

Table V. Treatments Which Exceeded Both Sensory and Analytical Criteria

| solvent | °C | raffi- nose, % | stach- yose, % | residual urease activ., % | residual alcohol, ppm |
|----------------------------|----|----------------------|----------------------|------------------------------------|-----------------------------|
| 70.0% ethanol | 60 | 0.1 | 1.4 | 0 | 5 |
| 90.0% methanol | 30 | 0.7 | 1.3 | 0 | 6 |
| 70.0% isopropyl alcohol | 60 | 0.8 | 2.9 | 1 | 7 |

products; consequently, those treatments that are most effective in the removal of these oligosaccharides would produce the more acceptable products. The test for urease is often used as an index for adequate heat treatment in the inactivation of antinutritional factors. Since the yield and product quality appeared to be moving in opposite directions, yield was not considered in the selection of treatments, only insofar as to assess the amount of yield loss to be tolerated for a given improvement in product quality. Likewise, the degree of protein denaturation as measured by NSI was not included because this would vary with the intended end use and could not be evaluated until the use was specified.

Applying the above criteria, three of the six remaining treatments were eliminated (Table V), two because of inadequate extraction of raffinose and stachyose (92.7% ethanol at 60 and 75 °C) and one for lack of enzyme inactivation (70% ethanol at 30 °C). Of the three remaining, it is interesting to note that each of the three alcohols is represented. In the event that the emphasis was placed on a different set of objectives by the dictates of economics or other factors, then the priorities might be weighed differently and some of the eliminated treatments might be acceptable.

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Use of Urea as Clathrate in Separation of Wax from Sorghum Bran Extracts

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S Soxhlet extraction of sorghum bran to obtain miscella was performed with eight separate solvents (absolute alcohol, acetone, ethylene dichloride, methanol, methyl ethyl ketone, *n*-butyl ether, *sec*-butyl alcohol, and Skellysolve B) and two azeotropic mixtures (ethylene dichloride-heptane-water, volume ratio 2:2:1, and methyl ethyl ketone-heptane-water, volume ratio 2:2:1). A batchwise Pfaudler distillation unit using Skellysolve B as an extracting solvent was used to obtain a sufficient amount of miscella for subsequent separation of wax from oil in the miscella by the acetone precipitative extraction and the urea clathrate methods. The crude wax yield was increased about 50% by lowering the temperature from 6 to -8 °C in the acetone method. At room temperature, the urea clathrate method yielded more than twofold increase in the crude wax over the acetone method at -8 °C.

For better and fuller utilization of agricultural products, the extraction of economically valuable lipid materials from the sorghum bran has been studied. All the soluble material that is extracted from the bran is referred to as "miscella". The fraction of miscella that remains in the

solid state at room temperature after the separation is referred to as "wax" and the other fraction is the "oil". Since the sorghum wax resembles the commercially valuable Carnauba wax (Bunger and Kummerow, 1951) in many of its physical properties, the objective of the study was to develop an economically feasible process for the separation of the wax and oil fractions in the miscella; the desired process must recover the wax fraction more completely and in purer form, with the yield and quality of the oil fraction being of secondary importance. The first

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Table I. Solvents Used in Soxhlet Extractions and Their Sources and Boiling Points

| solvent | sources | grade | bp, °C |
|---------------------------|---|-----------|--------|
| absolute alcohol | Fisher Scientific Co., St. Louis, MO | technical | 78 |
| acetone | Fisher Scientific Co., St. Louis, MO | technical | 56 |
| ethylene dichloride | Fisher Scientific Co., St. Louis, MO | technical | 84 |
| heptane | Fisher Scientific Co., St. Louis, MO | technical | 98.4 |
| methanol | Fisher Scientific Co., St. Louis, MO | technical | 67 |
| methyl ethyl ketone | Missouri Solvent and Chemical Co., St. Louis, MO | technical | 80 |
| <i>n</i> -butyl ether | Eastman Kodak Co., Rochester, NY | reagent | 142.4 |
| <i>sec</i> -butyl alcohol | Eastman Kodak Co., Rochester, NY | reagent | 99.5 |
| Skellysolve B | Skelly Oil Co., Kansas City, MO | technical | 69 |

criterion is its selectivity of miscella extracting solvent; that is, not only must it be able to extract large amount of miscella, but furthermore it must extract wax in preference to oil.

MATERIALS

The bran used in the entire work was obtained from Grain Products Company, Dodge City, KS. Average moisture content was 10% by weight of bran dried under 100 mmHg vacuum at 100 °C for 6 h. Screen analysis after 15 min of shaking showed the size distribution by weight as follows: below 20 mesh, 11.06 ± 1.46 wt%; 20 ~ 30 mesh, 13.37 ± 1.87; 30 ~ 40 mesh, 25.42 ± 2.69; 40 ~ 50 mesh, 29.11 ± 2.01; 50 ~ 70 mesh, 15.83 ± 1.81; above 70 mesh, 5.21 ± 1.35.

The solvents used and their sources are listed in Table I. Urea used as a clathrate was obtained from Merck, Rahway, NJ, in a reagent grade which has a melting point of 132 ~ 133 °C.

EXPERIMENTAL SECTION

Soxhlet Extraction. At the start of each run, about 30 g of bran was carefully weighed and charged into a fresh extraction thimble. The bran was extracted with 250 mL of solvent for 8 h. Next the flask was transferred into a vacuum oven and maintained at 90 °C about 8 min to drive off the last traces of solvent from miscella. After cooling over calcium chloride for 30 min, the flask and its contents were weighed to determine the amount of miscella obtained.

Eight single solvents (absolute alcohol, acetone, ethylene dichloride, methanol, methyl ethyl ketone, *n*-butyl ether, *sec*-butyl alcohol, and Skellysolve B) and two azeotropic mixtures (ethylene dichloride-heptane-water, methyl ethyl ketone-heptane-water, both volume ratio 2:2:1) have been tried for Soxhlet extraction. A total of 16 extractions was made with each solvent or solvent mixture.

Separation of Wax from Oil by the Acetone Method. Twenty-five milliliters of acetone was added to 1 g of the molten miscella which was obtained from each Soxhlet extraction run. Then another 25 mL of acetone was added to wash down any miscella adhering to the inside surfaces of the flask. The flasks were stoppered and placed in a refrigerator at 6 °C for at least 16 h. Then solvent containing the oil fraction was filtered from the wax through an asbestos-lined Gooch crucible into an evaporating dish. Two additional 10-mL volumes of cold (6 °C) acetone was used to rinse the flask and wash the filter cake. Then the crucible was placed in a vacuum drying oven at 90 °C for about 5 min or until the wax

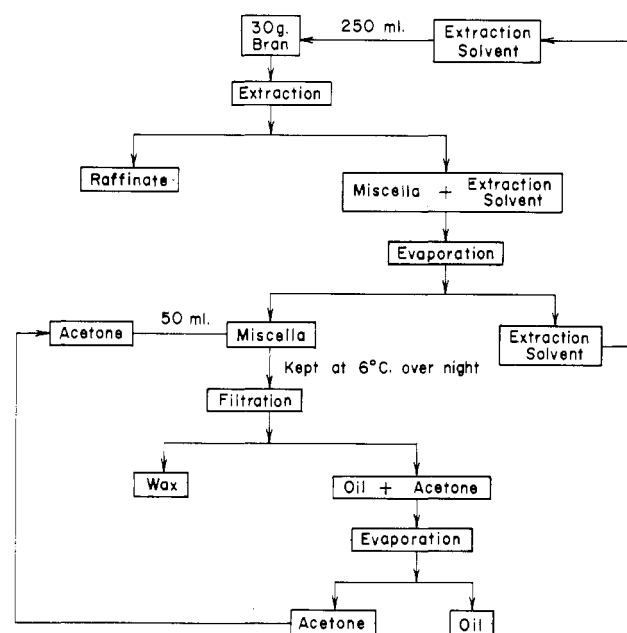


Figure 1. Schematic diagram illustrating the bench scale acetone method.

began to melt around the edge of the cake. After cooling for 30 min over calcium chloride, the crucible and wax were weighed.

The bulk of acetone in the evaporating dish was evaporated over a steam cone; the residual acetone and moisture were removed from the flask and evaporating dish by heating in a vacuum drying oven for 5 min at 90 °C. After cooling for 30 min over calcium chloride, the containers and contents were weighed. The wax content of a sample was determined from the weight in the crucible, while the oil was found in the evaporating dish. The procedure for this method is schematically shown in Figure 1.

Pfaudler Distillation Unit. In order to obtain a sufficient amount of miscella for the subsequent investigation of the separation of wax from oil, a large batchwise extraction was carried out with the Pfaudler distillation unit. It operates on the same principle as the bench scale, Pyrex glass Soxhlet extractor. Skellysolve B was used as the extracting solvent in all the runs.

In each batch, 9.1 kg (20 lb) of bran contained in a nylon cloth bag was placed in the basket extraction chamber and 75 L of Skellysolve B was charged into the steam-jacketed still pot. After about 8 h of operation, all the extract was

Table II. Summary of Results of Soxhlet Extractions and the Separation of Wax by the Acetone Method at 6 °C

| solvent | wt % of dry bran | | | wax/oil | wax mp range, °C | oil refractive index at 28 °C |
|---|---------------------------|-------------|-------------|-------------|------------------|-------------------------------|
| | miscella | wax | oil | | | |
| absolute alcohol | 12.93 (0.64) ^a | 7.86 (0.66) | 5.01 (0.43) | 1.59 (0.22) | 72 ~ 74 | 1.4652 |
| acetone | 8.56 (0.75) | 3.33 (0.37) | 5.31 (0.75) | 0.63 (0.14) | 73 ~ 76 | 1.4652 |
| ethylene dichloride | 7.35 (0.50) | 2.40 (0.21) | 4.94 (0.49) | 0.49 (0.08) | 82 ~ 84 | 1.4652 |
| methanol | 12.56 (0.58) | 7.32 (0.12) | 5.24 (0.57) | 1.41 (0.15) | 71 ~ 75 | 1.4652 |
| methyl ethyl ketone | 8.20 (0.53) | 2.83 (0.45) | 5.36 (0.37) | 0.53 (0.10) | 69 ~ 74 | 1.4661 |
| <i>n</i> -butyl ether | 8.88 (0.22) | 2.52 (0.08) | 6.34 (0.17) | 0.40 (0.01) | 61 ~ 65 | 1.4729 |
| <i>sec</i> -butyl alcohol | 10.15 (0.41) | 5.05 (0.40) | 5.12 (0.32) | 0.99 (0.13) | 72 ~ 76 | 1.4652 |
| Skellysolve B | 6.85 (0.54) | 2.76 (0.51) | 4.09 (0.31) | 0.72 (0.19) | 67 ~ 74 | 1.4698 |
| ethylene dichloride-heptane-water (2:1:1) | 7.08 (0.12) | 2.80 (0.18) | 4.28 (0.15) | 0.66 (0.06) | 63 ~ 69 | 1.4652 |
| methyl ethyl ketone-heptane-water (2:2:1) | 7.00 (0.17) | 2.88 (0.11) | 4.13 (0.15) | 0.70 (0.04) | 64 ~ 68 | 1.4652 |

^a The numbers in the parentheses are the standard deviation.

drained into the still pot and the receiver was disconnected from the rest of the still through proper valve arrangement for a by-pass line.

Then the solvent was distilled off as overhead and recovered, and the solvent-free miscella was withdrawn from the bottom of the still pot. The miscella was then placed on a steam cone to drive off the residual traces of solvent. In a typical run, the yields of miscella was about 850 g.

Temperature Effects in the Acetone Method for Wax Separation. Following the identical procedures described previously, 52 separation runs with 1 g of the molten miscella each were made using the same batch of miscella extract obtained from the Pfaudler distillation unit as the starting material. Twenty-six runs for the precipitative crystallization of wax from miscella using acetone as solvent were made at 6 °C and the rest at -8 °C.

Urea as Clathrate in Separation of Wax from Miscella. Fifty grams of urea were dissolved in 300 mL of about 50 °C methanol to prepare a saturated urea-methanol solution. Fifty milliliters of this saturated solution was mixed with the carefully weighed 1-g sample of molten miscella from the Pfaudler distillation unit. On standing, the crystalline complex separated quickly. After the mixture of miscella and urea-methanol solution reached room temperature, in order to complete the formation of the complex, the mixtures were kept in the refrigerator at 6 °C overnight. The complex precipitated and accumulated at the bottom of the flask. Then 250 mL of hot water (60 °C) was added to the cold complex mixture and stirred. As the complex formation was destroyed, the wax coalesced and floated on the surface of the liquid. The wax fraction was then filtered off with an asbestos-lined Gooch crucible mounted on a vacuum desiccator. The filtrate was boiled to release the oil fraction from the aqueous solution of urea and methanol. The oil fraction now floating on top of the solution was recovered by means of a separatory funnel. Fifty milliliters of methanol was used to wash the funnel and added to the oil fraction. It was found necessary to add a small amount of either methanol, acetone, or absolute alcohol to the oil to drive off the last traces of entrained water in the oil fraction by heating. Otherwise violent bumping or sputtering occurred toward the latter part of the evaporation period. The aqueous solution separated from the oil fraction was also evaporated to dryness to recover urea.

To check material balances, the weight of wax in the crucible was added to the weight of oil from the separatory funnel. If the combined weight did not approximate the weight of the starting miscella closely, the wax was again washed with hot (60 °C) water to make certain that all urea was released. The wax was allowed to air-dry for at least

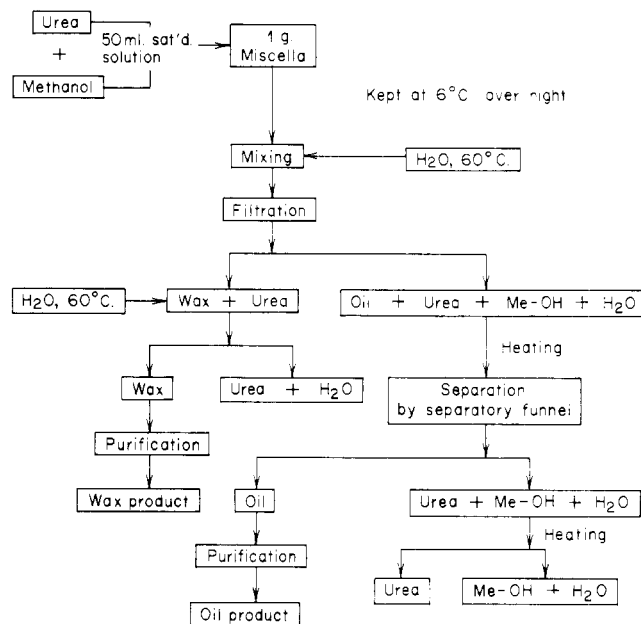


Figure 2. Schematic diagram illustrating the bench scale urea complex method.

24 h before weighing. A total of 26 runs were made. The procedure is schematically illustrated in Figure 2. Two additional separation runs were made with approximately 250 g of miscella. The same procedure as indicated previously was scaled up about 250 times from the 1-g sample size and followed.

Characterization of Extraction Fractions. The quality of the wax fraction was characterized by its melting point which was determined by the Fisher-Jones melting point apparatus, and the oil fraction by its refractive index which was measured with an Abbe refractometer at 28 °C.

RESULTS

The results of Soxhlet extraction runs with eight single solvents and two azeotropic mixtures and of the acetone precipitate method at 6 °C runs to separate the wax from oil were averaged and presented in Table II.

The largest amounts of miscella were obtained by using absolute alcohol and methanol as the extracting solvents and they also gave the highest weight ratio between wax and oil. The difference between these two extracting solvents is considered to be within the experimental error. The next high yield and the wax to oil ratio was obtained by using secondary butyl alcohol as the extracting solvent. From this observation, it may suggest that alcohols are good extracting solvents for the yields of miscella and of the wax and oil ratio. However, after cooling to room

Table III. Separation of Wax from Oil Using the Miscella Obtained by the Pfaudler Distillation Unit

| temp, °C | wt % of miscella | | wax/oil | mp, °C | refraction index at 28 °C |
|-------------|--|--------------|---------|---------|------------------------------|
| | wax | oil | | | |
| | Acetone Precipitative Crystallization Method | | | | |
| -8 | 26.43 (1.42) ^a | 73.57 (1.41) | 0.359 | 68 ~ 78 | 1.4672 |
| 6 | 16.78 (1.64) | 83.22 (1.64) | 0.200 | 67 ~ 74 | 1.4700 |
| | Urea Complex Method | | | | |
| room | 58.36 (0.50) | 41.64 (0.50) | 0.404 | 74 ~ 81 | 1.4661 |

Table IV.

| | wt, g | % based on total yield |
|-------------------|--------------------------------|------------------------|
| wax fraction | 150.2 | 58.2 |
| oil fraction | 108.2 | 41.8 |
| total yield | 258.4 | 100.0 |
| starting miscella | 269.9 | |
| wt loss | 11.5 or 4.28% of miscella used | |

temperature, some waxlike solid material was observed to float at the water-organic interface of the mixed solvents. The weight ratio between the wax and oil fraction was also the same as that obtained by single solvents, but the color of both fractions was a little darker. The color of the wax fraction from the Skellysolve B extract was grayish green and the oil fraction was dark green. Both wax and oil from the absolute alcohol and methanol extracts were deep tan. The products from all other solvents were tan.

The melting points of the wax fractions and the refractive indices of the oil fractions by separate Soxhlet solvent extractions are also listed in Table II. The higher melting points indicate better separations of wax from oil, and a narrow melting point range indicates the purity of the wax.

The miscella obtained by the Pfaudler distillation unit using Skellysolve B as an extracting solvent was dark green while in the molten state. Upon cooling to room temperature, it solidified into a light-green paste. This paste was used as the starting material in the subsequent studies on the temperature effect of the acetone method and the urea complex method in the separation of wax and oil from miscella. The results of the acetone precipitative crystallization method at two temperatures were obtained with their standard deviation and are summarized in Table III.

The wax fraction obtained by the urea complex method was light yellow and harder than that separated by the acetone method. The 26 runs of 1-g size batches were averaged and were also presented in Table III.

The results of two scaled-up runs using approximately 250 g of miscella were reproducible in larger batches which were scaled up about 250 times from the 1-g sample size. The results are presented in Table IV.

There was more than a twofold increase in the wax fraction yield when the urea complex method was used instead of the acetone method at -8 °C. The melting point also increased to 74 ~ 81 °C as compared to the melting point of the wax obtained by the acetone method which varied between 67 and 78 °C.

DISCUSSION

The urea complex method has not only an advantage of high wax yield over the acetone precipitative crystallization method, but also has several operational advantages that would make the urea complex method suitable for industrial process. The advantages of the acetone method are as follows: (1) it is necessary to have low temperature for crystallization; thus, the refrigeration cost could be considerable; (2) filtration of fine wax crystals would be difficult, since the size of crystals formed depends on the cooling rate during crystallization and the requirement for temperature control would be critical; and

(3) acetone is highly volatile and flammable; hence, the necessity of special provisions in handling and safety precautions would make the industrial process complicated. Furthermore, Sakurai (1954) reported that in the formation of urea complexes with fatty acid and methyl ester, respectively, in a methanol solution, no effects of agitation and temperature were observed. Therefore the refrigeration equipment and cost can be saved in the urea complex method.

It has been reported by Warth (1947) that Carnauba wax is composed of heptacosane, C₂₇H₅₆ (mp 59 °C), ceryl alcohol, C₂₆H₅₂OH [mp 76 °C (Weast, 1972: mp 80 °C)], carboceryl alcohol, C₂₇H₅₅OH (mp 80 ~ 82.5 °C), octacosanol, C₂₈H₅₇OH (mp 83.2 °C), triacontanol, C₃₀H₆₁OH [mp 86.4 ~ 86.8 °C (Weast, 1972: mp 88 °C)], 1-dotriacontanol, C₃₂H₆₅OH (mp 89.4 °C), dihydric alcohol, C₂₅H₅₀(OH)₂ (mp 103.5 ~ 103.8 °C), lignoceric acid, C₂₄H₄₈O₂ (mp 84.2 °C), cerotic acid, C₂₆H₅₂O₂ [mp 79 °C (Weast, 1972: mp 88 ~ 89 °C)], etc. Since the melting point of sorghum wax is slightly lower than that of Carnauba wax, the composition of sorghum wax may consist of more of those lower melting substances than Carnauba wax.

Urea is known to form channel inclusion compounds with many organic molecules: the urea host lattice is generally hexagonal (Fetterly, 1964), but some molecules (mainly cyclic) include the formation of a rhombohedral host lattice (Lenne, 1963) similar to that encountered in all channel-inclusion compounds of thiourea (Lenne, 1954). Coal liquids are known to contain a large amount of wax like materials known as asphaltines, which are mixtures of high-molecular-weight aromatic compounds. The urea clathrate method may be suggested as a method to remove those wax like materials in a coal liquification process, then thiourea may be a more effective separation agent than urea for the case.

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