of the whole seed. The linters had the lowest equilibrium moisture content of the various cottonseed products. Since the linters comprise only about 12% of the total weight of the seed, the hygroscopic equilibrium of this component contributes only a small fraction to the total hygroscopic equilibrium, *i.e.*, the whole seed and delintered seed have similar hygroscopic equilibria.

The results of the determinations of the distribution of moisture between kernels and lint-free hulls showed that the kernels constituted 63 to 64.5% of the weight of the delintered seed in equilibrium with air having a relative humidity ranging from 11 to 86%. Throughout this humidity range the ratio of the wet weights of the kernels to the wet weights of the lint-free hulls remained constant.

Summary and Conclusions

The hygroscopic equilibrium of a sample of the Stoneville 2B variety of cottonseed and its derived products has been determined over the relative humidity range of 11 to 93%. In a previous publication (1) the hygroscopic equilibrium of a sample of the D and PL variety of cottonseed was determined over the range of 31 to 93% relative humidity. Comparison of the previous and present results show that the whole seed and kernels of both varieties exhibit the same hygroscopic equilibrium behavior. A comparison of the aforementioned results with those reported by Simpson (3) on Stoneville 2B variety, and by Franco (4) on the 1A 7387 variety grown in São Paulo, Brazil, show that for intact cottonseed the hygroscopic equilibrium behavior is the same.

On the basis of the curves given in Figure 1 and on the assumption that the cottonseed used was representative of cottonseed in general, it is possible to calculate the equilibrium moisture content of the whole cottonseed or any component thereof. For example, cottonseed consisting of 12.1% linters, 87.9% delintered seed, 56.0% kernels, and 31.9% lint-free hulls, and containing 12% moisture on a whole seed basis, will yield linters, delintered seed, kernels, and lint-free hulls having 9.4, 12.7, 10.8, and 15.4% moisture, respectively.

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Studies on Olive Oil Standardization

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THE world's production of fats and oils, which before World War I (1914) was around 15 million tons, has now increased to 26 million tons. Since 1914 other changes also have occurred in the relative proportion of the world production of the various oils. Oils and fats from animal origin which previously represented more than two-thirds of the total amount now represent less than half. Of the plant origin fatty substances the greatest part (about three-fourths) are produced by yearly plants, mainly cotton seed, peanut, linseed, soya bean, and sunflower; the other one-fourth is derived from trees (olive, cocos, Elaeis, and others). Although olive oil production has been increased by 30% since 1914, the rate of increase of the other tree oils was much greater (almost tripled) during the same period. The reason for this increase is the lower cost of production due to the higher yield and the lower living standards in the tropics and Africa where these oils are mainly produced. The production of olive oil is practically limited to the Mediterranean countries and to California. The importance of olive oil however is not based only on the size of the world production of it but also on its superior quality and flavor. Olive oil, when well prepared, is highly appreciated as salad oil and usually is added in small proportions to other oils (refined seed oils) in order to impart to them a good flavor. This is the reason why extensive research work on olive oil is being conducted in almost every Mediterranean country as well as in California. Peanut and soya oils are produced in Asia and the main source of cotton seed oil is the United States. Of the plant oils, about one-third is used for industrial purposes (soap, etc.). The industrial use of plant oils is decreasing steadily because of the development of better methods of refining which permit a larger proportion of crude oil to be used for human consumption. Another factor is the increasing production of synthetic oils for industrial purposes. More than 50,000 tons of synthetic oils were produced in 1938 in Germany. Besides the synthetic process a biological process for fat production has been studied in Europe lately. This last method is based upon the culture of various microorganisms (Penicillum Zavanicum, Aspergillus niger, and others) on a medium containing carbohydrates and inorganic salts. Of the animal fats, fish oil (mainly whale oil) production received a new impetus due to the introduction by Germans and Japanese of better fishing methods. Asia and Africa supplied about 70% of all export of fats. United Kingdom, Germany, France, and the United States absorbed almost 90% of all imports. The production of butter before the last war was around four millions tons. Europe produced 48% but consumed more than 60%. Margarine, which was considered previously as an animal fat, is now made from hydrogenated plant oils to a proportion of 94% or more. Peanut butter, a fat which also contains proteins and carbohydrates, is produced almost exclusively in the United States. The consumption of fats and oils in the world varies greatly. Among the states with the highest consumption (before the war) were Denmark and Scandinavian countries with annual consumption of more than 28 kilos per capita, Great Britain, United States,* and Germany with 24

* Invisible fats not included.

kilos per capita. In Mediterranean countries the consumption was about 15 kilos per capita annually, in Russia, 11 kilos per capita, and in Asiatic countries, which are the last in the list, the consumption was less than 10 kilos per capita.

Two main methods at present exist for the extraction of the oil: the hydraulic pressure method and the solvent extraction method. For the olive oil industry the first method is generally used except for the extraction of the oil of the kernels. It is the belief of the author that the solvent extraction method could be successfully adapted for the treatment of the whole olive since it is more economical. An oil mill of daily input of 6 tons of olives would require the service of 7-8 workmen and mechanical force of 11 horsepower. A revolving extractor of the Buss type with a capacity of 12,000 liters would operate only with two workmen and needs 8-10 kilos of solvent daily and 5-6 kwh. per ton of olives. The cost of equipment and the space needed for the solvent extraction method is less; furthermore the extraction is more complete, since only 1 to 1.5% oil is left in the kernels, while with the use of presses the oil left in the kernels is more than 6%. The kernels as well as the solvent oil extracted keep better and contain more antioxidants which help to protect it from rancidity changes. For the refinement of the oils a new continuous process using monoethanolamine ($C_2H_5NH_2$) has been recommended as presenting several advantages, the most important of which is the decrease of loss in oil from partial saponification occurring in the present method (1).

The great variation in the quality of natural oils which is due to variation in the natural quality of olives and their preservation prior to extraction and various other factors affecting quality during the extraction process created the need for standardization in the olive oil industry. At present the so-called standard oils put on the market by various European firms are a mixture of refined kernel oils (or low grade olive oils) and seed oils with a certain proportion (about 15%) of a good quality virgin olive oil to give the natural flavor and aroma to the standard oil. These virgin oils are produced in special localities under better sanitary conditions and are more expensive. It is therefore highly important for the olive oil industry to study the quality and the natural properties of the olive oils and the factors affecting their development and preservation. Such a work has been undertaken for Italy by the Agricultural Experiment Station of Bari and other oil laboratories in Italy and by the Research Experiment Station for Agricultural Technology in Greece. Of this last station the author was in charge until 1945.

In our first publication on this subject (2) the effect of maturity and climatic conditions of the localities where olives are growing has been studied and proved to be quite important in determining the natural quality of the oil. With the advancement of maturity the proportion of liquid (unsaturated) fatty acids increased, a fact which has been observed before by other workers (3). In localities where the temperature is lower during the ripening period more liquid fatty acids are formed (4), a fact which proves for olive oil the validity of the Ivanov (5) climatic theory established on data obtained from seed oils. On another point concerning the effect of infection by the olive fly (6) (Dacus Olea) upon the variation in fatty acids of the oils produced, our results did not agree with the results obtained by Pantanelli and Brandonisio (7) of the Bari Experiment Station. Our opinion is that the results obtained by Pantanelli and Brandonisio, according to which the proportion of the saturated fatty acids in the oils is increased as a result of the olive fly infection, were due rather to the effect of the earlier state of maturity of the olives examined since infection by Dacus induces premature fall of the olive fruit. Another significant point was the discovery that oils from different localities differ greatly in their antioxidant value (8), which is related to the subsequent development of rancidity.

FIG. 1. The injection method of incorporating fertilizers in olive trees at the Recherche Experiment Station for Agricultural Technology, Athens, Greece.

In a study of the effect of fertilizers on the quality of the oil and in order to eliminate the effect of soil and tree variation and shorten the period of experimentation, the injection method, extensive use of which has been made for other purposes by workers of the East Malling Experiment Station, England, has been used (9). The idea of the method must be very old since there are known recipes of ancient Roman and Greek writers recommending the use of a semifluid mixture of sodium nitrate and water to smear the buds of vines for the production of early grapes (10). In its present form the injection method consists in incorporating slowly in the plant through its branches or trunk or even the leaves the solution of the chemical concerned. The solution is held in a container a little higher than the hole of injection and is connected with it by a rubber tube (Fig. 1). It takes about 24 hours for the liquid to be absorbed and the solution we have used was 0.25%. We started the injections when the olives were already formed, and we gave three injections of one kilos each at one-month intervals during the whole period. The first year of our preliminary experiments only the main fertilizers, nitrogen, phosphorus, and potassium, and their combinations were used. The next year the experiments were extended by the use of minor elements and phytohormones, but the civil war raging in Athens during the harvesting period ruined our experiment and no data were obtain for that year.

The preliminary experiments of 1944 showed however that a considerable increase in volume of fruit

occurred on the branches injected by ammonium sulfate and calcium phosphate. This was associated with a total increase in oil and protein content of the fruit. The average weight of olives was increased from 3.35 g. to 3.95 g., the protein content from 8.68% to 13.12%. The increase in yield of olive oil per acre was 27 kilos with nitrogen, 7.5 kilos with phosphorus and 8 kilos with potassium. We think that the injection method could be very useful for experimental purposes in many kinds of fertilization experiments.

Summary

Experiments were conducted in the Research Experiment Station for Agricultural Technology, Athens, Greece, to study the effect of climatic conditions, state of maturity, infection by olive fly, and the effect of various fertilizers upon the quality of olive oil.

Olive oil produced in localities where the temperature is low during the maturity period has more unsaturated fatty acids than oil produced in localities with higher temperatures. With the advance of maturity the proportion of unsaturated fatty acids increased. Infection by olive fly had no appreciable effect upon the relative proportion of saturated fatty acids.

For studying of the effect of fertilizers the injection method was used. Nitrogen fertilizers injected into the branches gave considerable increase in the volume of fruit, its protein content, and the total amount of oil produced per acre.

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The Determination of Tocopherol Content During the Commercial Processing of Soybean Oil*

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THE increasing study that is being given to the part that tocopherols (vitamin E) play in nutrition emphasizes the need for more information concerning the amount of the tocopherols in primary sources such as vegetable oils. This paper describes a procedure useful for assaying the tocopherol content of soybean oil and presents some data on the effect of commercial processing operations on the tocopherol potency.

The recent discovery of δ-tocopherol in soybean oil has made the accepted methods unapplicable (11). A thorough background of this field is found in other publications (1, 2, 12). A reasonably simple method for soybean oil has been developed by us by modifying the method of Rawlings (10) with these recent findings.

Directions for Modified Emmerie-Engel Procedure for Soybean Oil

A 1-ml. aliquot of sample containing 50 to 300 μg. of tocopherol in redistilled (over KOH and $KMnO_4$) absolute ethanol is added to an opaque 2-oz. glass stoppered bottle. One ml. each of 0.1% $FeCl_3 \cdot 6H_2O$ and 0.25% α,α'-dipyridyl solutions in ethanol are added, in order, followed by 22 ml. of purified ethanol. The color density is read in an Evelyn colorimeter (1.9-cm. cell) at 10 minutes from addition of $FeCl_3$ reagent using a 520 mμ. filter. The apparent tocopherol concentration is determined from a curve prepared from pure α-tocopherol (or pure hydroquinone calculated to read in molecular equivalents of α-tocopherol) and multiplied by 0.91 to obtain the correct assay of mixed tocopherols. (For colorimeters or spectrophotometers using a 1-cm. cell, use a 2-ml. aliquot of sample and 1 ml. each of 0.2% $FeCl_3 \cdot 6H_2O$ and 0.5% α,α'-dipyridyl reagents.)

The important modification of the method is the use of the factor 0.91 to correct the result obtained for the enhanced color due to δ-tocopherol. It was previously found (12) that the tocopherols in the soybean oils examined were a mixture of α-, γ-, and δ-tocopherols and that the ratio was approximately 10:60:30:, respectively. A milligram of such a mixture is found to reduce approximately 10% more ferric chloride than a milligram of α-tocopherol; therefore the correction factor of 0.91 is used to obtain the tocopherol content of Soybean Oil.

Previous work (1, 2) suggests that there are materials in soybean oil which will interfere with the assay method. Parker and McFarlane (7) have published a procedure for removing the carotenoids and phenols with 85% sulfuric acid. Samples so treated have been assayed with and without the addition of a known amount of a concentrate of mixed tocopherols. Corrections were made for inhibition by the graphical method of Kaunitz and Beaver (5). Some results are shown in Table 1, comparing the corrected assays with the original assay, and it is evident that the correction necessary for substances giving spurious color with the Emmerie-Engel reagent is largely compensated for by the repression of color due to the Kaunitz-Beaver effect. As a result, the corrected assays for the potency of crude and refined oils lay within ± 10% of the values obtained by direct assay.

*Communication No. 115 from the Research Laboratories of Distillation Products, Inc., Rochester, New York. (Presented at The American Oil Chemists' Society meeting in New Orleans, May 20-22, 1947.)