

Effect of different levels of palm oil on the compositional quality of Mozzarella cheese during storage

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Abstract

The effect of different levels of palm oil and storage period on quality of Mozzarella cheese was investigated. Four Mozzarella cheese samples were prepared from skim cow milk, standardized at four different levels of palm oil (0, 1, 2 and 3% respectively) and stored at 4°C for 40 days. The stored samples were examined for quality changes at 10 days interval. The results indicated that, pH values of Mozzarella cheese decreased while the total solids, fat, ash and titratable acidity were increased significantly ($P \leq 0.05$) as levels of palm oil increased and storage time progressed similarly. The total volatile fatty acids of cheese increased significantly ($P \leq 0.05$), the increase in sample 3% palm oil (17.0 mls 0.1N NaOH) was significantly ($P \leq 0.05$) more compare to sample 0% palm oil (15.0 mls 0.1N NaOH). The formol ripening index also showed increasing trend towards the the end of storage period.

Organoleptic quality of mozzarella cheese revealed that, 2% palm oil secured the best (4.60) acceptability, followed by 1% (4.30) and 3% (4.10) and the poorest (3.80) was recorded for sample 0% palm oil. Storage period significantly ($P \leq 0.05$) affected the acceptability. The best score was obtained at day 20, while the poorest score at the beginning of the storage period.

Keywords: Mozzarella cheese, corn oil, storage period

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Introduction

Mozzarella cheese has many health benefits; it is a good source of protein, vitamins and minerals. Consumption of Mozzarella cheese may protect against gout, a painful condition that results in the buildup of uric acid crystals in the joints. The calcium found in Mozzarella cheese also has its contribution in body weight loss and provides protection against breast cancer and metabolic syndrome, which is a group of conditions that increase the main sources of animal fat and dietary cholesterol. Even though cholesterol is essential for membrane structure, hormone and steroid biosynthesis (Mahann and Escott-Stump, 1996), it has been recognized that it is at an elevated level in the risk of developing heart disease or stroke (Ibrahim, 2003).

In Mozzarella cheeses, the flavour is of secondary importance where stretchability and melting characteristics are of primary importance. There is also a requirement for minimal fat leakage during cooking. Although low fat cheeses are available in the market, their textural qualities have been adversely affected by reduction in fat level or substitution of vegetable oil for milk fat (Malin *et al.*, 1993).

Vegetable oils are preferred over the solid animal fats because of health benefits. Oils contain higher proportion of unsaturated fatty acids, while solid fats contain more saturated fatty acids, which increased the low density lipoprotein (LDL) level of the blood, which is considered harmful for human health (Lucas, 2000).

Palm oil raises plasma cholesterol only when an excess of dietary cholesterol is present in the diet. It stimulates the synthesis of protective HDL cholesterol and the removal of harmful LDL cholesterol. Palm oil is rich in vitamin E which appears to reduce serum cholesterol concentrations and has potent anti-oxidant effects (Noronha *et al.*, 2007).

The objective of this work is to study the effect of palm oil and storage period on quality of Mozzarella cheese.

Material and methods

Milk: fresh cow milk obtained from the Khartoum University dairy farm was skimmed.

Milk fat replacer: palm oil obtained from local market Khartoum North. Sudan

Rennet: chr-Hansen's laboratory, Denmark.

Salt: from local market, Khartoum North. Sudan.

Preparation and manufacture of Mozzarella cheese

Four Mozzarella cheese samples were prepared from skim cow milk. Skimmed milk was divided into four equal portions. The first portion was the control, in the next three portions palm oil was added at 1%, 2% and 3% level, respectively. After standardization, the rennet powder were introduced at rate 0.05% (for each 20 liter of milk) and left for 30- 40 min for coagulation after coagulation, the curd was cut in to cubes about 3 centimeter with stainless steel knives and heated for 10 min before stirring and whey is drained. The curds were formed into blocks and left in open areas to drain off the remaining whey. The drained curd exposed to warm temperature until required acidity reached at critical pH (5.2- 5.3). The curd was then put in hot water at 75- 80C° and mixed properly for 5 min until a smooth elastic mass was obtained, stretched into proper forms using stainless steel container for 3 hour, slightly salted in 5% cold brine solution (sodium chloride) for 2 hours. The cheese removed from the brine, dried lightly, weighted and package in polyethylene. The resultant cheese was stored at 4°C to be analyzed for physicochemical, biochemical analyses and organoleptic properties, when fresh and during storage period of 0, 10, 20, 30 and 40 interval.

Physicochemical and biochemical analyses

Cheese sample were analyzed for total solids, fat, ash, titratable acidity and protein according to AOAC (1990). The pH values were measured by using pH meter (model puslmuchen 15-126017, Germany) as described by Newlander and Atherton (1964). Total volatile fatty acid were determined by the direct distillation methods of kosikowski, (1982). The formol ripening index were determined according to Abdel-Tawab and Hofi (1966).

Organoleptic properties of cheese

The sensory evaluation of mozzarella cheese was evaluated by scoring procedures described by Ihekoronye and Ngoddy (1985).

Statistical analyses

Statistical analysis were done using the statistical analysis system SAS (1997) using 5% level of significance. Means were separated using Duncan's Multiple Range Test.

Results and discussion

Physiochemical and biochemical properties of Mozzarella cheese

The physiochemical and biochemical properties of Mozzarella cheese manufactured from skim cow milk containing different levels of palm oil (0, 1, 2 and 3% respectively) are as follows.

Total solid

Table 1 shows changes in total solids (TS) of Mozzarella cheese as affected by levels of palm oil and storage period. The TS of cheese increased significantly ($P \leq 0.05$) with progress in storage period. Sample 3% palm oil recorded the highest TS (51.36%) compared to values 2% (50.05%), 1% (48.45%) and 0% palm oil (46.50%). Ismail *et al.*, (2010) reported that the total solid content of cheese with different levels of palm oil were higher value than control cheese. The increase in total solid content of cheese due to loss of moisture content was a result of high acidity of the cheese (Salma *et al.*, 1982, Collombo *et al.*, 1992 and Walstra *et al.*, 1999).

Fat content

Table 2 shows changes in fat content of Mozzarella cheese as affected by levels of palm oil and storage period. The levels of fat content of Mozzarella cheese increased significantly ($P \leq 0.05$) with storage time. Samples 3% palm oil had the highest content (8.60%) higher than respectively values of 2% (7.0%), 1% (5.35) and 0% palm oil (2.90%). The fat content increased

in all cheese samples due to the loss of moisture content during storage. The same trend of these results was reported by Mahran ,(2000) and Sameen *et al.*,(2008) who found the cheese showed increase in fat content during storage period.

Protein content

Table 3 shows changes in protein content of Mozzarella cheese during storage as affected by levels of palm oil. The protein content of cheese decreased as the levels of palm oil increased and increased significantly ($P \leq 0.05$) during storage. The protein content of 0% palm oil at the end of the storage (41.57%) was higher than 1% (37.25%), 2% (35.10%) and 3% palm oil (34.00%). Ismail *et al.*, (2010) using palm oil in cheese reported that, there were differences between the control cheese and the other treatment ($P \leq 0.05$). Ali and Abdel-Razig (2010) found that, sample 0% milk fat was highest in Mozzarella cheese made from different levels of milk fat.

Ash content

Table 4 shows changes in ash content of Mozzarella cheese during storage as affected by levels of palm oil. The levels of palm oil and storage period significantly ($P \leq 0.05$) affected the ash content of Mozzarella cheese. Sample 0% palm oil showed the lowest ash content (2.95%) and sample 3% palm oil was the highest (3.50%). During storage period the ash content increased as the storage period progressed. Ismail *et al.*, (2010) found that, the control cheese was the higher ash content than the cheese made with palm oil. Kebary *et al.*, (1998) conducted that, there was an increase in ash content of cheese with fat replacers. The increase in ash content during storage attributed to decrease in moisture content Amer *et al.*,(1978) Siber (1998); Kebary *et al.*,(1998); Osman(2000) Sameen *et al.*,(2008) and Abdel-Razig and Babiker(2009) reported that, there were increased in ash content of cheese during storage period.

Titrateable acidity

Table 5 shows changes in titrateable acidity of Mozzarella cheese during storage as affected by levels of palm oil, the levels of palm oil significantly ($P \leq 0.05$) affected the titrateable

acidity of Mozzarella cheese and storage period.. Sample 0% palm oil gave the lowest titratable acidity (0.37%) while sample 3% palm oil recorded the highest (1.45%). El-Koussy *et al.*, (1995) found the titratable acidity of Mozzarella cheese ranged from 0.69% to 0.67%. Ismail *et al.*, (2010) reported that, the cheese treatments with added vegetable oils indicated less acidity of cheese than control cheese. This leads to that the acidity cheeses are affected by vegetable oil types. These results are in agreement with those of Abo El-Naga *et al.*, (1994) and Abdel-Aty (2003). Sameen *et al.*, (2008) mentioned the acidity of Mozzarella cheese increased during storage period.

pH value

Table 6 shows changes in the pH values of Mozzarella cheese during storage period as affected by levels of palm oil and storage period. The pH of cheese decreased significantly ($P \leq 0.05$) during the storage time. Sample 3% palm oil had a significantly ($P \leq 0.05$) lowest pH (3.20) compare the other respective values of 0% (4.83), 1% (4, 05) and 2% palm oil (3.60). Sameen *et al.*, (2008) conducted that the pH of Mozzarella cheese using different milk fat levels ranged from 5.05 to 5.12%. Ismail *et al.*, (2010) found that, the pH of cheese containing palm oil ranged from 6.79 to 5.43%. El-Koussy *et al.*, (1995) and Kebary *et al.*, (1998) found the pH-value of Mozzarella cheese decreased during the storage. Increasing levels of milk fat substitution and storage period lead to slight decreased in the pH value (Ismail *et al.*, 2010).

Total volatile fatty acids

Table 7 shows changes in total volatile fatty acids (TVFA) of Mozzarella cheese during storage as affected by levels of palm oil. The TVFA of Mozzarella cheese increased significantly ($P \leq 0.05$) with storage time. Sample 3% palm oil produced the highest TVFA (17.0 mls 0.1N NaOH) which was significantly higher than respective values of 2% (16.50 mls 0.1 N NaOH), 1% (16.00 mls 0.1 N NaOH) and 0% palm oil (15.00 mls 0.1 N NaOH). Badawi *et al.*, (2006) mentioned that, the total volatile fatty acid of Mozzarella cheese increased during storage. Ali and Abdel-Razig, (2010) found that, the total volatile fatty acids increased with increasing level of fat milk. Ismail *et al.*, (2010) stated that, the TVFA of cheese containing palm oil increased during the storage.

Table1. Effect of levels of palm oil (%) on total solids content (%)* of Mozzarella cheese during storage period

Storage period (days)	Levels of palm oil (%)			
	0	1	2	3
0.0	46.50±0.11 ⁿ	48.45±0.16 ^m	50.05±0.19 ^k	51.36±0.14 ^{ij}
10	49.52±0.13 ^l	51.00±0.12 ^j	52.80±0.20 ^h	53.90±0.15 ^g
20	51.15±0.17 ^{ij}	53.20±0.16 ^g	54.50±0.18 ^e	56.10±0.12 ^c
30	51.60±0.19 ^j	53.45±0.17 ^{fg}	55.00±0.13 ^d	56.80±0.11 ^b
40	52.05±0.14 ^h	53.70±0.15 ^{fg}	55.20±0.15 ^{cd}	57.18±0.15 ^a

*Mean±SD having different superscript letters in columns and rows are significantly different (P≤0.05).

Table 2. Effect of levels of palm oil (%) on fat content (%)* of Mozzarella cheese during storage period

Storage period (days)	Levels of palm oil (%)			
	0	1	2	3
0.0	2.90±0.02 ^l	5.35±0.06 ⁱ	7.00±0.05 ^h	8.60±0.08 ^f
10	4.16±0.03 ^k	7.20±0.07 ^{gh}	9.15±0.02 ^e	10.40±0.09 ^{bc}
20	4.45±0.11 ^{jk}	7.45±0.08 ^{gh}	9.60±0.12 ^e	10.75±0.13 ^b
30	4.58±0.06 ^{jk}	7.85±0.04 ^g	10.00±0.05 ^c	11.50±0.07 ^a
40	4.65±0.08 ^j	8.15±0.13 ^f	10.35±0.11 ^{bc}	11.80±0.07 ^a

*Mean±SD having different superscript letters in columns and rows are significantly different (P≤0.05).

Table 3. Effect of levels of palm oil (%) on protein content (%)^{*} of Mozzarella cheese during storage period

Storage period (days)	Levels of palm oil (%)			
	0	1	2	3
0.0	38.83±0.16 ^c	34.30±0.11 ^{gh}	33.50±0.15 ^{jk}	31.00±0.18 ^l
10	40.52±0.13 ^b	36.40±0.09 ^e	34.00±0.08 ^h	32.80±0.19 ^k
20	40.91±0.12 ^b	36.60±0.14 ^e	34.30±0.16 ^{gh}	33.15±0.17 ^j
30	41.36±0.09 ^a	37.00±0.11 ^d	34.90±0.12 ^g	33.85±0.14 ⁱ
40	41.57±0.08 ^a	37.25±0.09 ^d	35.10±0.16 ^f	34.00±0.13 ^h

*Mean±SD having different superscript letters in columns and rows are significantly different (P≤0.05).

Table 4. Effect of levels of palm oil (%) on ash content (%)^{*} of Mozzarella cheese during storage period

Storage period (days)	Levels of palm oil (%)			
	0	1	2	3
0.0	2.95±0.18 ^j	3.00±0.13 ⁱ	3.30±0.16 ⁱ	3.50±0.12 ^{gh}
10	3.45±0.15 ^h	3.50±0.09 ^{gh}	3.60±0.17 ^{gh}	3.80±0.11 ^e
20	3.61±0.14 ^{gh}	3.65±0.19 ^g	3.75±0.08 ^f	3.90±0.15 ^{de}
30	3.73±0.12 ^f	3.85±0.13 ^{de}	4.00±0.18 ^c	4.10±0.20 ^b
40	3.95±0.16 ^d	4.00±0.18 ^c	4.20±0.14 ^{ab}	4.30±0.19 ^a

*Mean±SD having different superscript letters in columns and rows are significantly different (P≤0.05).

Table 5. Effect of levels of palm oil (%) on titratable acidity (%)* of Mozzarella cheese during storage period

Storage period (days)	Levels of palm oil (%)			
	0	1	2	3
0.0	0.37±0.05 ⁿ	0.38±0.02 ^m	0.40±0.09 ^l	0.42±0.06 ^l
10	0.64±0.03 ^k	0.67±0.04 ^k	0.70±0.07 ^j	0.73±0.08 ^f
20	0.86±0.11 ^f	0.88±0.09 ^f	0.90±0.13 ^e	0.95±0.02 ^e
30	1.08±0.15 ^d	1.10±0.07 ^{cd}	1.20±0.05 ^c	1.25±0.06 ^b
40	1.11±0.02 ^{cd}	1.25±0.08 ^b	1.35±0.14 ^{ab}	1.45±0.09 ^a

*Mean±SD having different superscript letters in columns and rows are significantly different (P≤0.05).

Table 6. Effect of levels of palm oil (%) on pH-value* of Mozzarella cheese during storage period

Storage period (days)	Levels of palm oil (%)			
	0	1	2	3
0.0	5.45±0.08 ^a	5.35±0.03 ^a	5.20±0.09 ^b	5.10±0.06 ^{bc}
10	5.13±0.06 ^{bc}	4.80±0.09 ^e	4.50±0.04 ^f	4.15±0.08 ^{gh}
20	5.03±0.04 ^c	4.60±0.11 ^f	4.20±0.05 ^{gh}	4.00±0.02 ^j
30	4.95±0.05 ^d	4.30±0.06 ^g	4.10±0.07 ⁱ	3.80±0.09 ^k
40	4.83±0.03 ^e	4.05±0.12 ^{ij}	3.60±0.11 ^f	3.20±0.05 ^m

*Mean±SD having different superscript letters in columns and rows are significantly different (P≤0.05).

Table 7. Effect of levels of palm oil (%) on total volatile fatty acids (mls 0.1N NaOH)* of Mozzarella cheese during storage period

Storage period (days)	Levels of palm oil (%)			
	0	1	2	3
0.0	15.00±0.16 ^m	16.00±0.15 ^l	16.50±0.09 ^l	17.00±0.14 ^k
10	19.60±0.14 ^j	21.00±0.17 ^h	21.60±0.16 ^{gh}	22.00±0.18 ^f
20	20.50±0.12 ⁱ	21.80±0.13 ^g	22.50±0.14 ^f	23.40±0.09 ^{de}
30	20.80±0.09 ⁱ	23.00±0.12 ^e	23.80±0.18 ^d	24.30±0.11 ^c
40	21.25±0.18 ^{gh}	24.30±0.16 ^c	25.60±0.11 ^b	26.20±0.14 ^a

*Mean±SD having different superscript letters in columns and rows are significantly different (P≤0.05).

Table 8. Effect of levels of palm oil (%) on formol ripening index* of Mozzarella cheese during storage period

Storage period (days)	Levels of palm oil (%)			
	0	1	2	3
0.0	20.50±0.18 ⁿ	22.00±0.13 ^m	23.50±0.12 ^l	25.50±0.17 ^j
10	23.80±0.16 ^l	25.50±0.14 ^j	25.80±0.18 ⁱ	27.10±0.11 ^h
20	25.40±0.12 ^k	27.10±0.17 ^{gh}	28.50±0.15 ^f	29.00±0.16 ^e
30	27.85±0.17 ^g	28.00±0.11 ^f	29.40±0.16 ^e	31.10±0.18 ^c
40	30.15±0.15 ^d	31.70±0.17 ^c	32.90±0.11 ^b	33.30±0.19 ^a

*Mean±SD having different superscript letters in columns and rows are significantly different (P≤0.05).

Formol ripening index

Table 8 shows the effect of levels of palm oil and storage period on formol ripening index (FRI) of Mozzarella cheese. Sample 0% palm oil was the lowest FRI (20.50%), while sample 3% palm oil was the highest (25.50%), sample 1% and sample 2% palm oil occupies an intermediate position. The FRI gradually increased till the end of the storage period ($P \leq 0.05$). Badawi *et al.*, (2006) found that, formol ripening index of Mozzarella cheese range from 12.08 to 19.98%.

The FRI of cheese increased with increasing levels of palm oil and storage time (Dinkci *et al.*, 2011). This result similar to this obtained by Kavas *et al.*, (2004) who concluded that, the formol ripening index of cheese made with palm oil increased during the storage.

Organoleptic quality of mozzarella cheese

Appearance

Table 9 shows changes in appearance of mozzarella cheese as affected by levels of palm oil and storage period. The score of appearance improved with storage time. Sample 2% palm oil gave the best score (4.5) significantly ($p \leq 0.05$) better than 1% (4.3), 3% (4.1) and 0% palm oil (4.1). Abdel-Rafee *et al.*, (2004) concluded that, the appearance score of mozzarella cheese improved with homogenization and during storage. Dinkci *et al.*, (2011) found that, the appearance of cheeses made with the vegetable fat blend were significantly lower ($P \leq 0.05$) than those of the cheeses containing only milk fat during the storage period.

Texture

Table 9 shows changes in texture of mozzarella cheese during storage as affected by levels of palm oil and storage period. The score of texture improved with storage time then decreased until the end of storage period, the best scores were obtained at the day 20 (Table 9). Sample 2% palm oil gave the best score (4.6) significantly ($P \leq 0.05$) better than 1% (4.5), 3% (4.2) and 0% palm oil (3.8). Abdel-Hamid *et al.*, (2001) and Sameen *et al.*, (2008) found that, the texture of mozzarella cheese improved during storage period. Kim and Yu, (1988) found that, the texture scores of Mozzarella cheese were highest with high fat levels. Fat plays a key role in the texture of cheese (O'connor, 1994).

Flavour

Table 9 shows changes in flavour of mozzarella cheese during storage as affected by levels of palm oil and storage period. The score of flavour of mozzarella cheese improved with storage time. Sample 2% palm oil gave best score (4.4) significantly better ($p \leq 0.05$) than 1% (4.3), 3% (4.2) and 0% palm oil (4.0) Sameen *et al.*, (2008) stated that, flavour of mozzarella cheese improved during storage. Dinkci *et al.*,(2011)stated that, the score of flavour of cheese containing palm oil improved with storage time. This result was similar to Azzam (2007).The improvement of cheese flavour, was mainly attributed to the production of acid by lactic acid bacteria (kosikowski, 1982).

Overall acceptability

Table 9 shows changes in overall acceptability of mozzarella cheese as affected by levels of palm oil and storage period. The score of acceptability of mozzarella cheese improved with storage period. Sample 2% palm oil gave the best score (4. 6) significantly better ($P \leq 0.05$) than 1% (4.3), 3% (4.1), and 0% (3.8) palm oil. Storage period significantly ($P \leq 0.05$) affected the appearance , texture , flavour and acceptability . The best score (4.50, 4.6,4.4 and 4.6 respectively) were obtained at the day 20 , and the lowest score (2.6, 2.5, 2.8 and 2.7 respectively at the beginning of the storage . Abdel Rafee *et al.*, (2004) observed that, the flavor and appearance score of mozzarella cheese improved during storage while body texture score decreased. Sameen *et al.*, (2008) concluded that, the sensory attribute of mozzarella cheese (appearance, texture and overall acceptability) were improved during storage period. Azzam, (2007) stated that, the acceptability of the cheese made with the vegetable fat blend improved during the storage.

Conclusion

Total solids, fat, protein, ash, titratable acidity, FRI, and TVFA increased during storage, while the pH values decreased. Overall acceptability of cheese containing 2% palm oil was the best than in the other samples followed by 1, 3 and 0% palm oil. Storage 20 days is found to be quite satisfactory for mozzarella cheese to obtain good quality.

Table 9. Effect of levels of palm oil (%) and storage period on organoleptic quality* of Mozzarella cheese

Storage period (Days)	Appearance				Texture				Flavour				Overall acceptability			
	Levels of palm oil (%)															
	0.0	1.0	2.0	3.0	0.0	1.0	2.0	3.0	0.0	1.0	2.0	3.0	0.0	1.0	2.0	3.0
	Scores															
0	2.60 ^l ±0.11	3.20 ^j ±0.09	3.30 ⁱ ±0.07	3.00 ^k ±0.08	2.50 ⁿ ±0.08	3.00 ^l ±0.11	3.10 ^k ±0.09	2.90 ^m ±0.06	2.80 ^m ±0.07	3.20 ^k ±0.06	3.40 ^j ±0.08	3.10 ^l ±0.09	2.70 ^k ±0.05	3.30 ⁱ ±0.06	3.50 ^h ±0.07	3.10 ^j ±0.08 _{ss}
10	3.40 ^{hi} ±0.08	4.00 ^d ±0.05	4.20 ^b ±0.07	3.80 ^f ±0.06	3.40 ^j ±0.06	4.30 ^d ±0.04	4.40 ^c ±0.03	4.10 ^f ±0.05	3.60 ⁱ ±0.06	4.10 ^d ±0.05	4.30 ^b ±0.04	3.80 ^g ±0.03	3.50 ^h ±0.07	4.20 ^d ±0.06	4.30 ^c ±0.04	4.00 ^f ±0.05
20	3.70 ^g ±0.06	4.30 ^a ±0.05	4.50 ^a ±0.03	4.10 ^c ±0.04	3.80 ^h ±0.06	4.50 ^b ±0.03	4.60 ^a ±0.02	4.20 ^e ±0.04	4.00 ^e ±0.06	4.30 ^b ±0.03	4.40 ^a ±0.02	4.20 ^c ±0.05	3.80 ^g ±0.06	4.30 ^c ±0.05	4.60 ^a ±0.02	4.10 ^e ±0.03
30	3.50 ^h ±0.05	4.10 ^c ±0.08	4.20 ^b ±0.06	4.00 ^d ±0.07	3.60 ⁱ ±0.06	4.30 ^d ±0.04	4.50 ^b ±0.03	4.00 ^{fg} ±0.05	3.70 ^h ±0.04	4.10 ^d ±0.07	4.20 ^c ±0.04	3.90 ^f ±0.08	3.50 ^h ±0.06	4.20 ^d ±0.04	4.50 ^b ±0.03	4.00 ^f ±0.05
40	3.10 ^{jk} ±0.09	3.90 ^e ±0.07	4.00 ^d ±0.08	3.70 ^g ±0.06	3.40 ^j ±0.08	4.20 ^e ±0.06	4.40 ^c ±0.05	3.90 ^g ±0.07	3.20 ^k ±0.06	3.80 ^g ±0.04	4.00 ^e ±0.07	3.60 ⁱ ±0.05	3.30 ⁱ ±0.07	4.10 ^e ±0.08	4.30 ^c ±0.06	3.80 ^g ±0.04

*Mean±SD having different superscript letters in columns and rows are significantly different (P≤0.05).

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