ORIGINAL RESEARCH

The effects of commercial starter culture and storage temperature on the oxidative stability and diacetyl production in butter

IHSAN BAKIRCI,[1]* SERAFETTIN ÇELIK[2] and CIHAT ÖZDEMIR[1]

[1]*Atatürk University, Agricultural Faculty, Department of Food Engineering, TR-25240, Erzurum, Turkey and* [2]*Harran University, Agricultural Faculty, Department of Food Engineering, Sanliurfa, Turkey*

In this study, the effects of four different direct-vat-set (DVS) commercial starter cultures (CHN11, CHN22, EZAL BT001 and MM100) and storage temperatures (4 ± 1 and −18 ± 2°C) on the oxidative stability and diacetyl production in butter during a 4-month period were investigated. Samples were taken from butter on days 3, 30, 60, 90 and 120. Analyses of titration acidity, peroxide value, free fatty acids and diacetyl contents of the samples taken were carried out. The effects of the starter cultures on the peroxide value and diacetyl contents were found to be significant ($P < 0.01$*) while the storage temperatures significantly affected all parameters tested. Interaction between the type of culture and storage temperature was significant for free fatty acids (*$P < 0.05$*) and diacetyl content (*$P < 0.01$*).*

Keywords Butter, Commercial starter cultures, Diacetyl, Oxidative stability, Storage temperature.

INTRODUCTION

Butter is mainly produced by concentrating milk fat from sweet cream or cream acidified with cultures or occasionally, as in Turkey, it is produced from yoghurt. Butter made from sweet cream is usually marketed without salting. However, the salty form is also marketed to achieve a long shelf life.[1–3]

Butter should be produced under controlled and hygienic conditions because the process is complex and thus great attention is needed.[4] Traditionally, manufacturers of fermented products maintained their own stock cultures and produced the bulk starter inoculum at the plant. This procedure, however, bears the risk of bacteriophage contamination. Therefore, direct-vat-set (DVS) cultures are mostly used and added into the cream directly. These methods of usage have the advantage of the correct balance and control of starter strains and supplying the optimum acidity and flavour to the product.[5,6]

The characteristic taste and flavour of fermented milk products such as yoghurt and butter depend on the concentrations of components such as acetaldehyde, diacetyl and acetoin produced by mesophilic and thermophilic lactic acid bacteria.[5,7] The flavour development in butter occurs through the action of a mixed starter culture that contains both acid and aroma-producing bacteria. The aroma-producing bacteria are responsible for fermentation of citrate into diacetyl, which gives a characteristic taste and flavour to butter even at very low concentrations (1–2 ppm).[1,8,9] However, diacetyl is reduced in the presence of diacetyl reductase to acetoin resulting in a substantial loss of the desirable aroma. Diacetyl reductase may be found in the starter cultures and is commonly associated with psychrotrophic bacteria.[10]

In determining storage stability and quality of butter, the peroxide value reflecting the oxidation of fatty acids, the amount of free fatty acid and the degree of lipolysis are taken into consideration.[11,12]

The amount of free fatty acids (FFAs) in the medium is defined as the amount of KOH (mg) needed to neutralize FFAs in 1 g of butter. It is a measure of the degree of lipolysis. When the amount reaches 1.8 mg KOH/g fat, there is a perceptible off-flavour in butter.[13] In a study carried out by Atamer and Sezgin[11] the relation between the amount of FFA and peroxide value against butter flavour was analysed. Samples with FFAs exceeding 3.3 mg KOH/g fat were recognized as rancid or spoiled by 59% of the panellists.

An off-flavour is also perceived in butter[13] when peroxide values reach 2 meq O_2/kg fat. Interestingly, the acceptable value is set at 10 meq O_2/kg fat by the Food Regulations in Turkey.[14]

Christensen and Holmer[15] produced butter by using two different packaging materials (polyethylene plastic and aluminium foils) and stored it at 4, 20 and −18°C for 14 weeks. Peroxide values of the butter samples stored at −18°C were found to be lower than peroxide values of samples stored at 4 and 20°C. In addition, butter samples in plastic

*Author for correspondence. E-mail: ibakirci@atauni.edu.tr

© 2002 Society of Dairy Technology

containers had higher peroxide values than those packaged in aluminium ($P < 0.05$). The researchers reported that packaging material and storage temperatures had significant effects on the storage stability of butter.

This study was planned to determine the effects of various commercial starter cultures and different storage temperatures on oxidative stability and diacetyl formation in butter.

MATERIALS AND METHODS

The cream used in this study was obtained from the pilot milk-processing plant of the Agricultural College of Atatürk University. Prior to butter production, the fat content of the cream was adjusted to 35%. Mesophilic DVS cultures were obtained from Peyma Hansen (CHN11, CHN22) and Mayasan Inc. (EZAL BT001, MM100).

Manufacture of experimental butters

A total amount of 12 kg cream was divided into four parts in 3-kg portions and pasteurized at 75 ± 2°C for 20 min using a batch pasteurizer, and then cooled to 21°C. Each part was inoculated with 1% of one of the cultures CHN11, CHN22, BT001 or MM100. The inoculated cream was ripened at about 21°C for 12 h and churned. The butters produced (as 100 g) were placed in sterilized jars and stored at 4 ± 1°C and −18 ± 2°C for 4 months. Samples were taken from the butters in parallel and analysed. Results represent averages of one trial.

Titratable acidity, free fatty acids, peroxide value and diacetyl content were determined on days 3, 30, 60, 90 and 120 of the storage period.

Fat and moisture content were measured according to the methods described by Kurt *et al*.[16] Titratable acidity (as lactic acid percentage) was determined according to Atamer.[13]

Determination of free fatty acids

About 5 g of melted butter was dissolved in 50 mL neutralized ethanol/diethyl ether mixture (1 : 1) and titrated with 0.1 N alcoholic KOH to obtain a light pink colour. Free fatty acid content (mg KOH/g fat) was obtained by calculation as described by Atamer.[13]

Peroxide value

Approximately 3 g of butter was weighed into an Erlenmeyer flask and dissolved in 10 mL chloroform. Then 15 mL acetic acid and 1 mL saturated potassium iodine (KI) were added and mixed for 1 min. The mixture was left in the dark at room temperature for 5 min. Distilled water (75 mL) was added and the mixture shaken vigorously. A prepared starch solution (1%, 1 mL) was added and the resulting solution titrated with 0.02 N sodium thiosulphate ($Na_2S_2O_3$) until the colour became clear. The peroxide value was calculated using the formula:[13]

$$PV = [(V_1 - V_0)N]/M$$

where V_1 is the amount of $Na_2S_2O_3$ used for titration (mL), V_0 is the amount of $Na_2S_2O_3$ used for the blank (mL), N is the normality of $Na_2S_2O_3$ and M is the amount of sample (g).

Diacetyl determination

The diacetyl content was analysed by the methods of Walsh and Cogan[17] and Xanthopoulos *et al*.[7] A 40-g sample of butter was transferred into a round-bottomed flask, and 10 mL of distillate were obtained by the steam distillation method. Aliquots (5-mL) of distillate were transferred into test-tubes. Then 1.5 mL hydroxylamine was added and the mixture held in a water bath at 75°C for 20 min. The contents of the test-tubes were mixed well while they are hot. Acetone phosphate (0.5 mL) was added, followed by mixing. Then the tubes were left to cool at room temperature. After cooling, 1.5 mL alkaline tartrate solution was added and mixed, and freshly prepared 0.1 mL $FeSO_4$ solution was immediately added and again mixed. The volume was adjusted to 10 mL with 33% dipotassium hydrogen phosphate solution and after 15 min absorbances were measured at 530 nm with a spectrophotometer (Miltonory Spectronic 20). Standard solutions of diacetyl were prepared as 1.0, 2.5, 5.0, 7.5 and 10 ppm and absorbances were taken at 530 nm to set up a standard curve.

Statistical analysis

Analysis of variance was performed according to the randomized block experiment design by using the MINITAB packet program.[18]

RESULTS AND DISCUSSIONS

All samples taken from the experimental butter had a fat content between 82 and 83% and a moisture content between 14 and 15%. The values were within the Turkish standards for butter samples. As there were no significant differences among the fat and moisture contents of the samples, they are not discussed further.

Titration acidity values obtained from the butter samples are presented in Table 1. General means of titration acidity, FFA, peroxide values and diacetyl contents of butters and their statistical evaluations in terms of culture type, storage temperature and storage periods are shown in Table 2. As seen from the tables, the type of culture had no effect on acid production. The reason might be that all the cultures used had similar growth properties. However, the samples stored at low temperature (+4 ± 1°C) had higher significantly ($P < 0.01$) values than those stored in a deep freezer (−18 ± 2°C). Different

© 2002 Society of Dairy Technology

Table 1 Titratable acidity values of butter samples (LA %)

	Storage period (days)				
Culture type	*3*	*30*	*60*	*90*	*120*
BT001-R	0.07	0.09	0.13	0.14	0.13
BT001-DF	0.07	0.08	0.10	0.11	0.10
MM100-R	0.07	0.10	0.13	0.15	0.15
MM100-DF	0.07	0.08	0.09	0.13	0.12
CHN11-R	0.09	0.10	0.10	0.13	0.16
CHN11-DF	0.09	0.09	0.10	0.11	0.11
CHN22-R	0.09	0.11	0.12	0.13	0.14
CHN22-DF	0.09	0.09	0.10	0.11	0.12

LA, lactic acid
R, stored in refrigerator (+4 ± 1°C)
DF, stored in deep freezer (−18 ± 2°C)

Table 3 The amounts of free fatty acids in butter samples (mg KOH/g fat)

	Storage period (days)				
Culture type	*3*	*30*	*60*	*90*	*120*
BT001-R	1.13	1.62	1.94	2.20	2.45
BT001-DF	1.12	1.27	1.44	1.56	1.75
MM100-R	1.30	1.39	1.64	2.19	2.62
MM100-DF	1.21	1.40	1.52	1.59	1.79
CHN11-R	1.19	1.52	1.75	2.01	2.58
CHN11-DF	1.14	1.23	1.26	1.29	1.54
CHN22-R	1.20	1.61	1.83	2.35	3.02
CHN22-DF	1.18	1.28	1.32	1.43	1.63

R, stored in refrigerator (+4 ± 1°C)
DF, stored in deep freezer (−18 ± 2°C)

storage temperatures, especially lower temperatures, might limit the activity of starters or enzymes involved in acid production. Moreover, interactions between culture type and storage period and between storage temperature and storage period were found to be significant at the level of $P < 0.01$.

Free fatty acids in fresh milk fat usually increase due to lipolysis by both natural milk enzymes and enzymes of bacteria. While the natural lipase is inactivated by pasteurization, microbial lipases of psychrotrophic bacteria such as pseudomonas are able to resist heat treatment.[19] Excessive amounts of FFAs are undesirable in milk products because the shorter-chain homologues contribute to an off-flavour typified by butyric acid.[12,20,21] The amounts of FFAs determined in butter samples during a storage period of 120 days are presented in Table 3.

During storage time, the levels of FFAs in samples kept at refrigerator temperatures were higher than in those stored at deep freeze temperatures. The differences were found to be statistically significant at the level of $P < 0.01$ (see Table 2). This was probably due to a higher lipolytic activity at the refrigerator temperature. The culture type had no effect on the amounts of FFAs. However, the amounts increased during storage, and the differences were found to be significant between different storage times ($P < 0.01$). Interaction of storage temperature with storage period was significant at a level of $P < 0.01$, and interaction of culture type with storage temperature was significant at a level of $P < 0.05$.

The peroxide value is accepted as an indicator for oxidative rancidity and is used to determine the oxidation level in butter. Oxidative rancidity is the result of oxidation of free unsaturated fatty acids (oleic and linoleic) to hydroperoxides, aldehydes, ketones, acids and epoxides, which have a rancid taste. The effects are caused by oxygen, light, heat, moisture and some elements.[3,11] Oxidation reaction rates increase with temperature, and can increase more than twofold with every 10°C rise depending on the composition of the oil.[12] Peroxide values of butter samples are shown in Table 4. According to the data, peroxide values continuously increase during the storage period in all of the butter samples. The highest values were produced by mesophilic culture MM100 in the samples, which means that oxidative stability was lowest in this type of culture. The same was true for the samples stored at refrigerator temperatures. The effects of culture type, storage temperature and storage times were found to be significant ($P < 0.01$).

Table 2 General means of titratable acidity, free fatty acid (FFA), peroxide values and diacetyl contents of butters, and their statistical evaluations in terms of culture type, storage temperature and storage period*

	Titratable acidity (LA %)	*FFA (mg KOH/g fat)*	*Peroxide value (meq O_2/kg fat)*	*Diacetyl (ppm)*
Culture type				
BT001	0.10 a	1.65 a	1.24 b	0.74 c
MM100	0.11 a	1.67 a	1.73 a	1.17 b
CHN11	0.11 a	1.56 a	1.40 b	1.70 a
CHN22	0.11 a	1.69 a	1.44 b	1.17 b
Storage temperature				
R	0.12 a	1.88 a	1.72 a	0.80 b
DF	0.10 b	1.40 b	1.18 b	1.60 a
Storage period (days)				
3	0.08 d	1.18 e	0.62 e	0.48 c
30	0.09 c	1.42 d	0.96 d	1.15 b
60	0.11 b	1.60 c	1.34 c	1.37 ab
90	0.13 a	1.84 b	1.79 b	1.49 a
120	0.13 a	2.17 a	2.55 a	1.49 a

*Means bearing different letters are significantly different from one another ($P < 0.01$)
R, stored in refrigerator (+4 ± 1°C)
DF, stored in deep freezer (−18 ± 2°C)

© 2002 Society of Dairy Technology

Table 4 Peroxide values of butter samples (meq O_2/kg fat)

	Storage period (days)				
Culture type	*3*	*30*	*60*	*90*	*120*
BT001-R	0.62	0.87	1.40	1.74	3.21
BT001-DF	0.58	0.61	0.76	1.12	1.44
MM100-R	1.07	1.46	1.76	2.45	2.82
MM100-DF	1.03	1.27	1.36	1.85	2.25
CHN11-R	0.44	0.89	1.64	2.06	3.01
CHN11-DF	0.32	0.72	1.23	1.60	2.08
CHN22-R	0.52	1.08	1.56	2.20	3.63
CHN22-DF	0.41	0.81	0.98	1.30	1.95

R, stored in refrigerator (+4 ± 1°C)
DF, stored in deep freezer (−18 ± 2°C)

Christensen and Holmer[15] reported similar results in their study. The interaction between storage temperature and storage period was found to be significant at a level of $P < 0.01$.

Diacetyl contributes the characteristic taste and flavour to butter and buttermilk.[22] It is formed by cultures of *Lactococcus lactis* ssp. *lactis biovar. diacetylactis and Leuconostoc mesenterioides* ssp. *cremoris*.[23] The highest diacetyl contents were produced by culture CHN11 while the lowest values were obtained with culture BT001 in those samples stored at refrigeration temperatures, as shown in Table 5.

Commercial culture, storage temperature and storage period had significant ($P < 0.01$) effects on the diacetyl contents of the samples. Diacetyl contents were higher in samples stored at deep freezer temperatures, and the amounts increased during storage. The data suggest a rapid equilibration in diacetyl levels at refrigeration temperatures, i.e. at 30 days, while the deep-frozen butter equilibrates at 90 days. The shift might be due to different thermal effects on the oxidation and reduction pathways. In a study carried out by Mortensen and Danmark,[24] the diacetyl content increased during the storage period. However, while the increase was not found to be significant in their study, it was significant in the present study. Interactions between culture type and storage temperature and between storage temperature and storage period were found to be significant ($P < 0.01$).

Table 5 Diacetyl contents of butter samples (ppm)

	Storage period (days)				
Culture type	*3*	*30*	*60*	*90*	*120*
BT001-R	0.31	0.52	0.56	0.56	0.57
BT001-DF	0.37	0.80	1.04	1.35	1.36
MM100-R	0.47	0.75	0.76	0.81	0.82
MM100-DF	0.39	1.51	1.93	2.12	2.14
CHN11-R	0.51	1.12	1.13	1.13	1.13
CHN11-DF	0.43	2.32	2.85	3.21	3.20
CHN22-R	0.75	0.98	1.01	1.00	1.00
CHN22-DF	0.63	1.23	1.63	1.73	1.72

R, stored in refrigerator (+4 ± 1°C)
DF, stored in deep freezer (−18 ± 2°C)

In conclusion, culture type and storage temperature and time affected titration acidity, hydrolytic and oxidative rancidity, and diacetyl content of the butter samples. It seemed that starter cultures were more active at refrigerator temperatures than at deep-freezer temperatures, resulting in higher titration acidity, free fatty acid contents and peroxide values at refrigeration temperatures. On the contrary, diacetyl contents were higher at deep-freezer temperatures, probably due to less breakdown. However, more detailed studies are needed to determine the effects of starter cultures and storage conditions on the storage stability and taste and flavour of butter.

ACKNOWLEDGEMENT

This work was financially supported by the Atatürk University Research Fund (Project no. 99/29).

REFERENCES

1 Atamer M (1993) *Tereyaðý Teknolojisi*. Ankara Üniversitesi Ziraat Fakültesi Yayýnlarý No. 1313. Ankara: Ankara Üniversitesi Ziraat Fakültesi Halkla Ýliokiler ve Yayýn Ünitesi.
2 Anon (1995) Butter and dairy spreads. In *Dairy Processing Handbook*, pp 263–278. Teknotext A B, ed. Lund, Sweden: Alfa Laval/Tetrapak.
3 Tekinsen O C (2000) *Süt Ürünleri Teknolojisi* (3. baský). Konya: Selçuk Üniversitesi Basýmevi.
4 Bruhn J C (1996) *Butter: Some Technology and Chemistry.* California: Dairy Research and Information Centre (DRINC).
5 Lane R (1992) Butter and mixed fat spreads. In *The Technology of Dairy Products*, pp 87–114. Early R, ed. New York: VCH.
6 Anon (1998) *Spotlight on Dairy Starter Cultures*. Food Ingredients Bulletin No. 11.
7 Xanthopoulos V, Picque D, Bassit N, Boquie C and Corrieu G (1994) Methods for the determination of aroma compounds in dairy product: a comparative study. *Journal of Dairy Research* **61** 289–297.
8 Yöney Z (1974) *Süt Kimyasý*. Ankara Üniversitesi Ziraat Fakültesi Yayýn No. 530. Ankara: Ankara Üniversitesi Basýmevi.
9 Baudot A and Marin M (1996) Dairy aroma compounds recovery by pervaporation. *Journal of Membrane Science* **120** 207–220.
10 Antinone M J, Lawless H T, Ledford R A and Johnston M (1994) Diacetyl as a flavor component in full fat cottage cheese. *Journal of Food Science* **59** 38–42.

© 2002 Society of Dairy Technology

11 Atamer M and Sezgin E (1984) Tereyaolarýnda Lipolitik ve Oksidatif Bozulmalarýn Saptanmasýnda Yararlanýlan Asit ve Peroksid Deðerleri Ýle Aroma Arasýndaki Ýliþki. *Gýda* **9** 329–334.
12 Munro D S, Cant P A E, MacGibbon A K H, Illingworth D, Kennett A and Main A J (1992) Concentrated milk fat products. In *The Technology of Dairy Products*, pp 117–145. Early R, ed. New York: VCH.
13 Atamer M (1993) *Tereyaðý Teknolojisi Uygulama Kýlavuzu*. Ankara Üniversitesi Ziraat Fakültesi Yayýnlarý No. 1314. Ankara: Ankara Üniversitesi Ziraat Fakültesi Halkla Ýliþkiler ve Yayýn Ünitesi.
14 Anon (1990) *Gýda Maddeleri Tüzüpü.* Hukuk Dizisi No. 21(39). Istanbul: Bayrak Matbaacýlýk.
15 Christensen T C and Holmer G (1996) GC/MS analysis of volatile aroma components in butter during storage in different catering packaging. *Milchwissenschaft* **51** 134–138.
16 Kurt A, Çakmakçý S and Çaðlar A (1996) *Süt ve Mamulleri Muayene ve Analiz Metodlarý Rehberi* (genioletilmip 6. baský). Atatürk Üniversitesi Yayýnlarý No. 252/D. Erzurum: Atatürk Üniversitesi Ziraat Fakültesi Ofset Tesisi.
17 Walsh B and Cogan T M (1974) Separation and estimation of diacetyl and acetoin in milk. *Journal of Dairy Research* **41** 25–30.
18 MINITAB (1993) MINITAB Inc., Enterprise Drive, State Collage, PA 6801-3008, USA.
19 Deeth H C and Fitz-Gerald C H (1976) Lipolysis in dairy products: a review. *Australian Journal of Dairy Technology* **31** 53–64.
20 Allen J C (1994) Rancidity in dairy products. In *Rancidity in Foods* (4th edn), pp 179–190. Allen J C and Hamilton R J, eds. London: Chapman & Hall.
21 Duncan S E, Christen G L and Penfield M P (1991) Rancid flavor of milk: relationship of acid degree value, free fatty acids, sensory perception. *Journal of Food Science* **56** 2.
22 Cogan T M (1972) Modification of the Prill and Hammer method for determining diacetyl. *Journal of Dairy Science* **55** 382–384.
23 Fix G J (1993) Diacetyl: formation, reduction, and control. http://www.brewingtechniques.com/library/backissues/issue1.2/fix.html.
24 Mortensen B K and Danmark H (1982) Manufacturing of cultured butter using lactic concentrates. *Milchwissenschaft* **37** 402–404.

© 2002 Society of Dairy Technology