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THE IMPACT OF THE INFUSION METHOD OF CHOKEBERRY POWDER IN WHITE TEA

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1.Introduction

Tea is a beverage with a global popularity that transcends geographical boundaries. It is consumed in a variety of forms, including hot and cold. The most commonly consumed teas (green tea, black tea, oolong tea, white tea, and yellow tea) are derived from the leaves and buds of the *Camellia sinensis (L.)* plant, belonging to the *Theaceae* family. These teas are distinguished by variations in harvesting, processing, and the degree of oxidation of the polyphenols present in fresh tea leaves (Sharangi, 2009; Unachukwu et al., 2010). As stated by some authors (Damiani et al., 2014), the two most prevalent grades, Silver Needle

and White Peony, are commercially accessible. However, there are numerous other varieties with diverse trade names.

The Silver Needle (Bai Hao Yinzhen in its traditional name) is manufactured exclusively from the unopened buds of the plant, without any leaves. As its name indicates, it is characterized by a silver-white hue and comprises long, thin needles. The buds are initially sun-dried on sieves or drying mats for a period of approximately 24 hours, representing the initial phase of the processing method. This is then followed by baking over a low fire until the buds are fully desiccated. The final product exhibits a subtle flavor profile and a pale-yellow

hue. The White Peony (traditional name Bai Mudan) is manufactured from the bud and one or two leaves derived from the plant's vegetative apex. The processing of the leaves involves two simple phases: withering (sun drying/airing/low temperature) and basket drying. The resulting tea exhibits a light golden-brown color and a pleasant roasted aroma. The flowers and leaves of the *Camellia sinensis* plant contain a variety of bioactive substances, including nutrients (carbohydrates, proteins, and minerals), alkaloids (methylxanthines), and phenolic compounds (phenolic acids, flavonoids, and tannins) (Sharma et al., 2021). White tea (WT) is described as "tea for one year, medicine for three years, and treasure for seven years" (Cheng et al., 2021), which indicates the increasing nutritional and functional values of aging WT. White tea (WT) has been demonstrated to exert beneficial effects on human health, including the prevention and treatment of diabetes, cancer, bacterial infections, and obesity (Olcha et al., 2022).

The chokeberry, also known as Aronia, is a *Rosaceae* shrub that is native to North America and was introduced to Europe approximately a century ago (Chrubasik et al., 2010; Sidor et al., 2019). Black chokeberries are a rich source of polyphenols, which contribute to their high biological activity. The polyphenols present in these berries include anthocyanins, flavonols, flavanols, proanthocyanidins, and phenolic acids (Tolić et al., 2017). A previous study conducted by Kokotkiewicz identified the phenolic chemicals present in chokeberry fruit, including procyanidins (0.7-5.2%) and anthocyanins (0.6-2.0%), as the primary classes with therapeutic characteristics (Kokotkiewicz et al., 2010). The high concentrations of anthocyanin and polyphenols may exert a protective effect against the development of cancer, diabetes, gastrointestinal disease, and cardiovascular disease (Burdejova et al., 2020). Given the popularity of chokeberry and its associated health benefits, a variety of preparations have been developed on an industrial scale, including concentrated extracts, juices, and Aronia food products. One

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particularly valuable product is fruit powder, which is obtained by drying and grinding the fruit. The primary advantage of fruit drying is the extension of the product's shelf life.

Given the numerous advantages offered by both white tea and chokeberry powder, our objective is to enhance the biological activity of white tea by developing a new product. This will be achieved by incorporating chokeberry powder into white tea, thereby investigating the impact of this addition on the tea's properties, including pH, acidity, and viscosity. Additionally, a sensory analysis of the teas was conducted.

2. Materials and methods

2.1. Materials

2.1.1. Raw materials

 The white tea utilized in the experimental research is a dry white peony tea procured from the supermarket and produced by the Basilur company. The chokeberry powder is an organic powder from the Aronia Charlottenburg company, procured from a commercial establishment. The nutritional information, as indicated on the product label, is as follows: The energy value is 1013kJ/242kcal, with fat comprising 2.5g-6.0g, of which saturated fatty acids account for 0.4g. Carbohydrates constitute 40-8.0g, with sugars amounting to 1.8-2.8g. Fiber is present in quantities of 70.0-80.0g, while proteins are found in amounts of 5.0- 10.0g. Salt is present in quantities of less than 0.01g.

2.1.2. Sample preparation

 In the experimental research, two infusion methods were employed in accordance with the methodology illustrated in Figure 1: hot infusion and cold infusion. Hot tea infusions were prepared by adding 100 mL of water at 70° C to 2.5 g of white tea and allowing the infusion to proceed for seven minutes (Damiani et al., 2014). The cold infusion was prepared by adding 100 mL of water at room temperature to 2.5 g of white tea and allowing the infusion to stand at room temperature (20–25 $^{\circ}$ C) for two hours, agitating continuously using a magnetic stirrer (IKA, RET basic, Germany) (Damiani et al., 2014). The same recipes were used for both types of infusion, with the same chokeberry powder concentrations. The concentrations of the chokeberry powder were 0%, 0.6%, 0.8%, and 1%. Prior to analysis, all samples were filtered through a 0.45 μm membrane filter.

Figure 1. Diagram of the infusion process of white tea with chokeberry powder. THC -White tea hot infusion; TH1- White tea with chokeberry powder 0.6% hot infusion; TH2- White tea with chokeberry powder 0.8% hot infusion; TH3- White tea with chokeberry powder 1% hot infusion; TCC -White tea cold infusion; TC1 - White tea with chokeberry powder 0.6% cold infusion; TC2 - White tea with chokeberry powder 0.8% cold infusion; TC3 - White tea with chokeberry powder 1 % cold infusion.

2.2. Methods

2.2.1. Total polyphenolic content

 Using gallic acid as a reference, the total polyphenolic content (TPC) was calculated using the Folin-Ciocalteu spectrophotometric method and represented as milligrams of gallic acid equivalents per milliliter (µg GAE/mL) (Singleton et al., 1999). Folin-Ciocalteu reagent (Merck, Darmstadt, Germany), 1.25 mL, was applied to a 0.5 mL sample after being diluted 1:10 (v/v) with distilled water. 1 mL of Na₂CO₃ 60 g/L was added after the mixture had been incubated for 5 minutes at room temperature. The sample absorbance at 750 nm was measured using a UV-VIS spectrophotometer (Specord 205, Analytik Jena Inc., Jena, Germany) after 30 min of incubation at 50 °C. Gallic acid was used as the standard, with concentrations ranging from 5 to 25 mg GAE/mL, to create the calibration curve.

2.2.2. Antioxidant activity (ABTS assay)

 The ABTS assay measurement of the different teas was performed according to the method described by (Damiani et al., 2014; Re et al., 1999). A 7.0 mmol/L aqueous ABTS [2,20-azinobis-(3-ethylbenzothiazoline-6) diammonium salt] solution was mixed in a 0.9:0.1 ratio with a 2.45 mmol/L aqueous solution of potassium persulfate as an oxidizing agent to quantify the radical cation (ABTS•+). Prior to use, the combination was stored at room temperature in the dark for 12–16 hours. To get an absorbance at 734 nm ranging from 0.6 to 0.8, the ABTS•+ solution stock was 80-fold diluted with water before to use. 0.025 mL of previously diluted tea, adequately diluted Trolox (6 hydroxy-2,5,7,8-tetramethylchro- man-2 carboxylic acid) standard ethanolic solution, or water as a control were added to 2.475 mL of this ABTS•+ solution, and the mixture was then added. The samples were kept at room temperature in the dark for two hours, and the samples' absorbance was measured against water at 734 nm. The following equation was used to compute inhibition percentage values (Equation 1):

*Inhibition of A*₇₃₄
$$
(\%) = \left(1 - \frac{A_C}{A_0}\right) \cdot 100
$$
 (1)

where Ac is an absorbance of the samples, A0 is an absorbance of the control. Antioxidant activity was expressed as mmol/L Trolox Equivalents (TE) using the linear regression value obtained from the Trolox calibration curve.

2.2.3. pH measurement

The pH was measured using the electrochemical method (Webster, 2003) with a potentiometer (Consort C1010, Consort, Turnhout, Belgium).

2.2.4. Viscosity

The viscosity $(p_{\text{c}}[cP])$ was determined using the Brookfield rheometer (Brookfield

Engineering Laboratories, Inc., Middleborough, MA, USA). The torque required to drive a disk, immersed at a predetermined depth, in the liquid whose viscosity is to be determined, into a rotational movement with a certain speed, will be measured. It is established that a thin layer of liquid adheres to the surface of a body in contact with a liquid due to adhesion forces. This layer, known as the boundary layer, moves as a single entity with the surface to which it has adhered, thus exhibiting the same velocity. The molecular cohesion forces exerted by the molecules in the layer under consideration will cause the molecules in the neighboring layer to move at a

slower speed due to the sliding between the layers of molecules.

The accuracy of the Brookfield viscometer was evaluated using a standard-viscosity liquid provided by the Cannon Instrument Company (State College, PA). The results demonstrated that the viscosity measurement data shear rate of 300 s^{-1} from the Brookfield viscometer was found to be within 5% of the standard viscosity value. The torque output became unstable at the shear rate of $300 s^{-1}$, which equaled 40 rpm. This instability is likely due to secondary flow caused by centrifugal force in the gap between the cone and plate geometries (Lee et al., 2012).

2.2.5. Sensory analysis

A total of one hundred evaluators, comprising both male and female participants between the ages of 21 and 60, were invited to score the tea samples on a 5-point hedonic scale, ranging from least favored (1, "dislike very much") to most liked (5, "like very much"). This was done in order to ascertain consumer preference for the tested tea samples. Infusions were evaluated considering the criterion of color, brightness, clarity, astringency, aroma and bitterness. The panelists received eight distinct tea samples in 150-ml tea cups, each bearing a randomly assigned number. Tea samples were interspersed with opportunities for participants to rinse their palates with water. The sensory analysis was conducted at room temperature in a facility equipped with LED lighting. The evaluators were selected from the personnel and student body at Transilvania University of Brașov.

2.2.6. Statistical analysis

Each tea sample was tested in triplicate, and the results of the three separate tests were averaged to yield a single value for each sample. All data are presented as the mean of the three replicates, followed by the standard deviation (SD). The significance of mean differences was assessed by one-way ANOVA. Tukey's test $(p \le 0.05)$ was used to compare mean differences. The correlation analysis was employed to estimate the degree of correlation between the data sets, while the regression analysis was utilized to model the relationship between the

predictor variables and the investigated parameters (JASP Team, 2023).

3.Results and discussions

As illustrated in Figure 1, eight distinct tea varieties were obtained through both cold and hot infusion methods. These teas were then subjected to comprehensive analysis, evaluating

their polyphenol content, antioxidant activity, viscosity, and pH. The findings from this experimental research were collated and presented in Table 1.

Analysis	Hot infusion				Cold infusion			
	THC	TH ₁	TH ₂	TH ₃	TCC	TC1	TC ₂	TC ₃
TPC	$4.05 \pm$	$8.28 \pm$	$9.11 \pm$	$11.13 \pm$	$8.25 \pm$	$19.4 \pm$	21.9 _±	$33.4+$
[mg/mL]	0.02 ^a	0.78^{b}	$0.56^{\rm b}$	0.27^{bc}	0.21 ^b	0.09 ^c	0.20 ^c	0.71 ^d
Antioxidant activity [mmol/L]	$14.98 \pm$ 1.76 ^a	$31.27 \pm$ 1.23 ^c	$33.03+$ 1.02 ^c	$36.31 \pm$ 1.76 ^c	26.3 [±] 1.01 ^b	$71.78 \pm$ 1.98 ^d	$74.32+$ 1.65 ^d	$82.45 \pm$ 1.89 ^d
pH	$7.82 +$	$7.78 \pm$	$7.75 \pm$	$7.73+$	$8.08\pm$	$7.97 \pm$	$7.95 \pm$	$7.92 \pm$
	0.76 ^a	0.98 ^{ab}	0.02 ^b	0.98 ^b	0.09 ^c	0.97 ^d	0.1 ^d	0.12 ^d
η [cP]	$1.11\pm$	$1.14 \pm$	$1.15 \pm$	$1.21 \pm$	$1.23 \pm$	$1.27 \pm$	$1.31 \pm$	$1.34 \pm$
	0.97 ^a	0.21^{ab}	0.05^{ab}	0.87 ^b	0.11 ^b	0.78c	0.23 ^d	0.55^d

Table 1. The properties of white tea

THC-White tea hot infusion; TH1-White tea with chokeberry powder 0.6% hot infusion; TH2- White tea with chokeberry powder 0.8% hot infusion; TH3-White tea with chokeberry powder 1% hot infusion; TCC-White tea cold infusion; TC1- White tea with chokeberry powder 0.6% cold infusion; TC2-White tea with chokeberry powder 0.8% cold infusion; TC3-White tea with chokeberry powder 1 % cold infusion; TPC-Total polyphenolic content; ɳ-Viscosity. The results are expressed as the mean value of the three replicates \pm the standard deviation (SD). Data with different superscripts reported in the same row are significantly different (one-way ANOVA, $p < 0.05$). Data within a row with the same superscripts are not significantly different (one-way ANOVA, $p > 0.05$).

3.1. Total polyphenolic content

Table 1 presents a summary of the total phenol contents (TPC) of the tea infusions, as determined by Folin-Ciocalteu's reagent. A comparison of the total phenol contents (TPC) of hot and cold teas reveals that the latter consistently exhibits a significantly higher TPC, a phenomenon particularly pronounced in the case of the higher concentration of chokeberry powder TC3, with a TPC of 33.4 ± 0.71 mg GAE/mL. A comparison of the control samples (THC and TCC) revealed that the TPC of white tea obtained by cold extraction (8.25±0.21 mg GAE/mL) was significantly different $(p<0.05)$ than that of white tea obtained by hot infusion $(4.05\pm0.02$ mg GAE/mL). This suggests that thermal treatment has a significant impact on

total phenol content. These values are consistent with those reported in the literature (Damiani et al., 2014; Dasdemir et al., 2023; Perera et al., 2015; Ramalho et al., 2013). The infusion method and fruit concentration have a significant impact on the total phenol content (TPC). Furthermore, an examination of the samples with varying chokeberry powder content revealed a significant increase (p˂0.05) in total polyphenol (TPC) content in those obtained through both hot and cold infusion methods.

Research on white and green tea infusions reveals that brewing conditions significantly impact the extraction of bioactive compounds and antioxidant capacity. Cold infusion (20- 25°C) was found to be more efficient in extracting bioactive compounds compared to hot infusion (80°C) (de Carvalho Rodrigues et al., 2015). However, brewing at 98°C for 7 minutes yielded optimal antioxidant polyphenols in white tea (Pérez-Burillo et al., 2018). White teas exhibited the highest concentrations of chlorophylls, carotenoids, and total phenolic compounds (Popoviciu & Mălureanu, 2022). Total catechin content varied widely among white and green teas, with some white teas containing comparable levels to green teas (Unachukwu et al., 2010). Particle size also influenced extraction, with milled leaves producing greater antioxidant activity than whole leaves (Castiglioni et al., 2015). Cold brewing for 120 minutes or hot brewing at 90°C resulted in maximum extraction efficiency, particularly for whole, large leaves (Castiglioni et al., 2015).

White teas exhibited the highest concentrations of chlorophylls, carotenoids, and total phenolic compounds (Popoviciu & Mălureanu, 2022).

3.2. Antioxidant activity (ABTS assay)

The antioxidant activity of the tea infusions was evaluated using the ABTS method. As evidenced in Table 1, the ABTS assay results demonstrate that all hot tea infusions exhibit significantly diminished antioxidant activity (14.98-36.31 mmol/L TE) in comparison to cold tea infusions (26.3-82.45 mmol/L TE). The highest antioxidant activity was observed in the case of the cold tea infusion with a 1% chokeberry powder concentration, which exhibited an antioxidant capacity of 82.45 ± 1.89 mmol/L TE. This evolution of antioxidant capacity in cold tea in comparison with hot tea was also observed by other authors (Damiani et al., 2014). Moreover, the antioxidant activity of the samples obtained through hot and cold infusion with an identical chokeberry powder content was found to be significantly different $(p<0.05)$.

Research on white tea's antioxidant activity in cold and hot infusions reveals varying results across studies. White tea generally demonstrates high antioxidant capacity, with some studies finding prolonged hot steeping or cold extraction to be most effective (Castiglioni et al., 2015; Hajiaghaalipour et al., 2016). However, one study reported optimal antioxidant activity at 70°C for white tea, decreasing at higher temperatures (Chernousova et al., 2018). Cold extraction $(20-25\degree C)$ was found to be more efficient in extracting bioactive compounds compared to hot extraction (80°C) in some cases (de Carvalho Rodrigues et al., 2015). Factors influencing antioxidant activity include steeping time, temperature, and particle size, with milled leaves generally yielding higher antioxidant activity than whole leaves (Castiglioni et al., 2015). White tea often exhibits greater antioxidant capacity than black tea and comparable or higher levels than green tea (Chernousova et al., 2018; Hajiaghaalipour et al., 2016). Additionally, some white tea extracts have shown bacteriostatic activity against S. aureus and E. coli (de Carvalho Rodrigues et al., 2015).

3.3. pH measurement

The pH values for both methods fell within the range of 7.73 to 8.08, exhibiting minimal discrepancy. A slight decrease in pH was observed in both types of infusion. The sample TCC exhibited the highest pH value. A reduction in pH was similarly documented by other authors (Lunkes & Hashizume, 2014; S. Zhang et al., 2023). The pH of the white tea hot infusion sample differs significantly from that of the white tea cold infusion sample $(p<0.05)$. The hot and cold infusion samples with chokeberry powder do not exhibit a significant difference in $pH (p > 0.05)$, whereas a significant difference is observed between the hot and cold infusion samples with the same addition of chokeberry powder (p˂0.05).

pH values of tea infusions were generally mildly acidic, ranging from 3.85 to 6.45 (Kaczmarek, 2004), with white teas showing similar pH levels to other teas, except for highly acidic hibiscus tea (Popoviciu & Mălureanu, 2022).

3.4. Viscosity

In regard to viscosity, it was determined that as the concentration of chokeberry powder increases, the viscosity of the tea for both infusion methods also increase. The lowest viscosity was observed in the case of the hot infusion, with a value of THC, 1.11 ± 0.97 cP. A comparison of the control samples from the hot and cold infusions reveals that the viscosity of the cold tea (TCC: 1.23 ± 0.11) is higher than that of the hot tea (THC: 1.11 ± 0.97 cP). Viscosity decreases as temperature increases. This is due to the fact that the powder particles increase the viscosity of the tea, thus demonstrating that the viscosity of the tea is directly proportional to the concentration of the powder (Pérez et al., 2022). Another explanation is that, as tea heats up, the molecules within the liquid move more quickly, reducing the friction between them, and thus decreasing viscosity. Cold tea, by contrast, will have a slightly higher viscosity than hot tea.

3.5. Correlation of infusion method and chokeberry powder concentration

The statistical analysis of the data indicates that the investigated parameters were affected by two factors: infusion method and chokeberry powder concentration, either independently or in combination. The analysis of variance conducted on the analytical parameters for different infusion methods and chokeberry powder concentrations showed significant differences in total polyphenolic content, antioxidant activity, pH, and viscosity.

It is noteworthy that the correlation coefficient (R) for TPC is 0.935, for pH it is 0.992, and for viscosity it is 0.995. Furthermore, the antioxidant activity also appears to exhibit variability, with a correlation coefficient of 0.958. The adjusted \mathbb{R}^2 for the two predictors, infusion method and chokeberry powder concentration, indicates that they can predict 70.8% of the variation in TPC results, 81% of the variation in antioxidant activity results, 96.3% of the variation in pH results, and 97.8% of the variation in viscosity results.

The results of the ANOVA indicate that the model is statistically significant. The predictors introduced into the model, both individually and in combination, exerted a notable influence on the analyzed parameters, as detailed below: The total polyphenolic content was found to be significantly influenced ($p < 0.05$) only by the infusion method, while antioxidant activity, pH, and viscosity were significantly influenced ($p <$ 0.05) by both the infusion method and the concentration of chokeberry powder.

3.6. Correlation of the analyzed parameters

Figure 2 illustrates a correlation heat map for Pearson r. Pearson's product-moment correlation coefficient is a measure of the linear relationship between two variables. The correlation analysis enables the estimation of the parameters of the correlation. As can be observed, the heatmap

is symmetric along the diagonal. Furthermore, the color blue represents positive correlation coefficients, while the color red represents negative correlation coefficients. The saturation of colors is indicative of the absolute value of the correlation coefficient. The significant correlations are marked with: $*p < 0.05$ if the correlation is significant at alpha=0.05 level; **p ≤ 0.01 if the correlation is significant at alpha=0.01 level and ***p < 0.001 if the correlation is significant at alpha=0.001 level.

3.7. Sensory evaluation of tea samples

The success of a novel product or formula is primarily contingent upon consumer demand and acceptability based on sensory perception. To ascertain consumer approval and identify any shortcomings in sensory attributes, a sensory evaluation was conducted in conjunction with an assessment of the product's intrinsic qualities.

The outcomes of the hot and cold infusion processes for all eight samples are presented in Figure 3. The graph demonstrated a positive correlation between color, brightness, clarity, astringency, aroma, and bitterness. The tea with the highest level of appreciation was the one with a 1% concentration of chokeberry powder cold infusion (TC3), which received the

maximum score (5 points) for the majority of the analyzed properties, with the exception of clarity, which received 4 points.

Figure 2. Heatmap for Pearson's r. TPC-Total polyphenolic content

Figure 3. Sensory evaluation of tea samples. THC -White tea hot infusion; TH1- White tea with chokeberry powder 0.6% hot infusion; TH2- White tea with chokeberry powder 0.8% hot infusion; TH3- White tea with chokeberry powder 1% hot infusion; TCC -White tea cold infusion; TC1 - White tea with chokeberry powder 0.6% cold infusion; TC2 - White tea with chokeberry powder 0.8% cold infusion; TC3 - White tea with chokeberry powder 1 % cold infusion

White tea infusions have been studied for their sensory properties and antioxidant content under various brewing conditions. Cold brewing for 120 minutes or hot brewing at 90°C for 7 minutes yielded the highest antioxidant activity, with milled leaves providing greater extraction (Castiglioni et al., 2015). However, whole leaf infusions were preferred in sensory evaluations, particularly for cold-brewed white teas (Castiglioni et al., 2015). Optimal conditions for both antioxidant content and sensory properties were found to be 98°C for 7 minutes (Pérez-Burillo et al., 2018). For Fuding white tea, a 3 minute infusion at 100°C with a 1:50 tea-towater ratio produced the highest sensory scores (H. Zhang et al., 2017). In cold infusions of Taiwanese teas, consumers could distinguish between unfermented/lightly fermented and heavily/fully fermented teas, with lightly fermented teas preferred for their balanced bitterness, astringency, fresh flavor, and late sweetness (Liu et al., 2021).

4. Conclusion

The findings of the experimental research indicated that cold tea infusion is an effective method for enhancing the active biological properties of tea. A comparison of the total phenol contents (TPC) of hot and cold teas indicates that cold teas consistently have a significantly elevated TPC, most notable in the greater concentration of chokeberry powder TC3, which has a TPC of 33.4 ± 0.71 mg GAE/mL.

It was shown that the application of heat treatment leads to a reduction of these compounds,obtaining a TPC value of 11.13±0.27 mg GAE/mL (TH3) for the highest concentration of aronia powder.

The ABTS assay results indicate that all hot tea infusions display markedly reduced antioxidant activity (14.98-36.31 mmol/L TE) relative to cold tea infusions (26.3-82.45 mmol/L TE). The pH values for both procedures ranged from 7.73 to 8.08, demonstrating negligible variation, with a small reduction in pH noted in both infusion modalities. A comparison of the control samples from the hot

and cold infusions indicates that the viscosity of the cold tea (TCC: 1.23 ± 0.11 cP) surpasses that of the hot tea (THC: 1.11 ± 0.97 cP), demonstrating that viscosity diminishes with rising temperature. In the context of sensory analysis, the tea infused with a 1% concentration of chokeberry powder (TC3) achieved the highest score, attaining the maximum rating of 5 points for most of the evaluated attributes.

In conclusion, chokeberry fruits represent a valuable resource for the tea industry, offering multiple health benefits and a distinctive taste and aroma. The exploitation of these fruits can bring advantages from both an economic and a health and sustainability perspective, through the development of a prosperous chokeberry tea sector and the promotion of a healthy and sustainable lifestyle.

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Conflicts of Interest:

The authors have no conflict of interest regarding the content of this paper.