# EXPERIMENTAL BEHAVIOR OF CRUDE SOYBEAN OIL IN ALCOHOLIC SOLVENTS: MISCIBILITY TEMPERATURE AND CHILLING SEPARATION EFFECTIVENESS

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*Abstract*−− **Experimental information about the behavior of crude soybean oil with alcohol solvents, useful in the design of oil extraction and separation processes, is provided. The miscibility temperature of crude soybean oil in ethanol, isopropanol and their mixture (M) at different oil/alcohol ratios (OAR), and both the oil equilibrium distribution coefficients ($k_{eq}$) between formed phases and the amount of oil recovered (OR) from the chilled miscellas were determined. In the analyzed OAR range, the highest miscibility temperatures were 78, 40 and 55 °C for ethanol, isopropanol and M, respectively. Generally, OR and $k_{eq}$ changed significantly with the solvent, the chilling temperature and the OAR. By using ethanolic miscellas, a more effective separation was reached, being OR slightly changed with the chilling temperature. The validation of the chilling separation, performed using miscellas obtained from the ethanol extraction of soybean oil, demonstrated that other extracted compounds did not affect the separation yield.**

*Keywords*−− **Ethanol, isopropanol, phase separation, oil recovery, alcoholic miscella, soybean.**

## I. INTRODUCTION

In an oil extraction conventional process from soybean and other oilseeds, hexane is the solvent commonly used because of its low cost and high solubilizing power, presenting positive economic aspects and high oil yields. However, as it is a flammable petroleum-based solvent, it poses safety, environmental, and health risks (Rapinel *et al.*, 2020; Russin *et al.*, 2011). Concerns about hexane availability, tighter restrictions on emissions and higher safety requirements have renewed interest in the study of alternative solvents in the scientific sector, anticipating the future requirements of the industry. However, the shift to other solvents from renewable sources or with less environmental impact must be supported by knowledge not only of the new parameters of extraction and the expected oil quality, but also of the modifications required in pre- and post-treatment of the oilseed material, among other factors. As hexane is still used and preferred by the oil industry, some of this required information is very scarce in the open literature, with only unspecific and incomplete data obtained in a non-systematic way.

Among alternative solvents, alcohols such as ethanol and isopropanol (IPA) are promising options since they are safer and less toxic than hexane, and they can be produced from renewable resources. Many studies have demonstrated that both alcohols are effective in the extraction of soybean oil (Gandhi *et al.*, 2003; Lusas *et al.*, 1997; Sawada *et al.*, 2014; Seth *et al.*, 2007; Toda *et al.*, 2016), obtaining higher oil yield or needing lower extraction temperature when ethanol or IPA are used, respectively. IPA is more expensive than ethanol. So, considering the benefit that each one can provide to the extraction process and their cost, solvent mixtures could also be an option to combine their advantages (Singer and Deobald, 1945; Comerlatto *et al.*, 2021). However, there is very scarce and no updated or specific information published on this important subject. As an approach for oil extraction, recent studies tested ethanol-hexane mixtures (Phan *et al.*, 2021) and ethanol-IPA mixtures, where all the extracted material was considered as oil (Comerlatto *et al.*, 2021). Moreover and from an economic analysis, it was indicated that mixtures 1.2:0.8 ethanol:IPA or enriched in ethanol would be preferred when extraction is performed at 50 °C, but the optimal condition would depend on the process temperature (Comerlatto *et al.*, 2021). Nevertheless, alcohols present lower selectivity towards triacylglycerols than hexane, with other compounds being extracted together with the oil (Baümler *et al.*, 2016; Citeau *et al.*, 2018; Mabona *et al.*, 2014). This is not necessarily a disadvantage, but it must be taken into account to properly characterize the oil yield and the product quality and also to evaluate the required post-treatment. Different studies have indicated that oils and meals extracted using alcohols as solvents have identical or better quality compared to those obtained with hexane (Baümler *et al.*, 2016; Gandhi *et al.*, 2003; Sawada *et al.*, 2014; Seth *et al.*, 2007).

One of the disadvantages associated with alcohol solvents compared to hexane is the high energy requirement due to the low solubility of oil at low temperatures (T~60 °C, typical extraction temperature using hexane), forcing to operate during extraction at or near their boiling points, and the higher latent heat of vaporization (to be considered when the solvent is recovered by distillation). However, as the oil solubility in IPA and ethanol decreases with temperature, both alcohols could be partially removed from the miscella by chilling, obtaining two phases. The formation of these phases would also allow a partial deacidification of the oil (Lusas *et al.*, 1997). This aspect has been studied from a thermodynamic point

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of view using refined oils and ethanol (Chiyoda *et al.*, 2010; Mohsen-Nia *et al.*, 2008; Sanaiotti *et al.*, 2010), but information using crude oils is scarce. On the other hand, hexane can only be removed by evaporation without also removing any minor oil component in this step. A techno-economic-environmental analysis of the ethanolic soybean oil extraction was recently presented, but only distillation stages were considered for miscella treatment (Potrich *et al.*, 2020).

The oil composition of the formed phases depends on the oil solubility at the temperature to which the miscella is cooled down. Thus, knowledge of the solubility of crude oils in alcohol solvents is necessary to determine the feasibility of oil separation by chilling after extraction. In the literature there are reports about the solubility of refined soybean oil in ethanol and IPA (Gandhi *et al.*, 2003; Rao and Arnold, 1957). However, information about alcoholic miscellas with crude oils in these solvents is scarce and, in some cases, not even current (Lusas and Hernandez, 1997; Mabona *et al.*, 2014; Rao *et al.*, 1955). Thus, the works found in the literature where solubility data is required to perform more comprehensive analyses of the extractive process have used the data available for refined oils. Crude soybean oils contain phospholipids, a surface-active agent that could hinder the phase separation by chilling. According to some authors, after the ethanol oil extraction it would be possible to achieve a proper separation into an ethanol-rich phase (1-4% oil content) and an oil-rich phase (90-95% oil content) after cooling the miscellas at low temperatures (4-25 °C) (Alloco *et al.*, 2014; Bueno-Borges *et al.*, 2017). Very brief results reported in an old patent showed that the separation process was more efficient in the cooling step when ethanol was used instead of IPA (Singer and Deobald, 1945). Other works have found that when large amounts of water were added to refined soybean oil-ethanol systems, the oil miscibility was slightly affected by temperature (Chiyoda *et al.*, 2010). However, the use of water in large proportions is not recommended in systems with crude soybean oils due to their high phospholipid content, which forms oil-water emulsions, making an efficient phase separation difficult. On the other hand, the time required for an effective phase separation has not been properly reported. Lusas *et al.* (1997) predicted a separation time of 6-8 h when IPA miscellas were chilled at 5 °C. Singer and Deobald (1945) suggested a settling period of between 16 to 20 h when mixtures of ethanol and IPA were used as solvents and cooled below 27 °C. However, although it is well known that the use of centrifugal force reduces phase separation time, to the best of our knowledge no study conducted on crude oils in alcohol miscellas has compared centrifugal with static separation in terms of its effect on time, oil recovery and phase composition.

As it was mentioned, the necessary information to evaluate the use of alcoholic solvents in the extraction of soybean oil and subsequent separation from the miscellas is scarce. Therefore, this aspect greatly justifies the present work whose aim was to determine, in a systematic study: a) the miscibility temperature of crude soybean oil in ethanol, IPA and their intermediate mixture (M, 1:1 v/v) at different oil to alcohol ratios (OAR) in order to establish appropriate extraction conditions, b) the equilibrium distribution coefficients of oil between oil-rich and alcohol-rich phases after chilling, and c) the amount of oil recovered from the miscellas after phase separation. It is worth mentioning that M was selected as an initial alcohol mixture to test if using mixed solvents would provide additional advantages to be explored in any future work. To sum up, the validation of the chilling separation was performed using “real ethanolic miscellas” (obtained by soybean oil extraction with ethanol) using gravitational and centrifugal forces.

## II. MATERIALS AND METHODS

### A. Sample Preparation

Ethanol (Dorwil, water content <0.2%), isopropanol (Anedra, water content <0.2%) and their mixture (M, 1:1 v/v) were used as solvents. Crude soybean oil (from hexane extraction) and disintegrated soybean collets were supplied by a local factory (OMHSA, Argentina).

Alcohols and oil densities were determined pycnometrically (Cc 10c-95; AOCS, 2009). Mixtures of crude soybean oil and solvent, hereinafter referred to as “laboratory miscellas”, at different OAR (20:80, 40:60 and 60:40 v/v, corresponding to 23:77, 44:56 and 64:36 w/w, respectively) were prepared in cylindrical glass cells and used for the miscibility and separation efficiency experiments. For validation assays, “real miscellas” were obtained by alcohol extraction and used at optimal conditions previously determined (solvent and chilling temperature). Batch extractions from soybean collets were carried out at 60 °C using a solvent to solid ratio of 3:1 (w/w) for 5 h (Toda *et al.*, 2016). At the end of the extraction, the miscellas were separated from the meal by filtration. In order to compare real and laboratory miscellas with similar compositions, some of the obtained real miscellas were concentrated by partial solvent evaporation until reaching an oil composition close to the lower level used in the previous assays (when crude soybean oil from the hexane extraction was used). Immediately after the miscellas were obtained, samples were collected to determine the oil composition and the content of other compounds extracted together with the oil (hereafter called defatted extract (DE)).

### B. Sample characterization

*Soybean Oil:* Standard AOCS (2009) and IUPAC (1992) methods were used to determine moisture (AOCS Ca 2d-55), total phosphorus (P) content (AOCS Ca 19-86 and Ca 12-55), peroxide value (AOCS Cd 8-53) and free fatty acid content (IUPAC 2.201). The phospholipid (PL) content was estimated from the P content using a conversion factor of 25 (Chapman, 1980; Dijstra, 2019).

*Soybean collets:* Their moisture and oil contents were determined according to IUPAC (1992) method 1.121 and 1.122, respectively. The particle size of the disintegrated collets was determined by digital image analysis using the software ImageJ® (Rasband, 1997) and expressed as the mean Feret diameter.

*Real miscellas:* Miscellas obtained by alcohol extraction were fractionated into two phases by hexane-washing and centrifugation: i) a hexane-soluble fraction containing the oil, and ii) a hexane-insoluble fraction containing the rest of the extracted compounds (DE). Solvent was removed from phases and oil and DE contents were quantified gravimetrically.

### C. Optical determinations of solubility

The solubility of the oil/alcohol solutions was optically determined using a vertical-scan macroscopic analyzer (TurbiScan Classic, Formulation Inc., USA). The TurbiScan analyzer has a reading head, consisting of a pulsed near infrared light source and two synchronous detectors (backscattering (BS) and transmission (T)), which move upward along a cell while scanning the whole sample height (acquiring data every 40 µm). The percentage of BS or T light was represented as a function of the sample height. The sample was considered stable when overlapping profiles were obtained after successive scanning. Additionally, a constant signal throughout the run indicates that the sample is homogeneous.

Flat-bottomed cells containing laboratory miscellas were subjected to increasing temperatures and mechanical agitation until a single phase was observed. Then, the stirring was stopped and the mixture was maintained at that temperature and scanned every 5 min for 30 min using the TurbiScan analyzer. The operating temperature was considered the miscibility temperature (Tm) when the solution was homogeneous and stable. If not, higher temperatures were tested until reaching those two conditions.

### D. Separation by chilling

Laboratory miscellas were prepared in cylindrical glass cells, heated at the corresponding Tm previously determined, and then vigorously agitated. Then, the mixtures were allowed to cool at two selected temperatures, 20 °C and 6 °C (Grimsby, 1984; Rao and Arnold, 1957; Rao *et al.*, 1955) for 44 h after those temperatures were reached, obtaining the separation of two phases by gravity (static condition): an oil-rich phase (lower phase, OP) and an alcohol-rich phase (upper phase, AP). Simultaneously, the miscellas were monitored optically using the TurbiScan equipment to determine the minimum time at constant temperature required to reach phase movement equilibrium. The stabilization of the phase separation was evaluated considering the changes of the mean %T values as a function of time at the OP zone of the glass tube.

*Oil distribution at equilibrium:* After reaching the equilibrium at 20 °C or 6 °C, samples of both phases were collected separately using pipettes to determine their oil composition. The oil content was determined by solvent evaporation. The distribution coefficients of oil at equilibrium ($\boldsymbol{k_{eq}}$) were calculated as follows:

$$k_{eq} = \frac{w_{Oil}^{OP}}{w_{Oil}^{AP}}, \qquad (1)$$

where $\boldsymbol{w}$ represents the mass fraction, and the superscript OP and AP represent the corresponding phases.

The oil recovery ($OR$) was calculated in order to determine the effectiveness of the separation. It was defined as the fraction of oil content in the original miscella ($OM$) recovered in $OP$, as follows:

$$OR(\%) = \frac{Amount\ of\ oil\ in\ OP(g)}{Total\ amount\ of\ oil\ in\ OM\ (g)} \times 100. \qquad (2)$$

### E. Validation of chilling separation

All validation assays were carried out with real miscellas using two oil compositions (a low-oil content and a concentrated miscella) at the temperature selected according to the experimental results. Experimental conditions were the same as those mentioned above, unless otherwise specified.

When working with real miscella, the temperature decrease contributed to the formation of a third phase (similar to a gum) between the OP and AP, hindering OP sampling. Therefore, AP was collected and the amount of oil recovered by chilling from the OM was calculated as the difference between the total oil content in the OM and the oil content in AP. The OR value reported in this case is the result of the oil present in both the third phase and the OP. In order to partially characterize the formed third phase and compare it with an industrial gum, its phosphorus content was determined and from this, its acetone insoluble matter (AI) value was calculated (List *et al.*, 1978). On the other hand, as the AP would also contain other compounds extracted together with the oil when the extraction solvent is more polar than hexane, the content of DE in AP samples was quantified.

Finally, in order to evaluate the retention of the other compounds extracted together with the oil in the AP, the defatted extract recovery ($DER$) was calculated as:

$$DER(\%) = \frac{Amount\ of\ DE\ in\ AP\ (g)}{Total\ amount\ of\ DE\ in\ OM\ (g)} \times 100. \qquad (3)$$

The results obtained at experimental equilibrium times (static separation) were compared to those obtained after 30 min of centrifugation at 1800 g (Avanti J-26XP, Beckman Coulter, USA) at 20 °C.

### F. Statistical analysis

All assays were performed at least in triplicate ($n = 3$), except in the case of the validation experiments performed with concentrated miscellas ($n = 2$). Except for equilibrium separation time, data were expressed as the mean ± standard deviation and statistically analyzed by analysis of variance using Infostat software (Di Rienzo *et al.*, 2011). Fisher's LSD method was used ($p \leq 0.05$). The quality of the experimental phase-equilibrium results was tested using the mass values of the phases obtained by a least-squares fitting of the corresponding set of component mass balances, and calculating the error between the calculated and the experimental total mass in the system (Marcilla *et al.*, 1995).

## III. RESULTS AND DISCUSSION

The characterization of the industrially obtained crude soybean oil gives the following results: free fatty acid value 1.04 ± 0.01%, peroxide value 0.98 ± 0.01, P content 1143.0 ± 30.4 mg/kg oil (by the colorimetric method) and 1156.9 ± 36.2 mg/kg oil (by the nephelometric method), PL content 28.6 ± 1.1 g/kg oil, moisture 0.15 ± 0.02%

Table 1: Miscibility temperature data for alcoholic miscellas at different oil concentrations.

| | **Miscibility temperature, Tm (°C)** | | |
|---|---|---|---|
| **$w_{oil}^{OM}$ (%)** | Ethanol | IPA | M |
| **64** | $75\pm1^{aB}$ | $30\pm1^{cB}$ | $50\pm1^{bB}$ |
| **44** | $78\pm1^{aA}$ | $30\pm1^{cB}$ | $55\pm1^{bA}$ |
| **23** | $75\pm1^{aB}$ | $40\pm1^{cA}$ | $55\pm1^{bA}$ |

w: mass fraction; OM: original miscella; IPA: isopropanol; M: Ethanol-IPA mixture 1:1 (v/v). Values with the same letter are not significantly different ($p > 0.05$). Lower case letters: comparison for a specific oil concentration miscella using different alcoholic solvents. Capital letters: comparison for a specific alcoholic miscella at different oil concentrations.

wb, and density 0.9202 ± 1.9 $10^{-4}$ g/mL (at 25 °C). It can be observed that the nephelometric method used to determine the P content, being a rapid procedure, has good accuracy compared with the conventional method. The industrially-obtained disintegrated collets presented the following characteristics: moisture 8.24 ± 0.04% db, oil content 22.55 ± 0.04% db, and mean Feret particle diameter 9.95 $10^{-2}$ ± 1.48 $10^{-2}$ cm.

### A. Solubility data

The temperatures needed to reach optically-stable unique phases (Tm) using different alcohol solvents and compositions are shown in Table 1.

For all the tested alcohol solvents, Tm changed due to oil composition. In general, the lowest Tm was obtained at the highest tested oil composition. This means that a low alcohol composition in the system is easier to keep in a unique phase than a higher alcohol composition. Moreover, a higher oil composition is related to a higher PL composition (amphiphilic compounds) in those systems, so phase stability could be reached at lower temperatures.

However, ethanol showed maximal Tm in the intermediate composition range. This unexpected value was checked by determining the oil composition in the solution (from the top and the bottom of the cell). A good agreement between the results was found since at 78 ± 1 ºC a unique phase (with similar oil composition than the original prepared miscella) was reached. However, two phases of different oil composition were obtained at lower temperatures (e.g. at T=75 ± 1 ºC, oil composition of OM and OP were 43.47 and 51.80 w%, respectively). This behavior could be related to a more unstable transition zone (intermediate oil composition miscella) between an ethanol-rich system (low oil composition miscella) and an oil-rich system, and so, requiring a higher temperature to get the condition of unique phase stability. A similar behavior was also reported by Magne and Skau (1953) for soybean and cottonseed oil ethanolic miscellas. Other authors have reported values near 70 ºC when crude (Rao *et al.*, 1955) and refined soybean oil-ethanol mixtures were used at high oil compositions (≥50%) (Follegatti-Romero *et al.*, 2010; Gandhi *et al.*, 2003). For the soybean oil-IPA miscellas, the highest Tm was observed at the lowest oil composition of the analyzed range, without exhibiting the previously mentioned unstable condition at the intermediate concentration. This difference could be related to the dissimilar solubilities of crude oil components in each tested alcohol. Results obtained at higher oil compositions in IPA miscellas are in accordance with previously reported data (Rao and Arnold, 1957). When hydrated IPA was used to form miscellas with soybean oil, and high oil compositions miscellas were tested, Tm near 40 ºC were reported (Lusas and Hernandez, 1997). Soybean oil-M systems showed Tm between those obtained for miscellas of each alcohol without distinguishing an intermediate unstable condition. This seems to show that the effect of IPA is greater than that of ethanol or at least it succeeded in avoiding the instability. No data about Tm have been found in the literature for these systems.

### B. Separation by chilling

The process of phase separation was monitored after reaching the established separation temperature (20 or 6 ºC), when two turbid phases were observed. Figure 1 shows representative profiles of %T along the tube height at different times (at 20 ºC) until the sample was stable. There can be observed how the phases %T was changing as the time proceeded. The %T of OP (lower phase) shows the highest differences increasing from very low and unstable values (related to the alcohol upward movement in an oil-rich phase) until almost constant values (homogeneous phase) close to 100%.

On the other hand, oil downward movement in AP (upper phase) did not show so big differences in its %T; however, some changes in its height (increment, at the expense of the diminution of an intermediate zone, IZ) were evidenced. It is worth mentioning that ethanolic miscellas showed the widest IZ (Fig. 1a), followed by those for ethanol-IPA miscellas (Fig. 1b) and IPA miscellas (Fig. 1c). This behavior could rise from the dissimilar solubilities of different crude oil components in each alcoholic system.

Figures 2 and 3 show the evolution of mean %T of OP over time for the laboratory miscellas at 20 °C and 6 °C, respectively. Generally, experimental data from approximately 6 h to 19 h were not obtained due to practical limitations (non-automatic measurements). However, an unexpected behavior was observed for OAR2 ethanol-IPA miscella; so, more data were obtained at intermediate time for this miscella (in an independent assay) confirming the previously obtained behavior. In almost all the miscellas at 20 °C (with the exception of OAR1 for IPA miscellas) (Fig. 2), that behavior could be represented with an exponential model ($\%T = a \times (1 - e^{-bt})$, where a and b are constant coefficients and the variable $t$ is the separation time) obtained by fitting the experimental data using SigmaPlot software (v.11.0, Systat Software, 2008), being all high significative regressions ($p<0.0001$, adjusted coefficient of determination $>0.87$). Taking into account that a 0.01 value of the exponential model derivative is close enough to zero to consider the equilibrium condition was achieved, the required times to reach that condition (equilibrium separation time) were estimated. In the case of OAR1 for IPA miscellas at 20 ºC, from 358 min no statistical differences

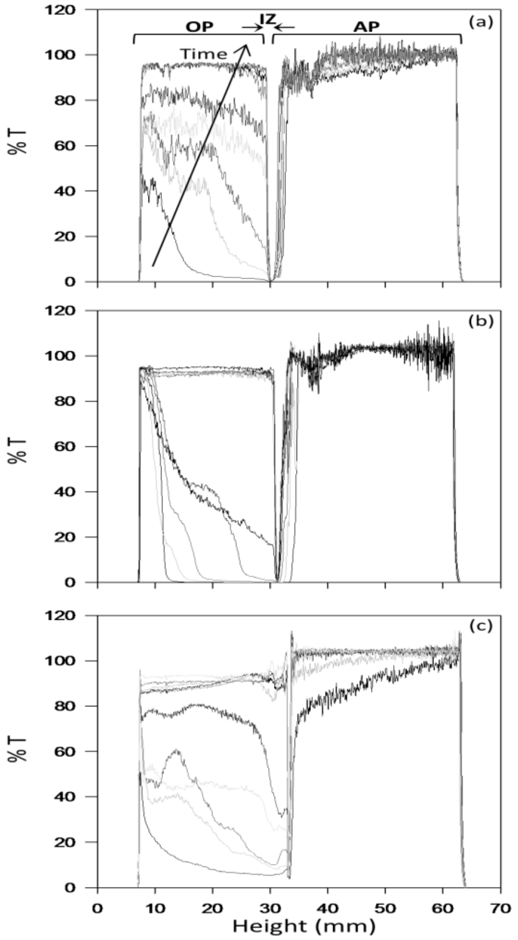

Figure 1. Percentage transmission values (%T) along the tube height at different times for the laboratory miscellas at 40:60 oil to alcohol volumetric ratio (20 °C): (a) Ethanol; (b) Ethanol-Isopropanol mixtures 1:1 (v/v); (c) Isopropanol. AP: alcohol-rich phase; OP: oil-rich phase; IZ: intermediate zone.

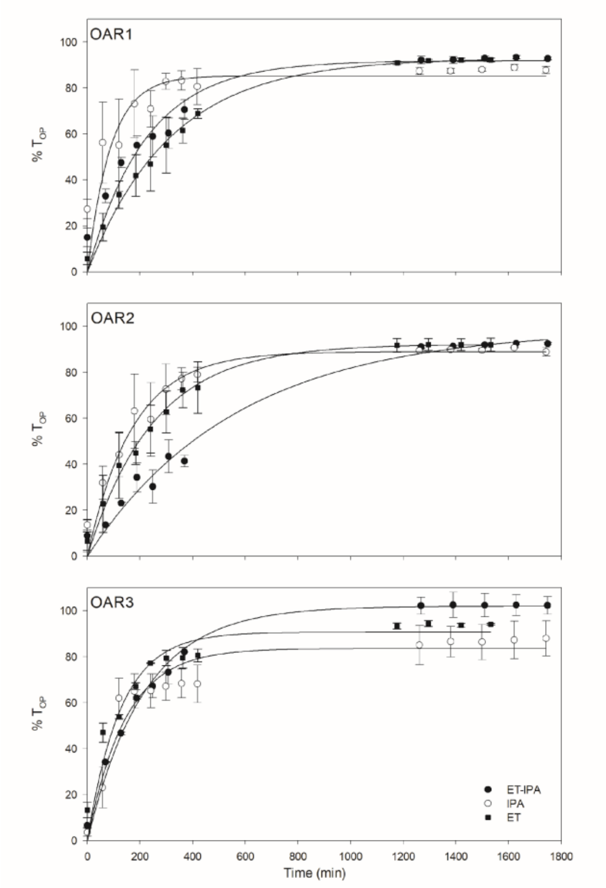

Figure 2. Mean percentage transmission values (%T) as a function of time at the oil-rich phase (OP) for the laboratory miscellas at different oil to alcohol volumetric ratio, OAR (1, 60:40; 2, 40:60; 3: 20:80) (20 °C). ET: ethanol; IPA: isopropanol.

Table 2: Equilibrium separation time at 20 ºC for alcoholic miscellas at different oil concentrations.

| | Equilibrium separation time (min) | | |
|---|---|---|---|
| $w_{oil}^{OM}$ (%) | Ethanol | IPA | M |
| **64** | 1050 | 358 | 830 |
| **44** | 890 | 690 | 1400 |
| **23** | 540 | 610 | 790 |

w: mass fraction; OM: original miscella; IPA: isopropanol; M: Ethanol-IPA mixture 1:1 (v/v).

were found between experimental %T; so, this was considered its equilibrium time.

Table 2 reports the obtained equilibrium times at 20 ºC. The values augmented with the oil composition in the original miscellas when ethanol was used as solvent, showing an increasing difficulty in component separation when oil is the major component. This could be related to the fact that ethanol droplets dispersed in oil would be more stable probably due to the distribution of PL between phases. Wu and Wang (2004) showed that PL fractionation from soybean lecithin using ethanol changed with both the lecithin oil content and the ethanol to crude lecithin ratio. When IPA was the solvent, the equilibrium time was lower at the highest tested OAR (OAR1) and slightly different for the other two OAR. This behavior was expected due to the higher solubility of oil in IPA than in ethanol (Gandhi *et al*., 2003), which would imply a lower separation of oil in globules. Additionally, An *et al.* (2001) showed that the lecithin/IPA ratio affects the phosphatidylcholine (PC) solubility. When the ethanol-IPA mixtures were used, the low and intermediate oil composition miscellas seemed to follow the ethanolic miscellas behavior (increasing the equilibrium time with oil composition), but showing greater difficulty to separate the components (requiring more time than ethanolic miscellas to reach equilibrium). This effect was probably due to the more affinity of oily components with IPA present in the alcoholic mixture. However, at high oil composition in ethanol-IPA miscella, the equilibrium time strongly diminished letting to think in a higher effect of the crude oil/alcohol ratio than the ethanol influence. Patil *et al.* (2010) studied the solvent fractionation of PC from soybean lecithin using ethanol and ethanol-IPA mixtures, between others. They suggested that the different overall polarity provided by the ethanol-IPA combination was responsible for the change in the PC solubility.

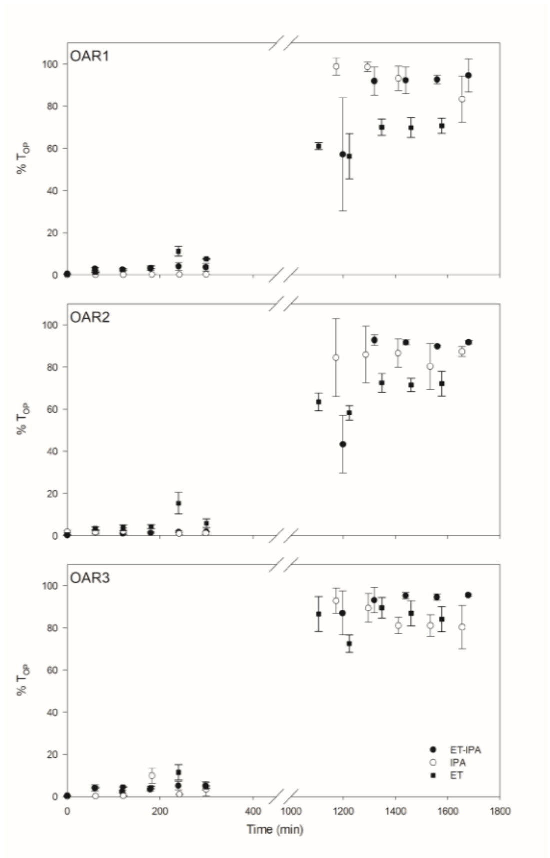

Figure 3. Mean percentage transmission values (%T) as a function of time at the oil-rich phase (OP) for the laboratory miscellas at different oil to alcohol volumetric ratio, OAR (1, 60:40; 2, 40:60; 3: 20:80) (6 °C). ET: ethanol; IPA: isopropanol.

Consequently, being PL the emulsifier in the present study, the expected change in their distribution when different solvents are used could be affecting the phase separation. Singer and Deobald (1945) reported a separation time between 16 and 20 h when a miscella from soybean oil extraction using an ethanol:IPA mixture of 75:25 was cooled below 27 ºC.

At a chilling temperature of 6 °C (Fig. 3), the observed experimental behavior could not be satisfactorily represented by the exponential model; so, intermediate data were not predicted. Statistical differences over time were obtained between data of the last period (>19 h). So, the required time to reach the equilibrium condition at 6 ºC was longer for almost all the analyzed cases (>20 h). The exceptions were IPA miscellas, which reached equilibrium before 19 h (in the zone without experimental information). Lusas *et al.* (1997) predicted a separation time of 6-8 h when cottonseed oil-IPA miscellas were chilled at 5 °C. As a whole and in accordance with Xu *et al.* (2001), OP and AP stability would depend not only on oil solubility but also on emulsifier distribution and alcohol content.

The quality of the phase-equilibrium experimental results was checked (errors between the calculated and the experimental total mass in the systems were always <0.2%). Thus, all experimental data of the phase composition were used to calculate $k_{eq}$ and OR (Table 3).

Values of $k_{eq}$ and OR changed significantly depending on the solvent used, the chilling temperature, and the initial oil miscella composition ($p<0.05$), except in the case of $k_{eq}$ for IPA at 20 °C. An increase in OP oil fraction, $k_{eq}$ and OR values, and a diminution in AP oil fraction were observed when the miscellas were cooled down to 6 °C, compared to those obtained at 20 °C. Only for high oil composition miscellas was OR not affected by the solvent used at 20 °C, or by the chilling temperature when ethanol and the ethanol-IPA mixture were used as solvents. It is worth mentioning that although similar OR could be obtained under the aforementioned conditions, OP with different oil mass fraction were obtained in all cases. Therefore, a better separation was reached when ethanolic miscellas were used. For diluted miscellas cooled at 20 °C, the results also suggest that using ethanolic miscellas (OR = 81.92 ± 1.07%) allows to obtain a more effective separation than using IPA miscellas (OR = 41.76 ± 1.22%) or ethanol-IPA miscellas (OR = 74.86 ± 1.68%). These findings could be related to the inability of the AP to retain more oil due to its oil saturation including differences in PL distribution, especially when ethanol is present in the solvent. Since the oil composition in the studied diluted miscella would be closer to the range of values expected in an industrial extraction operation (Bessa *et al.*, 2017), it would be possible to think that this methodology could be applied needing slightly additional evaporation. These results are in good agreement with reported data for ethanol and IPA miscellas (Singer and Deobald, 1945). Additionally, $k_{eq}$ data maintained a linear trend ($p<0.005$) with the studied oil composition for the original miscella.

### C. Validation

According to the obtained results, ethanol as extraction solvent and 20 °C as separation temperature were considered the optimal conditions (lower temperature slightly improved the separation but would highly affect the energy requirements). Therefore, the behavior of real miscellas was evaluated under these optimal conditions. It is worth mentioning that these real miscellas would have a maximum water content of 0.05% (contributed by collets). The soybean oil obtained by ethanol extraction exhibited a higher PL content than the oil used in the first experiments, being their P content 1506.77 ± 212.73 and 1064.43 ± 33.32 mg/L oil, respectively. The DE obtained together with the oil also presented PL: 0.76 ± 0.01%P, from which a PL content of 19 ± 0.49% was estimated (Chapman, 1980). Table 4 presents the experimental data obtained for the chilling separation of the real ethanolic miscellas at 20 °C. It was observed that both the oil mass fraction in AP and the OR obtained from concentrated real miscellas (16% oil mass fraction in OM, $w_{oil}^{OM}$) were similar to those obtained from laboratory ethanolic miscellas ($w_{oil}^{OM}$ = 23%).

Moreover, the third phase obtained from this miscella had 8.25 ± 0.83 mg P/g phase db (21% PL or 26% AI), demonstrating that it contained not only phospholipids but also other compounds as they could be occluded oil and carbohydrates (Baümler *et al.*, 2016).

Table 3: Chilling separation data for alcoholic miscellas at different oil concentrations, at 20 and 6 °C.

| **$w_{oil}^{OM}$ (%)** | **$w_{oil}^{OP}$ (%)** | **$w_{oil}^{AP}$ (%)** | **$k_{eq}$** | **OR** | **$w_{oil}^{OP}$ (%)** | **$w_{oil}^{AP}$ (%)** | **$k_{eq}$** | **OR** |
|---|---|---|---|---|---|---|---|---|
| | **20 °C** | | | | **6 °C** | | | |
| | | | | Ethanol | | | | |
| **64** | $87.83\pm0.48^{b\alpha A}$ | $7.75\pm0.54^{a\alpha C}$ | $11.36\pm0.86^{b\gamma A}$ | $96.33\pm0.23^{a\alpha A}$ | $90.29\pm0.62^{a\alpha A}$ | $6.54\pm0.26^{b\alpha C}$ | $13.83\pm0.61^{a\gamma A}$ | $96.74\pm0.13^{a\alpha A}$ |
| **44** | $86.34\pm0.30^{b\alpha A}$ | $6.55\pm0.10^{a\beta C}$ | $13.17\pm0.16^{b\beta A}$ | $92.02\pm0.15^{b\beta A}$ | $89.23\pm0.19^{a\alpha\beta A}$ | $5.04\pm0.04^{b\beta C}$ | $17.69\pm0.17^{a\beta A}$ | $93.79\pm0.04^{a\beta A}$ |
| **23** | $82.64\pm5.91^{a\alpha A}$ | $5.09\pm0.18^{a\gamma C}$ | $16.22\pm0.61^{b\alpha A}$ | $81.92\pm1.07^{b\gamma A}$ | $86.86\pm1.55^{a\beta A}$ | $3.86\pm0.03^{b\gamma C}$ | $22.51\pm0.31^{a\alpha A}$ | $86.30\pm0.17^{a\gamma A}$ |
| | | | | IPA | | | | |
| **64** | $69.86\pm0.72^{b\alpha C}$ | $17.98\pm1.11^{a\alpha A}$ | $3.89\pm0.27^{b\beta C}$ | $96.72\pm0.15^{b\alpha A}$ | $80.20\pm0.22^{a\alpha C}$ | $10.07\pm0.14^{b\alpha A}$ | $7.96\pm0.10^{a\gamma C}$ | $96.31\pm0.09^{a\alpha C}$ |
| **44** | $69.21\pm0.19^{b\alpha C}$ | $16.65\pm0.24^{a\alpha\beta A}$ | $4.16\pm0.07^{b\alpha\beta C}$ | $81.73\pm0.28^{b\beta C}$ | $80.52\pm0.22^{a\alpha C}$ | $9.15\pm0.03^{b\beta A}$ | $8.80\pm0.05^{a\beta C}$ | $89.30\pm0.01^{a\beta C}$ |
| **23** | $67.80\pm0.51^{b\beta B}$ | $15.41\pm0.23^{a\beta A}$ | $4.40\pm0.04^{b\alpha B}$ | $41.76\pm1.22^{b\gamma C}$ | $77.49\pm1.18^{a\beta B}$ | $7.82\pm0.17^{b\gamma A}$ | $9.91\pm0.32^{a\alpha C}$ | $72.99\pm0.59^{a\gamma C}$ |
| | | | | Ethanol-IPA mixture 1:1 (v/v) | | | | |
| **64** | $80.55\pm4.03^{a\alpha B}$ | $9.56\pm0.03^{a\alpha B}$ | $8.43\pm0.41^{b\gamma B}$ | $96.48\pm0.68^{a\alpha A}$ | $86.67\pm0.25^{a\alpha B}$ | $7.71\pm0.12^{b\alpha B}$ | $11.24\pm0.19^{a\gamma B}$ | $96.49\pm0.04^{a\alpha B}$ |
| **44** | $82.04\pm0.53^{b\alpha B}$ | $8.40\pm0.23^{a\beta B}$ | $9.77\pm0.25^{b\beta B}$ | $90.09\pm0.32^{b\beta B}$ | $85.94\pm0.12^{a\alpha\beta B}$ | $6.60\pm0.11^{b\beta B}$ | $13.01\pm0.24^{a\beta B}$ | $92.03\pm0.14^{a\beta B}$ |
| **23** | $76.49\pm3.10^{b\alpha A}$ | $7.33\pm0.37^{a\gamma B}$ | $10.44\pm0.27^{b\alpha C}$ | $74.86\pm1.68^{b\gamma B}$ | $84.16\pm2.00^{a\beta A}$ | $5.43\pm0.11^{b\gamma B}$ | $15.51\pm0.39^{a\alpha B}$ | $81.33\pm0.46^{a\gamma B}$ |

w: mass fraction; OM: original miscella; OP: oil-rich phase; AP: alcohol-rich phase; $k_{eq}$: equilibrium distribution coefficients of oil between oil-rich and alcohol-rich phases; OR: oil recovery; IPA: isopropanol. Solvent mass fraction (%) in OM, OP and AP results from subtracting to 100 the corresponding $w_{oil}$.

Values with the same letter are not significantly different ($p > 0.05$) by the LSD Fisher method. Lower case letters: comparison for a specific alcoholic miscella at different temperatures. Greek letters: comparison for a specific alcoholic miscella at different oil concentrations. Capital letters: comparison for a specific oil concentration miscella and temperature using different alcoholic solvents.

Table 4: Chilling separation data at 20 °C for miscellas obtained by ethanolic extraction.

| **$w_{oil}^{OM}$ (%)** | **$w_{DE}^{OM}$ (%)** | **$w_{oil}^{AP}$ (%)** | **$w_{DE}^{AP}$ (%)** | **OR** | **DER** | **$w_{oil}^{AP}$ (%)** | **$w_{DE}^{AP}$ (%)** | **OR** | **DER** |
|---|---|---|---|---|---|---|---|---|---|
| | | **44 h, GF** | | | | **1800 g, 30 min, CF** | | | |
| 7 | ND | $3.11 \pm 0.21^{b}$ | $1.17 \pm 0.08^{b}$ | $56.91 \pm 4.07^{b}$ | ND | $3.17 \pm 0.21^{b}$ | $1.21 \pm 0.05^{b}$ | $53.45 \pm 3.74^{b}$ | ND |
| 16 | 2.82 | $4.26 \pm 0.27^{a}$ | $2.85 \pm 0.03^{a}$ | $78.99 \pm 0.51^{a}$ | $80.12 \pm 0.40^{a}$ | $4.27 \pm 0.03^{a}$ | $2.88 \pm 0.10^{a}$ | $78.17 \pm 0.07^{a}$ | $82.17 \pm 1.61^{a}$ |

w: mass fraction; OM: original miscella; AP: alcohol-rich phase; DE: defatted extract; OR: oil recovery; DER: DE recovery; GF: gravitational force; CF: centrifugal force; ND: not determined. Solvent mass fraction (%) in OM and AP results from subtracting to 100 the corresponding $w_{oil}$ and $w_{DE}$.

For the same parameter, values with the same letter are not significantly different ($p > 0.05$) by the LSD Fisher method

The obtained AI value was lower than the reported for gums produced during aqueous degumming of crude soybean oils (54% db) (Ceci *et al.*, 2008). When centrifugal force was employed to accelerate the phase separation, OR, $w_{oil}^{AP}$ and third-phase AI values did not show statistically significant differences (p>0.1) from those obtained by static separation at equilibrium (gravitational force).

As the retention of compounds other than oil in the AP was demonstrated (DER≥80%), it can be inferred that the extraction of other compounds different from oil would not affect the oil quality because most of them are retained in the AP. This result was expected due to the polar characteristics of the extracted compounds, estimating that soluble carbohydrates are probably the major components of the DE (Baümler *et al.*, 2016).

## IV. CONCLUSIONS

Ethanol showed the highest miscibility temperature when mixed with crude soybean oil for all the evaluated proportions, compared to IPA and the equivolumetric IPA-ethanol mixture. These solvents were also effective in the separation process by chilling, with ethanol giving the best results, followed by the ethanol-IPA mixture. Thus, the higher energy requirements for evaporating these alcohol solvents after extraction, compared to the traditional hexane-based process, could be reduced by chilling separation. Since the oil recovery in the oily phase after chilling was similar when laboratory and real miscellas were used, it is concluded that other compounds extracted together with the oil when ethanol was used (real miscella) did not affect the separation yield; however, it must be considered that the formation of a third phase could generate oil losses and/or another interesting by-product that deserves further research. Centrifugation was as effective as equilibrium static phase separation after chilling the miscella, considerably reducing the time required to achieve the separation. To our knowledge, results presented in this work were obtained for the first time in a systematic way, which allows to arrive at appropriate and consistent findings and could be used as important input to continue with further studies at larger scales of processing to evaluate the feasibility of changing the extraction solvent in obtaining soybean oil. As was mentioned in the introduction, recent works presented a techno-economic-environmental analysis of the ethanolic soybean oil extraction considering for miscella treatment only distillation stages. It is expected that the information provided in the present work will serve to incorporate the miscella separation by chilling as an option to extend that type of analysis. Moreover, it is estimated that in the near future small and medium-sized industries could benefit the most from these results since they could use alcohols as renewable solvents to offer oils and meals of premium quality for human consumption.

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