EFFECT OF VISCOUS CULTURES ON THE RHEOLOGICAL AND SENSORY PROPERTIES, FLAVOR SUBSTANCES AND STARTER BACTERIA COUNTS OF SET YOGURT

KATI KIVAMLI YOĞURDUN REOLOJİK VE DUYUSAL ÖZELLİKLERİ, AROMA MADDELERİ VE STARTER BAKTERİ SAYILARI ÜZERİNE VİSKOZ KÜLTÜRLERİN ETKİSİ

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SUMMARY: Rheological, biochemical, bacteriological and sensory characteristics of set yogurt prepared by commercial freeze-dried starter cultures (A-set, B-viscous, C-viscous, A+B-combined, A+C-combined) were examined after 1,7 and 14 days of refrigerated storage. The maximum viscosity and the minimum whey separation were obtained in yogurt made with C culture. In addition, this starter culture resulted in higher acid development and starter bacteria counts in yogurt production than the others. As to sensoric properties, the maximum appearance, body and texture scores were obtained from the combined cultures with no decrease in storage. Yogurt prepared with culture A+C, owing to its lower acidity and its higher acetaldehyde and diacetyl content, was especially much more liked than the others in terms of flavor. Refrigerated storage resulted in a decrease in wheying-off and viable starter concentrations, and an increase in viscosity. In adition the level of lactic acid increased and different changes formed in the level of flavor substances during the storage.

ÖZET: Dondurarak kurutulmuş ticari starter kültürlerle (A-katı kıvamlı, B-viskoz, C-viskoz, A+B-kombine, A+C-kombine) hazırlanan katı kıvamlı yoğurtların reolojik, biyokimyasal, bakteriyolojik ve duyusal özellikleri soğukta depolamanın 1., 7. ve 14. günlerinde belirlenmiştir. En yüksek viskozite ve en düşük serum ayrılması C kültürüyle yapılan yoğurtta elde edilmiştir. Ayrıca bu starter kültür diğerlerine göre yoğurt üretiminde daha fazla asit artışı ve starter bakteri sayısının elde edilmesini sağlamıştır. Duyusal özellikler bakımından en yüksek yapı, tekstür ve görünüş değerleri kombine kültürlerle elde edilmiş ve bu değerler depolama sırasında azalma göstermemiştir. A+C kültürüyle hazırlanan yoğurt daha düşük asitlik ile daha yüksek asetaldehit ve diasetil miktarı nedeniyle tat ve koku bakımından diğerlerinden daha fazla beğenilmiştir. Soğukta depolama canlı starter sayısı ve su salmanın azalmasına, viskozitenin artmasına neden olmuştur. Ayrıca depolama sırasında laktik asit oranı artmış ve aroma maddeleri düzeyinde farklı değişimler meydana gelmiştir.

INTRODUCTION

Yogurt is produced from milk by lactic acid bacteria that produce large amounts of lactic acid through fermentation of the milk sugar, lactose. The type of bacteria used differentiates yogurt from other fermented products such as buttermilk, cottage cheese, or hard cheese (HANKIN and SHIELDS, 1980). LABRAPOULOS et al. (1982) observed that the selection of the lactic culture used in the production of yogurt plays an important role in the development of the desired body, acidity, viscosity and flavor of the finished product.

In general, any natural or processing influence that affects chemical or physical milk composition and constituents may be reflected in the consistency of the curd formed on coagulation. In addition, some strains of yogurt bacteria *(Streptococcus salivarius subs. thermophilus (S. thermophilus)* and *Lactobacillus delbrueckii subsp. bulgaricus (L. bulgaricus),* are distinguished by the slime formation that influences consistency. During fermentation, these strains synthesize different polysaccharides which contribute to the high viscosity of yogurt and called viscous cultures (RASIC and KURMANN, 1978). The exopolysaccharide production by yogurt bacteria is an important characteristic to be considered when selecting starter cultures for yogurt. At present, there is a tendency to incorporate strains with beneficial properties into the traditional yogurt starter cultures. In this respect, the use of slime-producing strains improve both the texture and body of the yogurt, preventing gel fracture and wheying-off, which are the common problems in the manufacturing of the product (GARCIA-GARIBAY and MARSHALL, 1991). The objective of this study was to compare traditional set and viscous yogurt cultures on the basis of rheological and sensory properties, flavor substances and culture growth of finished set yogurt product.

MATERIALS and METHODS

Cultures and their propagation: Commercial freeze dried ready set yogurt cultures, A-set, B-viscous, C-viscous, were obtained from Sanofi Bio-Industries. Prior to study each culture was transferred into 12% reconstituted skim milk medium using a 0.01% inoculum and incubated at 42°C for 4.5 hours.

Yogurt preparation: Raw whole milk (av. 3% fat) purchased from the dairy farm of Aegean University and standardized to 12% snf by skim milk powder was heated to 85°C for 30 min before fermentation and divided into five parts. The portions were then cooled to 48°C and individually inoculated with 3% each of active cultures of A,B,C,A+B (75%+25%) and A+C (75%+25%). The rates of combination were advised by the firm. Each of the inoculated yogurt mixes was divided into 200 ml portions which were poured into plastic containers, capped and incubated at 42°C until the pH was 4.65. After cooling to 4°C, all samples were stared at the same temperature for subsequent analysis.

Repeatability: All results presented in this paper are the mean of 3 separate trials.

Analysis and Measurements:

Total solids: (ANONYMOUS, 1982).

Titratable acidity: It was determined in 10 g of yogurt diluted with 90 ml of distilled water and was expressed as % lactic acid.

pH: It was determined by electromatic pH meter (Beckman Zeromatic SS-3 Model)

Viscosity: After a standard stirring of the yogurt samples the viscosity was measured by means of a Gerber viscometer. The average degree of flow of the sample for 100 ml was determined and reported in seconds as a mean value of the two observations at 20°C.

Whey separation: The amount of whey of a 15g sample taken after 30, 60 and 90 min was determined.

Flavor compounds: The volatiles were determined using the gas chromatographic method described by KANG et al. (1988).

Sensory evaluation: Sensory evaluations of yogurt samples were carried out on the basis of the Official Score Card of the American Dairy Science Association, modified slightly for this study. A technical panel of five experienced judges was selected for making score-card sensory evaluations for flavor, body and texture, and appearance. Refrigerated samples were served in randomly coded plastic containers.

Survival of starter bacteria: Enumeration of *S. thermophilus* and *L. bulgaricus* and *S. thermophilus,* respectively (ANONYMOUS, 1983). Results were expressed as colony forming units (cfu)/ml. Colony differentiation was based on the fact that *S. thermophilus* forms discrete high mass colonies (1-3 mm in diameter) while *L. bulgaricus* forms diffuse low mass colonies (2-10 mm in diameter).

RESULTS and DISCUSSION

In Table 1 dry solids, lactic acid, pH values and the number of starter bacteria of the yogurt samples after 1, 7 and 14 days of storage are tabulated. As expected, the variety of culture used in the fermentation did not affect dry solids of the samples. However, important differences were found in lactic acid and pH values among the samples. The highest mean of lactic acid and the lowest mean of pH values were obtained from yogurt fermented by C culture ($p<0.01$).

As can be seen from Table 1, pH decreased during cold-storage while acidity increased. These results confirm previous observations (ABRAHAMSEN and HOLMEN, 1980; BACKER and PUHAN, 1989; DESMAISON et al., 1990; SINHA et al., 1989). It is interesting to note that both higher lactic acid values and faster acid development was obtained from the samples in which viscous cultures (B,C) were used alone. In contrast, yogurt samples produced from viscous starter culture containing polysaccharide producing *S. thermophilus* have been reported to possess lower acidity and slower acid development during 8 days of storage (KREUDER et al., 1994).

TABLE 1. The Content of Dry Matter, Lactic Acid, the ph and the Number of Starter Bacteria in Yogurt Samples Manufactured With the Test Cultures After 1, 7 and 14 Days of Storage.[a]

Yogurt[b]	Days of storage	%Dry matter	%Lactic acid	pH	Number of starter bacteria ($x10^7$ cfu/ml) S.thermo philus	L.bul-garicus
A	1	15.16	1.09	4.63	84	37
	7	15.18	1.14	4.55	12	21.8
	14	15.21	1.17	4.48	8.5	9.4
	Mean	(15.18)	(1.13)[c]	(4.55)[c]	(34.8)[c]	(22.7)[c]
B	1	15.18	1.14	4.53	98	34
	7	15.20	1.21	4.48	16.5	19.3
	14	15.21	1.26	4.45	9.6	8.5
	Mean	(15.20)	(1.20)[d]	(4.49)[d]	(41.4)[d]	(20.7)[d]
C	1	15.10	1.18	4.50	124	41
	7	15.12	1.27	4.45	78	27.1
	14	15.17	1.30	4.42	0.9	9.2
	Mean	(15.13)	(1.25)[e]	(4.46)[d]	(71.1)[e]	(25.8)[e]
A+B	1	15.12	1.10	4.57	87	32
	7	15.18	1.14	4.52	9.1	14.1
	14	15.22	1.16	4.48	7.7	8.6
	Mean	(15.17)	(1.13)[c]	(4.52)[cd]	(34.5)[c]	(18.2)[f]
A+C	1	15.11	1.03	4.60	91	38
	7	15.14	1.09	4.50	62	17.3
	14	15.19	1.14	4.50	7.3	7.7
	Mean	(15.15)	(1.09)[f]	(4.53)[c]	(53.4)[f]	(21.0)[d]

a Data represent average of three separate trials and bracketed values are mean of data obtained during the storage

b A contains commercial strains of *S. thermophilus* and *L. bulgaricus*, 70% and 30% resp., B contains viscous strains of *S.thermophilus* and *L. bulgaricus*, 80% and 20% resp., C contains another viscous strains of *S. thermophilus* and *L. bulgaricus*, 80% and 20% resp.

c,d,e,f Bracketed means in row with unlike superscripts are significantly different ($P<0.01$) as determined by Student's t-test.

Table 1 also show the numbers of starter bacteria in yogurt. It is noteworthy to see that the yogurt prepared with C culture showed viable *S.thermophilus* and *L. bulgaricus* numbers higher than the others during the storage. ($p<0.01$). The higher lactic acid value observed in these samples may be attributed to the higher number of viable starter bacteria. This reflects perhaps that the activity of C culture was higher than the others. On the other hand, viable counts of *S. thermophilus* and *L. bulgaricus* started to decrease rapidly from the onset of storage period. But, the numbers of *S. thermophilus* decreased less rapidly in yogurts made with C and A+C culture especially in the first week of storage (Table 1). This situation supported again the idea that C cultures' activity was higher. The decrease in viable culture bacteria in yogurt during refrigerated storage was also observed by ABRAHAMSEN and HOLMEN (1980), KHEGIAS and DALLES (1984), SALINAS (1984), SİNHA et al. (1989), LAYE et al. (1993).

But, HAMANN and MARTH (1984) reported that there was a slight increase in numbers of *S. thermophilus* of the laboratory-manufactured yogurt samples during the storage of two weeks while the numbers of *L. bulgaricus* decreased rapidly from the onset of the storage period. Similar results were also observed by KIM at al. (1992).

In Turkey both set and stirred yogurt are manufactured commercially, with the exception that set yogurt is only made without flavor additives. The low amount of whey separated from the coagulum is an important consistency characteristic of set yogurt. One of the important factors that prevents leakage of whey is the use of viscous culture in the fermentation. Fig. 1, Fig. 2, Fig 3 show clearly that the maximum wheying-off values taken after 30, 60 and 90 min., respectively, were obtained in the samples made with culture A. In contrast to this, yogurt produced by culture C was more firm and the wheying-off in that coagulum was lower than the others in all cases. On the other hand, depending on the length of storage time wheying-off values were decreased in all samples.

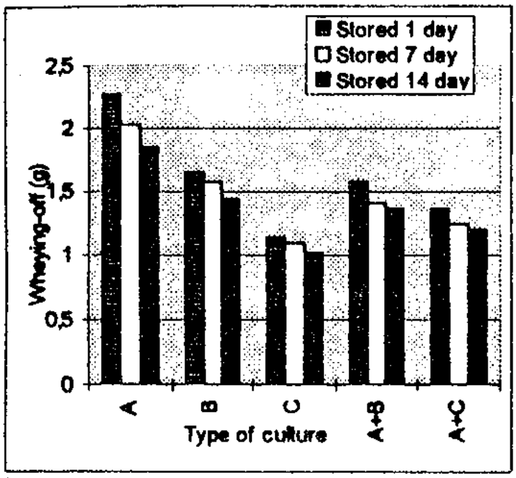

Fig. 1 **Influence of the culture type on the wheying-off values of yogurt coagulum taken after 30 min. (A=Set yogurt culture, B=Viscous yogurt culture, C=Viscous yogurt culture)**

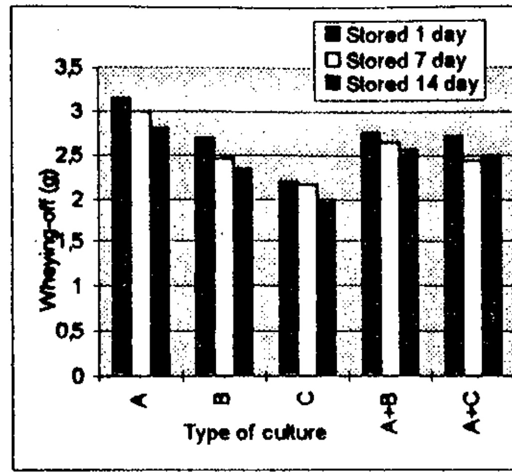

Fig. 2 **Influence of the culture type on the wheying-off values of yogurt coagulum after 60 min. (A=Set yogurt culture, B=Viscous yogurt culture, C=Viscous yogurt culture)**

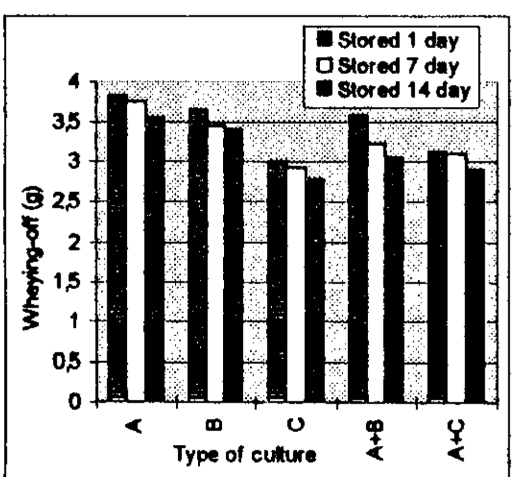

Fig. 3 **Influence of the culture type on the wheying-off values of yogurt coagulum taken after 90 min. (A=Set yogurt culture, B=Viscous yogurt culture, C=Viscous yogurt culture)**

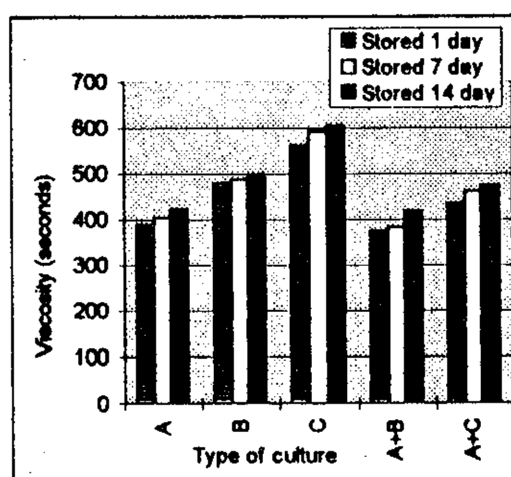

Fig 4. **The Influence of the culture type on the viscosity of yogurt (A=Set yogurt culture, B=Viscous yogurt culture, C=Viscous yogurt culture)**

A sufficient consistency and viscosity depends greatly on proper treatment of the milk and on optimal conditions covering the culture type during the manufacturing process. For this reason, it is of particular interest to use a culture that provides a higher viscosity to the final product. The results of the viscosity measurements are presented in Fig. 4.

The stored quality was again very high for yogurt made from C because samples had higher viscosity than yogurts made from other cultures and culture combinations. Statistical evaluation showed significant differences among the means of viscosity values for the samples made from the different cultures and combinations, obtained during the storage ($p<0.01$). The least value was obtained from A+B yogurt sample and the highest value from C-yogurt sample. On the other hand, a slight increase was obtained in all samples after 14 days, the greatest increase in viscosity was in yogurt from culture A+B.

Vanderpoorten and Waes (1972) examined the quality of yogurt prepared with 9 different cultures and found the yogurt from culture 1 had the highest viscosity, but it was too slimy to be used alone.

Results of sensory evaluation and aroma compound concentrations are presented in Table 2.

The flavor depends almost entirely on the starter culture and their metabolism during incubation. HİLD (1979) attributed typical yogurt flavor to the content of acetaldehyde and a certain level of diacetyl. Table 2 shows clearly that yogurt fermented with culture A+C which exhibited highest concentrations of acetaldehyde and diacetyl was the best liked product with regard to flavor on the 1., 7. and 14. day of storage. The mean flavor score of yogurt from culture A was significantly lower acetaldehyde content.

Yogurts made with the combined cultures had no decrease in texture and appearance scores during the storage. The over-syneresis especially from A-sample and excess slime production from B-sample could be the reasons for lower texture scores. As expected, depending on the storage time especially flavor scores gradually decreased. In this study yogurt prepared with the culture combination of A+C had the best sensory quality.

ROBINSON et al. (1977) reported that a close correlation was observed between acetaldehyde levels in natural, set yogurt, and "consumer" preference as recorded by a test pane. However it was concluded that sensory properties of yogurt were not only influenced by its acetaldehyde content, but also by acidity of the product (KNEFIEL et al., 1992). Correspondingly yogurt prepared with A+C, the best liked product with regard to flavor, had the lowest acidity.

KREUDER et al. (1994) found no important difference between yogurt prepared by the culture containing polysaccharide producing and non producing strains of *S.thermophilus* for flavor during 8 day of storage. But the texture was better in yogurt prepared by polysaccharide-producing-strain.

Different acetaldehyde concentrations were found in the first day of storage, ranging from 47 ppm to 65 ppm. These values were larger than those reported by other researchers. (ABRAHAMSEN, 1978; BOTTAZZI et al., 1973; ESTEVEZ et al., 1988; KNEFIEL et al., 1992; LAYE et al, 1993). Discrepancies may be due to factors such as the use of different starters, synergistic effects of the microflora itself, manufacturing process and fermentation conditions (BOTTAZZİ et al., 1973; DESMAZEUD, 1988; HUNGER, 1984). The highest amount of acetaldehyde, the most prominent compound of typical yogurt aroma, was attained by (81 ppm) the yogurt prepared with culture A+C after 7 day of storage. Therefore, it could be concluded that synergistic effects of its's microflora were stronger than the others. Yogurt from culture A had a lower acetaldehyde concentration than yogurt from viscous cultures and their combinations. Depending on the length of storage time the acetaldehyde amount of about all products increased in the first week of storage and then decreased. This change in acetaldehyde was also observed by other researchers (ABRAHAMSEN, 1978; BOTTAZZI et al., 1973; ESTEVEZ et al., 1988; HAMDAN et al., 1971; KANG et al., 1988). On the other hand, diacetyl and ethanol amounts of the samples were generally fonud in negligible amounts, lower than that obtained by HILD (1979) and KNEFIEL et al. (1992). Besides, minor variations were observed in the levels of diacetyl and ethanol during the storage. All samples had a maximum production of acetone in the seventh day of storage and then decreased.

Table 2. Sensory Characteristics and Aroma Compound Concentrations of Yogurt Samples Manufactured With the Test Cultures After 1, 7 and 14 days of Storage.[a]

Yogurt[b]	Storage time (days)	Sensory Characteristics[c]			Aroma compound concentrations (ppm)			
		Flavor	Texture	Appearance	Acetaldehyde	Diacetyl	Acetone	Ethanol
A	1	8.67	4.50	5.00	47.0	–	–	trace
	7	8.00	4.33	5.00	59.3	trace	3.5	trace
	14	8.00	4.17	4.50	60.7	trace	2.5	trace
	Mean	(8.22)[d]	(4.33)[d]	(4.83)[d]	(55.7)[d]			
B	1	9.00	4.80	5.00	51.0	–	–	trace
	7	8.50	4.50	5.00	68.3	trace	2.0	trace
	14	8.00	4.50	4.50	52.5	trace	1.0	trace
	Mean	(8.50)[e]	(4.60)[e]	(4.83)[e]	(57.3)[e]			
C	1	9.60	5.00	5.00	59.0	trace	–	2.3
	7	9.33	5.00	5.00	74.7	1.5	3.0	3.5
	14	9.00	4.50	4.50	63.7	1.3	1.5	7.5
	Mean	(9.37)[f]	(4.83)[e,f]	(4.83)[d]	(65.8)[e]			
A+B	1	9.50	5.00	5.00	61.3	–	–	trace
	7	9.50	5.00	5.00	74.3	trace	2.6	trace
	14	9.00	5.00	5.00	69.0	trace	1.4	2.5
	Mean	(9.33)[f]	(5.00)[f]	(5.00)[e]	(68.2)[e]			
A+C	1	10.0	5.00	5.00	65.0	trace	–	trace
	7	10.0	5.00	5.00	81.3	1.9	2.5	trace
	14	9.50	5.00	5.00	72.0	1.5	1.8	1.5
	Mean	(9.83)[g]	(5.00)[f]	(5.00)[e]	(72.8)[f]			

a **Data represent average of three separate trials and bracketed values are mean of data**

b **The yogurts were prepared by the test cultures as described in footnote to Table 1**

c **All values are averages of triplicates from a 5-member technical panel. Evaluation ratings were made on a 1-10 scale for flavor and on a 1-5 scale for texture and for appearance, respectively. Higher numbers indicate greater acceptability.**

d,e,f,g **Bracketed means in row with unlike superscripts are significantly different ($p<0.01$) as determined by Student's t-test.**

As a conclusion our results indicate that the use of viscous cultures offers new possibilities in the manufacture of set yogurt because parallel to the increase of acidity, number of starter bacteria, sensory quality and acetaldehyde concentration. Moreover, it is possible to manufacture yogurt with a lower syneresis and a significantly higher viscosity by the use of viscous cultures. This is of great importance because sensory characteristics and consistency of set yogurt are decisive for the preference of the final prodect.

On the other hand yogurt produced by viscous culture-C have superior properties than the other cultures with regard to the viscosity, the wheying-off, the production of acetaldehyde beside lactic acid and the number of starter bacteria. This culture is however too slimy to be used alone. Finally, it may be concluded that the culture combination of A+C is the most suitable culture for the preparation of yogurt because of the high sensoric quality and good rheological properties.

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