OLIVE 香川大学学術情報リポジトリ

FUNDAMENTAL STUDIES ON THE UTILIZATION OF OLIVE FRUITS

III The pickling of green olives (1) The biochemical changes during the fermentation of Spanish-type green olives

Kenji KATAKURA and Teiichi NARASAKI
(Laboratory of Agricultural Products Technology)
(Received May 30, 1955. Accepted June 10, 1955.)

INTRODUCTION

Olives are utilized in three principal ways, *viz.*, as ripe olives, as green olives, and for olive oil. There are several ways by which green olives may be utilized. In Japan, they are used mainly as fermented Spanish-type green olives, and as shown in the first paper of this series[1], the yield of the green olives tends to develop year after year accompanied by increasing biochemical problems to be solved.

VAUGHN, *et al.*[2] studied chemically and microbiologically the processes involved in the production of Spanish-type green olives, especially the fermentation process, and proposed valuable recommendations for the improvement of its production. They have divided the fermentation process into three stages, *i. e.*: the primary stage of fermentation, which persists normally for 7 to 14 days, and contains the microorganisms which may contribute to deterioration of the olives if the fermentation does not proceed in a normal manner, notably gram-negative bacteria of the genera *Aerobacter* and *Pseudomonas* and yeasts; the intermediate stage of fermentation, in which the predominating microorganisms are lactic acid bacteria, *Leuconostoc* and *Lactobacillus*, the total acidity increases rapidly, and the stage lasts for about two to three weeks; and the final stage of fermentation, in which the total acidity increases from about 0.3 to 1.0% as lactic acid, and the high acid-tolerant lactobacilli predominate. The importance of the first stage of fermentation has been pointed out, and the use of the starter and the addition of supplementary sugar to brine solutions have also been discussed. IZQUIERDO[3,4] also divided the fermentation process into three stages.

Researches on the fermentation processes of various-type green olives have been continued by considerable workers. BORBOLLA and his associates have studied the effects of pH and acidity of the brine for the fermentation[5,6], the use of pure culture of *Lactobacillus* in the fermentation[7,8], and the effect of the lye treatment followed by washing[9,10,11]. Chemical constituents were analyzed in the residual lye solution[12,13], and an acidic white crystalline substance, $C_{30}H_{48}O_3$ was separated[14]. A darkening of the brine solution of Spanish-type green olives had been studied[15], and a substance, which caused the darkening, was shown to be precipitated by 30% lead acetate. It was recommended to maintain the acidity of the brine solution at 0.5—0.9% as lactic acid to avoid the darkening. It has been also reported that the growth of natural yeast flora on the surface of brine solution was inhibited by 0.04—0.08% fennel oil[16], and that fennel oil could inhibit the growth of *Aerobacter aerogenes*, which causes the gaspockets in Spanish-type green olives[17]. In S.S.S.R., API[18] has obtained the acidity of 0.35% as lactic acid in a month with the addition of 1% sugar and 2% tomato juice, while the natural brine resulted only in the acidity of 0.1% as lactic acid even at the end of three months of fermentation.

OLIVE 香川大学学術情報リポジトリ

Thus the different phases of this industry, which were practiced originally in a purely empirical manner, seem to have today a firm scientific basis. However, the results reported by various authors are rather considerably different according to the authors, probably because the chemical compositions of the olives used in each study vary according to the locality where the olives had grown and the factors influencing the fermentation may also differ from each other. It seems, therefore, without meaningless to investigate the problems involved in the production of Spanish-type green olives in Japan. To facilitate the production of Spanish-type green olives, it seems to be necessary to investigate the chemical and microbiological changes occurring in the processes of the production, especially in the fermentation process, and the pretreatments should also be investigated in connexion with the fermentation process.

The authors will investigate the fermentation of Spanish-type green olives to establish the standard method of preparation in this country. Biochemical changes occurring in the natural brine solutions during the fermentation of Spanish-type green olives may be described in the present paper.

MATERIAL AND METHOD

The *Mission*, *Sevillano*, and *Manzanillo* varieties are all used for the production of Spanish-type green olives. In this country, the *Mission* variety is favored in its medium size and ease of preparation, and this variety was used in the present studies.

The olives were picked about November 20th, 1954, in Shōdo Island, covered with dilute sodium chloride solution, and sent to the authors' laboratory for the experiments.

The olives were placed in shallow vats and covered with 2% sodium hydroxide solution at room temperature. The solution was allowed to penetrate two-thirds of the way to the pits of the fruit. The treatment took about 12 hours. After the lye treatment, the olives were washed with water to remove sodium hydroxide for about 8 hours. Then the olives were packed into 2-L glass bottles, covered with 8% sodium chloride solution, and allowed to ferment.

Samples of brine (5 cc) were taken with pipettes from the lots of fermenting olives, which previously had been thoroughly agitated by rolling and shaking to insure uniform distribution of chemical constituents, diluted to 25 cc with distilled water in a 25-cc volumetric flask, and used for analyses.

Determination of pH of the brine solution. pH Value of the brine solution was determined directly by testing the original brine solutions with pH test paper (Toyo Filter Paper Co., Tokyo).

Determination of total acidity. A 5-cc sample of the diluted brine solution was pipetted into a 50-cc Erlenmeyer flask and titrated to the phenolphthalein end point with $0.01N$ carbonate-free sodium hydroxide solution. The total acidity, expressed as grams of lactic acid per 100 cc of brine solution, was obtained by mutiplying the number of milliliters of $0.01N$ sodium hydroxide solution used in titration by 0.09.

Determination of sodium chloride content. A 1-cc sample of the diluted brine solution was taken with pipette and titrated with $0.1N$ silver nitrate solution in the presence of a few drops of 10% potassium chromate solution until a slight permanent red color appears. The sodium chloride content, expressed as grams of sodium chloride per 100 cc of brine solution, was obtained by multiplying the number of milliliters of $0.1N$ silver nitrate solution used in titration by 5 X 0.585.

Estimation of reducing sugars. A 1-cc sample of the diluted brine solution was taken, deproteinized by adding with 9 cc of 10% trichloroacetic acid solution, and used for the determination of reducing sugars. The HAGEDORN-JENSEN method[19] was employed. The results were expressed as grams of glucose per 100 cc of brine solution.

Determination of lactic acid content. A 1-cc sample of the diluted brine solution was taken and used for the determination of lactic acid after deproteinizing with trichloroacetic acid and desugaring

OLIVE 香川大学学術情報リポジトリ

with the mixture of copper sulfate and calcium hydroxide. The determination was made colorimetrically according to BARKER and SUMMERSON[20].

Sampling was made at 3rd, 5th, 8th, 13th, 20th, 27th, 47th, 66th, and 85th days of fermentation and the analyses were completed in 24 hours after sampling at each time of sampling. After sampling was done, about 10 cc of 8% sodium chloride solution was added to each bottle to fill it up.

RESULTS AND DISCUSSION

The analytical results were summarized graphically in Figure 1. In Table I, the temperature variation in the fermentation room was given, for the variation of the room temperature, under which the experiments were performed, seems to be very important to consider the analytical results.

Table I. Temperature Variation in the Fermentation Room

Temperature (° C.)	Stage of Fementation (Days)								
	0—3	3—5	5—8	8—13	13—20	20—27	27—47	47—66	66—85
Maximum	18.6	20.5	19.0	17.0	17.5	14.5	11.0	8.5	14.5
Minimum	10.8	12.5	10.9	9.0	5.0	6.5	1.0	1.5	1.5

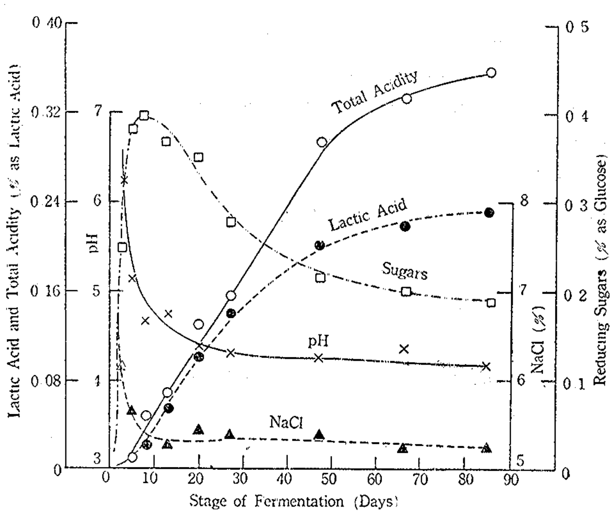

Figure 1. Changes of the Chemical Constituents in the Normal Brine Solution during the Fermentation of Spanish-type Green Olives

The results shown in Figure 1 are in close agreement with the description of VAUGHN, *et al.* [2], and it may be seen that the fermentation of the sample proceeded in a normal manner. Very active chemical changes were observed in the early part of the primary stage of fermentation, and the importance of this stage in the whole stages of fermentation will be easily understood. As indicated in Figure 1, the primary stage seems to persist for 7 to 14 days at room temperature in this country as in the United States. During the time the brine solution reached nearly the stabilization point; the reducing sugars became available for the bacteria in the brine solution and then began to decrease, coincidently, there were definite steady increases in total acidity and lactic acid, and a corresponding drop in pH. Sodium chloride decreased rapidly in the first five days and then reached the equilibrium. After the primary stage of fermentation, the pH and the reducing sugars of the brine solution decreased further, and the total acidity increased constantly up to about 0.25% as lactic acid in more two to three weeks of fermentation. The stage seems to correspond to the intermediate stage of VAUGHN, *et al.*[2] In the last stage of fermentation, the total acidity increased further, but the increase in lactic acid was comparatively small. DELMOUZOS, *et al.*[21] have reported that the normal brine solution contained only acetic and lactic acids as acidic constituents. Therefore, it may be concluded that the increases in the total acidity in the last stage is largely owing to the increase in acetic acid, and the highly acid-tolerant lactobacilli, which were shown to predominate the last stage of fermentation[2], seem to

OLIVE 香川大学学術情報リポジトリ

consist mainly of the heterofermentative type. In this stage, the total acidity increased at considerable rate, but the pH of the brine solution pemained at almost the same level, *i. e.*, pH 4.2. The fact seems to indicate the existence of a buffer system in the fermenting brine solution. The importance of the buffer system of the brine solution has been demonstrated by BORBOLLA, *et al.* [5,6,22] in the fermentation of Spanish-type green olives. They have found that the capacity of the buffer system increases as diffusion of the solution through the skin of the fruit and the lactic fermentation are proceeding, and its maximum was shown to be at pH 3.7—3.8[22]. The buffer system will differ according to the differences of the lye treatment, the washing, the varieties of olives used the localities where the ;olives had grown and the fermentation was performed, etc. From the Figure, it will be seen that the brine solution had an intense buffer action between pH4.4—4.0. The brine solution contained 0.2% reducing sugars at the end of the experiment. The substance expressed as the reducing sugars in the studies seems to include nonfermentable reducing substances, which may contain the bitter glucoside, oleuropein[23], and its decomposition products.

From the results of the present experiements, it may be concluded that supplementary sugar is necessary to obtain the sufficient level of acidity to inhibit the bacterial putrefaction.

SUMMARY

(1). The biochemical changes were studied in the normal brine solutions during the fermentation of Spanish-type green olives, and the results obtained were summarized in Figure 1 graphically.

(2). An intense buffer action was shown in the fermenting brine solutions about pH 4.0.

(3). It was found that the *Mission* variety resulted not sufficient enough acidity without the addition of supplementary sugars, *i.e.*, 0.4% as lactic acid.

(4). There seems little danger of deterioration at the room temperature under 20° C. without special control for preservation.

ACKNOWLEDGMENT

The authors wish to express their appreciations to Prof. Sin'itirô KAWAMURA, Laboratory of Biological Chemistry, this College, for his constant interest and many helpful suggestions in this work. They are also indebted to T. YAMAMOTO and O. YOSHIKAWA, both undergraduate students of this laboratory, for their participations in a significant portion of the analytical work in the studies.

LITERATURE CITED

(1) KATAKURA, K., NARASAKI, T.: *Tech. Bull. Kagawa Agr. Coll.*, **6**, 1(1954).

(2) VAUGHN, R.H., DOUGLAS, H.C., GILILLAND, J.R.: *Calif. Agr.Exp. Sta.*, **Bull. 678**,3(1943).

(3) IZQUIERDO TAMAYO, A.: *Grasas y aceites*, **3**, 79(1952); *C. A.*, **47**, 12692g(1953).

(4) ―――: *Ibid.*, **4**, 169 (1953); *C.A.*, **48**. 10251 (1954).

(5) BORBOLLA Y ALCALA, J.M.R. DE LA, *et al.*: *Anales real soc. espan. fis y quim.*, **47B**, 515(1951); *C.A.*, **46**, 7248c(1952).

(6) ―――: *Bol. inst. nacl. invest. agron.*, **12**, 115(1952); *C.A.*, **47**, 6570g(1953).

(7) ―――: *Grasas y aceites*, **3**, 91(1952); *C.A.*, **47**, 12692h(1953).

(8) ―――: *Anales real soc. espan. fis y quim.*, **50B**, 497(1954); *C.A.*, **48**, 14032a(1954).

(9) ―――: *Ibid.*, **48B**, 427(1952); *C.A.*, **46**, 9227h (1952).

OLIVE 香川大学学術情報リポジトリ

(10) ……: *Rev. cienc. aplicada*, **5**, 143(1951); *C.A.*, **46**, 5741f(1952).
(11) ……: *Ibid.*, **7**, 231(1953); *C.A.*, **48**, 4722b (1954).
(12) VIGUERA LOBO, J. Ma.: *Anales fis. y quim.* **41**, 520(1945); *C.A.*, **41**, 4251b (1947.)
(13) ……, BELLAPART, F.A.: *Anales real soc. espan. fis. y quim.*, **46B**, 143(1950); *C.A.*, **44**, 10953f(1950).
(14) ……: *Ibid.* **49B**, 317(1953); *C.A.*, **47**, 12692i(1953).
(15) MERZARI, A. H., MOLINA, J.S.: *Rev. argentina agron.*, **15**, 225(1948); *C.A.*, **43**, 5511c(1949).
(16) ……: *Ibid.*, **16**, 234(1949); *C.A.*, **44**, 9507a(1950).
(17) ……: *Ibid.*, **17**, 241(1950); *C.A.*, **45**, 6687f (1951).
(18) APT, F.S.: *Mikrobiologiya*, **20**, 415(1951) *C.A.*, **46**, 9227i(1952).
(19) HAGEDORN, H.C., JENSEN, B. N.: *Biochem. Z.*, **137**, 92(1923).
(20) BARKER, S.B., SUMMERSON, W.H.: *J. Biol. Chem.*, **138**, 535(1941).
(21) DELMOUZOS, J.G., STADTMAN, F.H., VAUGHN, R.H.: *J. Agr. Food Chem.*, **1**, 333(1954).
(22) BORBOLLA Y ALCALA, J.M.R. DE LA, *et al.*: *Ind. Eng. Chem.*, **44**, 2227(1952).
(23) CRUESS, W.V., ALSBERG, G.L.: *J. Amer. Chem. Soc.*, **56**, 2115 (1934).

オリブ果実利用に関する基礎的研究

Ⅲ オリブの緑果塩蔵 1. スペイン型 グリーンオリブの発酵中に於ける生化学的変化

片 倉 健 二 ・ 橧 崎 丁 市

緑果塩蔵はオリブ利用法の最も重要なものの一つである．従来は全く経験的に行われていた塩蔵方法も，CRUESS, VAUGHN, その他の多くの研究により，今日ではかなり科学的基礎が確立されて来たように見える．しかし乍らそれ等の研究結果の振れから理解される様に，他国の研究結果が必ずしも我が国の加工に適用し得るとは云えない．

著者等は，我が国の在来の方法を出来るだけ尊重し乍ら外国に於ける最近の研究結果を取入れた標準加工法を確立する為に，先ず正常な発酵過程に発酵液中に生起する生化学的変化を追究し次の点を明らかにした．

1）発酵初期に極めて活発な化学成分の変化が認められる．最初の7—14日間に，PHは7→4.6，NaClは8→5.5%と減少し，還元糖が 0→0.4%と溶出して来，酸が急激に生産されて来る．

2）発酵の中期に於て酸は最も多く生産されるが，PHの低下はそれ程著しくない．更に発酵後期の全酸度とPHとの両曲線の傾向から，発酵食塩水はPH 4—4.5にかなりの緩衝作用を持つている事が推定される．

3）ミッション種を用いた場合は全酸度は約 0.4%（乳酸として）で，腐敗を防止するに十分ではないから補糖が必要であると思われる．

4）発酵後期にも全酸度はかなり増加するが乳酸は余り増加しない．凡らく酢酸が主に生産されるものであろうが確認はしていない．

本実験に使用したのはミッション種のみで試料は小豆島試験場で生産さた果実を使用した．本研究を行うに当り引続き有益な助言を与えられつゝある本学川村信一郎教授，並びに実験を手伝われた山本・吉川両君に感謝する．