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# Effect of Commercial Lipase and Protease Enzymes from Microbiological Sources on Properties of White Cheese

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**Abstract**

In this study, effect of addition of commercial lipolytic (Piccantase A) and proteolytic (Fromase TL) enzyme preparation derived from *Mucor miehei* on the accelerated ripening of White cheese was investigated. Lipolytic and proteolytic enzyme preparation was added to cheese milk after rennet addition at the level of 0.5, 1.0, 1.5 and 1.0, 2.0, 3.0 g 100 $kg^{-1}$ of milk, respectively. White cheeses were ripened at 4±1 $^{o}$C for 30 days. WSN, NPN contents and ripening indice were significantly affected by the addition of different enzyme levels and especially using proteolytic enzymes. Cheeses P2 and P3 displayed the fastest ripening rate compared with control cheese. Enzyme treatments markedly increased the total VFA content of cheeses during ripening, particularly when lipase was used. It was determined that ripening period had no effect on the main chemical constituents of the cheeses, but caused significant increase in titratable acidity, WSN, NPN, CN, PPN, TVFA contents and ripening indice. It was cocluded that White cheese could be produced with a high acceptability when acid fungal protease, was used at level of 2 g 100 $kg^{-1}$ and lipase, was used at level of 1.5 g 100 $kg^{-1}$.

**Keywords:** Protease, lipase, enzyme, cheese, ripening period

**Mikrobiyolojik Kaynaklı Ticari Lipaz ve Proteaz Enzimlerinin Beyaz Peynirlerin Özellikleri Üzerine Etkileri**

**Özet**

Bu çalışmada, Beyaz peynirlerin olgunlaşması üzerine Mucor miehei' den elde edilen ticari proteolitik ve lipolitik enzim ilavesinin etkileri araştırılmıştır. Lipolitik ve proteolitik enzimler, peynir sütüne rennet ilavesinden sonra sırasıyla 0.5, 1.0, 1.5 ve 1.0, 2.0, 3.0 g 100 $kg^{-1}$ düzeylerinde ilave edilmiş ve üretilen Beyaz peynirler 30 gün süreyle 4±1 $^{o}$C' de olgunlaştırılmıştır. WSN, NPN ve olgunlaşma katsayısını farklı düzeylerde enzim ilavesi ve özellikle proteolitik enzim ilavesi önemli düzeyde etkilemiştir. Enzim uygulaması özellikle lipaz kullanılan örneklerde olgunlaşma süresince peynirlerin toplam uçucu yağ asitleri miktarını önemli oranda arttırmıştır. Olgunlaşma süresinin peynirlerin başlıca bileşenleri üzerine etkisinin istatistiksel açıdan önemli olmadığı fakat titrasyon asitliği, WSN, NPN, CN, PPN, TVFA içerikleri ve olgunlaşma katsayısında önemli düzeyde artışa neden olduğu belirlenmiştir ($p<0.05$). Beyaz peynirlerin 1.5 g 100 $kg^{-1}$ düzeyinde lipaz ve 2 g 100 $kg^{-1}$ düzeyinde proteaz enzimi kullanıldığında yüksek bir kabul edilebilirlikle üretilebileceği kanısına varılmıştır.

**Anahtar Kelimeler:** Proteaz, lipaz, enzim, peynir, olgunlaşma süresi

## Introduction

Most cheese varieties require a minimum ripening period to acquire a certain degree of flavour intensity, before they are acceptable to the consumer (Vafopoulou et al., 1989). Ripening itself is a costly and time-consuming process (Wilkinson et al., 1992) so that shortening the ripening period has several advantages in reducing the cost of cheese production. Elevated temperature, enzyme addition, modified starter have been investigated to reduce this period (Hayashi et al., 1990). Extensive studies in accelerated ripening have been reported by employing enzymes such as protease (Trepanier et al., 1992; Ardö and Petterson, 1988; Kim et al., 1994a; Nunez et al., 1991; Saldamlı and Kaytanlı, 1998; Lin and Jeon, 1987; Nasr et al., 1991), lipase (Kheadr et al., 2000a; Aydemir et al., 2000), and mixtures of enzymes (Kheadr et al., 2000b). Selection of types

and the amounts of enzymes to achieve a balance between accelerating and not to create undesirable flavour intensities is still difficult to obtain (Kim et al., 1994b).

White cheese is a soft white brined cheese made from ewes' and cows' milk and produced traditionally in most areas of the country. It is a ripened cheese which the time needed for its ripening is about 3 months, however it is also consumed freshly. Although White cheese is a widely consumed cheese in Turkey, there is some information about the possibilities of accelerated ripening. The aims of this study were to evaluate the effect of various levels and different enzyme preparations on ripening time and chemical and sensory attributes of White cheese.

**Materials and Methods**

Raw cow milk was received from the animal husbandry section of Cukurova University, Adana, Turkey. Wiesbyvac G 3 Mix 6 type mesophilic cheese culture (*Lactococcus lactis* ssp. *lactis* and *Lactococcus lactis* ssp. *cremoris*; Tonder, Denmark), fungal esterase lipase and acid fungal protease preparation commercially called "Piccantase A" and "Fromase TL" respectively, derived from *Mucor miehei*, was obtained from Gist-brocades (USA), calcium chloride (Merck, Darmstandt, Germany), rennet (Pınar, Izmir, Turkey) and NaCl (Merck) were obtained commercially.

Cheeses were made in the pilot plant of the food engineering department, faculty of agriculture, Cukurova University. Raw milk was pasteurised at 65 $^{o}$C for 30 min, cooled immediately to 30$\pm$2 $^{o}$C and then calcium chloride and starter culture were added at 0.02 % and 1 %, respectively. Rennet was added to coagulate the milk within 90 min. After 10 min, acid fungal protease at levels of 1.0 (P1), 2.0 (P2) and 3.0 (P3) g 100 kg$^{-1}$ of milk to the first three portions and fungal esterase lipase at levels of 0.5 (L1), 1 (L2) and 1.5 (L3) g 100 kg$^{-1}$ of milk to the second three portions were added. The last one was manufactured without the addition of microbial lipase or protease to serve as a control sample. The coagulum was cut into 2 cm$^{3}$ pieces. Whey was drained off and put into press. The curds were then cut into 7x7x7 cm cubes and put into brine prepared with NaCl which were adjusted 14 % and treated with salt for 6 h. Fresh cheeses in glass jars filled with the same brine were stored at 4$\pm$1 $^{o}$C for 4 weeks.

In raw milk, total solids were determined by gravimetric method according to Kaptan (1969); fat was determined by the Gerber method according to Turkish Standard TS 1018 (Anon., 1994); protein was determined according to Ling (1963); and lactose content was determined by the Lane Eynon method according to Anon. (1983).

In cheese samples, the amount of rennet to be added to milk was measured according to Gonç (1984). Total solids, salt and fat were determined by the methods stated in Turkish standard TS 591 (Anon., 1995). Acidity was determined according to AOAC (1965) and expressed as $^{o}$SH. Total nitrogen (TN), water-soluble nitrogen (WSN) and nonprotein nitrogen (NPN) were determined by the micro-Kjeldahl method of Gripon et al. (1975). The percentage of protein was calculated by multiplying the total nitrogen by 6.38. Ripening indices were found as the proportion of water–soluble nitrogen matter to total nitrogen matter. Protease-peptone nitrogen was calculated by subtracting the nonprotein nitrogen matter contents from the water-soluble matter contents. Casein nitrogen was calculated according to Lenoir (1979). Total volatile fatty acids (TVFA) were determined by the method described by Kosikowski (1982). A surberlin PNR 6 penetrometer (Germany) was used to measure penetrations of a conical spindle which was 95.5 g in weight (x 1/10 mm).

Organoleptic properties were evaluated 30$^{th}$ day of ripening by five dairy academic staf members from food engineering department, Cukurova University, according to the scoring card which stated in Turkish standard TS 591 (Anon., 1995).

White cheeses was examined on days 1 and 30 after manufacture. Data for each sampling stage was analysed statistically by one-way analysis of variance. Means with a significant difference ($p<0.05$) were compared by the least squares difference (LSD) test (Duzgunes et al., 1987).

**Results and Diccussion**

Dry matter, fat, protein and lactose content of the milk were found to be 11.22 %, 3.2 %, 3.47 % and 4.52 %, respectively. Dry matter content (5.40 %), fat (0.30 %), protein (0.69 %) and lactose (4.40 %) of the whey were determined.

Some chemical properties and composition of initial and 30 day stored White cheese are give in Table 1. Titratable acidity and pH of cheese samples were not significantly affected by the addition of enzyme ($p<0.05$). However, they were decreased during ripening period. The effect of the ripening period was significant ($p<0.05$). Guven and Konar (1997) demonstrated that neutral and alkaline components resulting from proteolysis and lipolysis caused the decrease in acidity.

Dry matter content was lower in protease-treated cheeses than lipase-treated cheeses at 30$^{th}$ days and changed irregularly during the ripening

period. The result indicate that the salt contents of all cheeses increased as ripening progressed and ripening period was not effected ($p>0.05$). The S/DM levels for all cheeses increased with the progress of ripening except L1 cheese; similar results for White cheese were reported by Saldamlı and Kaytanlı (1998). As shown in Table 1, fat content of protease-treated cheeses and lipase-treated cheeses showed a significant difference from the control cheese, A, at 30$^{th}$ days ($p<0.05$). The changes in fat content obtained during ripening were not found to be statistically significant ($p>0.05$).

In fresh cheeses, protein contents ranged from 17.86 % (P3) to 20.29 % (P1), decreasing to between 16.21 % (P3) and 18.82 (L1) by the end of the ripening. The effect of the ripening period on protein content was not significant ($p>0.05$). Protein content of the different treatments decreased during the ripening period. Total nitrogen contents decreased with ripening period. It was found that the ripening period was not effective on the total nitrogen content ($p>0.05$). Lowest total nitrogen content was consistently obtained for protease-treated cheeses at the the end of the ripening.

There was a significant increase in WSN and NPN during ripening ($p<0.05$). Highest levels of WSN were found in protease-treated cheeses at all stages of ripening. Control cheese resulted in slight increases in WSN and NPN levels. Increasing the enzyme ratios generally resulted in higher values for WSN and NPN in all cheeses. The percentages of WSN and NPN were significantly higher in the cheeses with added enzyme especially protease than control at 30$^{th}$ day ($p<0.05$). Statistically, the change that occurred during ripening was important ($p<0.05$). Yıldız et al. (1989) have reported that the WSN contents of White cheeses were between 0.23 and 0.64 % after 3 months of ripening. Chander et al. (1986) and Fulco et al. (1990) also showed that the WSN contents of cheeses increased throughout the ripening period.

During ripening, the CN contents of the cheeses decreased and this change was statistically significant ($p<0.05$). The effect of the different treatment on the CN contents was found to be significant ($p<0.05$). It was found that the PPN contents of cheeses P3 was considerably higher than that of the control cheese, K, at the end of the ripening period. The changes in PPN contents obtained during ripening were found to be statistically significant ($p<0.05$).

The ripening indice (water soluble N as a percentage of total N) of cheese with proteinase and lipase increased as the amount of enzyme increased. The ripening indices of cheeses K and L1 were found to be significantly lower than the other cheeses. Ripening period and use of enzymes were significantly effective on the ripening indice ($p<0.05$). The ratio of soluble nitrogen/total nitrogen which indicate the protein degradation of the cheese gradually increased during the ripening period ($p<0.05$). However, the increase in ripening indice in the cheeses with added lipase and protease were more greater than the control. Cheeses P2 and P3 displayed the fastest ripening rate compared with control cheese.

Total volatile fatty acids content (millilitres 0.1 N NaOH equivalent/100 g cheese) was greater in lipase and protease-treated cheeses than in untreated cheese after 30$^{th}$ day of ripening. Especially Piccantase A was more effective in this respect. TVFA content increased significantly in all cheeses ($p<0.05$). White cheese containing 1 (L2) and 1.5 g 100 kg$^{-1}$ (L3) *Mucor miehei* enzymes had higher TVFA content than the control after 30$^{th}$ day of ripening.

The organoleptic properties of the cheeses made with addition of three different levels of proteolytic and lipolytic enzymes are shown in Table 2. After 30$^{th}$ day, sample K was the best preferred cheese, followed consecutively by cheeses P1 and L3. The score for body and texture of cheese made with 1 g 100kg$^{-1}$ proteolytic enzyme was higher than control. The taste of protease-treated cheese (P1) was similar to lipase-treated cheese (L2).

**Conclusion**

Protease and lipase enzyme treatments markedly increased NPN, WSN, ripening indices and TVFA contents of the cheeses, respectively. Statistical analysis showed that, the ripening period affected all of components except the main chemical composition of White cheese. There was a more rapid breakdown of protein fractions in cheeses with added protease. It was concluded that the use of enzyme preparations especially protease could be recommended to accelerate the ripening of White cheese.

**Table 1.** Chemical and physical properties of White cheese

| | Da | K | P1 | P2 | P3 | L1 | L2 | L3 |
|---|---|---|---|---|---|---|---|---|
| TA | 1 | 22.80±0.57$^{a}$ | 27.20±0.26$^{a}$ | 26.80±0.67$^{a}$ | 25.60±0.39$^{a}$ | 21.60±0.66$^{a}$ | 24.80±0.13$^{a}$ | 24.80±0.39$^{a}$ |
| (°SH) | 30 | 12.00±0.13$^{a}$ | 14.80±0.57$^{a}$ | 14.80±0.70$^{a}$ | 14.80±0.09$^{a}$ | 11.20±0.53$^{a}$ | 13.20±0.70$^{a}$ | 11.40±0.98$^{a}$ |
| pH | 1 | 5.44±0.18$^{a}$ | 5.26±0.06$^{a}$ | 5.39±0.18$^{a}$ | 5.34±0.09$^{a}$ | 5.55±0.36$^{a}$ | 5.34±0.19$^{a}$ | 5.47±0.10$^{a}$ |
| | 30 | 5.76±0.13$^{ab}$ | 5.61±0.13$^{a}$ | 5.68±0.06$^{a}$ | 5.70±0.18$^{ab}$ | 5.93±0.14$^{b}$ | 5.76±0.07$^{ab}$ | 5.80±0.05$^{ab}$ |
| DM | 1 | 47.43±0.44$^{a}$ | 48.00±0.80$^{a}$ | 52.92±0.25$^{b}$ | 50.34±0.23$^{b}$ | 47.75±0.27$^{a}$ | 48.29±0.83$^{a}$ | 49.42±0.45$^{a}$ |
| (%) | 30 | 48.42±0.74$^{a}$ | 47.25±0.40$^{a}$ | 47.50±0.83$^{a}$ | 47.29±0.47$^{a}$ | 51.02±0.40$^{b}$ | 53.02±0.28$^{b}$ | 52.08±0.20$^{b}$ |
| Fat | 1 | 24.13±1.01$^{a}$ | 22.63±0.94$^{a}$ | 22.13±0.88$^{a}$ | 23.75±0.41$^{a}$ | 23.38±0.94$^{a}$ | 24.63±0.88$^{a}$ | 24.88±0.64$^{a}$ |
| (%) | 30 | 23.38±0.38$^{a}$ | 21.75±0.41$^{b}$ | 22.13±0.64$^{b}$ | 21.25±0.41$^{b}$ | 24.75±0.71$^{c}$ | 25.63±0.24$^{c}$ | 24.63±0.24$^{c}$ |
| P | 1 | 18.18±0.49$^{a}$ | 20.29±0.58$^{b}$ | 17.99±0.66$^{a}$ | 17.86±0.37$^{a}$ | 19.20±0.63$^{b}$ | 19.52±0.51$^{b}$ | 19.844±0.88$^{b}$ |
| (%) | 30 | 17.67±0.88$^{a}$ | 18.12±0.90$^{ac}$ | 17.55±0.39$^{a}$ | 16.21±0.26$^{b}$ | 18.82±0.71$^{c}$ | 17.29±0.98$^{ac}$ | 17.23±0.95$^{ac}$ |
| Salt | 1 | 4.32±0.37$^{a}$ | 4.20±0.50$^{ab}$ | 3.99±0.30$^{b}$ | 4.18±0.35$^{ab}$ | 5.25±0.19$^{c}$ | 4.11±0.20$^{ab}$ | 3.96±0.23$^{ab}$ |
| (%) | 30 | 5.54±0.37$^{a}$ | 5.80±0.59$^{a}$ | 5.61±0.15$^{a}$ | 5.75±0.45$^{a}$ | 5.39±0.48$^{a}$ | 5.00±0.4$^{a}$ | 5.26±0.31$^{a}$ |
| FDM | 1 | 50.87±1.02$^{a}$ | 47.15±1.27$^{a}$ | 41.82±1.04$^{b}$ | 47.17±1.60$^{a}$ | 48.96±0.34$^{a}$ | 51.02±0.70$^{a}$ | 50.34±0.05$^{a}$ |
| (%) | 30 | 48.28±1.10$^{a}$ | 46.03±0.60$^{b}$ | 46.59±0.88$^{ab}$ | 44.94±1.83$^{b}$ | 48.51±0.77$^{a}$ | 48.35±0.66$^{a}$ | 47.30±0.53$^{ab}$ |
| SDM | 1 | 9.11±1.02$^{ab}$ | 8.75±0.49$^{a}$ | 7.54±0.86$^{a}$ | 8.30±0.73$^{a}$ | 11.03±0.82$^{b}$ | 8.52±0.56$^{a}$ | 8.01±0.13$^{a}$ |
| (%) | 30 | 11.44±0.60$^{a}$ | 12.28±1.06$^{a}$ | 11.81±0.39$^{a}$ | 12.16±0.62$^{a}$ | 10.56±0.86$^{ab}$ | 9.43±0.38$^{b}$ | 10.09±0.51$^{ab}$ |
| PDM | 1 | 38.33±0.57$^{a}$ | 42.27±0.60$^{b}$ | 33.99±0.43$^{c}$ | 35.48±0.87$^{d}$ | 40.21±0.64$^{e}$ | 40.42±0.84$^{e}$ | 40.15±0.97$^{e}$ |
| (%) | 30 | 36.49±0.27$^{a}$ | 38.35±0.34$^{a}$ | 36.95±0.52$^{a}$ | 34.28±0.65$^{b}$ | 36.89±0.10$^{a}$ | 32.61±0.42$^{c}$ | 33.08±0.25$^{c}$ |
| PV | 1 | 59.40±3.33$^{a}$ | 54.10±5.80$^{a}$ | 46.00±1.70$^{b}$ | 49.60±3.64$^{b}$ | 46.70±3.54$^{b}$ | 38.40±1.23$^{c}$ | 49.00±5.20$^{b}$ |
| | 30 | 64.90±1.84$^{a}$ | 56.10±2.77$^{b}$ | 57.80±5.92$^{ab}$ | 52.00±4.28$^{b}$ | 41.00±0.57$^{c}$ | 43.20±4.15$^{c}$ | 44.30±2.49$^{c}$ |
| TVFA | 1 | 2.25±0.10$^{a}$ | 2.03±0.04$^{b}$ | 1.78±0.02$^{c}$ | 1.53±0.04$^{a}$ | 2.25±0.35$^{a}$ | 2.75±0.35$^{d}$ | 2.43±0.04$^{e}$ |
| | 30 | 2.50±0.19$^{a}$ | 3.10±0.04$^{b}$ | 3.39±0.16$^{a}$ | 3.25±0.04$^{a}$ | 3.24±0.35$^{b}$ | 4.15±0.35$^{c}$ | 4.13±0.04$^{c}$ |
| TN | 1 | 2.85±0.03$^{a}$ | 3.18±0.05$^{a}$ | 2.82±0.03$^{a}$ | 2.80±0.48$^{b}$ | 3.01±0.10$^{a}$ | 3.06±0.15$^{a}$ | 3.11±0.04$^{a}$ |
| (%) | 30 | 2.77±0.14$^{a}$ | 2.84±0.30$^{ac}$ | 2.75±0.50$^{a}$ | 2.54±0.06$^{b}$ | 2.95±0.11$^{c}$ | 2.71±0.07$^{a}$ | 2.70±0.62$^{a}$ |
| WSN | 1 | 0.34±0.01$^{ab}$ | 0.36±0.01$^{bd}$ | 0.39±0.01$^{c}$ | 0.38±0.02$^{cd}$ | 0.32±0.01$^{a}$ | 0.32±0.02$^{a}$ | 0.35±0.01$^{b}$ |
| (%) | 30 | 0.43±0.01$^{a}$ | 0.61±0.01$^{b}$ | 0.64±0.03$^{b}$ | 0.70±0.02$^{c}$ | 0.48±0.02$^{d}$ | 0.50±0.03$^{de}$ | 0.52±0.01$^{e}$ |
| NPN | 1 | 0.17±0.01$^{a}$ | 0.17±0.02$^{ab}$ | 0.15±0.00$^{b}$ | 0.16±0.01$^{ab}$ | 0.13±0.01$^{c}$ | 0.13±0.02$^{bc}$ | 0.13±0.02$^{bc}$ |
| (%) | 30 | 0.20±0.02$^{a}$ | 0.35±0.02$^{b}$ | 0.35±0.02$^{b}$ | 0.37±0.01$^{b}$ | 0.25±0.01$^{c}$ | 0.29±0.02$^{d}$ | 0.26±0.02$^{c}$ |
| PPN | 1 | 0.17±0.00$^{a}$ | 0.19±0.02$^{a}$ | 0.24±0.01$^{b}$ | 0.22±0.02$^{ab}$ | 0.19±0.01$^{a}$ | 0.19±0.02$^{a}$ | 0.22±0.01$^{ab}$ |
| (%) | 30 | 0.23±0.01$^{a}$ | 0.26±0.01$^{b}$ | 0.29±0.01$^{c}$ | 0.33±0.01$^{d}$ | 0.23±0.01$^{a}$ | 0.21±0.01$^{a}$ | 0.26±0.01$^{b}$ |
| CN | 1 | 2.51±0.04$^{a}$ | 2.82±0.01$^{b}$ | 2.43±0.02$^{c}$ | 2.42±0.01$^{c}$ | 2.69±0.05$^{d}$ | 2.74±0.02$^{d}$ | 2.76±0.04$^{d}$ |
| (%) | 30 | 2.34±0.03$^{a}$ | 2.23±0.05$^{b}$ | 2.11±0.06$^{c}$ | 1.84±0.07$^{d}$ | 2.47±0.05$^{e}$ | 2.21±0.06$^{b}$ | 2.17±0.03$^{bc}$ |
| RI | 1 | 11.93±0.75$^{a}$ | 11.32±1.28$^{a}$ | 13.83±0.72$^{b}$ | 13.57±0.29$^{b}$ | 10.63±1.29$^{a}$ | 10.46±0.64$^{a}$ | 11.25±0.14$^{a}$ |
| (%) | 30 | 15.52±0.43$^{a}$ | 21.48±0.70$^{b}$ | 23.27±0.88$^{c}$ | 27.56±0.24$^{d}$ | 16.27±0.58$^{a}$ | 18.45±0.29$^{e}$ | 19.63±0.82$^{f}$ |

$^{a,b,c}$ Means in the same letters followed by different column are significantly different (P<0.05)

TA: Titratable Acidity, DM: Dry Matter, P: Protein, FDM: Fat in Dry Matter, SDM: Salt in Dry Matter, PDM: Protein in Dry Matter, PV: Penetrations Value (x1/10 mm), TVFA: Total Volatile Free Acids (ml 0.1 N NaOH/ 100 g cheese), TN: Total Nitrogen, WSN: Water Soluble Nitrogen, NPN: Nonprotein Nitrogen, PPN: Protease-Peptone Nitrogen, CN: Casein Nitrogen, RI: Ripening Indice

**Table 2.** Organoleptic properties of White cheese

| | K | P1 | P2 | P3 | L1 | L2 | L3 |
|---|---|---|---|---|---|---|---|
| Appearance | 17.25±2.38$^{a*}$ | 17.75±1.28$^{a}$ | 17.25±1.04$^{a}$ | 18.00±1.85$^{b}$ | 17.50±1.77$^{a}$ | 18.25±1.28$^{b}$ | 20.00±0.00$^{c}$ |
| Body and Texture | 32.38±1.62$^{a}$ | 33.25±1.24$^{a}$ | 30.19±1.16$^{bc}$ | 27.56±2.43$^{b}$ | 29.75±1.95$^{bc}$ | 29.31±2.46$^{bc}$ | 30.63±2.62$^{c}$ |
| Smell | 9.75±0.71$^{a}$ | 9.38±0.32$^{ab}$ | 9.25±0.33$^{ab}$ | 9.50±0.43$^{b}$ | 9.00±0.07$^{b}$ | 9.00±0.06$^{b}$ | 9.50±0.23$^{a}$ |
| Taste | 34.56±1.24$^{a}$ | 30.63±1.21$^{b}$ | 29.75±0.20$^{b}$ | 26.69±0.98$^{c}$ | 29.75±1.95$^{b}$ | 30.63±1.25$^{b}$ | 27.13±.084$^{c}$ |
| Total Score | 93.88±2.69$^{a}$ | 91.33±3.91$^{a}$ | 85.78±2.98$^{b}$ | 81.59±3.70$^{b}$ | 86.22±1.18$^{b}$ | 86.64±1.36$^{b}$ | 88.02±3.46$^{a}$ |

$^{a, b, c}$ Means in the same letters followed by different column are significantly different (P<0.05)

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