Development and Structural Behaviour of Soybean Gelato

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SUMMARY

The aim of this study was to elaborate and evaluate structural characteristics of soybean gelato, varying the concentrations of soybean protein concentrate (2.95 to 17.05 %) and vegetable fat (7.95 to 22.05 %) using experimental design. The replacement of milk by hydrosoluble extract and soybean protein concentrate presented itself as an alternative to edible gelato production with distinct characteristics, especially in terms of protein, solubility, viscosity, melting point, overrun and acceptability. The addition of up to 5 % (m/V) protein concentrate, 14 % (by volume) soybean hydrosoluble extract, and 15 % vegetable fat on gelato formulations presented better structural characteristics, with viscosity ranging from 450-700 cP at 10 °C, a non-Newtonian behavior, protein stability (total protein 8.44 % and solubility 41 %). Soybean gelato structural analysis using X-ray diffraction presented 15° and 35° diffraction angles at 2θ, characterizing the crystalline part of the product. The thermal analyses showed four bands of mass loss in the temperature range of 40-600 °C, characterizing loss of moisture, decomposition of the soy protein and the fat/emulsifier of the formulations. Thus, the soybean gelato is one innovative product, lactose and milk protein-free with

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outstanding characteristics for the general public, mainly, for the populations with intolerance to such components.

Key words: gelato, soybean, viscosity, crystallinity, solubility, stability

INTRODUCTION

The food industry commercializes *in natura* soybean grains, it also produces various soybean-based products, such as soybean concentrates or isolates, textured soybean, oil, fermented foods (miso, soy sauce, and tempeh), hydrosoluble soybean extract, as well as non fermented foods such as tofu, flours, soybran, and okara (1-4). Protein and oil content are not only adequate but also rated as the best in terms of quality. Soy milk is cholesterol-free and has high unsaturated fatty acids levels, such as linoleic and linolenic acids, fats considered highly healthy oils (5).

Hydrosoluble extract and/or soybean protein concentrate are derivatives with great potential for creating distinctive products that could be used as a milk replacement for various formulations with several advantages. One of them is the non existence of lactose, which may cause food rejections due to intolerance issues regarding that compound (6-7), it also works as a preventive for degenerative chronic diseases through phytochemical (isoflavones) and for some cancer types, as well a cholesterol reduction (8-9).

However, limited reports exist on the role of soy proteins concentrate addition in ice cream. Soy proteins provide several functionalities such as high emulsifying properties, stability, water-holding, and binding (10-11), thus providing an array of food products, such as ice cream and/or gelato. The use of foods fortified with soy protein concentrate is an approach that could provide additional health benefits in a well-accepted food such as ice cream. The use of soy proteins to alter the ice cream products physicochemical and sensory properties has been reported (12-14).

Food industries such as ice cream have been expanding their market even with new products such as gelato, which has been constantly approved by the public. Gelato is ice cream variation with more creaminess and less fat. In this context, the preparation of soybean derivates products such as gelato is an innovative alternative to ice cream, adding value to soybean, with functional properties and specific structural characteristics.

MATERIALS AND METHODS

*Obtaining hydrosoluble extract and soybean protein concentrate*

The soybean (*Glycine max*) BRS 267 cultivar (2012/2013 harvest) used in this work was obtained from Embrapa Trigo – Passo Fundo-RS, Brazil.
Soybean hydrosoluble extract (SHE) was obtained according to the methodology of Mandarino et al. (15), with modifications. Initially, soybean grains were macerated in water at a 1:4 (m/V) ratio, kept at boiling point (97 °C) for 5 min. Then, soybean maceration water was drained out. After that, soybean grains were mixed in boiling water at 1:1 (m/V) ratio for 5 min, cooled to room temperature and dissolved using an industrial blender (Drink Machine, Equinox, Porto Alegre, Brazil). After disintegration, the contents were transferred to a basket centrifuge for separation (Eppendorf, 5403, Denkendorf, Germany), obtaining the soybean hydrosoluble extract.

The soybean protein concentrate was obtained using soybean hydrosoluble extract which was dried by spray dryer (LabPlant, SD-05, Huddersfield, England) using 190 °C air temperature, 47 m³/h air rate, and 600 mL/h extract inlet rate. The dried extract was stored in hermetically-sealed amber flasks until used.

Preparation of gelato formulations

Gelato formulations were prepared based on preliminary tests and following methodology by Maia et al. (16), with modifications. On formulations, soybean protein concentration of 2.95 to 17.05 % (m/V) and hydrogenated vegetable fat (Elegê, São Paulo, Brazil) of 7.95 to 22.05 % (m/V) using experimental design technique - 2² full factorial design. The fixed independent variables were soybean hydrosoluble extract (68 %), sucrose ( União, Rio de Janeiro, Brazil) of 10 %, powder glucose (Nutre, São Paulo, Brazil) of 5 %, stabilizer (Kerry, São Paulo, Brazil) of 2 % and emulsifier (Kerry, São Paulo, Brazil) of 2 %.

Water, soybean hydrosoluble extract, sucrose, glucose, soybean protein, and hydrogenated vegetable fat were mixed forming syrup that was then pasteurized (Model pp110, Arpírio, São Paulo, Brazil) at 90 °C for 25 s. Afterward, it was cooled to 4 °C for 24 h for maturation and subsequently, a strawberry flavor (Duas Rodas, Jaraguá do Sul, Brazil) was added to it. Thereafter, it was homogenized for 5 min using an industrial blender (Fortfrio, AA239 model, Betin, Brazil) and beating using an industrial ice cream maker and then cooled to -3 °C. The gelato batter was stored in polypropylene packaging (Valpri, Campinas, Brazil) at -20 °C.

The dependent variables (responses) on the 2² full factorial design were: protein content, fat, overrun, specific mass, viscosity, functional properties (solubility and protein stability), crystallinity, melting point, and thermogravimetric analysis.

Characterization of soybean protein concentrate and gelato formulations

Soybean powder protein concentrate was characterized in terms of protein, fat, and moisture contents. Gelato formulations were characterized in relation to total protein, solubility, and protein
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stability, fat, overrun, specific mass, viscosity, crystallinity, melting point, and thermogravimetric analysis.

Moisture was determined by oven (Marconi, MA035, Piracicaba, Brazil) drying at 105 °C using air circulation to a constant mass and lipids content was obtained by extraction with petroleum ether using a Soxhlet-type extractor (Nova ética, NT 340, Vargem Grande do Sul, Brazil) using the principles of gravimetric analysis. Total protein was calculated from the total nitrogen content using Kjeldahl method multiplied by factor 6.25, according to AOAC (17).

Solubility was determined by diffraction in a 10 mM phosphate buffer (Merk, Kenilworth, USA) at pH 7.0 with 0.1 % (m/V) ratio for 1 h at room temperature and constant speed. After that, the samples were centrifuged (Eppendorf, 5403, Denkendorf, Germany) at 10000 x g for 10 min at 4 °C. The protein content on the supernatant was determined using methodology by Lowry et al. (18) determined at 750 nm using bovine serum albumin 1 mg/L as a standard.

To determine the functional properties of gelato formulations were reconstituted in order to characterize the samples destabilization. Then, a 10 times dilution, weighing 4.6 g per sample adding 54 mL of pH 7 sodium phosphate buffer (Merck, Kenilworth, USA) was done. The emulsions were prepared at room temperature using an Ultraturrax T25 (IKA-Labortechnik, Staufen, Germany) homogenizer with an S25 N-10 dispersant accessory at 20,000 rpm for 1.5 min. Stability was analyzed using a vertical optical analyzer (Turbiscan, Classic MA2000, L'Union, France). The emulsions were placed in an 80 mm cylindrical glass cell to register destabilization, depending on the cell height, measuring it every 15 min for 5 h. Total destabilization (%D) was calculated at 40 mm of tube height through Eq. 1:

\[ \%D = \frac{RD_{\text{t=5h}} - RD_{\text{t=0h}}}{RD_{\text{t=0h}}} \]

Where, RD is retro dispersion at 40 mm tube height at times 0 and 5 h.

The gelato viscosity at temperatures of 10 and 20 °C was determined using a rotational viscometer (Brookfield Mark, Middleboro, USA).

Melting point was determined according to the methodology by Granger et al. (19), adapted. Initially, 100 g portions of ice cream were placed on a mesh grid (mesh size 1 x 1 cm) and the melted material was collected and weighed every 10 min.

Overrun was measured after batch freezing by carefully filling a capsule of known volume with the ice cream and weighed. Comparisons of the original ice cream mixture mass allow overrun calculation.

Ice cream specific mass (density relative to water) was determined by pycnometer (Labor Quimi, poá, Brazil). The pycnometer was first weighed empty, and then filled once with distilled water and once with ice cream at 25 °C and mass were recorded each time.
Diffractograms were obtained using an X-ray diffractometer (Shimadzu, XRD 6000, Kyoto, Japan) with Cu-1.54 transmitter tube, in order to observe crystallinity peaks.

The thermogravimetric analyzes were performed on TGA device (TA Instruments brand, Q500, New Castle, USA). The sample of 11.7 mg was placed in platinum pan and heated in nitrogen flow (40 cm³/min), with a temperature range of 40-600 °C with heating rates of 10 °C/min for each sample.

Consumers’ acceptability
Consumers’ acceptability of the ice creams were performed on a laboratory scale, with 36 panelists, professors, students, employees of the institution, male and female, from age groups ranging from 20 to 50 years. Sensory evaluation was performed by affective tests, which were employed to evaluate consumer acceptance. The tests were performed on the samples within 1 week of storage. The panelists were asked to indicate how much they liked or disliked the ice creams based on the attribute global acceptance, according to a 9-point hedonic scale (9 = extremely liked and 1 = extremely disliked). Each sample was randomly coded with three-digit numbers. As the research involved humans, tests were performed according to the Research Ethics Committee of the Regional Integrated University of Upper Uruguai and Missions according to Brazilian National Health Council ethical and scientific requirements.

Statistical analysis
The results were statistically treated using analysis of variance (ANOVA), Tukey test, Pearson correlation, and Principal Components Analysis (PCA), aided by Statistica version 5.0 online software (20) with 90 and/or 95 % confidence level.

RESULTS AND DISCUSSION
Soybean hydrosoluble extract and soybean powder protein concentrate presented 1.65 % protein contents (32.06 % total solids and 43.33 % dry basis) and 3.50 % fat contents (32.06 % total solids and 21 % dry basis).

Table 1 presents the 2² full factorial design matrix with coded (real) values of independent variables studied on gelato formulations and dependent variables (response). Gelato formulation from run 6 presented the highest total protein content and solubility. Such results are better viewed in Eqs. 2 and 3 and the contour curves (Fig. 1).

Table 1 HERE
The addition of protein concentrate influenced significantly (p<0.05) on protein contents and solubility of soybean gelato formulations. The non significant parameters were added to the lack of fit for the analysis of variance (ANOVA) test. Eqs. 1 and 2 presented a second-order coded model describing total protein and solubility in terms of independent variables, within the studied ranges. The models were validated by analysis of variance with a 0.93 and 0.95 coefficient correlation and F calculated values 12 and 15 times greater than the F tabulated ones, allowing the construction of contour curves shown in Fig. 1 (a-b), respectively. It is possible to see that the maximum for total protein and solubility is within the 15 % of soybean concentrated protein addition range and at any hydrogenated vegetable fat concentration.

\[ TP = 9.51 + 3.42X_1 \]  
\[ PS = 41.11 + 1.29X_1 \]

Where, TP is total protein, PS is protein solubility, \( X_1 \) is soybean protein concentrate (%), and \( X_2 \) is vegetable fat (%).

According to Zayas (21), the solubility index may be used as a parameter for protein functionalities. Protein is of great importance for ice cream quality, as it affects the beating, emulsification, and it improves the ice cream structure (22). It also contributes to functional properties such as the interaction with other stabilizers, emulsion stabilization after homogenization, on structure formation, and water retention capabilities, which improves the mixture viscosity, increases melting point and reduces ice formation (23).

Table 2 presents sedimentation, flotation, particles accumulation, and total protein destabilization observed along the measuring for gelato formulations. It is observed that the destabilization by flotation and sedimentation occurs, that is to say, it is a total protein destabilization measure. It was observed that F4 presented the lowest destabilization (D, %) but with sedimentation. On the other hand, F1 had a destabilization (D, %) higher than F4, however without sedimentation. Thus, F1 may be considered the formulation with the best stability and F5 had the lowest stability, without sedimentation. The higher stability of the protein provided a better overrun and melting point (Table 1 and 2; Fig. 2).

In terms of fat contents for gelato formulations, variations between 9.54 and 18.9 % occurred. It was noted that the vegetable fat variables and the interaction between variables had a significant positive
effect at the 95 % confidence level. Whereas, soybean protein concentrate presented a negative effect \((P<0.05)\), showing that by increasing the vegetable fat concentration and by interacting with the soybean protein concentrate, there is an increment on the formulations fat contents.

In terms of specific mass (Table 1) for soybean gelato formulations, there was a variation from 0.856 (run 5) to 1.12 g/mL (run 6). Run 1 presented the highest overrun value.

Eqs. 4, 5 and 6 presented second-order coded models that described the specific mass, melting point and protein destabilization as a function of independent variables, within the studied ranges. The models were validated by the analysis of variance with 0.97, 0.93 and 0.90 coefficient values and \(F\) calculated values 6.14, 2.84 and 2.46 times greater than the \(F\) tabulated ones, allowing the construction of contour curves shown in Fig. 1 (c-d), respectively. It is observed that the maximum for specific mass and minimum for melting point was at the range close to 12 % soybean protein concentrate and 15 % hydrogenated vegetable fat addition, respectively.

\[
\begin{align*}
\text{SM} &= 1.02 + 0.109.X_1 - 0.021.X_1^2 + 0.012.X_2 + 0.013.X_2^2 \quad /4/ \\
\text{MP} &= 0.266 - 15.X_1 + 11.89.X_1^2 - 0.426.X_2 \quad /5/ \\
\text{D} &= 25.20 - 9.66.X_1 + 12.94.X_1^2 - 19.27.X_1.X_2 \quad /6/ \\
\end{align*}
\]

Where, MP is melting point, SM is specific mass, D is protein destabilization, \(X_1\) is soybean protein (\%), and \(X_2\) is vegetable fat (\%).

The specific mass of ice cream is directly related to air incorporation, that is, the higher the air incorporation, the lighter the mass and thereby the lower this parameter value. Rodrigues et al. (24) evaluated the elaboration of chocolate flavour ice cream formulations by verifying the effect of powdered milk and milky cream replaced with whey powder and alternative fat (Dairy Pro ™) and noted that some formulations presented values of specific mass next to 0.475 g/mL. That could be due to whey proteins and alternative fat having functional properties that facilitate air incorporation, thus decreasing the specific mass value.

Cheaper ice creams usually contain more air than premium brand ones. One of the disadvantages of excessive air incorporation into ice creams is the tendency for it to melt more quickly. The amount of air has also a large effect on specific mass, where the best ice creams have a specific mass of up to 0.90 mg/L (25).

Oliveira et al. (26) evaluated ice cream air incorporation (overrun) of soybean and verified 80 % of overrun, and Passos et al. (27) found values of overrun close to the present study (29 %) in guava ice cream formulations.

The melting point behaviour of elaborated formulations, showing different characteristics among them with two groups of similar behaviour, one made by F1, F3, and F5 formulations, and the other one made by F2, F4, F6, F7, and F8 formulations. F1, F3, and F5 formulations presented a 40 % melting
for the first 5 min, distinctive from the other formulations that presented a 3% melting in 50 min. Passos et al. (27) evaluated three different guava ice cream formulations and observed 100% melting point in 35 min. However, milk-based ice creams presented 70 to 98% melting at times longer than 50 min (28).

Gelatos with high soy protein concentration are normally less stable, therefore with a smaller melting point. It was noted that F2, F4, F6, F7, F8, F9, and F10 formulations did not present a uniform and homogeneous melting due to their high protein and fat concentrations. Ice cream with high-quality must demonstrate limited resistance to melting when exposed to room temperature for a period of time. In this particular case, formulations from runs 1, 3, and 5 presented limited resistance, promoting a considerable amount of time to start melting, showing clots and phase separation during the experiment, which is related to the high level of solids on formulations due to the high contents of soybean protein concentrate. Other factors that contribute to this behavior are the excessive use of stabilizers/emulsifiers, high overrun, or even severe processing and interactions between components that promote highly stable gel formations (29).

There is a correlation between the variables observed in the analysis of principal components, where PC1 responded by 61.48% and PC2 to 16.15% of the total variance (Fig. 2). The protein presented a positive correlation (P<0.05) with respect to solubility (0.676) and specific mass (0.624) and negative (P<0.05) with melting point (-0.712) and fat (-0.586). There is a negative correlation, also, of the melting point with the solubility (-0.743) and specific mass (-0.886) and positive with the destabilization of the protein (0.540) and overrun (0.592). However, the overrun presented positive correction with the melting point (0.592) and fat (0.508) and negative with the specific mass (-0.738). The PCA (Fig. 2) also showed differentiation between the gelato formulations.

Fig. 2 HERE

The structural analysis by X-ray diffraction from soybean gelato formulations is seen in Fig. 3 (F1, F3, F5, and F9). Crystallinity patterns are established based on interplanar spacings and X-ray diffraction lines relative intensity (30), with gelato formulations presenting similar behavior; several main peaks near the 15° and 35° diffraction angles at 2Θ, which characterized the crystalline part (Fig. 3 – F1, F3, F5, and F9; other formulations Fig. not shown). A-type pattern presented higher intensity peaks at 2Θ equal to 15°, 17°, 18°, and 23° and B-type presented peaks at 2Θ equal to 5.6°, 15°, 17°, 22°, and 23°, where A-type pattern is more thermodynamically stable and denser than the B-type pattern, indicating greater cohesiveness between molecules (31). Soybean gelato formulations did not present
peaks lower than 15° at 2θ, characterized as an A-type pattern, demonstrating a tendency to cohesiveness between molecules.

Fig. 3 HERE

Fig. 4 presented rheological behaviour (viscosity versus shear rate) of soybean gelato formulations for F1 (a): 10 °C and (b): 20 °C; F3 (c): 10 °C and (d): 20 °C; and F5 (e): 10 °C and (f) 20 °C. In general, soybean gelato formulations presented a non-Newtonian fluid behavior. Such behavior is linked to the nonlinear relationship between shear rate and shear stress, in other words, a shear rate increase caused viscosity reduction on all analyzed samples. F1 formulation has more viscosity, of approximately 700 cP at 10 °C and 1,000 cP at 20 °C. This range of viscosity improved the overrun and the acceptability of the product. However, F3 formulation, which had greater fat contents, it showed lower viscosity and F5 had similar behavior to F3, with values around 450 cP at 10 °C and 680 cP at 20 °C. Oliveira et al. (26), observed that ice cream samples viscosity decreased with the shear rate, a behavior also noted in this study.

Fig. 4 HERE

The Fig. 5 show the traces of mass loss and its differential curves in the temperature range of 40-600 °C.

Fig. 5 HERE

The TGA curves (Fig. 5) for the formulations (F1, F3 and F5) showed similar behavior with four bands of mass loss. It can be seen that there is a small loss of mass at temperatures below 100 °C, which is due to the loss of moisture. The first band observed between 180-250 °C may be related to loss of adsorbed and binding water. The second band observed between 250-400 °C corresponds to the decomposition of the soy protein and the fat/emulsifier of the formulation. The third band, observed between 400-460 °C and the fourth of 460-540 °C which may be associated with components degradation (protein, fat and others).

By evaluating the melting point result and behavior of the gelato formulations functional characteristics, runs 1, 3 and 5 were evaluated in relation to the consumers acceptability (Table 3), with values of 80.55, 70.33 and 85.22 %, respectively. The runs 1 and 5 did not differ statistically (P<0.05), corresponding on the score scale of liked it regularly and really liked it.
In this way, it is possible to add up to 5 % (m/V) soy protein concentrate, 14 % (by volume) soybean hydrosoluble extract and 15 % vegetable fat to obtain a soybean gelato with functional characteristics.

CONCLUSION
The replacement of milk with hydrosoluble extract and soybean protein concentrates demonstrated to be an alternative on edible gelato preparation with distinguishing characteristics in terms of protein, solubility, viscosity, melting point, and overrun with consumers acceptability.
The addition of up to 5 % (m/V) protein concentrate, 14 % (by volume) soybean hydrosoluble extract, and 15 % vegetable fat on gelato formulations presented better structural characteristics, with viscosity ranging from 450-700 cP at 10 °C, a non-Newtonian behavior, protein stability (total protein 8.44 % and solubility 41 %). Soybean gelato structural analysis using X-ray diffraction presented 15º and 35º diffraction angles at 2 θ, characterizing the crystalline part of the product. The thermal analyses showed four bands of mass loss in the temperature range of 40-600 °C, characterizing loss of moisture, decomposition of the soy protein and the fat/emulsifier of the formulations. Therefore, replacing milk with soybean protein concentrate on gelato make it an innovative, distinguish product with outstanding and specific characteristics. It is a lactose and milk protein-free product that may be appreciated by the general public, and mainly, for the populations with intolerance to such components.

ACKNOWLEDGMENTS
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REFERENCES


Table 1. Full factorial design matrix $2^2$ (coded and real values) and responses on protein, solubility, fat, specific mass - SM, melting point – MP and overrun of the soybean gelato formulations

<table>
<thead>
<tr>
<th>Run</th>
<th>Independent variables*</th>
<th>Responses</th>
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<td>$X_1$</td>
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<tr>
<td>11</td>
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<td>0</td>
</tr>
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</table>

* $X_1 =$ soy protein concentrate (%), $X_2 =$ Hydrogenated fat (%), $^1$Expressed in dry basis.
Table 2. Sedimentation, flotation, particles accumulation, and total protein destabilization – D, behaviors observed along the measuring tube for gelato formulations

<table>
<thead>
<tr>
<th>Run</th>
<th>Sedimentation/mm</th>
<th>Flotation/mm</th>
<th>Particles accumulation/mm</th>
<th>D/%</th>
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<tbody>
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<td>1</td>
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<td>65 - 70</td>
<td>21.9</td>
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<td>-</td>
<td>10 - 60</td>
<td>60 - 70</td>
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<td>&lt; 15</td>
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<td>60 - 70</td>
<td>21.5</td>
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Table 3: Consumers´ acceptability of soybean gelato

<table>
<thead>
<tr>
<th>Runs</th>
<th>Panelists Score*</th>
<th>Acceptability/ % *</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>(7.25±1.34)\textsuperscript{a}</td>
<td>(80.55±14.89)\textsuperscript{a}</td>
</tr>
<tr>
<td>3</td>
<td>(6.33±0.62)\textsuperscript{b}</td>
<td>(70.33±6.89)\textsuperscript{b}</td>
</tr>
<tr>
<td>5</td>
<td>(7.67±1.12)\textsuperscript{a}</td>
<td>(85.22±12.44)\textsuperscript{a}</td>
</tr>
</tbody>
</table>

* (Means ± standard deviation) followed by the same lowercase letter in the row, did not differ by Tukey’s test (P <0.05); Hedonic scale (9 = extremely liked and 1 = extremely disliked)
Fig. 1. Contour curves for total protein (a), solubility (b), specific mass (c) and melting point (d) content as a function of soybean protein concentrate and vegetable fat soybean gelato, respectively.
Fig. 2. Principal Component Analysis (PCA) of gelato formulations

<table>
<thead>
<tr>
<th>Variables</th>
<th>Runs</th>
</tr>
</thead>
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</tr>
<tr>
<td>Fat</td>
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</tr>
<tr>
<td>Melting point</td>
<td>3, 5</td>
</tr>
<tr>
<td>Overrum</td>
<td>1</td>
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<tr>
<td>Solubility</td>
<td>8, 10</td>
</tr>
<tr>
<td>Specific mass</td>
<td>6, 9</td>
</tr>
</tbody>
</table>

Factor 1/61.48 %
Factor 2/16.15 %
Fig. 3. Soybean gelato formulations diffractograms (a: F1, F3; b: F5, F9)
Fig. 4. Viscosity behavior for F1 (a): 10 °C and (b): 20 °C; F3 (c): 10 °C and (d): 20 °C; and F5 (e): 10°C and (f) 20°C of soybean gelato formulations.
**Fig. 5.** Thermal analyses of gelato formulations in temperature range of 40-600 °C