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Original scientific paper

ANALYSIS OF PH VALUE AND WATER ACTIVITY AS KEY SAFETY PARAMETERS FOR KULEN SAUSAGE

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Abstract

Active acidity (pH) and water activity (*aw*) were analyzed in 14 samples (K01-K14) of kulen available in markets in the Republic of North Macedonia. These samples were produced under industrial conditions by various manufacturers from R.N. Macedonia and neighboring countries in the region. The obtained pH values ranged from 4.24 to 4.97, while the aw values of the Kulen sausages ranged from 0.789 to 0.903. The pH and *aw* values are critical parameters for assessing the stability and safety of fermented products. According to European regulations, fermented sausages do not support the growth of L. monocytogenes under conditions of pH ≤ 5.0 and *aw* ≤ 0.94. All analyzed samples of kulen sausages (K01-K14) fall within this range that does not support the growth of *L. monocytogenes*, indicating a well-controlled technological process.

Keywords: kulen, pH value, water activity (aw), microbiological stability.

1. Introduction

Kulen is one of the highest-quality products included in the group of fermented sausages (Keran et al., 2022). It is traditionally produced in Serbia (Sremski, Lemeški kulen, and Petrovac kobasica), Croatia (Slavonski kulen), and Hungary (Babić et al., 2011; Mastanjević et al., 2017; Ikonić et al., 2021; Šimunec et al., 2022). Kulen is produced from high-quality mature pork, which contains less water, exhibits a more intense red color, and has a firmer texture, (Vuković et al., 1988). 10-20% hard fat tissue is usually added to the Kulen Vuković et al., (2004), and ground red pepper lends its distinctive flavor to this type of sausage, while in some types, garlic is also added (Vuković et al., 2012).

According to the Rulebook (Official Gazette of the Republic of Macedonia No. 63/2013), kulen is a fermented, durable sausage made from coarsely ground pork of categories I and II, along with solid fatty tissue. Additionally, up to 10% of beef from categories I and II may be added to it. Other ingredients such as pepper and garlic can be included during production. The final product must not exceed 35% water content.

The industrial production of kulen involves the utilization of artificial coatings, nitrite salt, spice extracts, sugars, starter cultures, or acidifiers, such as Glucono-delta-lactone (GDL) (Vuković et al., 2011). Canning in this type of sausage is achieved through a method known as "preservation with obstacles" (Mastanjević et al., 2017). The method involves creating unfavorable conditions for the development of microorganisms through various means: fermenting sugars to lactic acid to lower the pH, reducing water activity (*aw*) through salting and dehydration, creating an anaerobic environment to inhibit the growth of aerobic bacteria, adding nitrates or nitrites to inhibit microbial growth, and preventing surface growth through fumigation or the application of specific coatings (Holck et al., 2017; Mastanjević et al., 2017). During fermentation, there is a reduction in both the pH value and water activity (aw), constituting a fundamental method for ensuring the safety of such fermented products (Bošković et al., 2017). The microbiological safety of these products primarily hinges on

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effectively managing pathogens from raw materials through post-processing stages (Serra-Castelló et al., 2021).
Regardless of whether fermented sausages undergo short, medium, or long ripening periods and whether they are packed in vacuum or modified atmosphere, ensuring microbiological stability and safety is paramount (Micheli et al., 2023). According to European Regulation, ready-to-eat foods, including fermented sausages, should not promote the growth of L. monocytogenes under specific conditions: food with a pH of ≤ 4.4 or aw of ≤ 0.92, as well as under conditions of pH ≤ 5.0 and aw ≤ 0.94. Additionally, the presence of L. monocytogenes must not exceed 100 CFU/g throughout the product's shelf life (European Commission, 2005). In North Macedonia, according to the rulebook (Official Gazette R.M No. 145/2014), a zero-tolerance policy is mandated for L. monocytogenes. This means that the presence of L. monocytogenes is not permitted in 25g of a product before it leaves the operator's control or in products available on the market within their shelf life.
This research was carried out to analyze the pH and aw values as basic parameters for ensuring proper technological processes and safety in commercially available kulen sausages in retail markets in the Republic of North Macedonia.

2. Material and methods

Kulen samples from industrial production, which are available in markets in the Republic of North Macedonia, were used as material for the research. Fourteen samples (designated as K01-K14) of Kulen from various producers (brands) were randomly selected for analysis. These samples originated from the Republic of North Macedonia (K05, K06, K07, K08 and K09) and neighboring countries including Serbia (K02, K11 and K12), Montenegro (K10), Slovenia (K01 and K14), and Croatia (K03 and K04). Sample K07 consisted of a whole sausage enclosed in vacuum packaging, whereas samples K08, K09, and K10 were presented unpackaged, in their primary casing (bulk). Additionally, samples K01 through K06 and K11 through K14 were sliced and packaged in a controlled atmosphere. From each sample of interest, five packages were purchased, from which a total representative sample was taken and analyzed after being divided into three subsamples (n=3).
The pH value was determined using a laboratory pH Meter PL-600, manufactured by MRC. Sample preparation for analysis followed the procedures outlined in (Capita et al., 2006). The water activity of the kulen samples was measured using Novasina LabTouch-aw, and sample preparation for analysis involved homogenization following the protocol outlined in (Beňo et al., 2023). Statistical analysis and calculations were conducted using Excel.
Sensory analysis was conducted following the methodology outlined by (Radovanovic and Popov-Raljic, 2000/2001). Twenty evaluators of various genders and age groups assessed the sensory attributes of appearance, color, smell, taste, and consistency. An importance coefficient was assigned to each attribute. The properties were rated on a scale from 1 to 5, and each rating was multiplied by its respective importance coefficient. The weighted scores were then summed and expressed as a percentage of the maximum possible quality. By dividing this percentage by the total sum of the importance coefficients (Σ=20), a weighted mean value was obtained, providing an overall quality assessment of the tested cultivars.

3. Results and discussion

According to Beňo, et al., (2023), water activity (a_w) is a key factor affecting the safety of dry fermented sausages. Food stability, modulation, microbial reaction and determination of the type of microorganisms present in food are dependent on the a_w value (Barbora-Cánovas et al., 2020).

From the results shown in table 1, it can be seen that the a_w of the tested samples is in the range of 0.789 to 0.903, which is very important from the point of view of the sustainability and safety of the product.

The lowest a_w values of 0.789 and 0.795 were recorded in samples K08 and K09, respectively. We hypothesize that the reduced a_w levels in these Kulen samples may be attributed to their exposure in their primary casing (bulk) within a cooler and the extended dehydration process. This observation aligns with the findings of Bersani & Cantoni (2006), who suggested that the a_w of salami decreases during outdoor storage due to prolonged dehydration.

Table 1. Water activity (a_w) in different samples of industrial kulen.

Water activity (a_w) - Kulen														
	K01	**K02**	**K03**	**K04**	**K05**	**K06**	**K07**	**K08**	**K09**	**K10**	**K11**	**K12**	**K13**	**K14**
x̄	0.874	0.903	0.891	0.883	0.881	0.805	0.871	0.789	0.795	0.850	0.852	0.866	0.887	0.846
min	0.873	0.901	0.890	0.882	0.880	0.804	0.869	0.787	0.794	0.848	0.851	0.865	0.886	0.844
max	0.875	0.905	0.892	0.884	0.882	0.806	0.872	0.790	0.796	0.853	0.854	0.867	0.889	0.849
Sd	0.001	0.002	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.002	0.001	0.001	0.001	0.002

n -3, number of samples, x̄ - mean value, min - minimum, max - maximum, Sd – standard deviation

According to Vuković (2006), the a_w of dry fermented sausages typically falls within the range of 0.80 to 0.90. Commercial samples, as reported by Keran et al., (2022), exhibit a water activity ranging from 0.844 to 0.890.

In fermented sausages of the varieties "Bečki," "Slavonski kulen," "Zimska," "Chajna," and "Sremski," produced under industrial conditions using commercial starter cultures, the a_w was reported to be 0.90, with the exception of “Sremski kolbas” where a_w was 0.89, (Frece et al., 2014).

In Slavonian kulen, the a_w value was reported as 0.91 after 20 days of fermentation, as documented by (Mastanjević et al., 2017). Conversely, in fermented sausages, the a_w after 21 days of fermentation ranged from 0.80 to 0.82, according to (Silins, 2014). It's noteworthy that in Mediterranean regions, a low a_w value is given more emphasis for ensuring product safety, in contrast to central and northern Europe where a pH value of 4.6 to 4.9 is considered more critical for the safety of fermented products, (Mitrović, 2016).

From the results shown in table 2, it can be concluded that for the analyzed samples, the lowest pH values of 4.24 and 4.29 were determined for samples K09 and K12, while the highest pH values of 4.95 and 4.87 were determined for samples K06 and K03, respectively.

Table 2. Active acidity (pH) in different samples of industrial kulen

pH value - Kulen														
	K01	**K02**	**K03**	**K04**	**K05**	**K06**	**K07**	**K08**	**K09**	**K10**	**K11**	**K12**	**K13**	**K14**
x̄	4.73	4.64	4.87	4.77	4.62	4.95	4.84	4.44	4.24	4.62	4.52	4.29	4.5	4.76
min	4.71	4.62	4.85	4.75	4.60	4.93	4.82	4.41	4.21	4.6	4.49	4.25	4.48	4.73
max	4.75	4.67	4.90	4.80	4.65	5	4.88	4.50	4.30	4.65	4.55	4.34	4.52	4.80
Sd	0.02	0.02	0.02	0.02	0.02	0.03	0.04	0.05	0.05	0.02	0.03	0.04	0.02	0.03

n -3, number of samples, x̄ - mean value, min - minimum, max - maximum, Sd – standard deviation

Compared to our results, during the analysis of industrial kulen, Keran et al., (2022), determined higher pH values that ranged from 5.43 to 5.53, while according to Vuković, (2006), the lowest measured value for pH in industrial kulen was 4.8. In the fermented sausages of the type "Vienna sausage", "Slavonski kulen", "Zimska", "Chajna" and "Sremski", which were produced in industrial conditions using commercial starter cultures, the pH value were 4.83, 4.57, 4.67, 4.76 and 4.79, respectively, (Frece et al., 2014). During fermentation at a temperature of 20ºC and added glucose of 0.8%, the pH value of Slavonian kulen decreased to 4.42, (Mastanjević et al., 2017).

According to the manufacturer's declaration, samples K06 and K03 were produced using starter cultures, while samples K09 and K12 were produced with the addition of glucano-delta-lactone (GDL). Organic acids and alcohols produced as a result of the growth and metabolism of lactic acid bacteria tend to lower the pH value of products (Austrich-Comas et al., 2023). Additives (acidifiers) are often used to reduce the pH value of sausages, which have the potential to quickly decrease the pH value (Kročko et al., 2016). The most commonly used acidulant is glucano-delta-lactone (GDL), whose use is justified in fermented sausages if the initial fermentation temperature is ≥ 24ºC (Vuković, 2006). GDL is a weak acid that, in contact with water, turns into gluconic acid and over time dissociates into hydrogen ions (Chang et al., 2009; Kročko et al., 2016).

In their study, Joshevska et al., (2021) produced two sets of fermented sausages supplemented with GDL and starter cultures. Sausages prepared with GDL exhibited a lower pH value of 4.83 compared to those made with starter cultures, which had a pH value of 4.96. Similarly, Gramatina et al., (2021) produced two batches of fermented sausages, one with starter culture fermented for four weeks, and the other with GDL fermented for three weeks. Post-fermentation, both batches had nearly identical pH values of 4.73 and 4.72 and aw values of 0.834 and 0.804, respectively. It's anticipated that with prolonged fermentation, the pH value would further decrease. Yim et al., (2015) noted an inverse relationship between the pH value and the amount of added GDL in fermented sausages.

According to Meloni, (2015), producers of fermented sausages face particular concerns regarding the emergence of pathogenic *L. monocytogenes*, which can thrive in a pH range of 4.5 to 9.0 (with optimal growth between pH 6 and 8) and can multiply in food at a a_w value of 0.92 and a sodium chloride (NaCl) concentration of 12%. These conditions are typically lethal to other microorganisms (EFSA, 2014). The pH and a_w values post-fermentation exert the most significant influence on the growth of *L. monocytogenes*. The utilization of lactic acid bacteria, coupled with a pH value below 5.1 and aw below 0.93, creates an inhospitable environment for the proliferation of *L. monocytogenes* (Brusa et al., 2021). In fermented sausages with an initial bacterial count of 105 CFU g-1, the growth of *L. monocytogenes* was inhibited until the 14th day, attributed to effective management of the technological process. Furthermore, in the final product, *L. monocytogenes* was below the detection limit (2 log10) (Zdolec et al., 2007). The inhibition of *L. monocytogenes* in Petrovac sausage was notably more effective and quicker in sausages produced under controlled industrial conditions compared to those produced using traditional techniques (Janković et al., 2017).

According to European regulations, fermented sausages should not promote the growth of of *L. monocytogenes*, especially under conditions where the pH is ≤ 5.0 and the water activity (a_w) is ≤ 0.94. In their analysis of 81 samples of dry sausages packed in vacuum to prove the presence of *Listeria spp*, Hristo et al. (2013) found that in 90.2% of the samples, the pH value exceeded 5, while only 9.8% had a pH value ranging from 4.4 to 5. Among the samples with a pH value greater than 5, four were contaminated with *Listeria spp*. The average a_w value for the tested sausages was 0.87. Considering the results presented in both tables for pH and a_w values in the analyzed industrially produced kulen, it can be deduced that all samples have a pH value lower than 5 and an a_w value lower than 0.94. Consequently, these products are expected to be safe

against *L. monocytogenes*. However, for definitive confirmation of their safety, additional microbiological analyses are necessary to ascertain the absence of *L. monocytogenes*.
Based on the results presented in Table 3, the sensory evaluation indicates that K02 kulen received the highest weighted mean score of 4.69, identifying it as the most sensory-acceptable kulen. In contrast, K03 kulen received the lowest score of 3.79. Specifically, K02 kulen received the highest ratings for the quality parameters of appearance, color, and taste. On the other hand, K08 received the highest ratings of 4.7 for smell and consistency.

Table 3. Sensory evaluation results of the different samples of industrial kulen

Quality indicator		**Appearance**	**Color**	**Smell**	**Taste**	**Consistency**	**Weighted mean score**	**% of max possible quality**
	CV Σ 20	**5**	**3**	**4**	**5**	**3**		
Samples								
K01		3.85	3.85	4.45	4.15	3.85	4.05	80.9
K02		4.75	4.8	4.6	4.8	4.4	4.69	93.75
K03		3.85	3.45	4.2	3.45	4	3.79	75.8
K04		4.15	4.4	4.45	3.85	4.3	4.19	83.9
K05		3.3	2.75	3.45	3.85	3.1	3.35	67.1
K06		4	4.25	4.4	4.4	4.5	4.29	85.85
K07		3.85	3.6	4.2	4	3.8	3.91	78.25
K08		4.4	4.7	4.7	4.7	4.7	4.62	92.5
K09		4.3	4.6	4.45	4.2	4.4	4.36	87.3
K10		4.4	4.7	4.5	4.4	4.2	4.43	88.7
K11		4.5	4.2	4.2	4	4	4.19	83.9
K12		4.2	3.85	4.2	4.2	4	4.11	82.35
K13		4.15	4	4	4	3.85	4.01	80.3
K14		3.85	3.85	3.9	3.8	3.7	3.82	76.5

4. Conclusions

The pH and a_w value was analyzed in 14 samples of (K01-K14) industrial production kulen available for sale in markets and produced by different manufacturers (brands) in the Republic of North Macedonia and the countries in the region.
From the sensory evaluation, it is evident that K02 kulen achieved the highest weighted mean score of 4.69, indicating it is perceived as the most sensory acceptable kulen. In contrast, K03 kulen obtained the lowest score of 3.79.
The results obtained for the pH value ranged from 4.24 to 4.97, while the a_w value ranged from 0.789 to 0.903. According to European regulations, fermented sausages should not promote the growth of *L. monocytogenes* under conditions of pH $\leq$ 5.0 and $a_w \leq 0.94$. All analyzed kulen samples exhibited a pH value below 5 and an a_w value below 0.92. This low pH and a_w values suggest that the studied Kulen products available in the market fall within the safe range in terms of pH and a_w concerning *L. monocytogenes*. However, to confirm their safety conclusively, additional analyses are required to verify the absence of *L. monocytogenes*.
It is crucial to acknowledge that the pH and a_w values obtained for the different kulen cultivars represent their current state. Further research is necessary to evaluate the safety of kulen and monitor changes in pH and a_w values based on variations in packaging, storage methods, and duration. These additional studies would provide a more specific understanding of the stability of pH and a_w values over the entire storage period. Such results would serve as a valuable guide for refining technological processes, optimizing packaging, and improving storage methods to ensure and sustain the microbiological safety of kulen.

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