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The effect of maturation conditions on physicochemical and viscoelastic properties of Kashkaval cheese

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Abstract. The equilibrium stress, decay stress, relaxation time, viscosity, modulus of elasticity, and decay modulus are major characteristics of viscoelastic food materials and therefore a modified mechanical model was used in this current research for the viscoelastic properties' evaluation of Kashkaval cheese. Also, the chemical composition (fat content, moisture, protein content, water activity, salt, and acidity), and inside-outside color of the Kashkaval cheese were studied. From the analysis of stress relaxation curves the analyzed cheese samples fall into the category of viscoelastic solids with equilibrium stress greater than 0. The decay stress and decay modulus of the maturated unpacked samples showed the highest values of 36.31 kPa and 121.05 kPa, while the relaxation time of cheese samples was greater than 112.35 s. To evaluate the fit of the applied mechanical model to the experimental data the determination coefficient ($R^2 > 0.937$) and the absolute average deviation coefficient were calculated (AAD < 10.324) and the evaluated cheeses' parameters with the modified Maxwell model were at statistically appropriate levels.

***Keywords*:** Kashkaval cheese; Maxwell model; decay stress; relaxation time; color.

1. Introduction

Cheese production has a long history and according to the latest research [1] probably dates back to the Neolithic, cheesemaking being an ancient art. Its manufacture has been started with the first domesticated animals. The classification of cheeses includes several factors such as milk type (bovine, ovine, caprine, or buffalo) fat content, moisture content (hard, semi-hard, semi-soft, soft), fabrication process, fermentation type, and ripening period; therefore, there are over 1,000 different variants and varieties of cheese worldwide [2]. Fresh or ripened cheese represents an important source of protein, fats, and minerals and in addition, it is easily digestible, which causes this product to be consumed frequently. Globally, cheese production is estimated at over 20 million tonnes (predominantly in Europe), being one of the most consumed milk products worldwide [1, 3]. About 35% of milk production is used for cheese fabrication in a great variety of colors, flavors, aromas, textures, and forms [4]. The pasta-filata cheese is one of the most common and consumed types of cheeses in the European region; mozzarella is the most known and consumed variety worldwide [5]. The pasta-filata cheese variety is produced from cows, sheep, or goat's milk and originates in the northern Mediterranean region (Italy, Greece, Turkey), and also Eastern Europe. This category of cheeses includes hard cheeses (Ragusano, Caciocavallo, Kashkaval, Provolone), which are ripened for a considerable period before consumption, and soft cheeses (Mozzarella, Scamorza), which are consumed fresh or after a short period of ripening [6]. Kashkaval is the most popular and typical pasta-filata hard cheese in many countries even in Romania, with very wide market popularity. The scalding, kneading, and stretching process of curd in hot brine (70-80 °C) leads to a product with unique texture characteristics [5, 7, 8]. Processed cheeses have viscoelastic properties which can be influenced by the chemical components, particularly by their fat concentration. To the author's knowledge, there are rheological studies based on Cheddar cheese viscoelastic evaluation [9], Arzua-Ulloa cheese [10], Gaziantep cheese [11], and mozzarella [12]. Consequently, the main idea of the current research was to study the textural-viscoelastic properties of the Kashkaval cheese during maturation in accordance with the chemical composition, using for the evaluation of viscoelastic properties a texture stress relaxation compression test and a modified Maxwell model. Also, the color of the Kashkaval cheese samples was evaluated.

2. Experimental

The Kashkaval cheese samples were obtained from a local producer (Suceava, Romania). The cheese-making technology was the classic one, using the same batch of cow's milk with 3.5±0.2% fat, chymosin (Chy-Max Extra, Chr. Hansen S.R.L. Brasov, Romania 1,8 g / 100 L), and manual scalding, kneading and stretching of the curd in hot brine (75 °C, 10% NaCl). The rectangular cheese samples had 150 ± 5 grams each and after 12 hours of drying (18 ± 2 °C and 60-70% humidity) were divided into three groups, each group containing three samples. One group was analyzed after drying (C1), one was packaged and vacuumed in polyethylene bags (C2) and subjected to maturation for two months, and the last group (C3) was maturated for two months unpacked.

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The maturation temperature was 10±2 °C and the relative humidity was between 70-80%.

2.1. Physico-chemical analysis

The fat content of Kashkaval samples was evaluated in accordance with the Van Gulik method - ISO 3433/2008 [13], which involved the dissolution of proteins with concentrated sulfuric acid and then the separation of fat in a Van Gulik butyrometer by centrifugation. The separation process assumed the addition of 1 mL of isoamyl alcohol and the fat level was read from the butyrometer scale. The moisture content was evaluated by the oven drying method at 102±2 °C [14], whereas the protein level was assessed by the Kjeldahl assay [15]. The salt concentration was evaluated based on the Mohr method [16]. The measurement of titratable acidity was expressed as Thörner degrees (°T) using NaOH (0.1 N) and phenolphthalein as an indicator [17]. The water activity measurements were achieved with a water activity meter (AquaLab Lite). All used reagents were analytical grade from Sigma Aldrich (Germany).

2.2. Color evaluation

The cheese color parameters were evaluated by applying the CIEL*a*b* - Commission Internationale de l'Eclairage uniform color space assay and using a Chroma meter model CR-400 (Konica Minolta, Japan). The used illuminant was C, and the measured parameters were luminosity or brightness (L*), red (+a*), yellow (+b*), green (-a*), and blue (-b*) [18]. In addition to the measured characteristics, it was also studied the hue angle (h^0 - Eq. 1), whiteness index (WI - Eq. 2), color intensity (C* - Eq. 3), yellowness index (YI - Eq.4), and color differences (ΔE* - Eq. 5) [19]. Both the measured and studied color properties were for inside and outside cheese evaluation.

$$h^0=\tan^{-1}(b^*/a^*) \quad (1)$$

$$WI=100-\sqrt{(100-L*)^2+a*^2+b*^2} \quad (2)$$

$$C^*=\sqrt{a*^2+b*^2} \quad (3)$$

$$YI=142.86\cdot b^*/L^* \quad (4)$$

$$\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2} \quad (5)$$

2.3. Viscoelastic evaluation

For the viscoelastic properties' determination measured by the uniaxial compression stress (σ_0, Pa) relaxation, a modified Maxwell model was applied [20]. For the stress-relaxation of Kashkaval cheese samples a Mark 10-ESM 301 Texture Analyzer, (Mark 10 Corporation, USA), equipped with a 100 N load cell and a flat probe of 50 mm was used. The sample had a cubic shape with sides (l_0) of 2 cm and the compression has been done until 6 mm (l = 1.4 cm) and the loading / unloading speed was set at 10 mm/min. The time of the deformation process (t) was set at 600 seconds [21] and the variation of stress during the time was registered by the MESUREgauge software (Mark 10 Corporation, USA). The speed of reading was 10 points per second (pps).

The mechanical model included in addition to the Maxwell element a spring mounted in parallel, which has the role of predicting the equilibrium stress (σ_e, Pa). This modified mechanical model is also known as the Zener model [22]. In the equation of the used mechanical model (Eq. 6) λ_{rel} represents the relaxation time and (σ_0 - σ_e) represents the decay stress (σ_d, Pa) [23]. Furthermore, by applying this mechanical model the specific viscosity ($\eta = \lambda_{rel}\cdot Ed$) [24], modulus of elasticity ($Ee = \sigma_e/\varepsilon$; $\varepsilon = \Delta l/l_0$), decay modulus ($Ed = \sigma_d/\varepsilon$) [25], and Deborah number (De) were determined [26]. The Deborah number was calculated as the ratio of the relaxation time and the time of the deformation process ($De = \lambda_{rel}/t$). As a measure of the mechanical model matching to the experimental data, the absolute average deviation (AAD) and coefficient of determination (R^2) were calculated (Eq. 7 and 8) [27].

$$\sigma(t) = \sigma_e + (\sigma_0 - \sigma_e)\cdot \exp\left(\frac{-t}{\lambda_{\text{rel}}}\right) \quad (6)$$

$$AAD = \frac{100}{N}\cdot \sum_{i=1}^{N}\left|\frac{y_{exp,i}-y_{prz,i}}{y_{exp,i}}\right| \quad (7)$$

$$R^2 = 1-\frac{\sum_{i=1}^{N}(y_{exp,i}-y_{prz,i})^2}{\sum_{i=1}^{N}(y\,exp,i-y_{prz,average})^2} \quad (8)$$

2.4. Statistical analysis

The differences between the obtained results were studied by analysis of variance ANOVA (α = 0.05), and multiple comparisons of means using the Fisher's least significant difference (LSD) at the 95 % confidence level with Statgraphics Centurion XVI software (Trial Version), while Principal Component Analysis (PCA) was performed by OriginPro software.

3. Results and discussion

3.1. Physico-chemical analysis

The mean physicochemical results of the analyzed Kashkaval samples, unripened-dried and after two months of maturation are reported in Table 1 and it can be observed that the moisture content varied from 52.92±2.25% to 44.73±0.98%; while the fat content in dry matter (d.m.) ranged between 41.11±0.52% and 48.25±1.31%.

Table 1. Physico-chemical parameters of Kashkaval samples.

Sample	Moisture (%)	Fat / d.m. (%)	Protein (%)	NaCl (%)	Acidity (°T)	a_w
C1	52.92a (2.25)	41.11c (0.52)	23.21b (0.35)	1.59b (0.20)	104c (2.49)	0.89a (0.02)
C2	44.73b (0.98)	43.52b (0.82)	23.9b (0.42)	1.92a (0.18)	151a (2.86)	0.88a (0.01)
C3	41.24c (1.51)	48.25a (1.31)	24.84a (0.25)	2.31a (0.32)	130b (2.62)	0.86a (0.02)

Mean values and standard deviations. Different letters (a–c) in a column show significant differences between samples ($p < 0.05$) evaluated with One-way ANOVA test.

The C3 group maturated unpacked, presented also higher protein (24.84%) and salt content (2.31%), whereas the water activity presented lower values. The lowest values of acidity (104 °T) were measured for dried and unripened samples (C1) and the highest values of 151 °T were measured for the cheese group packaged and vacuumed in polyethylene bags - C2 group.

The physicochemical parameters of Kashkaval samples were influenced by the maturation conditions, the unripened-dried group samples (C1) presented higher moisture content and therefore lower fat, protein, acidity, and salt, whereas the maturated groups (C2 and C3) showed lower moisture content and higher fat content, salt, and acidity.

3.2. Color evaluation

In addition to the visual texture, shape, and appearance, color represents also a quality attribute that influences customer preferences and purchasing decisions. Foods color offers information about a product's sensory characteristics (taste or flavor), about key brand attributes such as premium, natural, or healthy, also offers information related to food freshness and quality and in the case of cheeses, color provides information about maturation/age [28, 29]. Table 2 presents the evaluated color characteristics of the tested cheese samples for both inside and outside color evaluation. Color properties like luminosity (L*), red/green (+a*/-a*), and yellow/blue (+b*/-b*) were measured, whereas color characteristics like tone (h^0), color intensity (C*), whiteness index (WI), yellowness index (YI), and color difference (ΔE*) were calculated.

Table 2. ANOVA of Kashkaval color parameters.

Sample	L*	a*	b*	C*	h^0	YI	WI
	inside color parameters—mean (standard deviation)						
C1	86.23a (0.42)	-7.84a (0.09)	28.55b (0.11)	29.63b (0.18)	105.49a (0.08)	47.28b (0.22)	67.27a (0.18)
C2	81.11b (0.25)	-7.88a (0.19)	31.08a (0.41)	32.17a (0.38)	104.08b (0.61)	55.02a (0.84)	62.83b (0.58)
C3	80.82b (0.12)	-7.87a (0.11)	30.23a (0.15)	31.95a (0.22)	104.15b (0.12)	54.96a (0.32)	63.24b (0.28)
F-ratio	219	0.15	160	190	18	733	1317
p-value	p < 0.001	p > 0.05	p < 0.001	p < 0.001	p < 0.001	p < 0.001	p < 0.001
outside color parameters— mean (standard deviation)							
C1	84.54a (1.10)	-8.10b (0.12)	29.28b (0.24)	30.14b (0.71)	105.25a (0.18)	48.57b (0.62)	64.35a (0.23)
C2	79.57b (1.51)	-8.20b (0.25)	29.34b (1.19)	30.45b (1.20)	105.58a (0.35)	52.73b (1.51)	63.25b (0.80)
C3	71.76c (1.21)	-7.87a (0.18)	33.25a (1.18)	34.40a (1.20)	102.95b (0.40)	66.18a (2.45)	56.11c (1.85)
F-ratio	59	5	21	19	75	83	105
p-value	p < 0.001	p < 0.05	p < 0.001	p < 0.001	p < 0.001	p < 0.001	p < 0.001

Different letters (a–c) in a column show significant differences between the outside or inside measurements (p < 0.05) evaluated with One way ANOVA test. L* - luminosity, a* - red/green, b* - yellow/blue, C* - color intensity, h^0 - hue angle, YI yellowness index, WI - whiteness index.

The outside and inside color measurements highlighted that the dried and unripened group samples (C1) presented the highest values for brightness - 86.23 and 84.54, tone or hue angle - 105.49 and 105.25, and also for whiteness index - 67.27 and 64.35. The whiteness color parameter is a mathematical combination of luminosity, red-green, and yellow-blue and reflects the degree of cheese whiteness. The tone or hue angle results range from 0 to 360 degrees and represent the principal spectral component, such as red for 0 or 360 degrees, yellow for 90 degrees, green for 180 degrees, or blue for 270 degrees [29].

The calculated tone values for Kashkaval samples were above 102.95 and below 105.58, reflecting a yellow predominant spectral component and including also some green interferences. The results of the one-way ANOVA test pointed out that there are no differences between the inside brightness, yellow-blue color parameter, tone, color intensity, whiteness index, or yellowness index of samples maturated in polyethylene bags (C2 group) and the maturated unpacked Kashkaval samples from the C3 group; the samples belong to the same statistical group (p < 0.001). As regards the outside brightness and whiteness index, the one-way ANOVA statistical analysis separated the results into three statistical groups (p < 0.001) which assumes that the packaging materials influence these outside color parameters.

According to Khattab [30] the color evaluation also provides information about cheese defects, and some color parameters like brightness decreases during the maturation process, this finding is also valid in the case of the analyzed cheese samples. The inside and outside brightness of analyzed cheese samples decreased during maturation, with a greater decrease observed for samples maturated unpacked (C3), while the red/green color component presented negative values denoting a green influence. Some authors [31, 32] attribute this decrease in brightness to the lipolysis that occurs during the ripening process. The yellow/blue color parameter presents positive values between 28.55 for inside dried - unripened group samples (C1) and 33.25 for outside maturated unpacked samples (C3), observing an intensification of the yellow color during maturation. Similar results were stated also by other researchers [31, 33]; the intensification of the yellowish color of

Kashkaval samples was associated with the presence of carotene [31, 34].

Table 3. The color differences of Kashkaval samples.

Sample		C1	C2	C3	C1	C2	C3
		inside			**outside**		
C1	inside	-	5.48	5.46	1.66	6.25	15.39
C2			-	0.35	3.85	2.05	9.95
C3				-	3.86	1.73	9.93
C1	outside				-	4.78	13.78
C2						-	9.44
C3							-

To highlight even more the color differences between the cheese samples, another color parameter was used (ΔE), and the results are shown in Table 3. The total color differences calculated for Kashkaval samples varied between 0.35 and 15.39, the highest color difference was reported between the outside maturated unpacked samples (C3) and the inside dried - unripened group samples (C1), whereas the lower values were recorded between inside samples maturated in polyethylene bags (C2) and the inside samples maturated unpacked (C3).

Therefore, the inside total color difference between the Kashkaval samples maturated with or without packaging is not noticeable to the human eye, $\Delta E < 1$ [35, 36]. A minor color difference has been identified on the inside and outside evaluation of Kashkaval samples maturated in polyethylene bags (C2 group) ($1 < \Delta E < 3$); consequently, the samples maturated in polyethylene package have a uniform inside-outside color.

3.3. Viscoelastic evaluation

Table 4 presents the results of viscoelastic parameters measured by the stress relaxation tests and determined by applying the Maxwell mechanical model.

From the analysis of stress relaxation curves the Kashkaval samples fall into the category of viscoelastic solids with equilibrium stress greater than 0, whereas, the residual stress falls to 0 for viscoelastic fluids [37]. The equilibrium stress of Kashkaval ranged between 3.35 and 4.50 kPa, values much lower than those reported by Chakespari [25] for apples. Some authors [38] argued that the loading speed does not influence the equilibrium stress values of cheese, a higher speed of crosshead led to a slightly higher equilibrium stress, but the analysis of variance highlighted that the differences in equilibrium stress values were not significant.

As regards the decay stress and decay modulus, the C3 - maturated unpacked samples showed the highest values of 36.31 kPa and 121.05 kPa. The relaxation time is an important parameter of stress-relaxation tests and is related to food firmness. The relaxation time of cheese samples was greater than 112.35 s with a minor variation up to a maximum of 133.33 s. Furthermore, the viscosity measured in this study varied between 10.81 MPa·s and 13.60 MPa·s; additionally, cheese viscosity is related to its meltability one of the most significant physical characteristics that permit its use in toasted sandwiches, pizzas, and other foods [39]. The viscosity results of Kashkaval samples determined by the Maxwell model were in the same range as those reported by Sadowska [40] for full-fat Dutch-type cheese.

Table 4. Kashkaval viscoelastic properties.

Sample		**C1**	**C2**	**C3**
Maxwell viscoelastic parameters				
Equilibrium stress - σ_e	[kPa]	3.55b (0.02)	3.35c (0.03)	4.50a (0.01)
Decay stress - σ_d	[kPa]	24.34b (2.21)	26.90b (0.98)	36.31a (1.52)
Relaxation time - λ_{rel}	[s]	133.33a (5.25)	131.57a (7.56)	112.35b (3.82)
Viscosity - η	[kPa·s]	10.81b (0.25)	11.79b (0.58)	13.60a (0.98)
Modulus of elasticity - Ee	[kPa]	11.83ab (0.24)	11.16b (1.08)	15.00a (2.56)
Decay modulus - Ed	[kPa]	81.14c (3.44)	89.67b (2.01)	121.05a (4.58)
Deborah number - De	-	0.222a (0.007)	0.219a (0.01)	0.186b (0.005)
R^2	-	0.937	0.940	0.942
AAD	-	7.254	7.784	10.324

Different letters (a–c) in a row show significant differences between samples ($p < 0.05$) evaluated with One way ANOVA test.

The modulus of elasticity measured for Kashkaval samples (11.16 – 15 kPa) was similar to the values measured for mozzarella cheese (11.9 kPa) but greater than those reported for meat, 6.49 kPa [37]. Based on the results of the elasticity modulus it can be concluded that the maturated unpacked cheese samples are firmer than the unripened group samples and maturated in polyethylene bags samples. Deborah number represents a dimensionless parameter, which characterizes the viscoelastic materials, and as can be seen from Table 4 the values obtained were lesser than 1 (De < 1), with a decrease with maturation time, suggesting that the analyzed Kashkaval samples have more viscous than elastic behavior [23]. Deborah's number it's a measure of flowability [41] and tends to infinity for purely elastic bodies and to zero for Newtonian-viscous fluids [42]. The smallest Deborah number - 0.186 was registered for maturated unpacked Kashkaval samples.

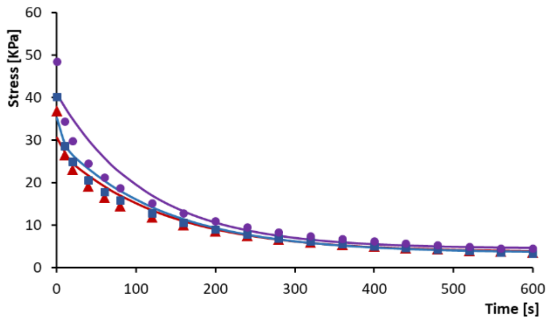

Figure 1. Relaxation curves of Kashkaval samples, experimental (▲, ■, ●) vs. Maxwell model predicted data (—): C1 (▲), C2 (■), C3 (●).

Figure 1 presents the stress-relaxation curves and the fit of the mechanical model to the experimental data of Kashkaval samples. It can be seen that the modified Maxwell model can be accurately used to evaluate the viscoelastic characteristics of Kashkaval samples in 600 seconds [43]. Furthermore, R^2 - the determination coefficient and AAD - the absolute average deviation coefficient were calculated to further highlight the fit of the model to the experimental data. The higher the values of R^2 and the lower the values of AAD, the better will predict the model the viscoelastic characteristics [44]. As can be seen, the determination coefficient values were greater than 0.937 whereas the absolute average deviation coefficient values were smaller than 10.324. Based on these we can conclude that the evaluated cheeses' parameters with the modified Maxwell model were at statistically appropriate levels.

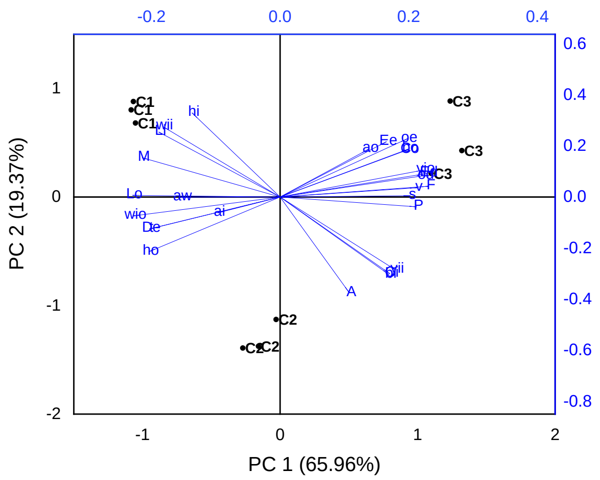

Figure 2. Principal Component Analysis Biplot (Score and Loading) of Kashkaval samples: Lo, ao, bo, Co, ho, yio, wio - outside color parameters, Li, ai, bi, Ci, hi, yii, wii - inside color parameters, F - fat, M - moisture, P - protein, s - salt, A - acidity, aw water activity, oe - equilibrium stress, od - decay stress, t - relaxation time, v - viscosity, Ee - modulus of elasticity, Ed - decay modulus, De - Deborah number.

The PCA biplot respectively Score and Loading, Figure 2, was performed to highlight the differences between Kashkaval samples, emphasize the relevant information from all evaluated parameters, and also to diminish the number of variables. The Kashkaval samples were separated into diverse quadrants based on the Score plot and according to the maturation condition category. The principal components PC 1 and PC 2 explain 85.33 % of data variation. The PC1 component describes 65.96% and separates the C1 and C2 groups from C3 based on fat, moisture, outside brightness, outside yellowness index, Deborah number, and relaxation time, whereas the PC2 component describes 19.37 % of data variation. The water activity, inside and outside red/green color parameters, and salt content displayed small influences in the Kashkaval samples projection. As can be seen the projection of the C3 - maturated unpacked samples was strongly influenced by fat, protein, yellow color parameter, and the majority of the viscoelastic properties except for Deborah's number and relaxation time, which along with moisture and inside brightness influences positively the projection of unripened group samples (C1). Regarding the projection of the C2 samples, it can be noticed that acidity and some inside color parameters (Ci, bi, yii) have a strong influence on their positioning in the PCA biplot.

4. Conclusions

The produced Kashkaval samples showed various physicochemical properties after two months of maturation compared to the initial ones and also presented a significant effect on color and viscoelastic properties. Compared to the maturated unpacked samples, the maturated samples in polyethylene bags presented a reduced moisture loss, respectively an increase in cheese yield. The results from the color evaluation highlighted that through the maturation process, the Kashkaval cheese loses its brightness and becomes more yellowish. The results of the one-way ANOVA highlighted that there are no differences between the inside brightness, yellow-blue color parameter, tone, color intensity, whiteness index, or yellowness index of samples maturated in polyethylene bags - C2 group, and the maturated unpacked Kashkaval samples from the C3 group; the samples belong to the same statistical group. The inside total color difference between the Kashkaval samples maturated with or without packaging is not noticeable to the human eye. The samples maturated in polyethylene package presented a uniform inside-outside color. Regarding the applied mechanical model and considering the calculated statistical indicators (AAD and R^2) it can be concluded that the modified Maxwell model can be successfully used to determine the viscoelastic parameters and the Kashkaval samples exhibit viscous and elastic comportment, specifically a viscoelastic solid one, with a decrease of stress with the time and with equilibrium stress greater than 0.

Conflicts of interest. The author declares no conflict of interest.

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