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学位論文題目　**A new manufacture method for set yogurt with low-temperature reduced dissolved oxygen fermentation**

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論文内容の要旨

Fermentation is one of the oldest methods for extending shelf life of milk. The combination *Streptococcus thermophilus* (*S. thermophilus*) and *Lactobacillus delbureckii* subsp. *bulgaricus* (*L. bulgaricus*) is traditionally used for the manufacture of yogurt. When both bacteria gain a mutual benefit, this association is known symbiotic relationship (or protocooperation). It was suggested that in yogurt the growth of *S. thermophilus* is stimulated by free amino acids and peptides liberated from the milk proteins by *L. bulgaricus* and *L. bulgaricus* is stimulated by formic acid produced by *S. thermophilus*. When in a mixed culture, acid production is much larger than the sum of a single culture. Symbiotic relationship between *S. thermophilus* and *L. bulgaricus* is very important for yogurt production because it reduces fermentation time.

The yogurt starter culture of *L. bulgaricus* and *S. thermophilus*, are facultatively anaerobic, so the fermentation of yogurt with these bacteria progresses well in the presence of oxygen. Although many studies have been conducted on the influence of oxygen on lactic acid bacteria, only a small number of studies have reported on the influence of oxygen on yogurt fermentation with a mixed culture of *L. bulgaricus* and *S. thermophilus* in yogurt manufacture. In this study, the author investigated the influence of dissolved oxygen (DO) on yogurt fermentation with the yogurt starter culture LB81, which was composed of *L. bulgaricus* 2038 and *S. thermophilus* 1131.

1. Development a new manufacture method for set yogurt of reduced DO fermentation (ROF) and reduced DO low-temperature fermentation (LT-ROF)

1-1. Bulgarian traditional home-made yogurt

The methods of general set yogurt preparation are as follows. The yogurt mix used was obtained by mixing raw milk, skim milk powder, butter, and water. After being homogenized at 15 MPa, the yogurt mix was heated to 95℃ and immediately cooled to 43℃. The yogurt bulk starter composed of *L. bulgaricus* and *S. thermophilus* was inoculated in the yogurt mix to a concentration of 2%. After mixing, the mixture was placed into polystyrene cups that were oxygen permeable. Fermentation was carried out at 43℃. The yogurt fermentation end point was set at 0.7 % acidity. The features of the Bulgarian traditional home-made yogurt are as follows. During fermentation, about 20% of water in the milk is absorbed into the pot. As a result, milk in the pot becomes about 1.2 times concentrated. The milk in the pot is gradually cooled by vaporization. As a result, fermentation pro-

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ceeds at relatively low temperatures. Low temperature fermentation creates smooth and creamy yogurt.

1–2. Trails of commercial production of the traditional “home-made” yogurt

The conditions of Bulgarian traditional yogurt are characterized as, first : the thick yogurt mix and second : low-temperature fermentation at 37℃. We attempted to mimic these conditions. The thick yogurt mix could be easily prepared with raw milk supplemented with butter, skim milk powder, etc.

1–3. Low-temperature fermentation

It is well known that yogurt made by low-temperature fermentation at 37℃ has a smooth texture, but the fermentation process takes a much longer time than the control fermentation at 43℃, and this longer time lowers the efficiency of yogurt manufacturing.

1–4. Reduced DO fermentation (ROF)

The change in DO concentration in the yogurt mix during fermentation at 43℃ was measured with the yogurt starter culture LB81. The starter culture LB81 actively began to 0 mg/kg beforehand. This resulted in shortened fermentation time. The time required for acidity in the yogurt mix to reach 0.7% was approximately 30 min less than in the control fermentation at 43℃. This new fermentation method was referred to as reduced DO fermentation (ROF).

1–5. Reduced DO low-temperature fermentation (LT-ROF)

The author applied this ROF to the low-temperature fermentation at 37℃. By combining ROF and low-temperature fermentation at 37℃, the time for the acidity of yogurt mix to reach 0.7% at 37℃ was decreased by approximately 40 min by reducing the DO.

1–6. Characteristics of set yogurt prepared by LT-ROF

As a general knowledge, low-temperature fermentation makes very smooth yogurt, because the fermentation proceeds for a longer time. However, it turned out that although acid production by LT-ROF was faster, it could make smoother yogurt. The Explanation for this may be as follows. The investigation revealed that yogurt curd formation began after the acidity reached approximately 0.4%, and because the yogurt fermentation end point was set at 0.7% acidity, the curds formed in the time during which the acidity of the yogurt mix changing from 0.4 to 0.7%. This period is referred to as the yogurt curd formation time and it is presumed that the longer curd formation time makes the texture of yogurt smoother.

The author compared 3 kinds of fermentation. They are LT-ROF, ordinary fermentation at 43℃ and low-temperature fermentation at 37℃.

Although the time of fermentation by LT-ROF was among the shortest of the 3 methods (180 min : equal to the control fermentation at 43℃), its curd formation time was the longest. The curd formation time with LT-ROF was 90 min, whereas with the other methods it was 50 min with the control fermentation at 43℃ and 70 min with low-temperature fermentation at 37℃. We hypothesized that this was the reason the yogurt made by LT-ROF method was very smooth in spite of its short fermentation time.

2. Effect of oxygen on symbiosis between *L. bulgaricus* 2038 and *S. thermophilus* 1131

2–1. Symbiosis between *L. bulgaricus* 2038 and *S. thermophilus* 1131 (LB81)

The two bacterial strains in yogurt, *L. bulgaricus* 2038 and *S. thermophilus* 1131, stimulate each other during their associative growth. In a mixed culture, acid production is much larger than the sum of a single culture (symbiotic relationship). It was observed that in yogurt the growth of *S. thermophilus* 1131 is stimulated by casein peptides liberated from the milk proteins by *L. bulgaricus* 2038 and *L. bulgaricus* 2038 is stimulated by formic acid produced by *S. thermophilus* 1131.

2–2. Symbiosis with LB81 accelerated by ROF

Acid production by *L. bulgaricus* 2038 or *S. thermophilus* 1131 in single culture were neither suppressed nor advanced by the DO concentration in the yogurt mix. While acid production by starter culture LB81 composed of *L. bulgaricus* 2038 and *S. thermophilus* 1131 was greatly accelerated by reducing DO to nearly 0 mg/kg in the yogurt mix (ROF).

The formate accumulation by the single yogurt culture *S. thermophilus* 1131 or LB81 was examined. In the case of symbiotic yogurt culture LB81, the starting point of formate accumulation in fermentation with the reduced DO (ROF) was approximately 30 minutes earlier than in the control fermentation.

2–3. Suppression of Symbiosis with LB81 by DO

Both acid production and formate production by LB81 was suppressed by the existence of more than 1 mg/kg of DO in the yogurt mix. In conclusion, these suggest that DO inhibit to the symbiotic relationship between *L. bulgaricus* 2038 and *S. thermophilus* 1131.

The author attributed the acceleration of acid production of LB81 by reduced DO mainly to the acceleration of formate production and the suppression of acid production of LB81 by DO mainly to the suppression of formate production.

3. NADH oxidase of *S. thermophilus* 1131 is required for effective yogurt fermentation with *L. bulgaricus* 2038

The results using H_2O-forming NADH oxidase (*nox-2*) defective mutant (Δ*nox*) of *S. thermophilus* 1131 revealed that Nox-2 played an important role for DO reduction during yogurt fermentation. Yogurt fermentation by a starter composed of Δ*nox* and *L. bulgaricus* 2038 was significantly slow, presumably because this starter could not reduce DO concentration to less than 2 mg/kg. These observations suggest that Nox-2 of *S. thermophilus* 1131 contributes greatly to yogurt fermentation.

4. Development of superior fat free set yogurt with LT-ROF

LT-ROF method has made it possible to manufacture excellent fat free set yogurt without using sugar, stabilizers and thickeners. The fat free set yogurt product prepared by LT-ROF had almost the same fatty taste, smooth texture, and sufficient hardness to stand up to the impact of shaking during transport as the Japanese normal fat set yogurt product containing 3.0% (wt/wt) milk fat prepared by control fermentation at 43℃.

5. Conclusion

The author found that reducing DO concentration in the yogurt mix with LB81 to nearly 0 mg/kg reduced the fermentation time at 43℃ compared with the control fermentation at 43℃ (ROF). The autho attributed the acceleration of acid production of LB81 by reduced DO mainly to the acceleration of formate production and the suppression of acid production of LB81 by DO mainly to the suppression of formate production.

The results using H_2O-forming NADH oxidase (*nox-2*) defective mutant (Δ*nox*) of *S. thermophilus* 1131 revealed that Nox-2 played an important role for DO reduction during yogurt fermentation.

Combining ROF with low-temperature fermentation (LT-ROF) reduced the time required for low-temperature fermentation. LT-ROF method has made it possible to manufacture excellent fat free set yogurt without using sugar, stabilizers and thickeners.

審査報告概要

平成26年2月10日（月）午後4時00分から，本専攻が11号館2階バイオサイエンス専攻大講義室にて開催した学位請求論文の公開本人口頭発表会で，学位請求者　堀内啓史氏は，40分間の口頭発表を行い，その後20分間の質疑応答を受けた。発表会終了後，主査，副査と専攻委員による審査会議を開催し，提出論文の内容と本人発表ならびに質疑応答について慎重に審査した。その結果，学位請求者の経歴や学術業績が学位記申請の要項を満たしており，質疑に対する応答が適切だと判断された。さらに，公表論文に関与した共同研究者との間で学位取得に関して問題が無いことを確認し，当該学位請求論文の内容が学位授与に相当することを全員一致で評決した。

よって，審査員一同は博士（バイオサイエンス）の学位を授与する価値があると判断した。