idly out of the column; kitol can be eluted without decomposition by an ethanol-ether mixture 1:1. Synthetic dyes which are sometimes used may, depending on their chemical composition, either be eluted from the upper column before putting the two columns in series, or they are adsorbed at the very top of the second column without showing any sign of moving down during subsequent elution.

Carotene can be recovered from the upper column eluate with the first 15 ml. of eluant and can accordingly be determined separately by colorimetry.

It is intended to check the method for the analysis of other materials which have a low content of vitamin A, e.g. butter, cattle feeds, blood, and faeces.

Acknowledgment

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Phase Relations Pertaining to the Solvent Winterization of Cottonseed Oil in Hexane and in Acetone-Hexane Mixtures

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COMPLETE phase relation data on the solvent winterization of cottonseed oil in acetone (1) showed that commercial C. P. acetone could be used as a winterization solvent. It was pointed out however that for certain oil-solvent ratios at a temperature about 5°C. below that required for adequate winterization, two liquid phases were present in addition to the solid phase. It was also indicated that moisture in the acetone might cause this separation into two liquid layers to take place at higher temperatures, thus causing interference with the efficient separation of the solid from the liquid. It has been found that this effect of moisture can be effectively counteracted by the presence of a small proportion of a hydrocarbon, such as hexane, to the acetone. Fifteen percent by weight of hexane was found sufficient to prevent liquid-liquid phase separation from interfering with the solvent winterization when less than about 1.4% by weight of moisture is present in the acetone as in the case of commercial grades of acetone.

The present report deals with the pertinent phase behavior in the solvent winterization of cottonseed oil in two additional solvents; namely, commercial hexane and a solvent mixture consisting of 85% by weight of acetone and 15% of hexane. With these additional data it is now possible to compare the relative advantages and disadvantages of acetone, hexane, and the acetone-hexane mixture for use as the winterization solvent for cottonseed oil.

Materials. Cottonseed oil No. 2, for which the complete data with acetone have already been published (1), was used in this investigation in order to permit direct comparison of the relative applicability of the different solvents. It was a commercial refined and bleached oil having an iodine value of 108.2. The commercial hexane used was Skellysolve B.[2] A commercial C. P. acetone was used containing 0.5% of moisture as measured by a special Karl Fischer reagent for ketones.[3]

Effect of Moisture in Acetone. To determine the approximate magnitude of the effect of moisture upon the formation of two liquid layers in the cottonseed oil-acetone system, 40% oil solutions were made up gravimetrically, using commercial acetone containing various amounts of added water. The solutions were slowly chilled to find the temperature at which two liquid layers appeared. The presence of 1.4% by weight of moisture in the acetone caused the break to take place at +5°C. instead of at −12°C., as previously reported for commercial acetone.

It was found that the addition of hexane counteracts the tendency of moisture to cause two liquid layers. Using as solvent a mixture of 15 parts by weight of hexane and 85 parts of acetone containing 1.4 weight percentage of water, a 40% cottonseed oil solution can be winterized at a chilling temperature of −8°C. without the appearance of two liquid phases. This suggests the use of an acetone-hexane mixture as a winterization solvent for cottonseed oil as a safety measure against the presence of moisture in the oil or the acetone. Fifteen weight percentage of hexane in the mixture should be more than adequate to counteract the amounts of moisture which would be expected to be present in commercial acetone.

Winterization Procedure. Laboratory bench-scale winterization tests were carried out by the same procedure as previously described except that the rubber stoppers for the centrifuge bottles were wrapped in tin foil to prevent the sorption of hexane from the samples. In brief, duplicate weighed samples of the oil in definite oil-solvent ratios were chilled in a bath of the desired temperature for a definite holding-time. They were then centrifuged for 10 minutes. The clear supernatant liquid was quickly decanted into a tared flask and the residual solid fraction weighed. From the weights and oil contents of the solid and super-

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[2] The mention of trade products does not imply that they are endorsed or recommended by the Department of Agriculture over similar products not mentioned.

[3] Mitchell, J., Jr., and Smith, D. M., "Aquametry," Interscience Publishers, New York, 1948, pp. 146-151.

natant fractions the total amount of solid which had separated during chilling was calculated. The "percentage of solid removed" is reported as the percentage of the total oil present in the original solution.

Effect of Oil-Solvent Ratio and Temperature. Winterizations were run on cottonseed oil-hexane mixtures between 10 and 75% oil by weight at several chilling temperatures, using a 3-hour holding time without agitation. The results are plotted in Figure 1. For any given chilling temperature there is only a slight increase in the percentage of solid separating as the concentration of oil is increased until the concentration reaches a certain value at which the curve breaks sharply and rises steeply. Because of the steepness of the curve these experimental points are less precise, having a reproducibility of only about 1.0% solid removed as compared to 0.3% for the acetone system (1).

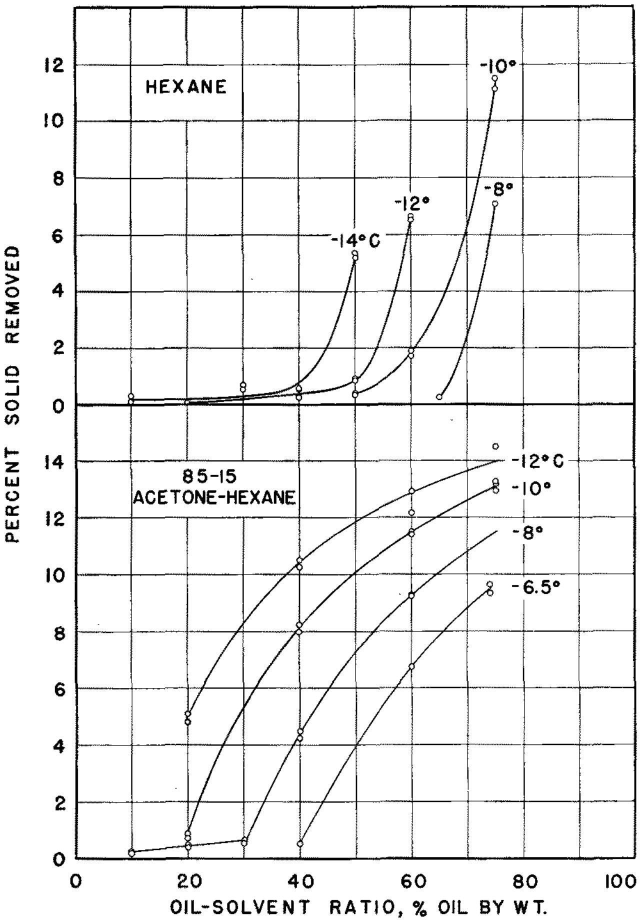

FIG. 1. Solvent-winterization data for cottonseed oil in specified solvents. (3-hour holding-time.)

The results of similar winterization experiments carried out in a solvent mixture consisting of 85% by weight of acetone and 15% of commercial hexane are also represented in Figure 1.

The marked differences in the phase-relation behavior of cottonseed oil in acetone (1), in acetone-hexane mixture, and in hexane are demonstrated by Figure 2, which shows the effect of chilling temperature on the percentage of solid removed when the oil-solvent ratio is kept constant, and by Figure 3, which shows the chilling temperatures required to cause the separation of specified percentages of solid as influenced by the oil-solvent ratio.

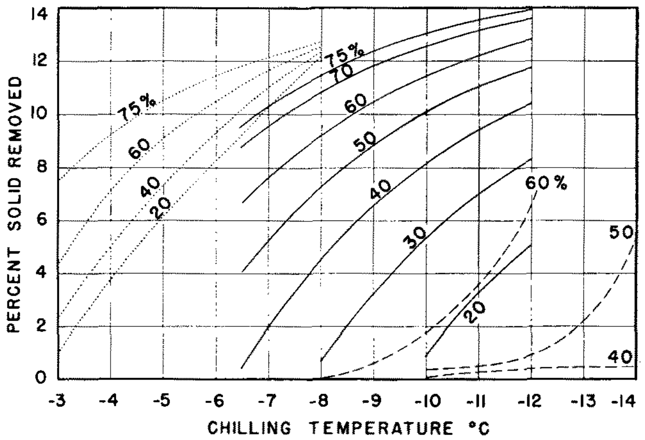

FIG. 2. Percentage of solid removed at various chilling temperatures at constant oil-solvent ratios for cottonseed oil with: ...., acetone; ————,85-15 acetone-hexane mixture; and — — — —, hexane. (3-hour holding-time.)

For oil-solvent ratios above the break in the hexane curves (Figure 1) a small change in the chilling temperature results in a large change in the percentage of solid separating. Thus to control the amount of solid to be removed it would be necessary to regulate the chilling temperature very acurately when hexane is used as the winterization solvent (Figure 2). Using a 3-hour holding-time, the chilling temperature required to remove a given percentage of solid is in general considerably lower in the hexane system than in acetone and is intermediate for the mixed solvent (Figure 3). Thus for a 50-50 oil-solvent ratio a chilling temperature of —4°C. for acetone, —7.5°C. for the mixed solvent, and —14°C. for hexane is required to remove 6% of solid from this oil, that is, to produce a winterized oil having a cold test of 6 hours (1). For lower oil-solvent ratios the differences would be even greater.

Figure 2 also shows that, for the acetone system at a given chilling temperature, the increase in the percentage of solid separating per 10% rise in concentration remains relatively constant over the entire range of concentration studied. In the case of the acetone-hexane solvent mixture this change for high oil concentrations is of the same order of magnitude as with acetone but becomes markedly greater as the concentration is lowered. Thus with the acetone-hex-

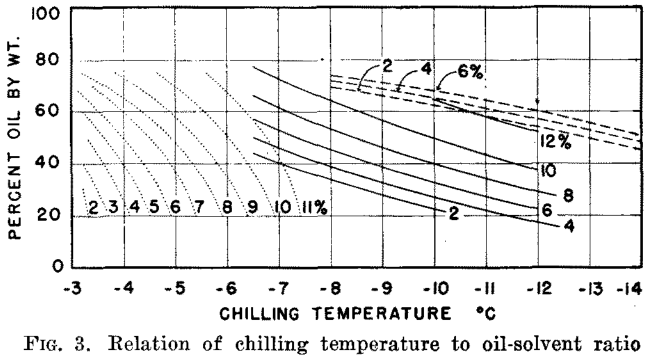

FIG. 3. Relation of chilling temperature to oil-solvent ratio for constant percentage of solid removed from cottonseed oil: .... in acetone; ————, 85-15 acetone-hexane mixture; and — — — —, hexane. (3-hour holding-time.)

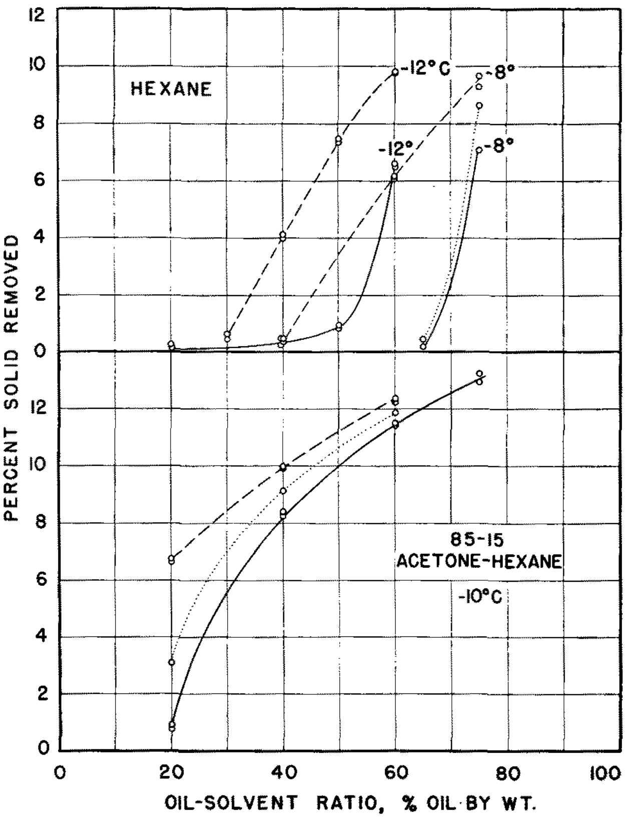

FIG. 4. Effect of holding-time and of agitation on the percent solid removed: ————, 3-hour holding-time; — — — —, 24-hour holding-time; and ...., 3-hour holding-time with gentle agitation.

ane mixture at —10°C. the percentages of solid removed from 20% and 30% oil solutions are 0.9 and 5.3%, respectively, a difference of 4.4%; and from 60% and 70% oil solutions they are 11.4 and 12.6%, respectively, a difference of 1.2%. This implies the necessity for a slightly more rigid control of the oil-solvent ratio in the solvent winterization of cottonseed oil in the acetone-hexane mixture over that of the acetone system except in very high concentrations of oil.

Effect of Moisture in Acetone-Hexane System. The presence of a small amount of moisture results in a slightly larger yield of solid separating at a given chilling temperature and also in better settling upon centrifuging. Winterization of a 40% cottonseed oil solution at —8°C. in an 85-15 acetone-hexane mixture, the acetone containing 1.4% of water, resulted in 4.9% solid removed and 92.8% by volume of clear supernatant liquid after centrifuging as compared to 4.4% solid, Figure 1, and 88% clear supernatant with 0.5% of water under the same conditions.

Cold Tests. The general agreement between the cold test data for the winterized oils obtained from these experiments with those of the acetone system (1) showed that regardless of which of the three solvents is used the same percentage of solid must be separated to attain a specified cold test value. For this cottonseed oil 6% of solid must be removed to give a winterized oil passing a six-hour cold test.

Effect of Holding-Time and Agitation. Using a 24-hour holding-time, winterization curves were run at —12°C. and —8°C. in hexane and at —10°C. in the acetone-hexane mixture. The data are plotted as broken lines in Figure 4. The full lines represent the corresponding 3-hour curves and the dotted lines 3-hour curves, in which the sample was gently agitated by swirling every 10 minutes during the chilling period. It is apparent that above certain oil concentrations considerably more solid separates from the hexane solutions in 24 hours than in 3 hours; the difference is small at low concentrations, increasing to a maximum just below the break in the 3-hour curve, and becoming smaller for higher concentrations. Thus for a 65% oil-solvent ratio at —8°C. only 0.2% of solid separates in 3 hours compared to 7.4% in 24 hours. For the 75% oil-solvent ratio the corresponding values are 7.1 and 9.5%, respectively. This indicates that in the hexane system a high degree of supercooling is necessary for the solid to form at a reasonable rate.

In the case of the acetone-hexane mixture the effect of increasing the holding-time is quite different. More solid separates at all concentrations so that the curve is shifted upwards; the change is greatest at low oil-solvent ratios and decreases gradually as the oil concentration is increased.

The effect of gentle agitation is also markedly different for the two solvents. The percentage of solid separating in the acetone-hexane mixture is definitely higher than without agitation so that the 24-hour curve is approached and the change is greatest at low oil concentrations. In hexane neither the shape of the curve nor the position of the break is appre-

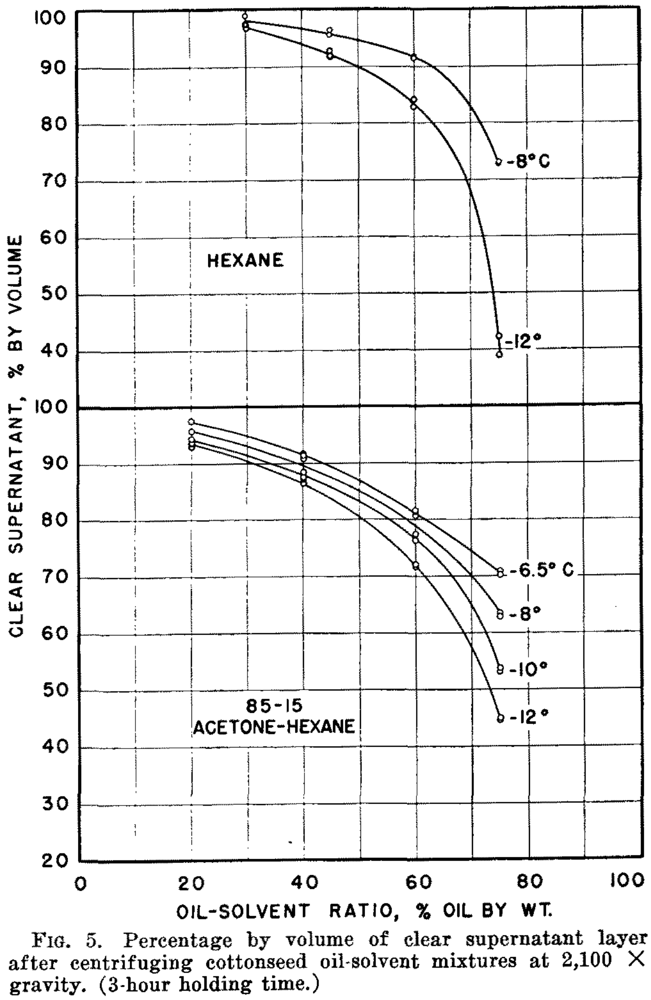

FIG. 5. Percentage by volume of clear supernatant layer after centrifuging cottonseed oil-solvent mixtures at 2,100 × gravity. (3-hour holding time.)

ciably changed, and there is no measurable effect at the lower concentrations.

Effect of Oil-Solvent Ratio and Temperature on Settling. Using the technique previously described (1) the relative settlabilities of the solid separating were determined for various oil-solvent ratios and chilling temperatures in hexane and in acetone-hexane mixture. Figure 5 summarizes the data obtained for a 3-hour holding-time, showing the percentage by volume of clear supernatant liquid after centrifuging at a speed corresponding to 2,100 times gravity. The curves for 8-hour, 16-hour, and 24-hour holding-times (not shown in the figure) fall farther and farther below the corresponding 3-hour curves, but the effect is only slight; the maximum difference in clear supernatant is only 2% at the 20% oil concentration and about 6% at the 75% oil concentration.

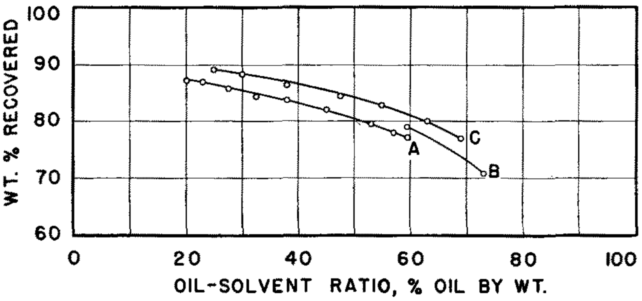

FIG. 6. Weight percentage of winterized oil recoverable from clear supernatant of cottonseed oil-solvent mixtures centrifuged at 2,100 × gravity after chilling to give a 6-hour cold test, 3-hour holding-time: A, 85-15 acetone-hexane mixture; B, hexane; and C, acetone.

Figure 6, obtained by calculations (1) from Figure 5 and the above winterization data, shows the percentage by weight of the original oil present in the clear supernatant liquid when 6% of solid has been removed. Thus it shows for different oil-solvent ratios the yield of winterized oil with a 6-hour cold-test recoverable by centrifugal separation of the above efficiency without washing the crystals. It is apparent that from this point of view acetone is the most favorable solvent.

Analysis of the data represented in Figures 1 and 5 indicates that for any of the three solvents the first portion of solid which separates does not settle as well as when larger amounts are involved. This is illustrated by Figure 7 which shows how the "degree of packing" is influenced by the oil-solvent ratio and by the percentage of solid removed, the degree of packing being defined as the weight in grams of solid separating divided by the volume in cc. it occupies at the bottom of the chilled solution after centrifuging. Curves for the acetone system, derived from data previously published (1) and for various 24-hour experiments have been included.

For the hexane system the curves show that the degree of packing increases sharply as the oil-solvent ratio increases and that for a given oil-solvent ratio it increases as the chilling temperature is lowered or as the holding-time is increased. With a 24-hour holding-time a maximum degree of packing is reached at about a 60% oil concentration in each case shown, or when 7% and 9% of the oil present is removed as solid at —8°C. and —12°C., respectively. For the acetone-hexane mixed solvent a maximum is obtained for each temperature with a 3-hour holding-time and the maximum shifts toward lower oil-solvent ratios as the chilling temperature decreases. In all cases the maxima correspond to a seperation of from 9 to 11% of the original oil as solid. In this solvent again a longer holding-time results in a marked improvement in the degree of packing of the solid. When acetone is used as the solvent the change in degree of packing for a given change in oil-solvent ratio is in general smaller. However, the maxima in the degree of packing as influenced by the percentage of solid removed become very sharp as the chilling temperature is decreased.

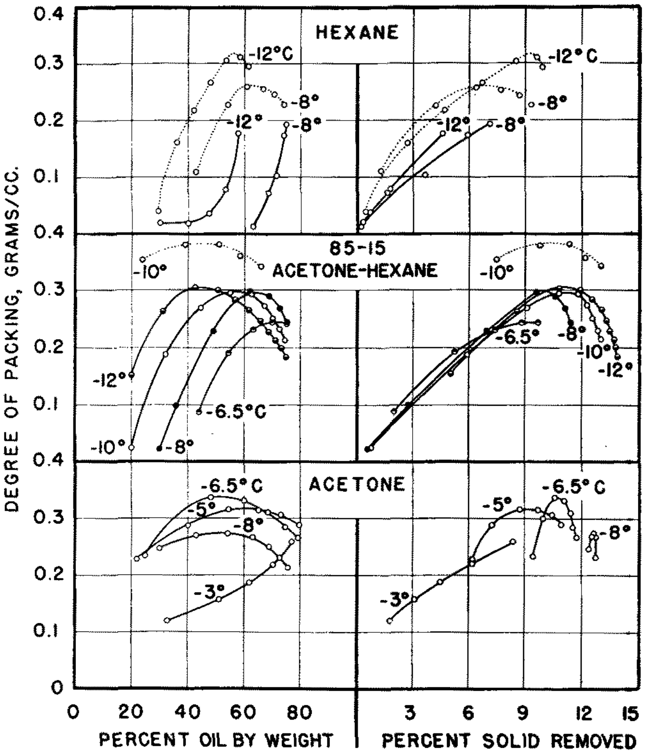

FIG. 7. Degree of packing of the solid for various chilling temperatures as influenced by the oil-solvent ratio and by the percentage of solid removed: ————, 3-hour holding time; and ...., 24-hour holding-time.

It is noteworthy that for all the solvents, oil-solvent ratios, and temperatures studied, the maxima in the packing curves occur when approximately 6 grams of solid have been separated from 100 grams of solution. This was confirmed by the settling data obtained coincidental to the various winterization experiments. In these the chilling and centrifugation were carried out in a conventional 250-ml. centrifuge bottle at a speed corresponding to 525 times gravity instead of in a 15-ml. conical centrifuge tube at 2,100 times gravity as in the above systematic settling experiments. At the lower speed and with the larger round-bottom cylindrical bottle the best settling is still obtained when 6% by weight of the oil-solvent mixture separates as solid. This indicates that the position of the optimum packing is not a function of the centrifugal speed, within the limits studied, or of the size or shape of the centrifuge vessel used.

Color. During the investigation of the winterization of cottonseed oil in hexane a slight difference in the color of the supernatant and the solid fraction was noticed. The solid was more reddish in color. A Lovibond color analysis gave the following results: 15 yellow and 1.4 red for the original oil and for the supernatant fraction; and 20 yellow and 2.3 red for the solid fraction.

Discussion

Under the conditions of these laboratory experiments hexane does not show promise as a practical solvent for use in the solvent winterization of cottonseed oil. A very close control of both the temperature and the oil-solvent ratio would be necessary in order to regulate the amount of solid separating in a 3-hour chilling period. At a given chilling temperature the initial rate of crystallization is very low up to a certain oil-solvent ratio. An increase beyond this ratio results in a sharp increase in the amount of solid separating. A high degree of supersaturation seems to be necessary before the initial rate of crystallization is appreciable. A longer holding-time results in more solid separating with a less sharp break in the curve. However a holding-time longer than 3 hours would probably be impractical for a continuous process. Gentle agitation does not result in any appreciable improvement. Another disadvantage of hexane as compared to acetone is the fact that a much lower chilling temperature would be required to produce a winterized oil, the difference being from 10° to 15°C. and, at oil concentrations less than 40%, even greater.

The use of acetone as a solvent has the advantage of a much more favorable chilling temperature and a definite improvement in the settling qualities of the crystals which form. It has the distinct disadvantage that the presence of a small percentage of moisture may cause the formation of two liquid phases in addition to the solid phase at the chilling temperatures required for adequate winterization, thus causing complications in the subsequent isolation of the solid.

The use of a mixture of acetone and hexane eliminates the major disadvantages experienced with either solvent alone. The mixture containing 15 parts by weight of hexane prevents the formation of two liquid layers at the temperatures required for adequate winterization, using acetone having a water content as high as 1.4% by weight, and it still does not cause the tendency toward supercooling exhibited when hexane alone is used. Compared with the acetone system the chilling temperature necessary to attain a specified degree of winterization is from 2° to 7°C. lower. The difference is greatest at low oil-solvent ratios. A slightly more rigid control of concentration is required to regulate the yield, but the temperature control is of the same order of magnitude for the mixed solvent as with acetone. If the moisture content of the acetone used is greater than 1.4% by weight, a larger proportion of hexane in the mixed solvent would be necessary.

The ability of the solid to pack out on centrifuging depends upon the oil-solvent ratio, the chilling temperature, and the solvent used, being very low at a given temperature for a small percentage of solid removed, increasing to a maximum, and then decreasing as the percentage increases. These maxima appear at different percentages of solid removed, calculated on the basis of the original oil present in any given solution. However they all correspond to the same number of grams of solid removed from 100 grams of solution.

Summary

Systematic phase relation data pertaining to the solvent winterization behavior of a refined cottonseed oil have been obtained for two additional solvents; namely, commercial hexane and a mixed solvent consisting of 85% by weight of acetone and 15% of hexane. Graphs have been constructed to show the effect of oil-solvent ratio, chilling temperature, holding-time, and agitation on the percentage of solid removed, the degree of winterization and the settling qualities of the solid separating.

These data, with those previously reported for acetone (1), afford a basis for the selection of the optimum conditions and procedures in the application of solvent winterization to cottonseed oil and bring out the relative advantages, disadvantages, and limitations of the three solvents. The acetone-hexane mixture seems to combine the advantages and eliminate the disadvantages of either of these solvents alone.

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ABSTRACTS . . . . Don Whyte, Editor

• Oils and Fats

R. A. Reiners, Abstractor

Report on crude fat or ether extract. H. H. Hoffman (Florida Dept. of Agr., Tallahassee). *J. Assoc. Off. Agr. Chem.* **34**, 558(1951). A possible alternate AOAC method was investigated in which the extraction time was reduced to 4 hours and the solvent flow increased. Insufficient collaborative data have been reported to judge the method.

Report on fat in fish meal. M. E. Stansby (U. S. Dept. Interior, Seattle, Wash.). *J. Assoc. Off. Agr. Chem.* **34**, 549(1951). Preliminary results obtained on extraction of fish meal by boiling in acetone indicate that the oil is extracted rapidly by this method. Further work is planned.

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Avocado oil from Kenya. E. Brown and M. W. Jarvis. *Colonial Plant and Animal Products* 1, 318(1951). Avocado oil obtained from the pulp of the Fuerte variety grown in Kenya was found to be very similar to that obtained from California fruit as shown by the following constants: sp. gr., 0.9155; n^{20}, 1.4688; acid value, 1.1; saponification value, 191.8; unsaponifiable matter, 0.7%; iodine number (Wijs), 82.6; acetyl value, 6.3.

Chemical engineering in the meat-packing industry. M. D. Sanders, A. W. DeVout, P. Bradford and W. F. Bollens (Swift &