Journal of Packaging Technology and Research (2021) 5:51–57
https://doi.org/10.1007/s41783-021-00108-1

RESEARCH ARTICLE

Performance Evaluation of a Biopolymer-based In-Package UV Activated Colorimetric Oxygen Indicator with Modified Atmosphere Packaged Mozzarella Cheese

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Received: 25 September 2020 / Accepted: 31 December 2020 / Published online: 21 February 2021

Abstract
Dye-based ultraviolet (UV) activated colorimetric oxygen indicator possess the potential for communication of package integrity to consumers. A research study was envisaged to evaluate the performance of biopolymer (sodium alginate, sodium caseinate and kappa-carrageenan) based ultraviolet activated smart oxygen sensor as spoilage or quality indicator for Mozzarella cheese. Mozzarella cheese samples were stored in a vacuum and 100% N_2 at refrigerated temperature for 16 days and gas composition, indicator colour values and physico–chemical parameters (moisture, pH, acid degree value, thiobarbituric acid value and water activity) changes were studied. The indicators showed significant colour change after photo-activation and during storage also. The oxygen indicator's colour was recovered at a very low oxygen concentration (0.381%). The indicator films could be potentially used for evaluating the purity of nitrogen and carbon dioxide gas cylinders used in modified atmosphere packaging of food.

Keywords Intelligent packaging · Mozzarella · Cheese · Oxygen indicator · Bio-polymer

Introduction

Packaging industry stands at third position globally, next to food and petroleum industries contributing nearly 2% of Gross National Product in developed nations [15]. Intelligent packaging involves the communication of product quality over a period of time using several indicators such as gas indicators, freshness indicators and time temperature indicators [19, 24]. With an increased focus on convenience aspect of consumer, gas sensors could be used to indicate the quality of the product at the consumer's end. In the past sensor based on various mechanism like fluorescence, conductance and luminescence have been used for the detection of oxygen but these indicators are expensive, requires skilled manpower and lacks movability [18]. Intelligent colorimetric indicators based on inexpensive dyes have been used to overcome these challenges and possess the potential to signpost the quality of the product depending on visible colour change which can be helpful for everyone present in the supply chain including producer, distributor, retailer and consumer [5, 23] developed a colorimetric oxygen indicator based on titanium dioxide nanotubes and methylene blue dye for evaluating the integrity of fish packages nondestructively. Some of the commercially available UV-activated oxygen indicators include Ageless Eye™ (Mitsubishi Gas Chemical Co.,), Shelf Life Guard (UPM) and Vitalon® (Toagosei Chemical Inc.) [11].

The global Mozzarella cheese market was valued at 33.09 billion US dollars in 2018 and growing with a CAGR of 5.2%. Approximately 4.5 billion pounds of Mozzarella cheese was produced in the United States during 2019 [21].

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Mozzarella cheese is mainly utilized for pizza and ready to eat items like snack foods, soups, dressings, etc. [13]. Furthermore, there exists a huge scope for some gas indicator which not only detects and indicates oxygen in MAP Mozzarella cheese packages with detectable colour change but also correlates the deteriorative changes of the Mozzarella cheese with the colour change of the indicator. The efficiency of UV activated colorimetric oxygen indicators have been mostly tested with meat products and intelligent packaging applications in the dairy industry are limited [4]. Most of the O_2 sensors function precisely in the oxygen concentration of 0 to 21% (0 to 21 kPa) with detection limits of 0.01 to 0.1 kPa, which is most appropriate for meat packaging applications [1]. It could be expected that with continuous advances in intelligent packaging and growing modified atmosphere packaged dairy products market, the demand for such type of intelligent packaging systems is expected to rise across the globe. Hence, the present research work on performance evaluation of biopolymer-based UV activated intelligent oxygen indicator for modified atmosphere (100% N_2) and vacuum packaged Mozzarella cheese was undertaken.

Materials and Method

The UV activated intelligent oxygen indicator based on sodium alginate containing methylene blue and neutral red dye named as SA-MB-NR, SC-MB-RSS for sodium caseinate containing methylene blue and resazurin sodium salt and CG-MB with kappa-carrageenan containing methylene blue were prepared according to the procedure mentioned by Deshwal et al. (2018) [7]. The oral acute toxicity estimate of methylene blue and neutral red dyes are 2055 and 22,081 mg kg^{-1}, respectively [17]. In our study, usage of these dyes was 0.12 g mL^{-1} which is very much below and the overall migration study carried out earlier [7] revealed that they are within the safe limits. Fresh, chilled, raw buffalo milk and cow milk was collected from the Experimental Dairy, ICAR-National Dairy Research Institute, Karnal. All the chemicals used were of analytical reagent grade and granular microbial rennet (Meito) *Mucorpusillus var. Lindt*, (M/s Meito Sangyo Co. Ltd., Japan) was used for cheese culturing. Gas composition in polystyrene trays sealed with ethylene-vinyl alcohol copolymer (EVOH) films containing Mozzarella cheese was assessed using CheckMate 9900 (PBI Dansensor, Ringsted, Denmark).

Procedure for the Manufacture of Mozzarella Cheese

Mozzarella cheese was manufactured using the methodology elaborated by McMahon and co-workers (2005) [14] with slight modifications. Mixed milk (cow and buffalo), standardized to 4% fat level was batch pasteurized (63 °C/30 min) with rapid cooling to 5 °C, acidified with citric acid to pH 5.4 and heated to 28 °C followed by addition of rennet (Meito) @ 0.7 g/100 L of milk at 27–28 °C. Horizontal and vertical cheese knives were used to cut the set curd after 15–25 min. After cutting, the temperature of the whey was raised to 37 °C to have a cooking effect on cheese cubes. Subsequently, whey was drained and curd was stretched in hot water (80–85 °C) having a volume of approximately 2 times of curd weight. Stretched curd was moulded into oval shape balls and placed in brine (20 % salt) at 4 °C for about 8–10 h. Mozzarella cheese met the regulatory standards prescribed by the Food Safety and Standards Authority of India [9].

Analysis of the Indicator Films in Terms of Colour Change

Original state of the indicator film refers to the film without UV activation. The photo-activated state of the indicator film refers to the oxygensensitive state achieved after UV activation and recovered state refers to the reversing back of the original indicator's colour after oxygen exposure. The $^{o, p, r}$ subscripts refers to the indicator's colour values in original, photo-activated and recovered state. Info palette in Adobe Photoshop software was used to determine the *L a b* distribution of the indicator samples and were further transformed to standard colour values (L^*, a^* and b^*) by following calculations [7].

$$L^* = \frac{L}{255} \times 100;\ a^* = \frac{(240a - 120)}{510} \times 100;\ b^* = \frac{(240b - 120)}{510 \times 100}$$

Determination of Physico–Chemical Properties of Mozzarella Cheese

Physicochemical properties of cheese like moisture [12], acid degree value [6] and thiobarbituric acid value [22] were determined using the standard procedure as mentioned in the references. The water activity of Mozzarella cheese during its storage was evaluated by AquaLab water activity meter (Decagon Devices Inc., USA). The pH of cheese slurry obtained by mixing cheese with distilled water in equal amount (w/v) was determined by pH Analyser (Labindia, New Delhi, Version I) after proper calibration.

Application of Oxygen Indicator to Modified Atmosphere Packaged Mozzarella Cheese

The prepared Mozzarella cheese was packaged in polystyrene trays filled with 100% N_2, and sealed using EVOH films and was stored at 5 ± 1 °C. Also, vacuum packaging was carried out in EVOH pouches (Table 1). All the three developed indicators were cut into a rectangular shape and applied inside the package under the sealant film in case of trays and facing outside, with an adhesive tape. The colour changes of the biopolymer-based UV activated intelligent oxygen indicator and physico–chemical changes of Mozzarella cheese were evaluated at 4 days interval. Mozzarella cheese stored under 100% N_2 was observed for changes in gas concentration with storage time. Vacuum packaging generates close contact of packaging material with food product due to near-complete absence of air inside the package. Vacuum packed samples were not studied for changes in gas concentration as on piercing the pack, the vacuum was lost and false reading was obtained every time, hence it was omitted. The data obtained were interpreted to meaningful results by one-way ANOVA at 5% level of significance using SAS Enterprise Guide (version 4.3).

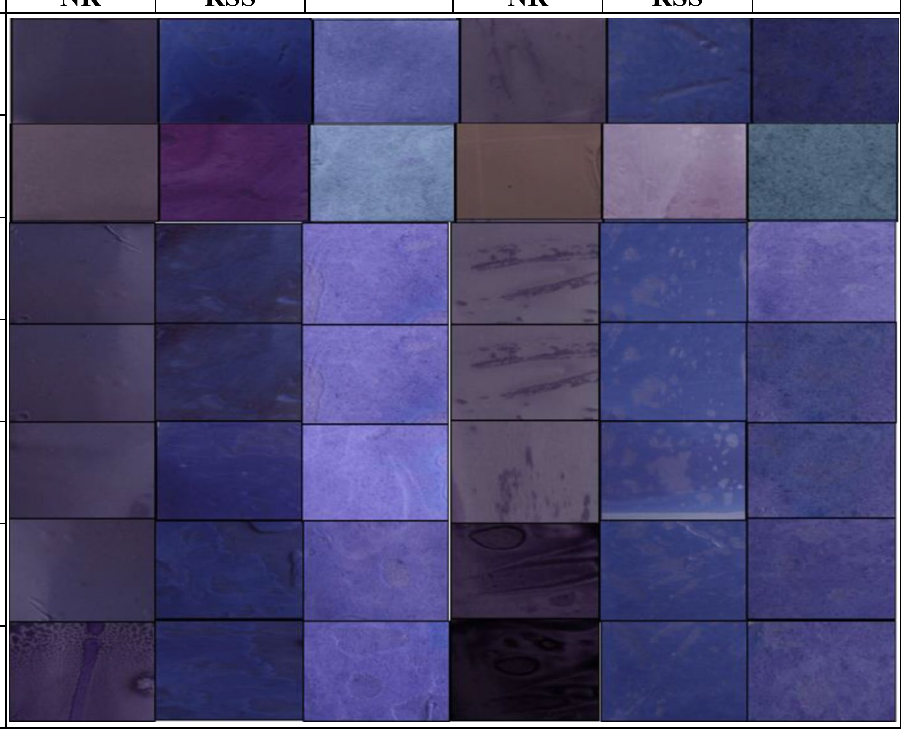
Results and Discussion

Variation in Gas Composition of Mozzarella Cheese Packages

The changes in the headspace gas composition of N_2 filled Mozzarella cheese during storage are presented in Table 3. It can be seen that an increase in oxygen and carbon dioxide was obtained, however, nitrogen content was found to decrease from 99.57 to 98.50%. The changes in all the gases composition were significant ($P < 0.05$). Increase in carbon dioxide concentration could be attributed to the growth of hetro-fermentative *lactobacilli*, aerobic and anaerobic microorganisms or due to yeast activity [3]. Oxygen increase could be attributed to trapped air between the slices and also due to permeability of packaging film and polystyrene trays. Alves et al. [2] stated an increase in oxygen concentration for Mozzarella cheese when packed under modified atmosphere of 100% CO_2. The increase in oxygen in Mozzarella cheese could also be due to increase in permeability of EVOH when stored at refrigerated conditions because EVOH permeability increases at higher humidity [19]. N_2 concentration is

Table 1 Colour changes of indicator films due to UV-activation and post-activation recovery applied to freshly packaged Mozzarella cheese stored under different conditions

Modified atmosphere packs	Storage Interval (in days)	Modified Atmosphere Packaging (100% N_2)			Vacuum Packaging		
		SA-MB-NR	SC-MB-RSS	CG-MB	SA-MB-NR	SC-MB-RSS	CG-MB
	Original						
	Photo-activated						
	1						
Vacuum Packaging	4						
	8						
	12						
	16						



affected by the partial pressure difference between the package and the atmosphere.

Changes in Instrumental Colour Values of Indicator Films Applied to Packages

Mozzarella cheese applied with three different indicator films in their original state (L_o*, a_o* and b_o*) were having significantly different ($P < 0.05$) colour values after photo-activation (L_p*, a_p* and b_p*) both qualitatively and quantitatively under packaging systems of 100% N_2 and vacuum (Table 2). Further, from Table 2, it can be seen that with increasing storage period significant differences ($P <$ 0.05) were observed in the values of recovered (L_r*, a_r* and b_r*) colour. Sodium alginate-based indicator (SA-MB-NR) showed the presence of darker colour after 16 days under vacuum which may be due to the contact of the indicator film with the product and its moisture. Under 100% N_2 also some loss of film colour was observed. However, it was not as high as under vacuum. SC-MB-RSS and CG-MB indicator film were more stable and they didn't show any distinct colour changes both under vacuum and 100% N_2 on a qualitative basis (Table 1). However, on quantitative analysis all three indicator film colour values in recovered state *viz.* L_r* a_r* and b_r* changed during storage (Table 2).

Changes in Physico–Chemical Parameters of Mozzarella Cheese During Storage

pH

The physico–chemical changes in Mozzarella cheese stored under different packaging conditions are presented in Table 3. The pH of Mozzarella cheese increased from 5.32 to 5.60 for nitrogen flushed and to 5.52 for vacuum packaged sample. Samples stored in 100% N_2 atmosphere were having significant changes in pH ($P < 0.05$). Release of ammonia owing to transformation of acidic amino acids like aspartic acid, glycine etc., consumption of lactic acid by yeast and moulds and more pronounced release of basic amino acids could be the reason for increase in the pH of Mozzarella cheese. Garabal et al. [10] had correlated the pH increase of cheese with proteolysis and to the formation of amines and ammonium compounds.

Water Activity and Moisture Content

The initial a_w of Mozzarella cheese increased from 0.954 to 0.996 in 100% nitrogen samples and to 0.975 for vacuum packaged product. The analysis of water activity data using ANOVA revealed the significant effect ($P<0.05$) of the storage period on a_w of Mozzarella cheese (Table 3). As compared to vacuum, 100% N_2 samples were having a higher increase in water activity. Moisture loss is vital from the economic standpoint and can also be an indicator of cheese freshness. The moisture content of Mozzarella cheese did not had any significant effect ($P>0.05$) in vacuum packaged samples as it marginally increased from 55.09 to 55.72%. However, in nitrogen packed samples it significantly increased from 55.09 to 56.54% ($P < 0.05$) at the end of 16 days of storage. Greater amount of water contributes to elevated proteolysis and an increase in Maillard browning reactions in presence of residual sugars during cheese baking [8].

Acid degree Value

Acid degree value (ADV) can be used as an index of lipolysis. Lipolytic enzymes in cheese arise from milk, rennet and microorganisms causing off-flavor and shelf-life reduction. During storage, ADV content of Mozzarella cheese significantly increased from 1.848 to 3.286% ($P < 0.05$) for nitrogen packed product and to 3.123% for vacuum packed product ($P < 0.05$). Various authors had correlated the increase in acid degree value with the oxidation of methyl ketones and decomposition of free fatty acids [16].

Thiobarbituric Acid Value

Lipid present in foods undergoes oxidative degradation in the presence of oxygen or some catalyst like metal or sunlight. Polyunsaturated fatty acids (PUFA) get oxidised, which causes the formation of compounds such as aldehydes, ketones, and alcohol, thus finally resulting to oxidative rancidity [20]. Thiobarbituric acid (TBA) value of Mozzarella cheese significantly increased from 0.027 to 0.135 after a period of 10 days in 100% N_2 samples ($P <$ 0.05) while in vacuum packaged samples it significantly (P < 0.05) increased from 0.027 to 0.107.

Conclusion

The indicator films were efficient to detect very low oxygen inside cheese packages. However, owing to very high sensitivity and rapid colour recovery of the indicator film, spoilage or change in physico–chemical properties of the Mozzarella cheese could not be correlated with indicator's colour. These type of indicators could be used for detecting the purity of gas cylinders or with non-food contact applications. The future work on the development of oxygen indicator changing colour according to the oxygen concentration in the packages and its evaluation with oxygen-sensitive dairy products could be undertaken. Also, efforts could be made for binding the ingredients of the indicator to avoid leaching on moisture contact.

Table 2 Changes in the instrumental colour values of indicator films on photo-activation and recovery applied to Mozzarella cheese packaged under different packaging systems during storage

MAP	SA-MB-NR						SC-MB-RSS						CG-MB					
	L_o*	L_p*	a_o*	a_p*	b_o*	b_p*	L_o*	L_p*	a_o*	a_p*	b_o*	b_p*	L_o*	L_p*	a_o*	a_p*	b_o*	b_p*
100% N_2	8.41 ± 0.91^{a}	12.48 ± 0.48^{b}	3.59 ± 0.47^{a}	4.00 ± 0.37^{b}	− 7.47 ± 0.94^{a}	− 4.29 ± 0.40^{b}	7.33 ± 1.34^{a}	8.92 ± 0.83^{b}	5.32 ± 0.75^{a}	11.65 ± 0.81^{b}	− 15.85 ± 1.42^{a}	− 11.88 ± 0.81^{b}	15.91 ± 0.92^{a}	21.86 ± 1.24^{b}	4.97 ± 0.54^{a}	− 0.50 ± 0.47^{b}	− 16.68 ± 0.45^{a}	− 10.59 ± 0.67^{b}
Vacuum	9.12 ± 1.18^{a}	13.26 ± 0.46^{b}	3.35 ± 0.52^{a}	3.85 ± 0.36^{b}	− 7.00 ± 1.28^{a}	0.97 ± 0.33^{b}	10.69 ± 0.64^{a}	19.53 ± 0.83^{b}	3.85 ± 0.84^{a}	6.12 ± 0.67^{b}	− 15.15 ± 0.66^{a}	− 7.24 ± 0.55^{b}	7.75 ± 0.63^{a}	15.49 ± 1.47^{b}	4.71 ± 0.73^{a}	− 2.06 ± 0.47^{b}	− 13.03 ± 1.25^{a}	− 7.50 ± 0.41^{b}
Storage (in days)	L_r*		a_r*		b_r*		L_r*		a_r*		b_r*		L_r*		a_r*		b_r*	
Modified atmosphere packaging (100% N_2)																		
1	8.90 ± 1.12^{AB}		3.62 ± 0.67^{A}		− 7.68 ± 0.84^{A}		8.11 ± 0.60^{B}		4.44 ± 0.68^{A}		− 12.62 ± 1.11^{A}		16.10 ± 1.10^{A}		4.65 ± 0.76^{A}		− 16.68 ± 1.06^{A}	
4	9.71 ± 1.14^{BC}		3.88 ± 0.42^{A}		− 7.68 ± 0.69^{A}		8.06 ± 0.57^{B}		4.21 ± 0.58^{A}		− 12.35 ± 0.89^{A}		18.41 ± 1.45^{B}		8.26 ± 0.70^{BC}		− 20.32 ± 0.54^{C}	
8	9.93 ± 0.81^{C}		3.85 ± 0.46^{A}		− 7.91 ± 0.54^{A}		7.75 ± 0.70^{AB}		6.50 ± 0.52^{C}		− 16.53 ± 0.80^{B}		20.71 ± 1.04^{C}		7.85 ± 1.07^{B}		− 21.18 ± 0.93^{D}	
12	9.49 ± 0.56^{BC}		3.79 ± 0.44^{A}		− 8.97 ± 0.54^{B}		7.77 ± 0.71^{AB}		5.00 ± 0.70^{B}		− 14.47 ± 1.37^{C}		15.32 ± 1.41^{C}		7.68 ± 1.12^{B}		− 18.82 ± 0.94^{B}	
16	8.36 ± 1.82^{A}		5.79 ± 1.18^{B}		− 8.97 ± 1.47^{B}		7.38 ± 0.75^{A}		5.44 ± 0.56^{B}		− 15.12 ± 1.29^{C}		15.59 ± 1.12^{C}		8.85 ± 0.74^{C}		− 20.85 ± 1.12^{CD}	
Vacuum packaging																		
1	13.92 ± 0.75^{A}		3.50 ± 0.42^{A}		− 5.76 ± 0.56^{A}		11.30 ± 0.70^{A}		3.79 ± 0.64^{A}		− 14.47 ± 1.13^{A}		16.59 ± 1.28^{A}		6.68 ± 0.54^{A}		− 17.09 ± 1.27^{A}	
4	13.75 ± 0.90^{A}		3.44 ± 0.48^{A}		− 5.88 ± 0.55^{A}		11.50 ± 0.60^{A}		4.09 ± 0.73^{AB}		− 14.29 ± 0.68^{A}		12.38 ± 0.78^{B}		4.44 ± 0.84^{B}		− 14.88 ± 1.21^{B}	
8	14.85 ± 0.75^{B}		3.62 ± 0.69^{A}		− 5.53 ± 0.70^{A}		11.47 ± 0.79^{A}		5.29 ± 0.54^{C}		− 15.56 ± 1.42^{B}		12.38 ± 0.76^{B}		4.32 ± 0.68^{B}		− 15.38 ± 0.97^{B}	
12	5.10 ± 1.78^{C}		4.74 ± 0.47^{B}		− 6.71 ± 0.59^{B}		10.64 ± 0.91^{B}		4.32 ± 0.70^{B}		− 14.74 ± 1.29^{AB}		11.79 ± 0.80^{B}		6.47 ± 0.70^{A}		− 15.26 ± 0.79^{B}	
16	2.05 ± 0.78^{C}		2.32 ± 1.03^{C}		− 3.65 ± 1.18^{C}		9.71 ± 0.77^{C}		4.47 ± 0.82^{B}		− 15.41 ± 1.10^{B}		12.55 ± 1.07^{B}		6.18 ± 1.01^{A}		− 15.65 ± 0.92^{B}	

Mean ± SD

^{ABC}Mean values within an attribute (column) with similar superscripts do not differ significantly (P>0.05)

^{abc}Mean values within an attribute (row) with a similar superscript do not differ significantly (P>0.05)

$^{o, p, r}$Subscripts refer to original, photo-activated and recovered, respectively.

Table 3 Physico–chemical and headspace gas composition changes during storage of Mozzarella cheese stored under different packaging conditions

Storage interval (in days)	Water activity	Moisture	pH	Acid degree value	Thiobarbituric acid value	Headspace GAS COMPOSITION (%)		
						O_2	CO_2	N_2
Modified atmosphere packaging (100% N_2)								
1	0.954 ± 0.00^{a}	55.09 ± 0.52^{a}	5.32 ± 0.02^{a}	1.848 ± 0.03^{a}	0.027 ± 0.00^{a}	0.381 ± 0.01^{a}	0.048 ± 0.01^{a}	99.57 ± 0.01^{a}
4	0.971 ± 0.01^{b}	55.55 ± 0.45^{ab}	5.35 ± 0.03^{ab}	1.915 ± 0.03^{b}	0.057 ± 0.01^{b}	0.406 ± 0.01^{a}	0.083 ± 0.01^{b}	99.51 ± 0.01^{ab}
8	0.989 ± 0.01^{c}	56.20 ± 0.08^{ab}	5.42 ± 0.01^{b}	2.188 ± 0.02^{c}	0.096 ± 0.03^{c}	0.587 ± 0.03^{b}	0.124 ± 0.01^{c}	99.29 ± 0.03^{b}
12	0.995 ± 0.01^{cd}	56.38 ± 0.24^{b}	5.52 ± 0.03^{c}	2.826 ± 0.01^{d}	0.106 ± 0.01^{d}	0.815 ± 0.01^{c}	0.205 ± 0.01^{d}	98.95 ± 0.04^{c}
16	0.996 ± 0.02^{d}	56.54 ± 0.16^{b}	5.60 ± 0.01^{d}	3.286 ± 0.02^{e}	0.135 ± 0.03^{e}	1.197 ± 0.01^{d}	0.295 ± 0.02^{e}	98.50 ± 0.03^{d}
Vacuum packaging								
1	0.954 ± 0.00^{a}	55.09 ± 0.52^{a}	5.32 ± 0.02^{a}	1.848 ± 0.03^{a}	0.027 ± 0.00^{a}	–	–	–
4	0.959 ± 0.01^{b}	55.63 ± 0.01^{a}	5.35 ± 0.02^{ab}	1.913 ± 0.12^{b}	0.042 ± 0.01^{b}	–	–	–
8	0.966 ± 0.02^{c}	55.64 ± 0.01^{a}	5.40 ± 0.01^{b}	1.969 ± 0.01^{c}	0.063 ± 0.02^{c}	–	–	–
12	0.968 ± 0.01^{c}	55.67 ± 0.01^{a}	5.48 ± 0.02^{c}	2.271 ± 0.01^{d}	0.091 ± 0.01^{d}	–	–	–
16	0.975 ± 0.00^{d}	55.72 ± 0.01^{a}	5.52 ± 0.02^{c}	3.123 ± 0.01^{e}	0.106 ± 0.03^{e}	–	–	–

Mean ± SD

^{abcde}Values with different superscripts within a column differ significantly ($P<0.05$)

* Since it was difficult to measure oxygen concentration of vacuum packaged Mozzarella cheese, it was considered as 0

Compliance with Ethical Standards

Conflict of Interest The authors declare no conflict of interest.

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