<0.05)
-: Statistically insignificant difference according to Scheffe's test (P>0.05)

The representation of MFF in samples of chickpea spreads was minimal. Only one sample, a spread with sunflower oil and fresh lemon juice analyzed after 24 hours of storage at 4 °C (D4a), showed the presence of MFF (1.10^1 CFU/g). In the other samples, this group of microorganisms did not occur at all (<1.10^1 CFU/g). The legislation states that out of five samples from the total set, two samples can reach the amount of mold (other than *Geotrichum candidum*) 5.10^2 and three samples cannot show the presence of mold. The chickpea spreads that were analyzed in this research met the stated requirements.

Regarding the presence of yeast, their amount was not large in individual samples, even in several they were not present at all. Their highest number was found in sample B (fresh chickpea spread with sunflower oil), namely $4.9.10^2 \pm 1.2.10^2$ CFU/g. The legislation states that out of five samples from the total set, two samples can reach a yeast count of 5.10^3 and three samples can reach a value of 5.10^2. All chickpea spreads met the stated requirements.

Tuytschaever *et al.* (2019) focused their research on vegetarian spreads and dips with vegetables or herbs as a basic ingredient, such as pesto, salsa, guacamole and evaluating their microbiological quality and safety. They subjected 40 chilled and 8 shelf-stable vegetable spreads to microbiological analysis. The number of yeasts and filamentous fungi ranged from <1 to 6.67 log CFU/g.

Coliform bacteria represent an important group of microorganisms, which includes the *Enterobacteriaceae* family and many of its genera, e.g.: *Citrobacter, Enterobacter, Escherichia, Klebsiella, Arsenophonus, Erwinia, Shigella, Hafnia, Kluyvera, Pantoea, Proteus, Salmonella, Serratia, Yersinia a Yokenella.*

It follows from the performed analyzes that the fewest coliform bacteria were found in chickpea spreads with olive oil (<4.10^1 CFU/g). On the contrary, the most coliform bacteria were detected in the group of spreads with sunflower oil (B), depending on the temperature and storage time (from $1.3.10^2$ - B to 1.10^3 CFU/g - B8b). The results were again compared with the legislative regulation, which states that out of five samples from the total set, two samples can reach a value of 10^4 and three samples can reach a value of 10^3 coliform bacteria. In this case too, all chickpea spreads met the stated requirements.

In most of the analyzed samples, in addition to typical colonies of coliform bacteria (distinct deep pink balls), pinkish colonies bordered by yellow pigment also grew. Using the MALDI-TOF BIOTYPER method was performed identification of colonies. The genus *Enterobacter*, which is classified as coliform bacteria, specifically the species *Enterobacter cloacae* (*Cronobacter sakazakii*), is a distinct, yellow-pigmented variant of a motile, gram-negative, non-spore-forming, rod-shaped coliform bacterium from the *Enterobacteriaceae* family **(Yan and Gurtler, 2014)**. Researchers **Segars *et al.* (2016)**, evaluated the growth of different strains of the genus *Cronobacter*. The results of this work were compared with our analyses. Colony morphology and soil color change in the vicinity of a grown *C. sakazakii* colony, in the work of Segars *et al*. was visually identical to our results.

The last bacteria that were monitored in chickpea spreads was the spore-forming bacterium *Bacillus cereus*. The results showed that none of the analyzed samples showed the typical characteristics of *B. cereus* bacteria - rough and dry colonies with a pale pink background surrounded by egg yolk precipitate (<1.10^1 CFU/g). What is interesting, however, is that yellow-colored colonies grew on all the agar plates examined. This coloration was the result of the fermentation of mannitol caused by the bacterium *B. subtilis*. All plate spreads were overgrown over the entire bowl, or *B. subtilis* did not form separate colonies but merged into one large, massive colony, so colonies could not be counted.

Currently, no legislation requires systematic screening of food for contamination by the pathogen *B. cereus*. Within the EU, the only regulation that results in a safety limit for *B. cereus* in food concerns dry infant formula with a maximum limit of 50 CFU/g **(Commission Regulation (EC) No. 1441/2007)**. In France, the regulatory limit for the presence of *B. cereus* in food, especially food rich in starch, was set at 1.10^5 CFU/g food. Most cases of foodborne illness caused by *B. cereus* were associated with concentrations above 10^5 CFU/g of food material, but some cases were associated with bacterial abundances of 10^3 CFU/g **(Ramarao *et al.*, 2020)**.

Physic-chemical parameters of chickpea spread hygienic quality

Variability of physic-chemical parameters of hygienic quality of fresh, marinated, and grilled poultry meat in relation to its storage conditions researched **Zeleňáková *et al.* (2022)**. Chickpea spreads, whose main raw material is chickpea, can be considered acidic in terms of pH value. Chickpea themselves has a pH in the range of 6.48-6.80 **(Trznadel, 2021)**. Adding lemon juice made the spreads even more sour.

Average pH values ranged from 6.16±0.02 (C8b) to 6.45±0.03 (B). Figure 2 shows that the lowest average values were measured for chickpea spreads with olive oil and lemon juice that were stored at 8 °C (C8a and C8b). Storage of the spreads at 4 °C had no significant effect on the pH value analyzed after 24 and 48 hours compared to the measurement of freshly prepared spreads. On the contrary, storage of the spreads at 8 °C decreased the pH value compared to all other measurements (immediately after preparation and storage at 4 °C). The storage time (24 and 48 hours) of the chickpea spreads did not have a significant effect on the value ph. Overall, the presence of fresh lemon juice visibly reduced pH, by an average of 0.21.

Statistical analysis using the Scheffe´s test showed that there was a statistically significant difference (P<0.05) between the spreads without the addition of lemon juice (all combinations of groups A and B) and the samples to which it was added (all combinations of groups C and D).

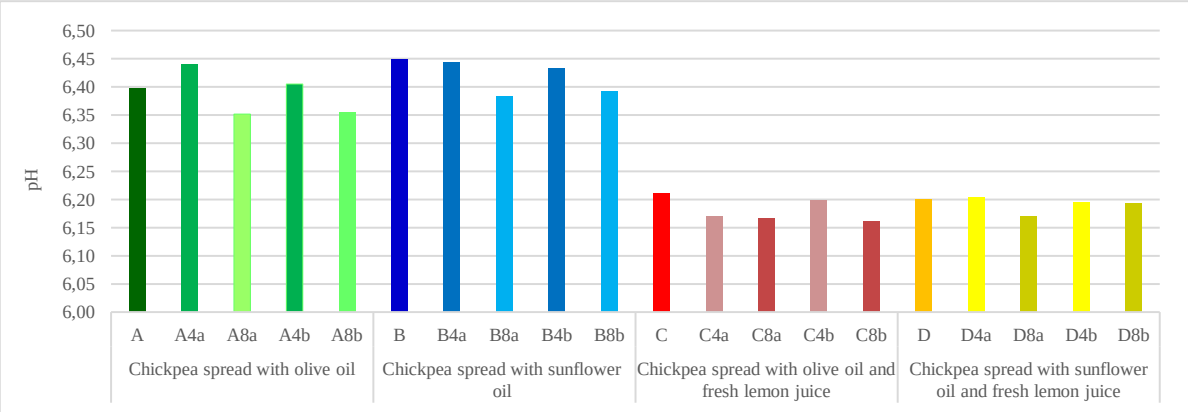

Figure 2 pH in individual combinations of chickpea spreads

Kirse-Ozolina *et al.* (2016b), found that chickpea spreads had higher pH values than pea spreads. **Olaimat *et al.* (2022)**, measured the pH value of chickpea hummus without garlic extract and citric acid at 6.49. Adding 0.5; 1.0 and 1.5% citric acid significantly reduced the pH value to 4.7; 4.1 and 3.7. Conversely, the addition of 1-3% garlic extract reduced the initial hummus pH values to 6.2-6.3. However, these pH changes were not significant.

Water activity (a_w) is a major factor in preventing or limiting the growth of microorganisms. In some cases, a_w is the primary parameter responsible for food stability, modulating the microbial response and determining the type of microorganisms present in food **(Tapia *et al.*, 2020)**. Water activity values that make food susceptible to spoilage by bacteria, pathogens, yeasts, and filamentous fungi are >0.85 **(Kirse-Ozolina *et al.* (2016b)**.

The average values of water activity in chickpea spread samples ranged from 0.95 (sample D4a) to 0.99 (sample D). For samples A and B (samples without lemon juice) after 24 hours of storage, a slight decrease of water activity was detected for samples stored at 4 °C, while it decreased further at 8 °C. On the contrary, in the analyzes performed after 48 hours, increased values of water activity were measured, which corresponded to fresh samples. For samples that contained lemon juice, storage temperature of 8 °C for 24 hours had the opposite effect on water activity compared to samples without lemon juice.

Statistical analysis revealed that chickpea spreads of group A showed a significant difference (P<0.05) with samples of groups B and C. Chickpea spreads with sunflower oil (samples of group B) showed the most differences with chickpea spreads with sunflower oil and lemon juice (samples of group D). The most statistically significant differences were between samples of chickpea spreads with olive oil and lemon juice (group C) and samples of chickpea spreads with sunflower oil and lemon juice (group D)

Salt acts as a preservative by reducing the availability of water in food, thereby depriving microorganisms of the opportunity to use the available water as a nutrient and reducing enzymatic activity. The growth of pathogens and spoilage microorganisms in the presence of salt is prevented or delayed. Among the main mechanisms responsible for the inhibition of microorganisms by salt are cell plasmolysis, inhibited respiration, utilization of glucose, prevention of transport of substrate into cells through cell membranes, limitation of oxygen solubility and interference with enzymes **(Elias *et al.*, 2020)**. Slovak legislation states **(Ordinance of the Ministry of Health of the Slovak Republic No. S08975-OL-**

2014) that ready meals intended for direct consumption may contain a maximum of 1.3 g/100 g of added salt.
The average values of NaCl in fresh chickpea spreads were from 0.624 g/100 g (D) to 0.783 g/100 g (B).

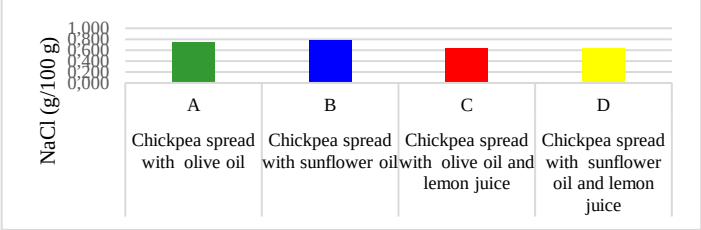

Figure 3 NaCl content in individual combinations of chickpea spreads

A statistically significant difference (P<0.05) was found between samples of group A and samples of group C, as well as between samples of group A and samples group D (D, D4a, D9a, D4b, D9b). Statistical significance was also noted between groups B and C; B and D. These results reflect the ingredients used in the production of chickpea spreads - the addition or not adding lemon juice.

Table 4 represents all correlations between quality indicators in chickpea spreads. Overall, it can be concluded that from fresh spreads, a statistically significant difference (P<0.05) between two indicators was observed only in these samples: A and D between pH and TVC and in sample C between a_w and TVC. Correlation relations between indicators in spreads kept for 24 hours at 4 °C showed a statistically significant difference (P<0.05) only for sample B4a (pH – a_w). In spreads stored for 24 hours at 8 °C, statistical significance (P<0.05) was found between a_w and salt (sample A8a) and between a_w and TVC (sample D8a). A statistically significant difference (P<0.05) was also noted between pH and salt in the spread kept for 48 hours at 4 °C (B4b). Finally, a statistically highly significant difference between two variables - pH and a_w (P<0.01) was observed for sample A8b, a chickpea spread with olive oil stored for 48 hours at 8 °C. For two samples, namely C and C8b, there was no linear relationship between salt and TVC. All other interrelationships between indicators in chickpea spreads were not statistically significant (P>0.05). A statistically very highly significant difference between the two variables (P<0.001) was not confirmed.

Table 4 Correlation relationships between parameters a_w, pH and TVC in chickpea spreads in relation to the used ingredients and storage conditions

A				**A4a**				**A8a**				**A4b**				**A8b**			
	a_w	NaCl	TVC		a_w	NaCl	TVC		a_w	NaCl	TVC		a_w	NaCl	TVC		a_w	NaCl	TVC
pH	0.213$^-$	-0.248$^-$	**-0.821**$^+$	**pH**	-0.282$^-$	-0.064$^-$	-0.535$^-$	**pH**	0.312$^-$	0.643$^-$	0.346$^-$	**pH**	0.200$^-$	0.360$^-$	0.772$^-$	**pH**	**0.956**$^{++}$	0.388$^-$	0.431$^-$
a_w		0.482$^-$	-0.449 -	**a_w**		0.042$^-$	0.176$^-$	**a_w**		**0.905**$^+$	0.185$^-$	**a_w**		0.078$^-$	0.175$^-$	**a_w**		0.366$^-$	0.277$^-$
NaCl			0.400$^-$	**NaCl**			0.188$^-$	**NaCl**			0.313$^-$	**NaCl**			0.260$^-$	**NaCl**			0.313$^-$
B				**B4a**				**B8a**				**B4b**				**B8b**			
	a_w	NaCl	TVC		a_w	NaCl	TVC		a_w	NaCl	TVC		a_w	NaCl	TVC		a_w	NaCl	TVC
pH	0.515$^-$	0.648$^-$	-0.251$^-$	**pH**	**0.837**$^+$	0.804$^-$	-0.491$^-$	**pH**	0.594$^-$	0.516$^-$	0.149$^-$	**pH**	-0.198 -	**0.864**$^+$	0.090$^-$	**pH**	0.416$^-$	-0.310 -	0.183$^-$
a_w		-0.108 -	-0.318 -	**a_w**		0.473$^-$	-0.426$^-$	**a_w**		0.011$^-$	0.638$^-$	**a_w**		0.178$^-$	0.499$^-$	**a_w**		-0.418 -	0.183$^-$
NaCl			0.344$^-$	**NaCl**			-0.196$^-$	**NaCl**			-0.710 -	**NaCl**			-0.027 -	**NaCl**			-0,310 -
C				**C4a**				**C8a**				**C4b**				**C8b**			
	a_w	NaCl	TVC		a_w	NaCl	TVC		a_w	NaCl	TVC		a_w	NaCl	TVC		a_w	NaCl	TVC
pH	0.350$^-$	-0.533 -	0.426$^-$	**pH**	-0.426$^-$	0.083$^-$	-0.433$^-$	**pH**	-0.435$^-$	0.029$^-$	-0.398 -	**pH**	-0.245 -	-0.128 -	-0.191 -	**pH**	-0.461 -	0.279$^-$	0.403$^-$
a_w		-0.158 -	**-0.822**$^+$	**a_w**		0.213$^-$	0.739$^-$	**a_w**		-0.164$^-$	0.674$^-$	**a_w**		0.213$^-$	-0.426 -	**a_w**		0.115$^-$	0.133$^-$
NaCl			**0.00**$^-$	**NaCl**			-0.144$^-$	**NaCl**			0.076$^-$	**NaCl**			-0.083 -	**NaCl**			**0.00**$^-$
D				**D4a**				**D8a**				**D4b**				**D8b**			
	a_w	NaCl	TVC		a_w	NaCl	TVC		a_w	NaCl	TVC		a_w	NaCl	TVC		a_w	NaCl	TVC
pH	0.287$^-$	0.274$^-$	**-0.818**$^+$	**pH**	-0.067$^-$	0.378$^-$	0.380$^-$	**pH**	-0.430$^-$	-0.077$^-$	0.707$^-$	**pH**	-0.739 -	0.093$^-$	-0.066 -	**pH**	-0.076 -	0.438$^-$	0.164$^-$
a_w		0.517$^-$	-0.459 -	**a_w**		0.280$^-$	0.150$^-$	**a_w**		0.211$^-$	**-0.911**$^+$	**a_w**		0.158$^-$	0.651$^-$	**a_w**		0.190$^-$	0.567$^-$
NaCl			-0.131 -	**NaCl**			0.113$^-$	**NaCl**			-0.110 -	**NaCl**			0.165$^-$	**NaCl**			0.084$^-$

r_{xy} value:
+++: Statistically very highly significant difference between variables (P<0.001)
++: Statistically highly significant difference between variables (P<0.01)
+: Statistically significant difference between variables (P<0.05)
-: Statistically insignificant difference between variables (P>0.05)

CONCLUSION

The aim of research was to verify variability of selected parameters of hygienic quality of chickpea spreads in relation to the used ingredients and storage conditions. High water activity (0.95 to 0.99) together with pH range (6.16 to 6.45), chickpea spreads are an ideal medium for the growth of various microorganisms. The average values of NaCl in fresh chickpea spreads were from 0.624 g/100 g (D) to 0.783 g/100 g (B). Most correlations between individual indicators in chickpea spreads were not statistically significant (P>0.05). A statistically highly significant difference between the two variables - pH and a_w (P<0.01) was detected in sample A8b. The presence of MFF and yeast in samples of chickpea spreads was minimal. A higher amount of yeast was detected in the spread with sunflower oil (B - on average of $4.9.10^2$ CFU/g). The least amounts of coliform bacteria were found in the group of chickpea spreads with olive oil (<4.10^1 CFU/g). The presence of the bacterium *B. cereus* was not proven (<1.10^1 CFU/g). On the contrary, in chickpea spreads there was a higher representation of TVC (from 3.08 to 4.52 log CFU/g). The results showed that the chickpea spread with olive oil and lemon juice had the best properties in terms of safety.
A major problem in the processing of food products such as vegetable dip or spreads is their limited shelf life, as they are susceptible to spoilage and quality degradation. Refrigeration slows the growth of microorganisms but does not stop it. Therefore, it is important to prevent microbial contamination in food during preparation. Prevention includes: Maintaining proper personal hygiene, including hand washing, wearing food-safe gloves during food handling and preparation; using proper cleaning and hygiene practices; using proper temperature control, refrigerated foods with high humidity should be kept at 4 °C or less; purchasing ingredients from approved, reputable sources.

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REFERENCES

Ahmed T. & Uddin A. M. (2020). Microbiological analysis of popular spreads used in restaurants inside Dhaka city, Bangladesh. *Stamford Journal of Microbiology*, 10(1), 16-19. https://www.banglajol.info/index.php/SJM/article/view/50727
Al-Qadiri H., Amr A., Al-Holy M. A. et al. (2021). Effect of gamma irradiation against microbial spoilage of hummus preserved under refrigerated storage. *Food Sci Technol Int*., 7, 598-607. https://doi:10.1177/1082013220975891
Amin S. & Borchgrevink C. P. (2022). *A Culinology ® Perspective of Dry Beans and Other Pulses.* Dry Beans and Pulses (eds M. Siddiq and M.A. Uebersax). https://onlinelibrary.wiley.com/doi/10.1002/9781119776802.ch18
Barker B. (2018). *Post-Harvest Storage of Pulses*. Saskatchewan Pulse Growers. https://saskpulse.com/files/technical_documents/Post-Harvest_Storage_of_Pulses.pdf
Boyer R. R., Wells E. P., Eifert J. et al. (2020). *What do I need to know to sell Refrigerated dips, spreads, dressings, and salads, at the farmers market?* VCE

Publications, FST-300P (FST-363P). https://www.pubs.ext.vt.edu/content/pubs_ext_vt_edu/en/FST/FST-300/FST-300.html

Cohen, J. (1988). *Statistical power analysis for the behavioral sciences*, 2nd Ed. New York: Academic Press. 567 p. ISBN 9780203771587. https://www.taylorfrancis.com/books/mono/10.4324/9780203771587

Commission regulation (EC) No 1441/2007 of 5 December 2007 amending Regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs.

Decree of the Ministry of Health of the Slovak Republic no. 125/2017, by which amends decree no. 533/2007 on requirements for common facilities catering.

Dugesar V., Chaurasia A. K. & Mishra S. N. (2021). *Loss of pulses seed during storage*. The Pharma Innovation Journal, 10(11), 2798-2805. https://www.thepharmajournal.com/archives/2021/vol10issue11S/PartAC/S-10-11-263-518.pdf

Elias M., Laranjo M., Agulheiro-Santos A. C. et al. (2020). *The role of salt on food and human health*. Salt in the Earth, Cengiz et al. IntechOpen. UK, 19-43. ISBN:978-1-83880-212-7.

Haddad M. A., Yamani M. I., Jaradat D. M. M. et al. (2021). Traditional Foods in Jordan and Traceability. Hummus and Related Variations. Food Traceability in Jordan. *SpringerBriefs in Molecular Science*, 25-40. https://link.springer.com/chapter/10.1007/978-3-030-66820-4_2

Jackson J., Kinabo J., Lekalake R. et al. (2022). *Processing and Utilization of Dry Beans and Pulses in Africa*. Dry Beans and Pulses (eds M. Siddiq and M.A. Uebersax). https://onlinelibrary.wiley.com/doi/10.1002/9781119776802.ch16

Kirse-Ozolina A., Karklina D. & Muizniece-Brasava S. (2016a). Consumer acceptance of new pulse spreads before and after sous vide treatment. *Int. Scientific Publications: Agriculture & Food*, 4, 104-114. https://www.researchgate.net/publication/303898384_Consumer_acceptance_of_new_pulse_spreads_before_and_after_sous_vide_treatment

Kirse-Ozolina A., Karklina D. & Muizniece-Brasava S. (2016b). Physicochemical Stability of Pulse Spreads during Storage after Sous Vide Treatment and High-Pressure Processing. *International Journal of Biological, Biomolecular, Agricultural, Food and Biotechnological Engineering*, 10(12). https://www.researchgate.net/profile/Asnate-Kirse-Ozolina/publication/310495042_Physicochemical_Stability_of_Pulse_Spreads_during_Storage_after_Sous_Vide_Treatment_and_High_Pressure_Processing/links/58303f6508aef19cb817c81d/Physicochemical-Stability-of-Pulse-Spreads-during-Storage-after-Sous-Vide-Treatment-and-High-Pressure-Processing.pdf

Klug T. V., Martínez-Hernández G. B., Collado, E. et al. (2018). Effect of Microwave and High-Pressure Processing on Quality of an Innovative Broccoli Hummus. *Food Bioprocess Technol*, 11, 1464-1477. https://link.springer.com/article/10.1007/s11947-018-2111-8

Martínez-Preciado A. H., Ponce-Simental J. A., Schorno A. L. et al. (2020). Characterization of nutritional and functional properties of "Blanco Sinaloa" chickpea (Cicer arietinum L.) variety, and study of the rheological behavior of hummus pastes. *Journal of Food Science Technology*, 57, 1856-1865. https://link.springer.com/article/10.1007/s13197-019-04220-8

Olaimat A. N., Al-Holy Y M. A., Abu Ghoush M. H. et al. (2022). Use of citric acid and garlic extract to inhibit Salmonella enterica and Listeria monocytogenes in hummus. *International Journal of Food Microbiology*, 362(109474), ISSN 0168-1605. https://www.sciencedirect.com/science/article/abs/pii/S0168160521004335?via%3Dihub

Ordinance No. 06267/206-SL of the Ministry of Agriculture of the Slovak Republic and the Ministry of Health of the Slovak Republic, which issues the title of the Food Codex of the Slovak Republic regulating the microbiological requirements for foods and their packaging.

Ordinance of the Ministry of Health of the Slovak Republic No. S08975-OL-2014 for salt content in food.

Ramarao N., Tran S. L., Marin, M. et al. (2020). Advanced Methods for Detection of Bacillus cereus and Its Pathogenic Factors. *Sensors (Basel)*, 20(9), 2667. https://www.mdpi.com/1424-8220/20/9/2667

Segars K., Simpson S., Kerdahi K. et al. (2016). Evaluation of Cronobacter Growth and Phenotypic Variation Under Modified Culture Conditions. *Curr Microbiol*, 72, 190-197. https://link.springer.com/article/10.1007/s00284-015-0936-1

Tapia M.S., Alzamora S. M. & Chirife J. (2020). Effects of Water Activity (a_w) on Microbial Stability as a Hurdle in Food Preservation. *Water Activity in Foods.* https://onlinelibrary.wiley.com/doi/10.1002/9781118765982.ch14

Tayyarcan E. K., Evran S., Akin P. A. et al. (2022). The use of bacteriophage cocktails to reduce Salmonella Enteritidis in hummus. *LWT*, 154(112848), ISSN 0023-6438. https://www.sciencedirect.com/science/article/pii/S0023643821020016?via%3Dihub

Trznadel N. (2021). *Are Chickpeas Acidic or Alkaline?* Food FAQ. https://foodfaq.org/are-chickpeas-acidic-or-alkaline/

Tuytschaever T., Jacxsens L., De Boeck E.et al. (2019). Microbial Quality and Safety of Pesto, Salsa, Guacamole and Tapenades at Retail Market. *Food Microbiology*, https://biblio.ugent.be/publication/8633771

Wallace T. C., Murray R. & Zelman M. K. (2016). The Nutritional Value and Health Benefits of Chickpeas and Hummus. *Nutrients*, 8(12), 766. https://www.mdpi.com/2072-6643/8/12/766

Zetochová E. (2020). *Ascochyta rabiei* (Pass.) Labr. - hubovité ochorenie cícera baranieho. https://www.agroporadenstvo.sk/rastlinna-vyroba-strukoviny?article=1679

Zetochová E. (2021). Hrach siaty (*Pisum sativum* L.) - pestovanie, ochrana a využitie. https://www.agroporadenstvo.sk/rastlinna-vyroba-strukoviny?article=20849

Zeleňáková L., Rybnikár S., Angelovičová M., Fikselová M. & Kolesárová A. (2022). Variability of selected parameters of hygienic quality of fresh, marinated, and grilled poultry meat in relation to its storage conditions. *Journal of Microbiology, Biotechnology and Food Sciences*, 1(9233), ISSN 1338-5178. https://office2.jmbfs.org/index.php/JMBFS/article/view/9233

Yan X. & Gurtler J. B. (2014). Editor(s): Carl A. Batt, Mary Lou Tortorello. *Encyclopedia of Food Microbiology* (Second Edition), Academic Press, 528-532, ISBN 9780123847331. https://www.sciencedirect.com/science/article/abs/pii/B9780123847300003827?via%3Dihub