Studies on the Crystal Growth of Rice Bran Wax in a Hexane Medium

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ABSTRACT: Rice bran oil (RBO) is well known for its high wax content (2–4%). A good separation of waxes must be ensured through the formation of crystals that can be removed with a minimal retention of oil to maintain high yields of refined oil. In the present study, the form and size distribution of RBO wax crystals were investigated using a laser diffraction technique. An attempt was made to study the effect of cooling on growth and size distribution of RBO wax crystals in hexane medium, and it showed that high cooling rate and low temperature induces the formation of a great number of small nuclei. In addition, experiments were performed to evaluate the effect on the growth of wax crystals of successive additions of gum and pure TG to the medium. The entire experiment was designed to optimize the temperature and incubation time of wax crystallization to facilitate the efficient separation of wax from crude RBO–hexane miscella using membrane technology.

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KEY WORDS: Crystal growth, crystal size, RBO wax.

Rice bran oil (RBO), which is high in free fatty acids (FFA), is also known for its high content of unsaponifiable matter, including waxes. These waxes are esters of long-chain saturated FA (C_{20}–C_{24}) and alcohols (C_{24}–C_{28}). They have low solubility in oil at low temperatures and therefore produce turbidity in the oil through crystallization. For the past several years, much attention has been given to developing the proper technology for dewaxing RBO as well as for reducing its total unsaponifiable matter quite significantly to enhance its use as an edible or cooking oil. The dewaxing process commonly used for this vegetable oil is winterization followed by centrifugation or filtration, which is usually done during the refining process. This winterization process is based on the crystallization of waxes present in the oil at a definite temperature (isothermal crystallization), and the overall crystallization process can be divided into two steps: nucleation and growth. Once the nuclei are formed, they grow and develop into crystals. Different processing factors, such as the cooling rate and crystallization temperature as well as the supersaturation of the system, may influence the crystallization behavior of the system (1,2). Moreover, the crystal size distribution is very important because the mode of particle transport and subsequent adhesion to the filter/membrane wall are strongly related to it. This information is also required to identify and model the mechanisms involved in the deposition of wax crystals in oil or miscella (3). Studying the influence of parameters such as temperature, cooling rate, and incubation time on wax crystallization is quite useful since it leads to efficient dewaxing of the studied oil, in this case, RBO.

RBO can be dewaxed in the miscella phase as well as in the oil phase, but because information on economic and infrastructure conditions in Indian industries is lacking, the choice between dewaxing in the miscella phase or the oil phase is still a matter of debate. Dewaxing in the miscella phase has the major advantage of incorporating a low amount of oil in the separated wax compared with dewaxing processes in the oil phase. Because we are working on the purification of RBO miscella by membrane technology, this study was conducted in the miscella phase. The original wax content of crude RBO varies from 2–4% (w/w). But in the miscella phase, with a 30% (wt/vol) concentration of oil, it is around 0.5–1.5% (wt/vol) in a hexane medium. Considering the average concentration of wax in RBO and the capacity of the instruments, we conducted the study with a 1% (wt/vol) solution of RBO wax in hexane, taking into account that for usual wax concentrations in RBO, the morphology of the crystals does not undergo great modification.

MATERIALS AND METHODS

Oil, gum, and wax samples. Crude RBO was collected from Sethia Oil Mill (Burdwan, West Bengal, India). All solvents used were of analytical reagent grade and were purchased from Merck India Ltd. (Mumbai, India).

Approximately 1 g of RBO waxes in pure form was isolated from about 50 g of crude RBO oil by an acetone-insoluble method followed by crystallization from isopropanol. Waxes were dissolved in hot isopropanol solvent (100 mL) and then separated by centrifuging at $3{,}000 \times g$ for 30 min at 5°C (4). RBO gum (approximately 1 g) was extracted from 100 g of crude oil by a water degumming method and was purified by repeated chloroform–methanol extraction (5) (using 50 mL of solvent in each extraction). Oil with no acetone-insoluble matter was obtained in the laboratory by successive extractions with acetone (200 mL of acetone was used to purify 50 mL of crude RBO), after which the oil was stored at 5°C for 10 d and then filtered through a Büchner filter (1). The purity of the samples was tested by TLC analysis (6).

Wax solutions. A 1% solution of pure RBO wax in hexane was prepared. A 100-mL quantity of hexane was initially filtered

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through a 0.1-μm cellulose nitrate membrane filter paper (Whatman, Maidstone, United Kingdom) to remove any dust and particulate matter. The solutions were first heated to 50°C to solubilize the waxes (1 g) and then stored at 0°C. Solutions were also prepared with RBO wax in hexane (100 mL) in the presence of RBO gum (0.1 g) and both RBO gum (0.1 g) and TG (30 g). Three sets of each solution were prepared and used for analysis. The viscosity of the sample was taken as that of hexane at that particular temperature, and the default refractive index value (1.52) was taken for analysis. The shape of the particle was considered to be spherical.

Crystal/particle size analysis method. A laser particle size analyzer (Zetasizer 1000HSA; Malvern Instruments Company, Malvern, United Kingdom) with a particle size analysis range of 0.003–3 μm that was equipped with a photon co-relation spectroscope was used for the study (7). The equipment used a helium–neon laser as the light source and allowed the detection of optically anisotropic wax crystals that developed in the sample. The sample was contained in a glass cell and placed on a sample holder, which was equipped with a thermocouple to control the temperature. The glass cell was filled with approximately 5 mL of wax solution and placed inside the instrument. The laser was fixed at 90° angles. Three consecutive particle size distribution analyses were performed, and before each analysis, the solution was heated to 50°C for at least 30 min to erase any previous thermal history. After the analysis of each sample, the cell was thoroughly rinsed with hot hexane, refilled with another sample, and placed back in the instrument. Because mass yield measurements could not be performed very rapidly, these data are not included in this text. After adjusting all the parameters, the sample cell was placed in the instrument and the target temperature was achieved within 5 min. Accordingly, data were taken after a definite time interval for the time study, and for the temperature study, the sample was held at that particular temperature for 1 h and the reading was then taken.

Reproducibility of the crystallization protocols. The results from two samples crystallized in different batches under identical conditions yielded virtually identical particle size distributions, with the mean particle diameter differing by only 0.1–0.2 mm. This confirmed that both the crystallization procedure and the particle size distribution method were reproducible.

RESULTS AND DISCUSSION

Nucleation and growth of wax. Pure RBO wax solutions were crystallized at 5, 10, 15, and 20°C. The size of the wax crystals seemed to attain a maximum at 10–15°C (Fig. 1). When the crystallization temperature was increased (e.g., for crystallization at 20°C), the driving force (in this case, the only driving force was the difference in chemical potential) was lowered and kinetic factors became the limiting step in the crystallization process. But when the experiment was performed at a low temperature and high viscosity, the wax molecules in the solutions migrated with difficulty, resulting in a higher induction time of crystallization (defined as the time at which the crystallized mass started to increase markedly), since they now had more difficulty in crossing the interface between the solid and liquid phases. This time included the time taken for the temperature in the glass cell to reach the target temperature of crystallization (approximately 5 min for this instrument).

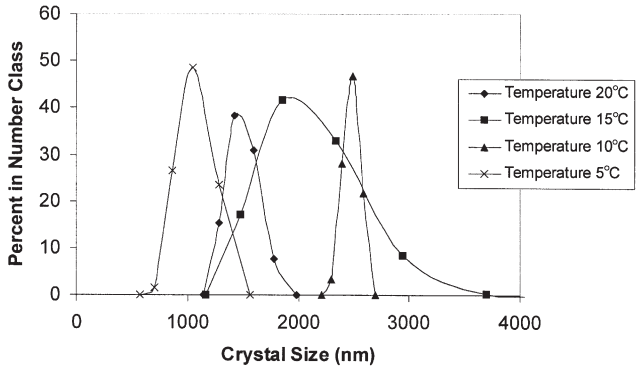

FIG. 1. Crystal size distribution of rice bran oil (RBO) wax (1% wt/vol) in hexane after cooling for 1 h at different temperatures.

Effect of the incubation period. The optimal incubation period could be taken as 60 min for all temperatures, as after this period no substantial increase in crystal size was observed (see Fig. 2, where a single temperature of 10°C is presented).

Effect of gum on the hexane solution of wax. When 1% (wt/vol) of RBO gum was added to the hexane solution of RBO wax, the crystals were significantly reduced in size, *viz.* from 3700 to 2200 nm max at 15°C, 2692 to 1617 nm max at 10°C, and 1550 to 900 nm max at 5°C (Fig. 3). This observation is in ageement with those reported by Morrison and Thomas (8). The trend in reduction of crystal size at all temperatures was nearly similar. The size distribution obtained at 20°C was nearly similar to the distribution obtained at 10°C.

Effect of TG and gum together on the hexane solution of wax. When 30% (wt/vol) RBO TG (with no acetone-insoluble matter) was added to the above solution, i.e., to the hexane solution of wax and gum (the usual concentration of oil in miscella

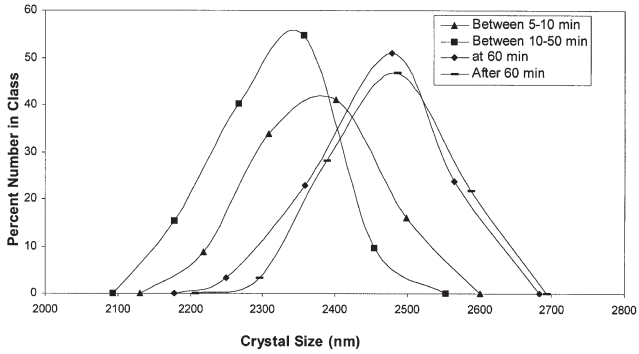

FIG. 2. Crystal size distribution of RBO wax (1% wt/vol) in hexane at a typical temperature of 10°C for a period of 1 h and more. For abbreviation see Figure 1.

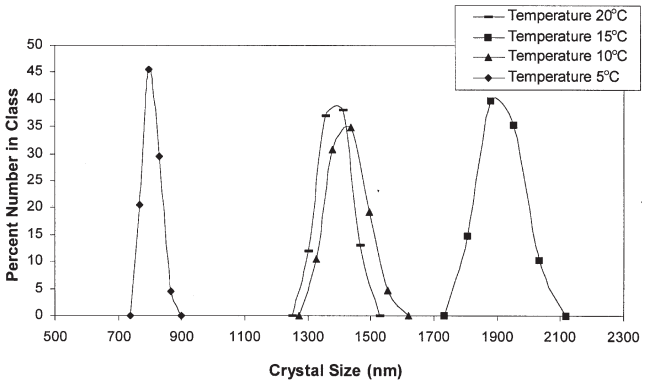

FIG. 3. Crystal size distribution of RBO wax + gum (1% wt/vol) in hexane after cooling for 1 h at different temperatures. For abbreviation see Figure 1.

obtained from RBO in a solvent extraction plant), the mean crystal size of the waxes increased slightly to a certain level at temperatures ranging from 5–20°C (Fig. 4). But in this case, the size distributions were very wide and the minimal and maximal values were almost identical or slightly lower than the size values obtained with the addition of gum alone. The reduction in mean size attributable to the presence of gum was probably partly neutralized here because of the hydrophobic nature of the TG. The differences in distribution at 20 and 15°C were negligible.

When the maximal modal size of wax crystals in the three different conditions was plotted against temperature (Fig. 5), we found that the maximal crystal size was obtained at temperatures between 10 and15°C; we also observed that pure wax in hexane medium produced the largest crystals. The inhibiting effect of gum was established in this study, as was reported previously by Petruccelli and Añón (9) for sunflower oil wax. The presence of TG partly nullified the inhibiting effect of gum, and in every case, we observed that at 5°C, the maximal number of smaller-sized crystals was formed.

From the foregoing observations, we concluded that in the case of RBO wax, low-temperature crystallization in a hexane medium facilitates the formation of a maximal number of

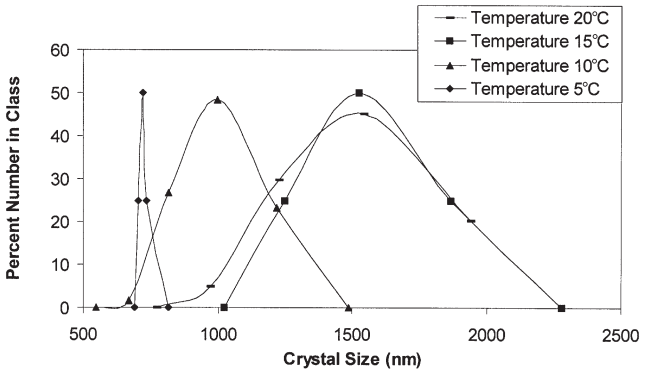

FIG. 4. Crystal size distribution of RBO wax in the presence of gum (1% wt/vol) and oil (30% wt/vol) in hexane after cooling for 1 h at different temperatures. For abbreviation see Figure 1.

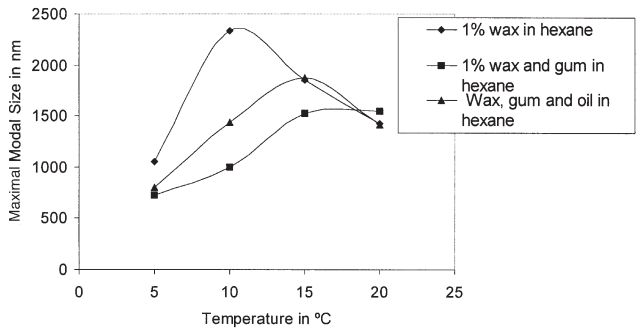

FIG. 5. Maximal modal size of RBO wax crystals at a temperature range of 5–20°C. For abbreviation see Figure 1.

smaller-sized crystals but that temperatures between 10 and 15°C produce crystals of an optimal size and number. The study will help to pre-fix the temperature and time of crystallization of RBO wax in a hexane medium to separate it from RBO–hexane miscella by a filtration process, by passing it through membrane, or by using a hermetically sealed, airtight, evaporation-proof centrifuge.

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