# VARIATIONS IN CONTENT OF SUGARS AND RELATED SUBSTANCES IN OLIVES[1]

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## INTRODUCTION

In connection with a study of the composition of California olives it was found necessary to know something of the nature and amounts of the sugars and to select suitable methods for their determination. The fruit of olives of 17 varieties grown in California was therefore examined for the purpose of ascertaining (1) the amount and nature of the reducing substances in the flesh, and (2) the relation of these substances to the maturity and variety of the fruit.

## REVIEW OF THE LITERATURE

Few references to the amount or nature of the sugars in olives appear in the literature. Power and Tutin,[2] studying the alcoholic extract of dried olive bark and leaves, reported the presence of sugar yielding *d*-phenylglucosazone. Bourquelot and Vintilesco [3] reported the presence in olive flesh of a phenolic compound causing Fehling's solution to indicate more than the actual amount of sugar. Scurti and Tommasi [4] in an attempt to explain the formation of oil in olives found no notable quantities of carbohydrates.

## MATERIAL STUDIED

The varieties studied may be divided into three groups: (1) Varieties well established in California; Mission, Manzanillo, Sevillano, and Ascolano. These varieties are grown primarily for pickling and only the Mission is used for oil making. (2) Several varieties less widely grown—the Barouni, Columbella, Lucca, Nevadillo, Picholine, and Uvaria. (3) Varieties but recently introduced [5] into California—the Bidh el Hammam, Chemlali, Chitoni, P. I. G. 27172, Saiali Magloub, Salome, and S. P. I. 27173.

The relation between composition and maturity was studied in the Ascolano, Columbella, Manzanillo, Mission, Nevadillo, and Uvaria. In each of these varieties a series of samples was obtained from a single tree except in two instances where fruit was no longer available on the selected tree, and final samples were taken from neighboring

[1] Received for publication July 24, 1929; issued June, 1930.

[2] POWER, F. B., and TUTIN, F. THE CONSTITUENTS OF OLIVE LEAVES. Jour. Chem. Soc. [London] 93: 891–904. 1908.
——— and TUTIN, F. THE CONSTITUENTS OF OLIVE BARK. Jour. Chem. Soc. [London] 93: 904–917. 1908.

[3] BOURQUELOT, E., and VINTILESCO, J. SUR L'OLEUROPÉINE, NOUVEAU PRINCIPE DE NATURE GLUCOSIDIQUE RETIRÉ DE 'L'OLIVIER (OLEA EUROPÆA L.). Compt. Rend. Acad. Sci. [Paris] 147: 533–535. 1908.

[4] SCURTI, F., and TOMMASI, G. SULLA FORMAZIONE DEL GRASSO NEI FRUTTI OLEAGINOSI. [FORMATION OF FAT IN OLEAGINOUS FRUITS (OLIVES)]. Ann. R. Staz. Chim. Agr. Sper. Roma (II) 4: 253–286. 1910. [Abstract in Jour. Soc. Chem. Indus. 30: 1021. 1911. [Original not seen.]]

[5] The Chico, Calif., Plant Introduction Garden of the Bureau of Plant Industry, United States Department of Agriculture kindly cooperated by furnishing these varieties.

trees. In all cases an effort was made to minimize sampling errors by picking approximately half of each sample from opposite sides of the tree, and by picking all the fruit on the branches or twigs chosen at each sampling. The picking dates, at somewhat irregular intervals, ranged from October 1, 1927, to February 13, 1928, according to the maturing habits of the variety and the lateness at which sufficient fruit remained on the trees. In most instances the first picking was made when the fruit had reached about three-fourths its full size, was distinctly immature for pickling and of low oil content, and the last picking when it was rapidly falling from the trees, past its prime for oil making.

## METHODS OF ANALYSIS

### AMOUNTS OF SUGAR

The sugars were extracted from ground samples of the flesh by boiling with distilled water in a 500 c. c. volumetric flask for approximately 30 minutes, making up nearly to the mark, allowing to stand overnight, and then filling up to the mark. The decoction was then thoroughly shaken and strained through dry cheesecloth, and portions of the filtrate were set aside for clarification. Determinations of the amounts of sugars present were made by the picric-acid reduction method of Thomas and Dutcher.[6] Reducing sugars present both before and after hydrolysis were reported as dextrose in all cases, and regarded as simple and total sugars, respectively.

### NATURE OF THE REDUCING SUBSTANCES

On a few samples dry basic lead acetate, subsequently removed by sodium oxalate, was compared as a clarifying agent with mercuric nitrate, removed by sodium bicarbonate and powdered zinc. The nature of the reducing substances present was further studied in a few samples by adding one-fifth of a commercial compressed yeast cake to a 50 c. c. portion of the filtrate prepared as previously described and brought to 37° C. The sample was thoroughly stirred to break up the yeast cake and then incubated for 20 minutes at 37° F., being shaken at 5-minute intervals. It was then clarified and examined in the usual way.

## EXPERIMENTAL DATA

### CLARIFICATION

Basic lead acetate was found to leave a distinctly greater reducing power in the water extract than did mercuric nitrate, as shown in Table 1. Not only were the percentages of reducing substances, both before and after hydrolysis, greater when lead was used, but the increase on hydrolysis by picric acid was also always greater. This would indicate either that the mercury removed some sucrose or that the lead failed to remove nonsugar substances, of which hydrolysis by picric acid produced or increased the reducing power. From the

[6] THOMAS, W., and DUTCHER, R. A. THE COLORIMETRIC DETERMINATION OF CARBOHYDRATES IN PLANTS BY THE PICRIC ACID REDUCTION METHOD. I. THE ESTIMATION OF REDUCING SUGARS AND SUCROSE. Jour. Amer. Chem. Soc. 46: 1662–1669. 1924.

results obtained from yeast treatment it is assumed that the lead failed to remove completely all the nonsugar substances that had reducing power either before or after hydrolysis.

TABLE 1.—*Comparison of basic lead acetate and mercuric nitrate as clarifying agents in the determination of sugars in olives*

[Reducing substances and sucrose reported as dextrose]

| Variety | Date picked | Clarifying agent | Reducing substances before hydrolysis | Reducing substances after hydrolysis | Sucrose |
|---|---|---|---|---|---|
| | 1927 | | *Per cent* | *Per cent* | *Per cent* |
| Ascolano | Oct. 4 | Lead | 5.00 | 6.92 | 1.92 |
| | | Mercury | 4.62 | 5.42 | .80 |
| | | Difference | .38 | 1.50 | |
| Columbella | Oct. 1 | Lead | 3.46 | 4.34 | .88 |
| | | Mercury | 1.58 | 1.70 | .12 |
| | | Difference | 1.88 | 2.64 | |
| Manzanillo | do | Lead | 4.52 | 5.18 | .66 |
| | | Mercury | 2.04 | 2.28 | .24 |
| | | Difference | 2.48 | 2.90 | |
| Mission No. 1 | do | Lead | 4.20 | 4.88 | .68 |
| | | Mercury | 1.88 | 2.16 | .28 |
| | | Difference | 2.32 | 2.72 | |
| Mission No. 3 | Oct. 4 | Lead | 6.40 | 9.20 | 2.80 |
| | | Mercury | 4.92 | 5.62 | .70 |
| | | Difference | 1.48 | 3.58 | |
| Do | Nov. 1 | Lead | 4.42 | 5.48 | 1.06 |
| | | Mercury | 3.42 | 4.02 | .60 |
| | | Difference | 1.00 | 1.46 | |
| Nevadillo No. 1 | Oct. 1 | Lead | 4.14 | 5.02 | .88 |
| | | Mercury | 1.62 | 2.02 | .40 |
| | | Difference | 2.52 | 3.00 | |
| Sevillano No. 2 | Oct. 4 | Lead | 6.32 | 8.00 | 1.68 |
| | | Mercury | 5.54 | 6.90 | 1.36 |
| | | Difference | .78 | 1.10 | |
| Uvaria | Oct. 1 | Lead | 2.98 | 6.02 | 3.04 |
| | | Mercury | 1.04 | 1.20 | .16 |
| | | Difference | 1.94 | 4.72 | |

## YEAST TREATMENT

The yeast treatment was given to six olive samples, to a 0.4 per cent dextrose solution, and to distilled water, none of which had reducing power after clarification with mercuric nitrate. Three portions of one sample were clarified in three ways, namely, one with mercuric nitrate, one with basic lead acetate, and one with yeast followed by basic lead acetate. The results obtained are given in Table 2.

TABLE 2.—*Reducing substances remaining in an extract from olives after fermentation with yeast, and after clarification with different defecating agents*

[Reducing substances reported as dextrose]

| Treatment | Reducing substances— | |
|---|---|---|
| | Before hydrolysis | After hydrolysis |
| | *Per cent* | *Per cent* |
| Clarified with mercuric nitrate | 1.58 | 1.70 |
| Clarified with basic lead acetate | 3.46 | 4.34 |
| Treated with yeast, then clarified with basic lead acetate | 2.42 | 3.02 |

It is apparent from Tables 1 and 2 that clarification by basic lead acetate for the determination of sugars in olives by the picric-acid method appears unsatisfactory. On the other hand, reducing substances and substances having reducing power after hydrolysis that are left in the extract by mercuric nitrate appear to be limited to fermentable sugars. Mercuric nitrate was therefore used in the routine determinations of simple and total reducing substances. In the remainder of this paper the substances so determined will be considered as sugars.

## RATIO OF SIMPLE TO TOTAL SUGARS

The ratios of simple to total sugars, expressed as dextrose, determined in several sets of samples representing the extremes of maturity studied are given in Table 3. The early-season mean ratio is 0.86, ranging from 0.80 to 0.89. The late-season mean ratio is 0.77, ranging from 0.67 to 0.94. Excluding the two samples not from the trees originally selected, the late-season mean ratio is 0.74, ranging from 0.71 to 0.77. The irregularity of the values obtained from samples not from the tree originally selected as shown in Table 3 and also in Table 4 indicate that the sampling error was probably large. From the ratios given it appears that the amounts of sugars principally or entirely sucrose, hydrolyzed by heating with picric acid, constitute usually from 15 to 25 per cent of the total reducing substances.

TABLE 3.—*Ratio of simple to total sugars in olives*

| Variety | Date picked | Ratio |
|---|---|---|
| Ascolano | Oct. 4, 1927 | 0.85 |
| | Jan. 3, 1928 | .77 |
| Manzanillo | Oct. 1, 1927 | .89 |
| | Feb. 13, 1928 | .75 |
| Mission No. 1 | Oct. 1, 1927 | .87 |
| | Feb. 13, 1928 [a] | .67 |
| Mission No. 3 | Oct. 4, 1927 | .88 |
| | Nov. 1, 1927 | .73 |
| | Jan. 2, 1928 | .71 |
| Nevadillo | Oct. 1, 1927 | .80 |
| | Feb. 13, 1928 [a] | .94 |

[a] Fruit not from tree originally selected.

## MATURITY AND SUGAR CONTENT

Only the total sugars were considered in comparing different stages of maturity and different varieties. In Table 4 are shown the per-

centages of total sugars in six varieties of olives picked on the dates indicated. In addition to the figures as determined on the fresh basis there are given the corresponding figures after calculation to the dry basis.

TABLE 4.—*Total sugars* [a] *in olives during ripening*

| Variety | Date picked | Total sugars on— | | Grams per 100 grams pits (fresh basis) |
|---|---|---|---|---|
| | | Fresh basis | Dry basis | |
| | | *Per cent* | *Per cent* | |
| Ascolano | Oct. 4, 1927 | 5.42 | 16.9 | 36.2 |
| | Nov. 1, 1927 [b] | 2.28 | 8.8 | 17.6 |
| | Nov. 10, 1927 | 5.54 | 21.2 | 54.8 |
| | Jan. 3, 1928 | 5.66 | 16.5 | 36.5 |
| Columbella | Oct. 1, 1927 | 1.70 | 6.6 | 5.1 |
| | Nov. 5, 1927 | 2.70 | 11.0 | 8.4 |
| | Dec. 13, 1927 | 3.25 | 12.7 | 12.4 |
| Manzanillo | Oct. 1, 1927 | 2.28 | 8.5 | 11.2 |
| | Nov. 5, 1927 | 2.68 | 10.2 | 11.5 |
| | Dec. 13, 1927 | 4.22 | 13.5 | 18.7 |
| | Feb. 13, 1928 | 2.68 | 7.1 | 16.6 |
| Mission No. 1 | Oct. 1, 1927 | 2.16 | 6.5 | 5.4 |
| | Nov. 5, 1927 | 2.70 | 8.6 | 10.0 |
| | Dec. 13, 1927 | 3.82 | 10.6 | 16.4 |
| | Feb. 13, 1928 [c] | 3.56 | 6.7 | 6.3 |
| Mission No. 3 | Oct. 4, 1927 | 5.62 | 15.4 | 15.2 |
| | Nov. 1, 1927 | 4.02 | 12.1 | 14.7 |
| | Jan. 3, 1928 | 4.66 | 9.3 | 11.5 |
| Nevadillo No. 1 | Oct. 1, 1927 | 2.02 | 5.2 | 8.9 |
| | Nov. 5, 1927 | 3.02 | 7.6 | 14.4 |
| | Dec. 13, 1927 | 2.68 | 7.0 | 12.8 |
| | Feb. 13, 1928 [c] | .96 | 1.9 | 4.6 |
| Uvaria | Oct. 1, 1927 | 1.20 | 5.0 | 1.6 |
| | Nov. 5, 1927 | 2.25 | 10.2 | 3.6 |
| | Dec. 13, 1927 | 2.30 | 6.7 | 3.0 |

[a] Reported as dextrose.
[b] Not considered in mean, Tables 5 and 6.
[c] Not from tree originally selected.

The trend, especially in the figures on the dry basis, is for the sugar content to increase in October and November, and to decrease in December and January.

Previous investigations [7] having shown that through the range of maturity studied the average pit weight remains constant, the figures were also calculated in terms of grams of sugars per 100 g. of pits in order to indicate the absolute amounts present. Since this eliminates the distortion of percentage resulting from coincident marked changes in the oil content of the flesh, the quantitative variations are different, but the direction of the trend is the same.

Variations apparently resulting from varietal differences in maturing time have been adjusted in Figure 1 by shifting some of the curves horizontally to bring the maximum values observed to a common vertical axis. The means of the values grouped according to vertical axes were used as a basis for the smoothed curves.

## VARIETY AND SUGAR CONTENT

In view of the relationship which appeared to exist between maturity and sugar content, the varieties were compared with respect to

[7] NICHOLS, P. F. RECENT INVESTIGATIONS ON OLIVE OIL. Ann. Tech. Conf. Calif. Olive Assoc. (San Francisco) Proc. 6: 73–84. 1927.

sugar content in early season, in late season, and for the entire season. In Table 5, the relative positions of the varieties are arranged according to mean sugar content on the fresh basis for the entire season. It will be noted, however, that the positions of varieties differ slightly

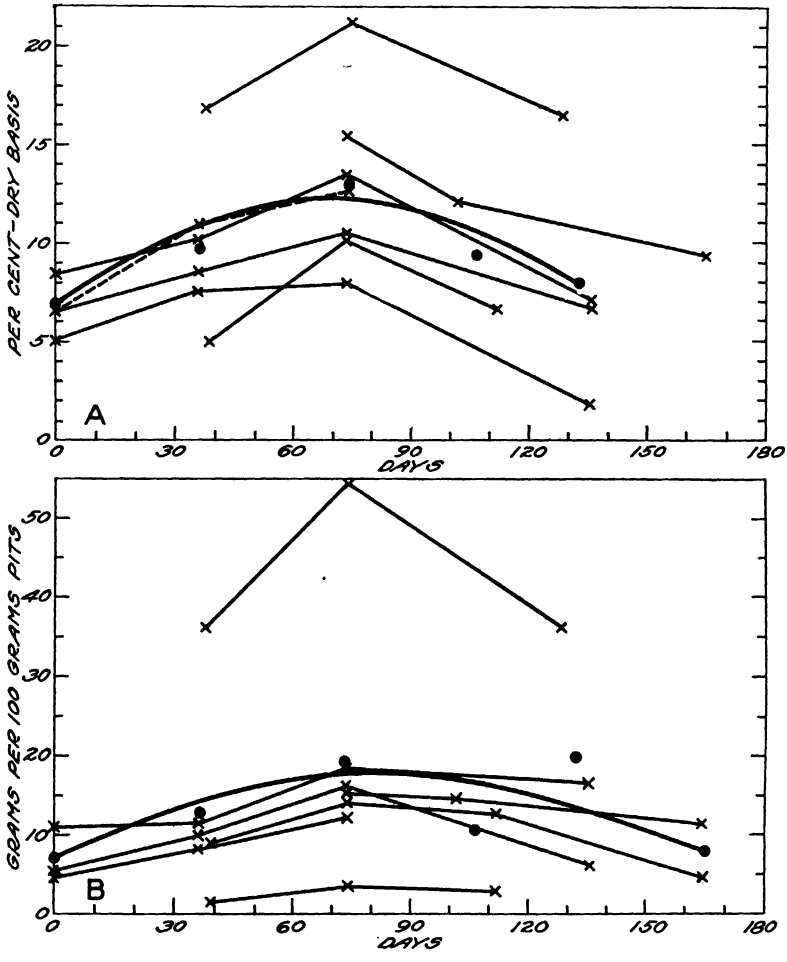


FIGURE 1.—A, Total sugars in olives during ripening, expressed as per cent on a dry basis. Maximum values adjusted to same vertical axis. Mean values indicated by circles and heavy curved line. B, Total sugars in olives during ripening, expressed as grams per hundred grams of pits. Maximum values adjusted to same vertical axis. Mean values indicated by circles and heavy curved line

from that order in the early and late seasons. The same is true in Table 6, in which the varieties are similarly arranged with respect to mean sugar content calculated on the dry basis. In almost all cases, however, the relative position remains approximately the same.

TABLE 5.—*Total sugars in olives of different varieties expressed in percentage on a fresh basis, in the early, late, and entire season*

| Variety | Mean sugar content for entire season | Early season, October-November | | Late season, December-February | |
|---|---|---|---|---|---|
| | | Samples | Mean Sugar content | Samples | Mean Sugar content |
| | *Per cent* | *Number* | *Per cent* | *Number* | *Per cent* |
| Ascolano | 5.54 | 2 | 5.48 | 1 | 5.66 |
| P. I. G. 27172 | 4.89 | | | 2 | 4.89 |
| Barouni | 4.59 | 1 | 3.56 | 4 | 4.84 |
| Sevillano | 4.09 | 3 | 4.87 | 2 | 2.92 |
| Mission | 3.68 | 4 | 3.62 | 4 | 3.74 |
| Chitoni | 3.57 | 1 | 3.22 | 2 | 3.75 |
| Manzanillo | 2.96 | 2 | 2.48 | 2 | 3.45 |
| Chemlali | 2.72 | 1 | 2.15 | 2 | 3.00 |
| Columbella | 2.55 | 2 | 2.20 | 1 | 3.25 |
| Nevadillo | 2.55 | 3 | 2.67 | 4 | 2.46 |
| Bidh el Hammam | 2.39 | 1 | 2.04 | 2 | 2.56 |
| Saiali Magloub | 2.06 | 1 | 1.48 | 2 | 2.35 |
| Uvaria | 1.92 | 2 | 1.72 | 1 | 2.30 |
| S. P. I. 27173 | 1.54 | 1 | 1.54 | | |
| Lucca | 1.52 | 1 | 1.52 | | |
| Picholine | 1.16 | | | 1 | 1.16 |
| Salome | 1.14 | 1 | 1.14 | | |

TABLE 6.—*Total sugars in olives of different varieties expressed in percentage on a dry basis, in the early, late, and entire season*

| Variety | Mean sugar content for entire season | Early season, October-November | | Late season, December-February | |
|---|---|---|---|---|---|
| | | Samples | Mean Sugar content | Samples | Mean Sugar content |
| | *Per cent* | *Number* | *Per cent* | *Number* | *Per cent* |
| Ascolano | 18.2 | 2 | 19.0 | 1 | 16.5 |
| Sevillano | 16.8 | 3 | 21.1 | 2 | 10.4 |
| Barouni | 13.3 | 1 | 15.2 | 4 | 12.8 |
| P. I. G. 27172 | 10.6 | | | 2 | 10.6 |
| Columbella | 10.1 | 2 | 8.8 | 1 | 12.7 |
| Manzanillo | 9.8 | 2 | 9.4 | 2 | 10.3 |
| Mission | 9.5 | 4 | 10.6 | 4 | 8.4 |
| Chitoni | 9.0 | 1 | 8.2 | 2 | 9.0 |
| Uvaria | 7.3 | 2 | 7.6 | 1 | 6.7 |
| Bidh el Hammam | 7.2 | 1 | 6.6 | 2 | 7.4 |
| Chemlali | 7.0 | 1 | 6.3 | 2 | 7.4 |
| Nevadillo | 6.5 | 3 | 10.2 | 4 | 6.2 |
| Saiali Magloub | 6.0 | 1 | 4.7 | 2 | 6.6 |
| S. P. I. 27173 | 5.3 | 1 | 5.3 | | |
| Lucca | 5.3 | 1 | 5.3 | | |
| Salome | 4.4 | 1 | 4.4 | | |
| Picholine | 3.0 | | | 1 | 3.0 |

In view of the wide and inconsistent variations shown in Table 4 these means can not be used for close comparisons of varieties. In a relative way, however, the varieties may be divided into three groups with respect to sugar content: Ascolano, P. I. G. 27172, Barouni, and Sevillano, which were high; Mission, Chitoni, Manzanillo, Chemlali, Columbella, Nevadillo, Bidh el Hammam, Saiali Magloub, and Uvaria, which were medium; and S. P. I. 27173, Lucca, Picholine, and Salome, which were low.

SUMMARY

Basic lead acetate was found to be unsatisfactory as a clarifying agent, and to give too high results in the picric acid determination of reducing sugars and sucrose in the water extract of olive flesh.

Reducing substances before and after hydrolysis, remaining in the water extract after clarification by mercuric nitrate were found to be removable by a short treatment with yeast and are believed to be sugars.

The early-season mean ratio of simple to total sugars, expressed as dextrose, usually declined as the season progressed.

The total sugar percentage on the dry basis and in absolute amounts was found to increase in early season and to decrease in late season.

Of the varieties studied Ascolano, P. I. G. 27172, Barouni, and Sevillano were high in sugar content; S. P. I. 27173, Lucca, Picholine, and Salome were low; and the Mission, Chitoni, Manzanillo, Chemlali, Columbella, Nevadillo, Bidh el Hammam, Saiali Magloub, and Uvaria were intermediate.

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