

FUNDAMENTAL STUDIES ON THE UTILIZATION OF OLIVE FRUITS

V The pickling of green olives (3) Effect of the use of pure culture of lactobacilli on the fermentation of Spanish-type green olives

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Initiation and maintenance of the favorable lactic fermentation are of primary importance for the production of high quality fermented green olives. A satisfactory content of the natural acidity (0.5-1.0%, as lactic acid) produced by the desirable lactic acid bacteria was shown to be essential not only for keeping quality but also for superior color as well as flavor of green olives⁽¹⁾.

The necessity of supplementary sugar was discussed in the previous paper⁽²⁾, and the satisfactory acidity was obtained with the addition of 0.9 per cent glucose to the green fermenting *Mission* olives in authors' laboratory⁽³⁾.

However, olives are sometimes difficult to ferment. Many processors realize that the olives do not very often cure, but deteriorate without a proper control of fermentation.

CRUESS^(4,5) has recommended the use of artificial heat to accelerate the fermentation, the addition of supplementary fermentable material, and the use of "starters". His recommendations may be followed with assurance, after precise studies of fermentation process and controlling measure⁽¹⁾.

The important species of lactic acid bacteria found to predominate in the fermenting green olives have been reported to be *Leuconostoc mesenteroides*, *Lactobacillus plantarum*, *L. bucherei*, and *L. brevis*⁽¹⁾; the use of the pure culture of *L. plantarum* was recommended for starting lactic fermentation^(1,3). CRUESS used the starter of actively fermenting normal brine in the first⁽⁴⁾, and later he suggested the use of pure culture of lactic acid bacteria for green olive fermentation⁽⁵⁾.

The addition of pure culture of lactobacilli speeded up the formation of acids and lowering of pH⁽⁶⁾. Deterioration of fermenting green olives, which occur very often when supplementary sugar is added, could be avoided by the addition of pure culture of favorable lactic acid bacteria⁽⁵⁾. Treatments with pure lactobacillus cultures and fermentable sugars not only accelerated the rate of fermentation but also improved the quality of freshly pickled olives⁽⁷⁾.

The present experiments were undertaken to study the effect of the use of pure cultures of lactobacilli on the fermentation of green olives with *Mission* fruits in the presence of added supplementary sugar.

EXPERIMENTAL

Material and Method. The experiment was conducted with *Mission* fruits, treated as in the previous paper⁽²⁾, added with 2% (vol/vol.) pure culture on the third day of fermentation, and supplemented with 0.9% glucose on the eighth day.

The stage of inoculation was taken as the time after rapid decrease of sodium chloride concentration and before maximum elution of fermentable materials into the brine solution from the flesh of fermenting green olives.

Starters were prepared with *L. plantarum* and *L. mesenteroides*, separately; they were inoculated into ordinary malt extracts containing 3% of sodium chloride and incubated at 30° C. for 48 hours before seeding the olive brine.

Three lots of fermenting green olives in duplicate were prepared with: (A) 2% pure culture of *L.*

plantarum, (B) 1% pure culture of *L. plantarum* and 1% pure culture of *L. mesenteroides*, and (C) no starter.

The pH, total acidity, lactic acid, sodium chloride, and reducing sugar were determined in the brine solution of each lot of experiments up to about three months of fermentation. Analytical methods were quite the same as before⁽²⁾.

Result and Discussion. The results obtained were given in Figures 1 and 2. No data were described for the lot B (seeded with *L. plantarum* and *L. mesenteroides*), for the results gave no difference with the lot A (seeded with *L. plantarum* alone). The findings are very similar to those reported by VAUGHN et al.⁽¹⁾, who showed that *L. mesenteroides* alone gave satisfactory results, but it played no part in completion of the olive fermentation and gave no additional effect on the fermentation in the presence of added pure culture of *L. plantarum*.

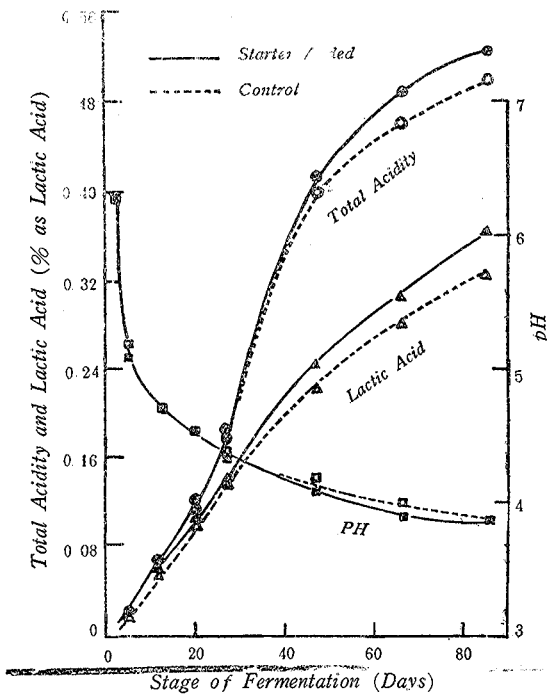


Figure 1. Effect of the use of starter on the production of acids and the lowering of pH of the brine solution during the fermentation.

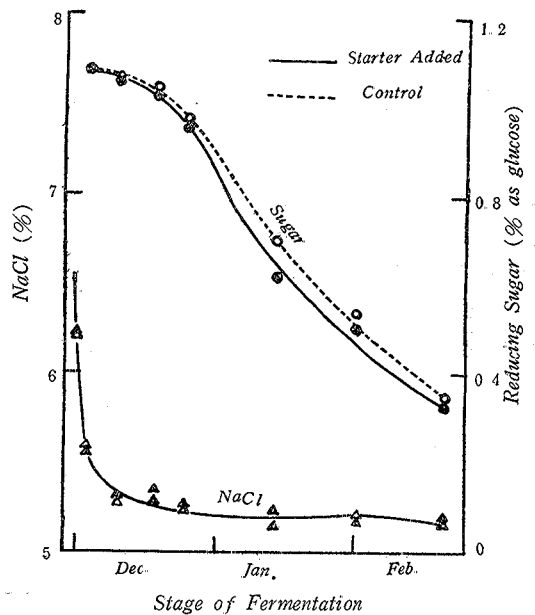


Figure 2. Effect of the use of starter on the change of NaCl and the decrease of reducing sugar in the brine solution during the fermentation.

As shown in the figures, the use of starter seems to effectuate certainly good results in the fermentation. However, the statistical analyses of the data in the Table 1 show that the differences in total acidity, lactic acid, and residual sugar were all insignificant at probability of 0.05.

Inoculation alone was shown to have little effect on the acceleration of acid production. However, when corn sugar was made available, acid production increased significantly⁽¹⁾. On the basis of the result obtained in this experiment and the result given in the preceding paper⁽³⁾, it would, therefore, appear that the use of starter is of value in controlling the undesirable bacteria present during the primary stage of fermentation and of initiating the lactic fermentation, but has no marked effect on the absolute amount of acid produced. The limiting factor for acid production seems to be the amount of fermentable materials available in the brine solution and the availability of necessary growth-promoting substances for the favorable

Table 1. Effect of the use of starter on the production of acids during the fermentation of Spanish-type green olives (Statistical analyses made on the data taken on the 85th day of fermentation).

Test	Trial	Total Acidity %, as lactic	Lactic Acid %	Residual Sugar %, as glucose
Starter added	1	0.518	0.360	0.331
	2	0.538	0.365	0.327
	Mean	0.528	0.363	0.329
Control	1	0.497	0.318	0.336
	2	0.504	0.342	0.341
	Mean	0.501	0.330	0.339
t-Distribution		t=2.46*	t=2.74*	t=3.03 ₄

* Insignificant at probability of 0.05 ($t_{0.05}$ (df=2) = 4.303).

lactic acid bacteria. Therefore, the effect of the use of starter could be made conspicuous in the case of incipient spoilage or under the conditions in which the bacterial spoilage had been very liable to occur.

There was no indication of shortage of growth-promoting substances during the whole period of fermentation, while tomato juice has been used extensively to stimulate growth of lactic acid bacteria^(1), 5-8).

The concentration of sodium chloride in the fermenting brine solution is known to be one of the factors which influence the acceleration of the fermentation by the starter⁽¹⁾. As seen from Figure 2, the concentration of sodium chloride was about 6% at the stage of adding starter. The value seems to be safe for the growth of *L. plantarum*. It would be a problem, whether one should wait the rapid decrease of the concentration of sodium chloride or should start with lower concentration from the first.

VAUGHN et al.⁽¹⁾ reported that the effect of inoculation was most noticeable on the rate of acid production and no decrease in the pH value during the first two months of the fermentation period, but in the present experiment none of the effect was observed in the first month of the fermentation. The difference is probably due to the lower temperature in the fermentation room in this experiment, which was shown in the preceding paper⁽³⁾.

As already discussed⁽³⁾, the rate of fermentation seems to be very slow in these experiments, and the acceleration of the fermentation by artificial heating should be studied further.

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オリブ果実利用に関する基礎的研究

V オリブの緑果塩蔵

(3) 乳酸菌の純粹培養がスペイン型グリーンオリブの発酵に及ぼす効果

榎崎 丁市, 片倉 健二

L. plantarum 及び *L. mesenteroides* の純粹培養がミッションオリブの緑果塩蔵に及ぼす効果について試験した。乳酸菌は3%の食塩を含む麦芽汁に培養し、塩蔵3日目の発酵食塩水に2%の割合で添加し、更に8日目にグルコースを0.8%添加して室温で発酵させグルコースのみを添加した対照と比較した。

約1カ月頃から試験区の生酸が対照より多くなって来たがその差は必ずしも統計的に有意なものとは認められなかった。しかし乍ら発酵経過は何等の腐敗等の異常もなく極めて好適に進行しているものと思われた。

VAUGHN等も指適しているようにスターターの使用は補糖の場合のように発酵結果に顕著な効果を示すものでなく、その効果の最も重要な点は腐敗菌を抑えて発酵を安全にする事であると思われる。従って今迄の実験では腐敗現象が余り認められないので、スターターの効果が現れて来なかったであろう。

しかし乍ら多くの研究者はスターター使用の必要性を指摘しているし、その操作は決して困難ではないからスターターを使用する事は推奨し得ると思う。

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