Gamma-oryzanol Solubility and Effect of Solvents Mixture
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ABSTRACT
Gamma-oryzanol is a nutraceutical compound that can be obtained from rice bran using organic solvents extraction. In this work, pure solvents (hexane and hexanol) as well as two binary mixtures of them (with pre-set mass ratios of 1:1 and 3:1) were investigated in the gamma-oryzanol solubilization process, in the range of temperature from 283.2 to 323.2 K. This strategy was based on the Scatchard-Hildebrand theory which suggests the increasing of the solubility of a solid solute when it is dissolved in a mixture of two carefully selected solvents.

The composition of gamma-oryzanol in the liquid phase was determined using a gravimetric method. The sample was dried at 378.2 K until constant mass. After that, the solid-liquid equilibrium data of gamma-oryzanol were correlated using the Apelblat equation. It can be observed that the solubility of gamma-oryzanol increases with the increasing of temperature. In addition, it was observed that the binary mixture of solvents presented a synergistic effect in the solubility of gamma-oryzanol. In relation to the modelling approach, it can be mentioned that Apelblat equation shows good agreement with the experimental values. It can be concluded that solubility of gamma-oryzanol in organic solvents increases with rising temperature and with the use of binary mixtures of the selected solvents, making possible to obtain fractions of oil enriched with this nutraceutical compound.

Keywords: Scatchard-Hildebrand Theory; solubility; gamma-oryzanol.

INTRODUCTION
Gamma-oryzanol is a complex mixture of triterpenic alcohols and phytosterols esterified with ferulic acid and composes 1.1% to 2.6% of rice bran oil [1]. This compound has been reported as a powerful antioxidant and as hypocholesterolemic agent in several scientific studies [2,3,4]. Besides, the intake of this compound may decrease the early atherosclerosis [5], treats disorders of menopause [6], and acts on inflammatory processes [7]. This nutraceutical compound can be extracted from rice bran oil through oil saponification followed by solvent extraction and crystallization [8]. Despite the functional importance of this compound, there are no studies reporting the gamma-oryzanol solubility in organic solvents that could allow the extraction or preservation of this substance in the rice bran oil.

Hexane is commonly used as solvent for rice bran oil extraction and the knowledge of gamma-oryzanol solubility in this solvent is an important step to preserve rich fractions of oil. In addition, mixture of solvents can increase the gamma-oryzanol solubility, as suggested by Scatchard-Hildebrand theory [9]. The main aim of this study was to investigate the gamma-oryzanol solubility in pure solvents (hexane and hexanol) as well as in two binary mixtures of these solvents with mass ratios of 1:1 and 3:1 from 283.2 to 323.2 K. The solid-liquid equilibrium data were correlated using the modified Apelblat equation.

MATERIALS & METHODS
Solubility – apparatus and procedure
Gamma-oryzanol was kindly supplied by Tsuno Rice Fine Chemicals Co. (Japan). Hexane and hexanol were purchased from Merck (Germany).

The methodology to obtain the experimental data of solubility was based on the isothermal method [10]. Glass jacketed cells of about 50 cm$^3$ each were charged with the desired solvent and kept inside an insulated box. The temperature inside the box was controlled in order to maintain it constant. The temperature in each cell was controlled by circulating thermostatic water in the jacket and was considered to be accurate within ±
0.1 K (thermostatic bath from Cole Parmer, model 12101-55, USA). Dried gamma-oryzanol was added to the solution until a small excess of solid remained not solubilized. The components of the system were weighed on an analytical balance with a precision of 0.0001 g (Adam, model PW 254, Milton Keynes, UK). After adding the components, in a pre-set temperature (283.2-323.2 K), the mixture was stirred vigorously with a magnetic stirrer (Ika, model Lab disc, Germany) for at least 30 min and allowed to stand for about 24 h at constant temperature to enable any finely dispersed solids to settle down. From each equilibrium cell, samples of the clear supernatant liquid were carefully withdrawn using pipettes at a slightly higher temperature than the solution temperature in order to avoid any precipitation. After that, the composition of gamma-oryzanol in the liquid phase was measured by gravimetric procedure. The sample was dried at 378.2 K until constant mass. The saturated mole fraction solubility of gamma-oryzanol can be obtained using Equation 1:

\[
x_2 = \frac{\frac{m_2}{M_2}}{\frac{m_1}{M_1} + \frac{m_2}{M_2}}
\]

Considering a pure solvent system, the subscripts 1 and 2 refer to the solvent and solute, respectively. Where \( x \) is the mole fraction, \( m \) represent the mass and \( M \) represent the molar mass (86.18 g·mol\(^{-1}\) for hexane, 102.18 g·mol\(^{-1}\) for hexanol, and 602.10 g·mol\(^{-1}\) for oryzanol) [11].

**Modeling Approach**

The modified Apelblat equation was used to correlate the solubility data. In this equation \( A, B \) and \( C \) are adjustable parameters and \( T \) is the absolute temperature.

\[
\ln x_2 = A + \frac{B}{T} + C \ln(T)
\]

The difference between experimental and calculated data was evaluated using the root-mean square deviation (RMSD), as shown in equation 3.

\[
\text{RMSD} = \left[ \frac{\sum_{i=1}^{N} (x_{i}^{\text{calc}} - x_{i}^{\text{exp}})^2}{N} \right]^{1/2}
\]

where \( N \) is the number of experimental data, and the superscripts calc and exp refer to the values calculated from equation 2 and to the experimental values, respectively.

**RESULTS & DISCUSSION**

Figure 1 shows the experimental data obtained in the temperature range from 283.2 to 323.2 K and calculated data by modified Apelblat equation. It can be observed that the solubility of gamma-oryzanol increases with the increasing of temperature. It is also possible to note that the compound studied presented lower solubility in hexane than in hexanol. In addition, it is observed that gamma-oryzanol has its solubility increased when mixed solvents are used, especially in the 1:1 mass ratio of hexane: hexanol. Scatchard Hildebrand theory predicts that the solubility of a solid has a maximum value in a solvent whose solubility parameter is the same as that of the solute. To better understand the behavior of the system investigated, experimental solubility was plotted versus solvent composition, as can be seen in Figure 2. In this Figure it is possible to note a maximum point of the gamma-oryzanol solubility when a mixed solvent with 1:1 mass ratio is used.
Figure 1. Experimental and calculated solubilities of gamma-oryzanol in different organic solvents.

Figure 2. Experimental solubility of Gamma-oryzanol in a mixed solvent containing hexane and hexanol.

The values of adjustable parameters for Apelblat equation, the RMSD and the determination coefficient (R²) are shown in Table 1.
Table 1. Parameters of modified Apelblat equation

<table>
<thead>
<tr>
<th>Solvent</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>RMSD</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>hexane</td>
<td>177.07</td>
<td>-12058.55</td>
<td>-25.11</td>
<td>0.01</td>
<td>0.99</td>
</tr>
<tr>
<td>hexanol</td>
<td>-156.68</td>
<td>4145.17</td>
<td>24.29</td>
<td>0.05</td>
<td>0.99</td>
</tr>
<tr>
<td>hexane-hexanol 1:1</td>
<td>-611.59</td>
<td>25000.48</td>
<td>91.93</td>
<td>0.08</td>
<td>0.99</td>
</tr>
<tr>
<td>hexane-hexanol 3:1</td>
<td>50.90</td>
<td>-5402.05</td>
<td>-6.51</td>
<td>0.13</td>
<td>0.97</td>
</tr>
</tbody>
</table>

The low values of the RMSD, calculated by equation 3 and shown in Table 1, can confirm the good description of the temperature dependence of solubility using the modified Apelblat equation. In general, this equation can describe the temperature dependence of solubility of gamma-oryzanol in the proposed solvents.

CONCLUSION

Solubility data of gamma-oryzanol in pure solvents (hexane and hexanol) and in two binary mixtures with mass ratios of 1:1 and 3:1 in the temperature range from 283.2 to 323.2 K were determined. It was observed that the solubility of nutraceutical compound increase with rising temperature and in the alcohol solvent. It was also observed that the mixture of the two pure solvents presented a synergetic effect improving the solubility of the compound, being that this behavior can be explained by the Scatchard Hildebrand theory. In general, the modified Apelblat model can describe the temperature dependence of solubility of gamma-oryzanol.

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REFERENCES