https://doi.org/10.17221/99/2023-CJFS

Water activity of Czech dry-cured meat products: Influence of sampling point and sample preparation method

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Citation: Beňo F., Kostlán J., Pivoňka J., Pohůnek V., Ševčík R. (2023): Water activity of Czech dry-cured meat products: Influence of sampling point and sample preparation method. Czech J. Food Sci., 41: 340–347.

Abstract: Water activity (a_w) is a key factor influencing dry-cured meat products' safety. However, the standards for determining a_w do not specify how the laboratory sample for self-analysis should be prepared and adjusted for determination in this type of food. This work aimed to verify whether the place of sampling and method of sample preparation of dry-cured meat products can influence the measured value of a_w. For this purpose, samples of dry-cured heat-treated and fermented meat products were purchased from the local market. Samples before analysis were taken from the edges and centre of the meat products, and preparation consisted of: *i*) homogenisation; *ii*) dicing (4 × 4 × 4 mm); *iii*) slicing. The results of this work indicate that a_w is significantly affected by both the part of the product from which the sample is taken and the method of preparation of the sample itself ($P < 0.05$). The highest measured values of a_w were determined in samples prepared by slicing, and the lowest values were determined in homogenised samples. The place of sampling significantly affects the a_w, especially for dry-cured heat-treated products.

Keywords: available water; safety control; sampling method; stability

An important group of meat products are dry-cured meat products, which are traditional foods produced and consumed in different regions worldwide. Their importance in the market is widely known because of the strict requirements of legislation and the high demands of consumers who expect high quality, assured safety, and durability in these products (Lippolis et al. 2016). In addition to the technological processes used to produce meat products to ensure stability, there are other obstacles, such as adding chemical additives (salt, nitrites), competing microorganisms, and antimicrobials in spices or smoke (Gomez et al. 2020).

One of the basic indicators of the shelf life of dry-cured meat products is the knowledge of water mobility, and its availability for undesirable microbial and nonmicrobial spoilage processes is the so-called water activity (a_w). Water activity is the main parameter for nutritional stability, modulation, microbial reaction, and determination of the type of microorganisms present in food (Barbora-Cánovas et al. 2020). Knowledge

Supported by the grant of Specific university research at University of Chemistry and Technology, Prague (Project No. A1_FPBT_2023_004).

https://doi.org/10.17221/99/2023-CJFS

of the a_w value of dry-cured meat products, whether cooked or dried and fermented, helps producers control safety, as a_w significantly impacts the possible growth of undesirable microbial pathogens or spoilage microorganisms (Rao 1997; Nielsen et al. 2012).

Determination of a_w can be performed on almost all foods of various forms (liquid, solid, powder, granules), in most cases without complex sample modification. The sample is placed directly in the measuring cell, or the sample requires simple modification in the form of a size reduction. If the sample comprises multiple components, care must ensure that all components are proportionally represented in the measuring cell as in the product. Before measuring, it is also necessary to have the sample heated to laboratory temperature (frozen products) to eliminate possible moisture condensation in the measuring cell (Cazier and Gekas 2001; Nollet 2004; Barbora-Cánovas et al. 2020).

In the Czech Republic, a_w is the main safety parameter of dry-cured meat products, which must not exceed the value of 0.93 in dry-cured meat products, according to Decree No. 69/2016 Coll. (2016). However, this Decree does not specify how the sample should be prepared before the analysis. It also does not specify the location from which it is necessary to take a sample for a representative evaluation. As far as we know, no decree describes such a methodology; any handling, time delay, change in storage, preparation, or sample handling conditions can affect the resulting measured a_w of meat products, as confirmed by previous studies (Troller and Christian 1978; Harper et al. 2010; Little 2010).

This study aims to investigate the effect of different sample preparation methods and the influence of the sampling location on the a_w value of dry-cured meat products. The results of this work could help producers and inspection authorities verify the safety of meat products compared with current legislation.

MATERIAL AND METHODS

Dry-cured meat products

Two types of dry-cured meat products from the Czech market were selected to evaluate the place of sampling and the sample preparation method before determining a_w. These included dry-cured heat-treated meat products (Vysočina salami) and fermented meat products (Poličan salami, Gombasecká sausage) made by Czech producers. Storage occurred at constant temperature in the laboratory thermostat POL-EKO ST2B40 (POL-EKO, Poland) to prevent the a_w in the sample from changing. The storage temperature was set according to the type of meat product (Decree No. 69/2016 Coll.) at 20.0 ± 0.1 °C. Samples of meat products were stored before analysis in a whole package. Before each determination of a_w, the meat products were removed from the thermostat well in advance and left to temper at laboratory temperature.

Determination of a_w

A_w was determined using AquaLab 4TEV (Meter, USA) with a dew point sensor with a measurement accuracy of ± 0.003. The instrument uses dew point detection techniques to measure the a_w of the sample by using a cooled mirror. The sample is brought into equilibrium with the main body of the sealed chamber, which contains a mirror and means for detecting condensation on the mirror. At equilibrium, the relative humidity of the air in the room is equal to the a_w of the sample. A thermoelectric (Peltier) cooler precisely regulates the mirror's temperature in the device. The detection of the exact point of the beginning of condensation on the mirror is monitored by a photoelectric cell. A beam of light is directed at the mirror, which is reflected to a photodetector, which records the change in reflectance when condensation occurs on the mirror. A thermocouple attached to the mirror records the temperature at which condensation occurred. In addition to the abovementioned technique, AquaLab uses an internal fan to circulate air inside the measuring cell to reduce the time required to reach equilibrium. When measuring a_w, working with a clean sample dish filled with the product to a maximum of 1/2 height was always necessary. Calibration was performed with salt calibration standards based on the expected range of meat products a_w value. In our case, calibration solutions with a_w = 0.920 and a_w = 0.760 were used.

Sample collection location

As part of our work, it was necessary to standardise the sampling method, i.e. from which part of the product it is appropriate to take a sample for analysis, as the methodology for sampling from meat products is unavailable in both Czech and foreign literature. For this part of the experiment, a sample was divided the product into four parts, in which the a_w was determined. The proposed sampling points can be seen in Figure 1. Such a division is beneficial for products whose diameter is more significant than 3.5 cm. Up to a width of 3.5 cm of the product, a slice of the entire width of the product is used for analysis, especially for determining a_w. An area that occupies 6/10 of the length of the product and 1/3 of its width was chosen as the core (A area) of the product for the

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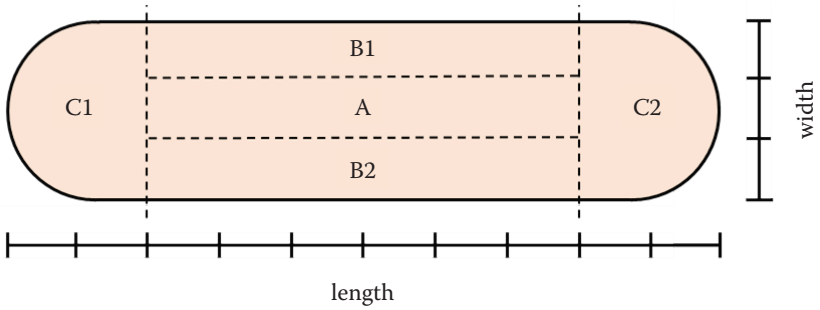

Figure 1. Indicative product partitioning for a_w analysis of salamis with a diameter over 3.5 cm

a_w – water activity; A – product core; B1, B2 – edges around the core; C1, C2 – narrowed edges

experiment. This area was chosen based on knowledge comparable to the heat treatment of the sample, where it is important to know the worst heated spot. Edge parts B1 and B2, surrounding the core (A), make up 6/10 of the length of the product. It should be noted that areas B1 and B2 are only one area that creates a cylinder with a wall width equal to 1/3 of the product's width. Areas C1 and C2 are narrow edges of the products. During drying and fermentation, the products hang behind the C1 area (the upper part).

Sample preparation

This work proposes 3 sample preparation methods to measure a_w in meat products – slicing, dicing, and homogenisation (grinding) (Figure 2). After cutting and preparation of the representative sample, the remaining whole meat product is always wrapped with food-grade foil between measurements to prevent the sample from drying out. To test the effect of sample preparation on a_w determination, all types of sample preparation were carried out in all parts of the sausages. Before each measurement, the steel dishes were thoroughly washed, dried, and tempered to laboratory temperature. The prepared samples were stored in closed containers at 20 °C before analysis to prevent humidity changes.

Slicing. Samples up to 3.5 cm in diameter were sliced to fill no more than 1/2 the height of the steel dish. In the case of a sausage width > 3.5 cm, only the centre of the sample – area A – was sliced.

Dicing. During dicing, a 4 mm thick slice was first cut. The slices cut off were then cut into cubes of cube size 4.0 × 4.0 × 4.0 mm. The cubes were then placed in a steel dish prior to analysis.

Homogenisation (grinding). Homogenisation was performed in a Grindomix GM 200 (Retsch GmbH, Germany) at 4 000 rpm for 10 s. From each homogenised sample, a quantity was transferred into a steel dish such that each tray was filled to a maximum of 1/2 its height.

Dry matter and pH determination

The dry matter of the meat products was determined by drying in an oven UF110m (Memmert GmbH, Germany) at 105.0 ± 1.0 °C to a constant weight (Helrich 1990). The pH value was measured using a pH-me-

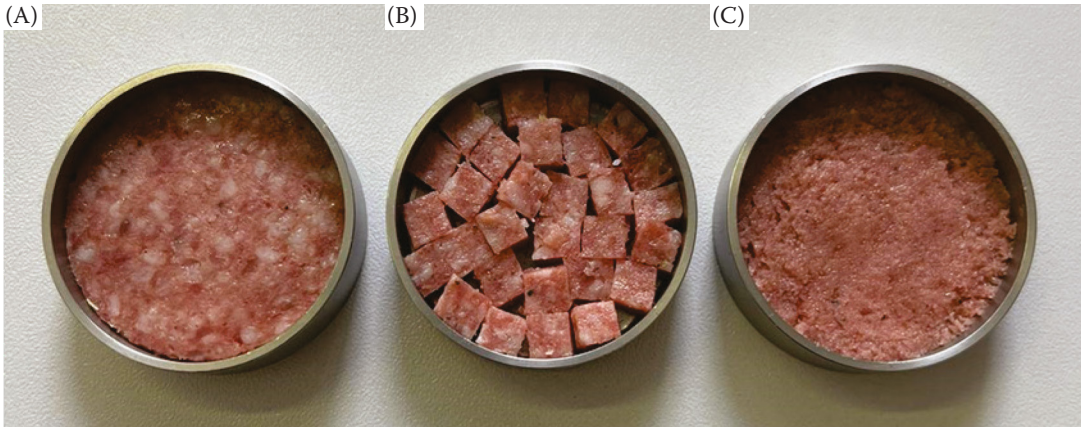

Figure 2. Methods for sample preparation – (A) slicing, (B) dicing, and (C) homogenisation

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ter pH8+ DHS (XS Instruments, Italy) with pH glass electrode XS Sensor Flat (XS Instruments, Italy) for surface measurements.

Statistical analysis

The data collected were used for statistical evaluation in STATISTICA 12.0 software (StatSoft Inc., USA). First, one-way analysis of variance (ANOVA) was used to compare the effect of sampling location on the a_w value. Then, a post hoc HSD Tukey multiple comparison test was used to see the different data groups. The data was then statistically processed in the same way each sample was prepared. The significance level was established at $P = 0.05$. Before the statistical evaluation, the Dean-Dixon test was performed to exclude outliers.

RESULTS AND DISCUSSION

The time between sample preparation and measurement should be as short as possible to avoid moisture exchange with the room air (Barbora-Cánovas et al. 2020). The actual measurement should also be performed quickly and accurately. Obtaining the expected results also depends on how the samples are prepared (Nielsen et al. 2012; Barbora-Cánovas et al. 2020).

Effect of sample preparation and location. The first tested sample was a heat-treated dry-cured meat product – Vysočina. This was a diameter product with a larger than the diameter of the steel plate. Therefore, it was divided into areas (Figure 1). Table 1 shows the results of the determination of a_w in the different regions and compares the other sample preparation methods for analysis.

After statistical processing of the a_w data, it can be concluded that there was a statistically significant difference in the sampling locations of the slicing method between area A and all other areas (B, C1, C2) ($P < 0.05$). In the case of the dicing sample preparation method, there was also a statistically significant difference between area A and all other areas (B, C1, C2) ($P < 0.05$). Similar to slicing, no statistically significant effect was confirmed between the other areas when compared ($P > 0.05$). Regarding the homogenisation of the sample before analysis, area A differed significantly ($P < 0.05$) only with area C (the upper part of the product). Thus, in the case of homogenisation, there is a disturbance of the integrity of the product that practically balances the value of the a_w from the different parts of the product. Consequently, the highest water activity values were in the samples prepared by slicing. Similar trends have been reported by Harper et al. (2010) in their work on testing the effect of sample preparation on determining water activity in jerky products. In their work, they compared samples prepared by cutting slices in a hexagonal shape (3.2 cm diameter), which had a significantly lower water activity value ($P < 0.001$) compared to cubed samples (0.4 × 0.4 cm). Therefore, their work's conclusions are identical to ours, with the recommended method of preparing an intact sample to preserve its integrity.

Although there were no statistically significant differences between the sampling areas in the case of slicing and dicing, again, the highest a_w values were measured in the product's core in all cases. This again confirms that the product's core is the most suitable location to determine a_w values. Also, regarding statistical data processing, area A (core) emerges as the most appropriate sampling area for measuring a_w, which came out as the highest in all cases. It should also be noted that in the case of this sample, the a_w value was not < 0.93 in either case. The samples were stored accord-

Table 1. Dependence of sample preparation and area on a_w – Vysočina salami

Area	a_w			One-way ANOVA (sample preparation)
	sample preparation			
	slices	dicing	homogenisation	
A (n = 60)	$0.956^{bcdBC} \pm 0.005$	$0.951^{bcdAC} \pm 0.003$	$0.941^{cAB} \pm 0.004$	1.11×10^{-16}
B (n = 30)	$0.952^{aBC} \pm 0.006$	$0.947^{aAC} \pm 0.003$	$0.940^{AB} \pm 0.006$	1.03×10^{-13}
C1 (n = 18)	$0.950^{aBC} \pm 0.006$	$0.946^{aAC} \pm 0.003$	$0.938^{aAB} \pm 0.006$	5.43×10^{-9}
C2 (n = 18)	$0.950^{aBC} \pm 0.005$	$0.946^{aAC} \pm 0.002$	$0.939^{AB} \pm 0.003$	4.09×10^{-12}
One-way ANOVA (areas)	1.67×10^{-6}	8.04×10^{-11}	0.0286	–

$^{a–d}$ means in a column (difference between sampling location), respectively $^{A–C}$ means in a row (difference between sample preparation), with a different superscript letter differing ($P < 0.05$) as analysed by one-way ANOVA; values are shown as mean ± standard deviation (SD); n – number of determinations; A – product core; B – edge around the core; C1, C2 – narrowed edges; a_w – water activity

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Table 2. Dependence of sample preparation and area on a_w – Poličan salami

Area	a_w			One-way ANOVA (sample preparation)
	sample preparation			
	slices	dicing	homogenisation	
A (n = 60)	$0.779^{cdC} \pm 0.024$	$0.784^{cdC} \pm 0.028$	$0.756^{cAB} \pm 0.024$	1.19×10^{-8}
B (n = 30)	$0.770^{c} \pm 0.022$	$0.781^{C} \pm 0.030$	$0.757^{cB} \pm 0.025$	0.0027
C1 (n = 18)	$0.748^{ab} \pm 0.029$	$0.760^{aC} \pm 0.032$	$0.731^{abB} \pm 0.029$	0.0231
C2 (n = 18)	$0.755^{a} \pm 0.024$	$0.761^{a} \pm 0.033$	0.742 ± 0.025	0.1200
One-way ANOVA (areas)	1.06×10^{-5}	0.0042	0.0010	–

$^{a–d}$ means in a column (difference between sampling location), respectively $^{A–C}$ means in a row (difference between sample preparation), with a different superscript letter differing ($P < 0.05$) as analysed by one-way ANOVA; values are shown as mean ± standard deviation (SD); n – number of determinations; A – product core; B – edge around the core; C1, C2 – narrowed edges; a_w – water activity

ing to the recommended storage conditions indicated on the product label at temperatures up to 20 °C. Therefore, according to Decree No. 69/2016 Coll. (2016), the correct technological procedure was not followed.

In addition to dry-cured heat-treated meat products, the effect of sampling location and the sample preparation method for measuring the a_w of fermented meat products was investigated. The first representative product was a sample of Poličan salami. The measured a_w values and the influence of the sampling point for the analysis can be seen in Table 2.

The a_w value of the fermented meat product was again the highest in the core of the product (area A). This trend was observed mainly for the sliced and diced samples before analysis. The lowest values were measured in areas C1 and C2, where the a_w values were statistically significantly lower than the product's core ($P < 0.05$) and other areas, respectively. The a_w values of the sample between areas A and B were insignificant in any of the preparation methods. This is probably due to the product manufacturing process itself, where the fermentation process during the manufacturing process is more uniform for this type of product compared to the heat treatment and subsequent drying of the product compared to the heat treatment and subsequent drying of the product. Regarding the individual sample preparation methods for analysis, the dicing method showed the highest average a_w values. Thus, this fact does not correlate with the above results for the heat-treated product, suggesting that the slicing method is the most suitable sample preparation method. A problem may arise in drying dry-cured heat-treated meat products, as confirmed by a study by Benli (2017), where the a_w values for these products were also higher (0.916–0.940) than fermented products.

Table 3 shows the a_w of the last product (Gombasecká sausage). It was a fermented product with a small diameter of about 3.5 cm. The sample was divided into tenths, with the first 2/10 representing its tighter upper end (area C1) and the last 2/10 representing the area of C2. The remaining 8/10 were designated as area A. Although there were no statistically significant differences between the sampling points

Table 3. Dependence of sample preparation and area on a_w – Gombasecká sausage

Area	a_w			One-way ANOVA (sample preparation)
	sample preparation			
	slices	dicing	homogenisation	
A (n = 30)	$0.876^{b} \pm 0.019$	0.873 ± 0.019	$0.869^{c} \pm 0.009$	0.0283
C1 (n = 10)	$0.860^{a} \pm 0.011$	0.865 ± 0.003	$0.863^{c} \pm 0.010$	0.1631
C2 (n = 10)	$0.866^{C} \pm 0.004$	$0.864^{C} \pm 0.004$	$0.851^{abAB} \pm 0.007$	1.24×10^{-5}
One-way ANOVA (areas)	0.2720	0.4731	2.36×10^{-6}	–

$^{a–c}$ means in a column (difference between sampling location), respectively $^{A–C}$ means in a row (difference between sample preparation), with a different superscript letter differing ($P < 0.05$) as analysed by one-way ANOVA; values are shown as mean ± standard deviation (SD); n – number of determinations; A – product core; B – edge around the core; C1, C2 – narrowed edges; a_w – water activity

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in all cases, it was confirmed that the highest measured values of a_w were again in area A, the product's core. There was a statistical difference only in the slicing method ($P < 0.05$).

In all samples tested, it was confirmed that the highest a_w was in the core of the product (area A). Similarly, the water content was highest in area A. Although the water content and the water activity value are not directly related, it can be presumed that a high water content in the product's core will also result in a higher water activity value. Petit et al. (2014) similarly conclude from their data that a_w decreases with decreasing water content. We can also hypothesise that the higher water content in area A of the product results in a lower salt concentration than in areas B, C1, and C2. As reported by Gurtler et al. (2014), the salting process significantly reduces the free water content of the product with a consequent reduction in water activity. Bjarnadottir et al. (2020) also confirmed that a higher concentration of salt results in higher water activity value in dry-cured ham, where increasing the salt concentration by 2.5% decreased the water activity from 0.921 to 0.856. Salt and water create osmotic changes in the meat product, causing osmotic dehydration and removing water from the meat (Dimakopoulou-Papazoglou and Katsanidis 2017; Barat et al. 2011).

The preparation of the samples before analysis by homogenisation resulted in the lowest measured a_w values. Therefore, it can be hypothesised that this sample preparation method is unsuitable for this type of product and heat-treated dry-cured meat products. The pH values of all products were comparable to those of other studies. By the requirements of Commission Regulation (EC) No. 2073/2005 (Commission Regulation of 15th November 2005 on microbiological criteria for foodstuffs, Official Journal of the European Union L 338, 22nd December 2005: 1–26) for microbial controls, it can be concluded that the fermented meat product Poličan, which has been found (in all areas) to have pH values > 5 and $a_w < 0.94$, supports the growth of bacteria *Listeria monocytogenes*. On this basis, microbial controls should be carried out by that Regulation.

Dry matter and pH value. The dry matter content in the core of all tested meat products was always significantly higher ($P < 0.05$) (Table 4). This fact is closely related to the higher a_w value, which was, in fact, the highest in area A. Similarly, the high water content of the core supports our contention that samples for the determination of a_w should be prepared from the product's core. The dry matter content of the Vysočina product ranged from 57 to 62%, consistent with Válková et al. (2006). On the contrary, the dry matter content of the fermented products (Poličan, Gombasecká) was more than 20% higher. This may be related to the lower water activity of fermented products combined with a lower pH value. However, no significant differences ($P > 0.05$) were observed for pH values, only for the Gombasecká sausage sample, where the pH value in the core was the lowest. The average pH values ranged from 5.78 to 5.84 of Vysočina salami are in line with those obtained by Válková et al. (2006) and Węglarz (2010). As in foreign publications, the average pH values of the fermented products did not exceed pH 5.2 (Hughes et al. 2002; Herrero et al. 2007).

In the case of fermented meat products, microbiological activity (including LAB – lactic acid bacteria) is determined by the level of available water, which correlates with a_w, which is then a safety criterion (Stadnik et al. 2022). In general, a water activity value

Table 4. Dry matter content and pH value in different areas of meat products

Area	Dry matter (%) (n = 8)			pH (n = 15)		
	Vysočina	Poličan	Gombasecká sausage	Vysočina	Poličan	Gombasecká sausage
A	$57.51^{bcd} \pm 2.37$	$79.57^{bcd} \pm 2.11$	$76.51^{bc} \pm 0.87$	5.84 ± 0.20	5.11 ± 0.14	$4.84^{bc} \pm 0.13$
B	$60.92^{a} \pm 2.62$	$81.62^{a} \pm 2.04$	–	5.78 ± 0.24	5.13 ± 0.16	–
C1	$61.70^{a} \pm 3.04$	$83.01^{a} \pm 2.17$	$77.61^{a} \pm 1.92$	5.84 ± 0.23	5.11 ± 0.16	$4.93^{a} \pm 0.17$
C2	$61.26^{a} \pm 2.51$	$82.33^{a} \pm 2.72$	$77.93^{a} \pm 0.83$	5.78 ± 0.19	5.16 ± 0.17	$4.92^{a} \pm 0.19$
One-way ANOVA	2.27×10^{-5}	0.0001	0.0083	0.1766	0.0921	0.0050

$^{a–d}$ means in a column (difference between sampling location) with a different superscript letter differing ($P < 0.05$) as analysed by one-way ANOVA; values are shown as mean ± standard deviation (SD); n – number of determinations; A – product core; B – edge around the core; C1 – C1, C2 – narrowed edges

https://doi.org/10.17221/99/2023-CJFS

of 0.980–0.995 is reported to be most conducive to the growth of microorganisms. Inhibition of the growth of microorganisms then starts at water activity less than 0.85, as Dave et al. (2011) reported, which does not correlate with the results of the water activity determination of Gombasecká sausage. This could be due to the higher water content of Poličan salami, where the water content was lower in all products.

CONCLUSION

As a safety parameter, the water activity of meat products is influenced by sample preparation before analysis, in addition to the production and raw materials used. The slicing method was found to be the most suitable method for sample preparation of our data, as it ensures the integrity of the product. Dicing and homogenisation result in fat spreading, distorting the resulting water activity value. Our data show that the lowest water activity values were found for the homogenisation method. It is also necessary to take the sample for analysis from the product's core, where the production processes (drying, fermentation) are the slowest. The water content, which was also highest in the core of the products, also supports our hypothesis that the samples for water activity analysis should be from the product's core.

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Received: July 3, 2023
Accepted: September 6, 2023
Published online: September 13, 2023