Development of a zero trans margarine from soybean-based interesterified fats formulated using artificial neural networks

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1. INTRODUCTION

Evidences of the harmful effects of trans fatty acids (TFA) on human health has led the food industry to seek alternatives for production of trans-free products. The demand for trans-free fats has encouraged research into different raw materials and processes for the production of fat for varied purposes. However, although many of the currently used raw materials do not contain trans fatty acids,
they do contain high amounts of saturated fatty acids. Saturated fatty acids are also considered unhealthy because they contribute to an increased risk of cardiovascular disease (Keys et al., 1965; Mensink et al., 2003).

Interestereified fats are an alternative to produce low trans products because interesterification produces desirable physical changes in the fats without the production of trans isomers (Ribeiro et al., 2007). Despite this advantage, interestereified fats may suffer from limitations due to the difficulty of achieving the same performance as trans fats.

For a fat to achieve the desired characteristics for use in certain products, blends of oils and fats from different sources is often needed. When mixed in the appropriate proportions, a fat exhibiting the specific necessary characteristics is produced. This is the basis for how fats are prepared using various raw materials worldwide. For products in which a hard consistency is required, palm-based fats are usually employed. Palm-based fats contain a high content of saturated fatty acids, and they are not readily available in Brazil; this limitation increases the cost of products that utilize them. In contrast, soybean-based fats and soybean oil are highly available in the Brazilian market. If soybean-based fats are used as the majority of the raw materials, the cost of the products is significantly reduced. In addition, their use results in healthier fatty foods, with a low saturated fat and high polyunsaturated fat content.

The formulation systems of fats in the food industry are normally based on the experience of the formulator. Conventional methods require multiple trial-and-error procedures, which may result in economic losses and consume considerable time and raw materials (Block et al., 1997). Artificial neural networks (ANN) are computational techniques to develop a mathematical model based on the neural structure of intelligent organisms and acquired knowledge through experience (Britto, 1994). The use of ANN as an alternative to conventional methods for the formulation of specialty fats has been previously demonstrated. Block et al. (1997) formulated specialty fats from hydrogenated fats and refined soybean oil using an ANN, and they developed a computer program specifically for this purpose: Software Mix (Barrera-Arellano et al., 2005). ANN were highly efficient for the development of margarines produced from hydrogenated fats in a pilot plant, demonstrating that ANN may be used to formulate products with the same characteristics as those formulated using conventional methods (Block et al., 1999; Block et al., 2003).

The use of ANN trained with soybean-based interestereified fats may allow for the formulation of trans-free products similar to commercial products that are currently formulated using blends of fats from sources that are frequently costly in Brazil. Starting from the desired profile, the ANN used in this study proposes various formulations that consist of different amounts of raw materials, and allows the user to choose the formulations of interest. Thus, it becomes possible to choose formulations with reduced quantities of saturated and trans fatty acids, as well as higher quantities of polyunsaturates.

The aim of this study was to develop a low-trans hard margarine using soybean fats and soybean oil (sources available in the Brazilian market). Furthermore, an ANN was used to formulate these products.

2. MATERIALS AND METHODS

2.1. Material

Two soybean-based interestereified fats, Fat A (soybean/palm kernel) with a MP of 53°C and Fat B (soybean/cottonseed/palm kernel) with a MP of 47.3°C, provided by Triângulo Alimentos (Itápolis, SP, Brazil), and a commercial refined soybean oil, were used for training the ANN and preparing the blends. Commercial fats for hard margarines were used as the standard fats: a zero-trans fat (Standard fat 1) and a high-trans fat (Standard fat 2) provided by Triângulo Alimentos (Itápolis, SP, Brazil) and Bunge Alimentos (Gaspar, SC, Brazil), respectively.

2.2. Methods

2.2.1. Fatty Acid (FA) composition

Esterification was carried out according to the method of Hartman and Lago (1973), and the fatty acid methyl esters were separated according to the AOCS method Ce 1-62 (AOCS, 2004) using a DB - Agilent, (Agilent, Santa Clara, United States) 23 capillary column (50% cyanopropyl-methylpolysiloxane), 60 m in length, 0.25 mm internal diameter, and 0.25 µm film. The analyses were performed using an Agilent 6850 Series GC system equipped with a capillary column and a flame ionization detector (FID). The oven temperature program was a 5 min hold at 110°C, increasing from 110 to 215°C at a rate of 5°C·min⁻¹, and a hold for 24 min at 215°C. The detector temperature was 280°C, the injector temperature was 250°C, the carrier gas was helium, the split ratio was 1.50, and the injection volume was 1.0 µL. The qualitative composition was determined by comparing the peak retention times with those of fatty acid standards. All samples were analyzed in duplicate.

2.2.2. Triacylglycerol (TAG) composition

The TAG compositions were determined according to method Ce 5b-89 (AOCS, 2004) using capillary column gas chromatography (CGC Agilent 6850 series GC system). We used a DB-17HT (50% phenyl-methylpolysiloxane) capillary column, 15 m in length, 0.25 mm internal diameter, and 0.15 µm film. The column temperature was
250 °C, increased up to 350 °C at 5 °C·min⁻¹, 1:30 split injection ratio, helium carrier gas at 1.0 mL·min⁻¹ flow rate, 360 °C injector temperature, 375 °C detector temperature, 1.0 µL injection volume, and 100 mg·5 mL⁻¹ in tetrahydrofuran sample concentration. TAG identification was carried out by comparing retention times according to the methods of Antoniossi Filho et al. (1995). All samples were analyzed in duplicate.

2.2.3. Iodine index and saponification index

The iodine and saponification values were calculated from the fatty acid composition according to methods Cd 1c-85 and Cd 31-94, respectively (AOCS, 2004).

2.2.4. Solid fat content (SFC)

The SFC was determined according to method Cd 16b-93 (AOCS, 2004) using nuclear magnetic resonance (NMR). A Bruker PC120 Minispec spectrometer (Bruker, Bremen, Germany) was used to read samples in series at 15, 20, 25, 30, 35, 37.5, and 40 °C, and samples were analyzed in duplicate.

2.2.5. Melting point (MP)

The MPs were determined according to method Oc 18-80 (AOCS, 2004) using a Mettler FP90 Control Unit (Mettler, Pasadena, Maryland, United States), and samples were analyzed in triplicate.

2.2.6. Consistency

Margarine samples were tested for consistency using the penetration test with an acrylic cone with an apex angle of 40° and no truncation in a TA-XT2i texture analyzer (Stable Micro Systems, Haslemere, United Kingdom) according to Ribeiro et al. (2009). The penetration data were converted to calculate the yield value (Haighton, 1959). Data were obtained at 10, 20, 25, 30, 35, and 40 °C. All samples were tested in triplicate, and the means were used to calculate the yield value.

2.2.7. Margarine Emulsion Stability

Emulsion stabilities were measured in triplicate with two temperature cyclization steps using the industry methodology (Danisco, Cotia, São Paulo, Brazil). In the first step, the samples remained at 5 °C for 48 h to allow for full complete crystallization. They were then placed at 35 °C for 24 h and immediately tested. The samples were placed in a cooling temperature for another 24 h and tested again. In the second step, the samples were stored at 35 °C for 48 h, analyzed, cooled (to 5 to 10 °C) for an additional 72 h, and tested again.

2.2.8. Statistics

To detect significant differences, we applied Tukey’s test (p<0.05) using Statistica ® 7.0 software (Statsoft, United States).

2.3. Experimental Procedure

2.3.1. Formulation Process by ANN

The ANN used in the present study was a multilayer perceptron with sigmoid-type activation functions. It consisted of an input layer with three variables representing a blend of the example formulations (a percentage of each raw material), two intermediate layers containing 15 neurons each, and an output layer that consisted of eight variables representing the SFC at seven different temperatures and the MPs of the example formulations.

The fat blends for margarine were formulated using the ANN trained by Gandra et al. (2009), using soybean oil and two soybean-based interesterified fats with different MPs, using the Software Mix (Barrera-Arellano et al., 2005). During training, 62 blends were formulated with different proportions of the three raw materials. The input data utilized was the SFC and the MP of each formulation, as well as the proportion of each raw material, according to Block et al. (1997).

Raw materials (interesterified fats A, B, and oil) and commercial fats (1 and 2) were characterized for SFC at 10, 20, 25, 30, 35, 37.5, and 45 °C, as well as for their MP, their fatty acid composition, and their triacylglycerol composition. After supplying the data to the software Mix (the SFC and MP of the commercial fats were used as the standards for margarine fats) the search for mixtures (containing different proportions of the three raw materials) with similar SFC and MP values was performed. Of the proposed formulations for each commercial fat, three formulations with SFC and MP values close to the desired values were selected.

The blends suggested by the ANN for each commercial fat were formulated and analyzed for SFC, MP, fatty acid composition, and triacylglycerol composition. The consistency of the commercial fats and the ANN blends were determined and compared. To test the effectiveness of the ANN formulations under real processing conditions, a commercial fat and a formulation proposed by the ANN with similar profiles were selected for production in a pilot plant. The margarines were analyzed for consistency and stability by temperature cyclization.

2.3.2. Margarine Production

Margarines were prepared at Danisco’s (Cotia, SP, Brazil) pilot plant using the model Perfector (1+1) X 57 (Gerstenberg & Agger A/S, Copenhagen, Denmark) encompassing an 8 kg capacity emulsion tank, two cooling units (crystallizers), and intermediate...
pin units for mechanical work. The margarines were produced under the same conditions, using the same ingredients and additives, and standardized to 67% fat content according to the base formulation suggested by Danisco: 31% water, 1.5% sodium chloride, 0.05% sodium benzoate, 0.05% potassium sorbate, 0.10% powdered skim milk, 0.6% Dimodan® UP/B (Danisco, Cotia, São Paulo, Brazil), 0.003% beta-carotene (FMC Química do Brasil Ltda, Campinas, São Paulo, Brazil), 0.04% butter flavor (Kerry, Campinas, São Paulo, Brazil), and 0.03% Grindox™204 antioxidant (Danisco, Cotia, São Paulo, Brazil).

3. RESULTS AND DISCUSSION

The fatty acid composition of the raw materials and commercial samples is shown in Table 1. Raw materials A and B had compositions characteristic of soybean products. Commercial fat 1 is likely to be a palm fat or palm oil-based fat due to the levels of palmitic and oleic acids it contained. The fatty acid composition of commercial fat 2 indicated the use of partially hydrogenated fats due to high levels (11.28%) of trans isomers. Palmitic and lauric acid values also indicated the presence of palm fat and palm kernel oil.

According to the TAG composition (Table 2), interesterified fats A and B had TAG values similar to those measured by Ribeiro et al. (2009) and were characteristic of soybean-based fats, and the soybean oil also had the expected values. The TAG values for the commercial fats are presented in Table 2.

The formulations proposed by the ANN are listed in Table 3 (the proportions of each raw material). The SFC of the interesterified fats A and B used for training the ANN are listed in Table 4, as are the SFC and MP of each commercial fat and the blends suggested by ANN.

Fats 1 and 2 had high SFC and MP, which are characteristic of fats used in cooking or industrial margarines (Hoffmam, 1989). After soliciting responses (ANN formulations) with SFC similar to the commercial fats, the SFC values predicted for each formulation were experimentally tested. The values showed major differences at 10 and 45°C and minor differences.

Table 1

<table>
<thead>
<tr>
<th>Fatty Acid</th>
<th>Fat A*</th>
<th>Fat B**</th>
<th>Soybean Oil</th>
<th>Commercial Fat 1</th>
<th>Commercial Fat 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>C6:0</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>0.02</td>
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<td>C8:0</td>
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<td>0.18</td>
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<td>0.77</td>
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<td>3.46</td>
<td>2.58</td>
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<td>C14:0</td>
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<td>0.40</td>
<td>0.07</td>
<td>1.95</td>
<td>1.56</td>
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<tr>
<td>C16:0</td>
<td>15.77</td>
<td>11.64</td>
<td>10.05</td>
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<td>32.66</td>
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<tr>
<td>C16:1</td>
<td>0.18</td>
<td>0.08</td>
<td>0.10</td>
<td>0.13</td>
<td>0.15</td>
</tr>
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<td>0.10</td>
<td>0.09</td>
<td>0.10</td>
</tr>
<tr>
<td>C18:0</td>
<td>46.06</td>
<td>29.46</td>
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<td>9.24</td>
<td>9.00</td>
</tr>
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<td>9.78</td>
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<tr>
<td>C18:2</td>
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<td>17.40</td>
<td>21.14</td>
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<td>24.54</td>
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<td>0.61</td>
<td>0.19</td>
<td>0.38</td>
<td>1.33</td>
</tr>
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<td>16.80</td>
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<td>0.41</td>
<td>0.36</td>
</tr>
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<td>0.15</td>
<td>0.16</td>
<td>0.15</td>
<td>0.08</td>
</tr>
</tbody>
</table>

Saturated: 66.87  43.71  14.96  49.26  46.98
Monounsaturated: 9.38  17.63  21.4  33.36  24.79
Polyunsaturated: 22.49  37.4  62.9  15.54  16.96
Total trans: 1.26  1.26  0.74  1.84  11.28
Saponification index: 196.91  195.12  193.94  202.86  223.41
Iodine index: 47.2  82.9  134.0  49.26  50.8

*Fat A= Interesterified fat A, **Fat B= Interesterified B
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between 25 and 35°C from the predicted SFC. The correlation between the predicted and desired SFC values resulted in coefficients of determination ($R^2$) between 0.87 and 0.91 for the ANN formulations and the commercial fat 1, and $R^2$ between 0.90 and 0.92 for commercial fat 2 and the ANN formulations. The SFC and MP values predicted by the ANN for the proposed formulations were similar to the

### Table 2

<table>
<thead>
<tr>
<th>Carbon Number</th>
<th>TAG* (%)</th>
<th>Inter. Fat A</th>
<th>Inter. Fat B</th>
<th>Soybean Oil</th>
<th>Commercial Fat 1</th>
<th>Commercial Fat 2</th>
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<td>C42</td>
<td>LaMP</td>
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<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>LaOLa</td>
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<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>C44</td>
<td>MMP</td>
<td>–</td>
<td>2.94</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>LaPP</td>
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<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>LaOM</td>
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<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>C46</td>
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<td>0.33</td>
<td>–</td>
<td>4.35</td>
<td>1.66</td>
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<tr>
<td></td>
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<td>–</td>
<td>2.17</td>
</tr>
<tr>
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<td></td>
<td>LaOST</td>
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<td>–</td>
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</tr>
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<td>PLL</td>
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<td>PLLnL</td>
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<td>C54</td>
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<td>–</td>
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<td>4.75</td>
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<td>1.67</td>
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<tr>
<td></td>
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<td>SiLO</td>
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<td>OLL</td>
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<td>–</td>
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<td></td>
<td>LLnL</td>
<td>–</td>
<td>2.00</td>
<td>5.02</td>
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<td>–</td>
</tr>
<tr>
<td></td>
<td>Other</td>
<td>–</td>
<td>–</td>
<td>4.51</td>
<td>2.92</td>
<td>–</td>
</tr>
</tbody>
</table>

*La = lauric acid; M = myristic acid, P = palmitic acid, St = stearic acid, O = oleic acid, L = linoleic acid, Ln = linolenic acid. -: not detected.
abundant in soybean fats, and stearic acid, which is characteristic of soybean products interesterified with fully hydrogenated soybean oil. Palmitic acid, which largely determines fat plasticity, was also prevalent in the blend compositions. Palmitic acid is also responsible for crystallization in the $\beta'$ form, an essential characteristic in most products, especially margarines (Jeyarani and Reddy, 2003).

values obtained experimentally; these results are acceptable because the industry works within a range of variation of up to $4^\circ C$. The correlation between the predicted and experimentally values for all formulations proposed by the ANN were $R^2 > 0.99$ at a significance level of 5%.

Table 5 lists the FA and TAG compositions of the formulations proposed by ANN. There was a prevalence of oleic acid and linoleic acid, which are abundant in soybean fats, and stearic acid, which is characteristic of soybean products interesterified with fully hydrogenated soybean oil. Palmitic acid, which largely determines fat plasticity, was also prevalent in the blend compositions. Palmitic acid is also responsible for crystallization in the $\beta'$ form, an essential characteristic in most products, especially margarines (Jeyarani and Reddy, 2003).
Table 5
TAG composition and majority fatty acids (FA) of the formulations proposed by the neural network

<table>
<thead>
<tr>
<th>Carbon Number</th>
<th>TAG (%)</th>
<th>1A</th>
<th>1B</th>
<th>1C</th>
<th>2ª</th>
<th>2B</th>
<th>2C</th>
</tr>
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<tbody>
<tr>
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<td>0.08</td>
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Majoritary Fatty acids (%)

| C16:0         | 11.64 | 11.71 | 11.32 | 13.11 | 12.86 | 12.95 |
| C18:0         | 25.11 | 23.10 | 19.51 | 33.16 | 33.50 | 32.86 |
| C18:1         | 17.54 | 17.46 | 18.29 | 14.56 | 15.00 | 14.85 |
| C18:2         | 37.81 | 39.62 | 42.51 | 31.78 | 31.31 | 31.93 |
| C18:3         | 4.06  | 4.35  | 4.96  | 2.59  | 2.58  | 2.66  |
| Other FA      | 3.85  | 3.76  | 3.41  | 4.80  | 4.75  | 4.75  |

La = lauric acid, M = myristic acid, P = palmitic acid, St = stearic acid, O = oleic acid, L = linoleic acid, Ln = linolenic acid. -: not detected.
*Means of two measurements.
Table 6 presents the total values of saturated, monounsaturated, polyunsaturated, and trans fatty acids in the commercial fats and their respective blends formulated by ANN with soybean-based fats. The ANN blends had higher polyunsaturated contents than the commercial fats. In addition, ANN blends (1A, 1B and 1C) formulated for commercial fat 1 had lower saturated contents, while the ANN blends (2A, 2B and 2C) formulated for commercial fat 2 had similar saturated contents.

The TAG composition of the commercial fats and the ANN formulations is presented in Table 6 according to the degree of saturation of the AG. The SSU and SUU TAG are the main components in the interesterified products, and the UUU TAG contained high values due to the presence of soybean oil in their formulations. The properties of fatty foods may be related to the TAG composition of the fat used in their formulation (O’Brien, 2004). SSS and SSU TAG are responsible for their structure, and SUU TAG are important due to their melting properties at body temperature and their plasticity at room temperature. The increase in SSU and SUU levels due to chemical interesterification is associated with increased functionality and improved sensory characteristics, which increases their application in foods (Ribeiro et al., 2009; Karabulut et al., 2004). Bomaz et al. (1993) evaluated TAGs and butter hardness and concluded that the SUU fraction decreases hardness, while SSS and SSU fractions increase butter hardness.

Figure 1 shows the consistency values (yield values) for the commercial fats and ANN formulations (commercial fat 1 and ANN formulations (a), commercial fat 2 and ANN formulations (b)). The commercial fat 1 was significantly different \((p > 0.05)\) than the ANN formulations at all temperatures tested; however, the commercial fat 2 and the formulated ANN blends were similar at 10 and 20 °C. The commercial fat 2 and the respective formulated ANN blends had similar SSS TAG values, which may have played a role in their similar consistencies. In contrast, the commercial fat 1 and its respective ANN formulations showed greater differences in SSS and others TAG groups, as well as in their consistencies.

At the pilot plant scale, margarines with the commercial fat 1 and ANN blend 1A were produced.
DEVELOPMENT OF A ZERO TRANS MARGARINE FROM SOYBEAN-BASED INTERESTERIFIED FATS FORMULATED...

ANN with soybean-based fat resulted in improved nutritional characteristics, containing low saturated fats, zero trans fats, and high polyunsaturated fats.

4. CONCLUSIONS

The use of the soybean-based fats as raw materials is an alternative for the production of low-saturated and low trans fat foods with lower costs in South American countries, especially in Brazil. Moreover, a specific ANN for the formulation of fats can be a very efficient tool for the development of formulations with profiles highly similar to those currently utilized in industry.

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REFERENCES


