Abstract
In this study are described the protocols for the manufacture of miniature version of Fresh and Chihuahua cheese. But also, it was analyzed the usefulness of these miniature cheeses determining instrumental Texture Profile Analysis (TPA). Portions of 250g of pasteurized milk were used for the manufacture of miniature Fresh-type and Chihuahua-type cheeses. The miniature cheeses had 4 cm of diameter with a range weight of 28g (Fresh cheese) to 22g (Chihuahua cheese). Miniature cheeses had the proper size to perform TPA, obtaining three cheese samples from each miniature cheese. The composition and texture characteristics of miniature cheeses were similar than the observed in commercial Fresh and Chihuahua cheeses. Miniature cheeses can be used for the assessment of new ingredients and their effect on the cheese texture, saving space, time and costs.

Keywords: mini-cheeses protocol, cheese texture, Fresh cheese, Chihuahua cheese.

Introduction
Nowadays, there is a growing interest in the development of new dairy products. A number of new ingredients and technologies from around the world are helping to improve the manufacture of cheese. Some examples of ingredients are the cheese-ripening enzymes, lactic acid bacteria that produces exo-polisaccharides, acidulants (like sodium acid sulfate), anticaking agents and enzymes that improve the cheese yield.
Recently, there also have been widely explored new products for cheese added with ingredients or modified to produce a benefit for health. Some examples of these products are the low-fat, and low-salt cheeses, and the cheeses added with functional ingredients like vitamins, minerals, probiotics, prebiotics, soluble dietary fiber and omega-3 fatty acids (Pszczola, 2006; Johnson et al., 2009). Nevertheless, one of the main concerns in the use of new ingredients or technologies for the manufacture of cheese is the possible change in its texture characteristics.

The cheese texture is influenced by the ingredients used for its manufacture (composition and heat treatment of the milk, lactic acid bacteria used, type of coagulant, addition of gums, etc.), the manufacturing procedures (acidification rate of the milk and curd, cutting size, stirring and stretching conditions of the curd, washing steps, etc.), the chemical composition of the cheese (water, protein and fat content, pH, calcium, sodium and phosphate content, etc.) and the conditions of ageing (time and temperature, residual enzyme activity, relative humidity, etc.) (Lucey et al., 2003).

The texture, along with the flavor and appearance of the food are the main factors in the sensory acceptability of a food product (Bourne, 2004). According to Bourne (2002) the food texture is defined as …»that group of physical characteristics that arise from the structural elements of the food, are sensed primarily by the tactile perception, are related to the deformation, disintegration, and flow of the food under a force, and are measured objectively by functions of mass, time and distance». The texture in the food can be measured by sensory tests like descriptive sensory analysis with trained judges, and by using instrumental methods (Bourne, 2004; Foegeding et al., 2011). One of the most used instrumental methods for solid food is the Texture Profile Analysis (TPA). In this method, a sample of food is compressed and decompressed two times, imitating the first two chews on a food. The results obtained with this probe give different texture notes that have good correlation with the obtained by sensory methods (Bourne, 1978; Bourne, 2004). The instrumental TPA was developed 40 years ago and it is a very popular and used method not only in research but also in the industry as quality control of the food texture (Pons and Fiszman, 1996). Currently, the instrumental TPA remains to be among the most broadly used instrumental measurement for cheese-texture evaluation (Gunasekaran and Ak, 2003).

The assessment of new ingredients or changes in the manufacture procedures over the cheese texture usually requires the production of experimental cheeses. Sometimes due to the number of treatments and its corresponding replicates the experiments use to be, even on a pilot scale, expensive, time-consuming, require large space for its storage, and use to be of low reproducibility (Shakeel-Ur-Rehman et al., 2001). Some alternatives for the production of cheese on a pilot scale have been suggested for different uses. Salles and others (1995) reported a cheese model for evaluation of sensory attributes and flavor compounds. Milesi et al. (2007) reported the use of mini soft cheeses for monitoring microbial populations and proteolysis during the cheese ripening. Shakeel-Ur-Rehman et al. (1998) described the methodology for the manufacture of miniature cheese with similar composition and flavor of Cheddar cheese. This miniature cheese has been used for the assessment of the proteolytic activity and the heat resistance of some lactic acid bacteria (Shakeel-Ur-Rehman et al., 1999; Jeanson et al., 2003). Hynes et al. (2000) modified the methodology of Shakeel-Ur-Rehman et al. (1998) to produce a miniature washed-curd cheese, under controlled microbiological conditions. These authors used this methodology for testing single bacterial strains in a washed-curd cheese environment. Nevertheless, almost all the cheese models described have been used for microbiology or sensory studies, but have been scarcely used for texture analysis.
In this study are described the protocols for the manufacture of miniature version of two of the most consumed cheeses in Mexico and USA by the Hispanic community, the Fresh and Chihuahua cheese. This miniature Fresh and Chihuahua cheeses were compared in composition and texture with commercial samples of Fresh and Chihuahua cheese, to assess its usefulness determining the texture of these kinds of cheeses by instrumental TPA.

Materials and Methods

Manufacture of miniature Fresh-type cheese.

Portions of 250g of standardized (3% fat) milk were placed in four glass beakers of 500 mL and pasteurized at 63 °C for 30 minutes. The milk portions were cooled at 32 °C and added with 100 μL of a CaCl$_2$ solution 6.6 M. Afterward, 13.75 μL of chymosin (Chy-Max, Chr Hansen, Horsholm, Denmark) was added to the milk and incubated (incubator Shel Lab, Oregon, USA) by one hour at 32 °C. The coagulum formed was cut with a stainless-steel spatula in small cubes of 0.8 cm$^3$, held for 10 minutes and then stirred at 150 rpm for 60 minutes in an orbital shaker. The whey was drained, and the curd was salting with 1% NaCl (w/w) and transferred into polypropylene tubes (4.3 cm diameter and 9 cm height). The tubes were centrifuged at 1700 x g for 30 minutes at room temperature. The expelled whey was drained and the curd was centrifuged again for 60 minutes at 1700 x g. Mini cheeses were removed from the tubes, and stored in refrigeration unpacked at 4 °C and 75% Relative Humidity (RH) for 48 hours. Finally, mini cheeses were wiped with tissue paper, packed in hermetic polyethylene bags (16.5 cm x 14.9 cm) and stored at 3 °C for one week before its analysis (Figure 1).

Manufacture of miniature Chihuahua-type cheese.

Portions of 250 g of standardized (3% fat) milk were placed in four glass beakers of 500 mL and pasteurized at 63 °C for 30 minutes. Milk portions were cooled at 32 °C and inoculated with a freeze-dried starter culture that contained Lactococcus lactis spp. lactis, Lactococcus lactis spp. cremoris and Lactococcus lactis spp. lactis biovar diacetylactis (Choozit, Danisco, Niebüll, Germany). The milk was incubated for one hour at 32 °C and afterward added with 100μL of CaCl$_2$ 6.6 M and 13.75μL of chymosin, Chy-Max (Chr Hansen, Horsholm, Denmark). After one hour of incubation at 32 °C, the coagulum was cut with a stainless-steel spatula in small cubes of 0.8 cm$^3$, held for 10 minutes and then stirred at 150 rpm for 60 minutes in an orbital shaker. The whey was drained, and the curd was warmed to 38 °C in a water bath (IsoTemp 210, Fisher Sci, Iowa, USA) until the pH reached 5.6. Then the curd was salting with 1% NaCl (w/w) and transferred into polypropylene tubes (4.3 cm diameter and 9 cm height). The tubes were centrifuged at 1700 x g for 30 minutes at room temperature. The expelled whey was drained and the curd was centrifuged again for 60 minutes at 1700 x g. Mini cheeses were removed from the tubes, and stored in refrigeration unpacked at 4 °C and 75% Relative Humidity (RH) for 48 hours. Finally, mini cheeses were wiped with tissue paper, packed in hermetic polyethylene bags (16.5 cm x 14.9 cm) and stored at 3 °C for one week before its analysis (Figure 1).
Figure 1. General procedures for the manufacture of Fresh-type and Chihuahua-type miniature cheeses.
flat-ended probe (P/2 SL) of 2.5 cm of diameter. Three cylindrical sections (1.2 cm diameter and 1.5 cm height) from each cheese sample (Figure 1) were compressed by 75% twice using a crosshead speed of 0.05 cm/sec. The second compression was delayed 5 seconds from the first compression. Cheese samples were allowed to equilibrate at room temperature (25 ºC) prior to testing. Hardness, fracturability, adhesiveness, springiness and cohesiveness were calculated by the software instrument called Texture Exponent (Stable Micro System, London, England).

Statistical analysis.

A completely randomized design was used to compare rheological and proximal data obtained from the mini cheeses with the information acquired from samples of Fresh and Chihuahua cheese brands. Additionally, a Tukey test was performed for multiple-comparison of means. On the other hand, a principal component analysis (PCA) using a correlation matrix was carried out to determine similarities or clusters among the cheese samples. PCA was obtained with the data collected from the TPA and compositional analysis. These analyses were performed using the Minitab Release 14.12.0 software (Minitab Inc. USA).

Results and Discussion

Composition of miniature cheeses.

Miniature Fresh cheeses had an average size of 4 cm of diameter and 2.0 cm of height, with a weight of 28 ± 1.04 g. The fat content of miniature Fresh cheeses was the same that the observed in the three brands of Fresh cheese. However, their protein and moisture content was similar to two of the three brands of Fresh cheese (Table 1). The pH of miniature Fresh cheeses and the samples of the different Fresh cheese brands were statistically different. Fresh cheese is a curd-like product with high moisture (46-57%) and low fat (18-29%) content and a nearly neutral pH (6.1). The shelf life of this type of cheese is only two weeks in refrigeration (VanHekken and Farkye, 2003; Walstra et al., 2006). The high moisture and neutral pH of the Fresh cheese allow the growth of different microorganisms (even at low temperatures) during its storage, acidifying the pH of the cheese. The pH variability among the samples of the different brands of Fresh cheese and the miniature cheeses could be due to differences in the time of manufacture before its analysis. Nevertheless, in general, it was observed that the composition of the different Fresh cheese brands, and the miniature Fresh-type cheeses were similar to the reported by other authors (Table 1) for Fresh cheese (VanHakken and Farkye, 2003).

Miniature Chihuahua cheeses showed a lower weight (22 ± 0.98g) and height (1.8 cm) than the observed in the miniature Fresh cheeses. The fat contents of the miniature Chihuahua cheeses were the same that the observed in the samples of the four brands of Chihuahua cheese. The protein content of miniature Chihuahua-type cheeses was equal to three of the four commercial samples of Chihuahua cheese. However, the moisture content was significantly higher in the miniature cheeses, and the second brand (Table 1) than the observed in the other three brands of Chihuahua cheese. Chihuahua cheese is a semi-hard product made from either raw milk or pasteurized milk by the Mennonite communities settled in the state of Chihuahua, Mexico. The main compositional difference between Chihuahua cheese and Cheddar cheese is the high moisture content, usually as high as 45% (Gutierrez-Mendez and Nevarez-Moorillón, 2009). The difference in moisture content among the Chihuahua cheese brands assessed in this study (Table 1) is probable due to differences in the process methods or the ripening time of each cheese. The miniature cheeses had only seven days of ripening and thus, high moisture content, whereas some brands of Chihuahua cheese use to ripen the cheese at least one month. As well as miniature Fresh cheeses, the miniature Chihuahua cheeses had similar compositional characteristics than the reported for Chihuahua cheese brands (Gutierrez-Mendez and Nevarez-Moorillón, 2009).
Table 1. Summary of the composition observed in commercial samples of Fresh and Chihuahua cheeses and miniature Fresh-type and Chihuahua-type cheeses.

<table>
<thead>
<tr>
<th>CHEESE TYPE</th>
<th>Fat (%)</th>
<th>Protein (%)</th>
<th>Moisture (%)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh-type miniature cheeses</td>
<td>16.13 ± 1.93 a</td>
<td>17.18 ± 2.57 b</td>
<td>54.92 ± 1.01 a</td>
<td>6.00 ± 0.02 a</td>
</tr>
<tr>
<td>Fresh cheese (Brand 1)</td>
<td>18.25 ± 1.50 a</td>
<td>19.24 ± 3.07 ab</td>
<td>50.60 ± 1.78 b</td>
<td>5.91 ± 0.02 c</td>
</tr>
<tr>
<td>Fresh cheese (Brand 2)</td>
<td>18.63 ± 1.11 a</td>
<td>22.87 ± 1.19 a</td>
<td>54.73 ± 0.66 a</td>
<td>6.33 ± 0.06 a</td>
</tr>
<tr>
<td>Fresh cheese (Brand 3)</td>
<td>16.50 ± 1.91 a</td>
<td>15.72 ± 0.90 b</td>
<td>55.63 ± 0.73 a</td>
<td>5.84 ± 0.06 d</td>
</tr>
<tr>
<td>Fresh cheese*</td>
<td>18-29</td>
<td>17-21</td>
<td>46-57</td>
<td>6.1</td>
</tr>
<tr>
<td>Chihuahua-type miniature cheeses</td>
<td>27.25 ± 2.99 b</td>
<td>24.43 ± 1.42 a</td>
<td>42.3 ± 1.44 a</td>
<td>5.35 ± 0.05 c</td>
</tr>
<tr>
<td>Chihuahua cheese (Brand 1)</td>
<td>28.75 ± 2.22 ab</td>
<td>25.97 ± 0.80 a</td>
<td>37.54 ± 1.43 b</td>
<td>5.43 ± 0.02 b</td>
</tr>
<tr>
<td>Chihuahua cheese (Brand 2)</td>
<td>30.75 ± 2.06 a</td>
<td>24.18 ± 2.21 a</td>
<td>43.34 ± 0.70 a</td>
<td>5.23 ± 0.01 d</td>
</tr>
<tr>
<td>Chihuahua cheese (Brand 3)</td>
<td>27.00 ± 2.16 a</td>
<td>20.42 ± 1.30 b</td>
<td>37.50 ± 0.73 b</td>
<td>5.48 ± 0.02 a</td>
</tr>
<tr>
<td>Chihuahua cheese (Brand 4)</td>
<td>27.00 ± 1.41 b</td>
<td>23.63 ± 0.61 a</td>
<td>38.74 ± 0.41 b</td>
<td>5.50 ± 0.01 a</td>
</tr>
</tbody>
</table>

| Chihuahua cheese**          | 21-36     | 22-28        | 33-45         | 5.0-5.5     |

a,b,c,d, Superscripts with different letter in the same column indicate significant differences (p < 0.05).

*Composition of commercial Fresh cheeses reported by VanHekken and Farkye (2003).

**Composition of commercial Chihuahua cheeses reported by Gutierrez-Mendez and Nevarez-Moorillón (2009).

Texture profile of miniature cheeses.

The fracturability (force observed at the first significant break during the first compression cycle) of miniature Fresh cheeses was equal to the observed in all the commercial brands of Fresh cheese (Table 2). Nevertheless, also miniature Fresh cheeses were similar, at least in two of the three brands of Fresh cheese, in hardness (peak force during the first compression), adhesiveness (work necessary to pull the compressing plunger away from the sample), springiness (the height that food recovers), chewiness (work necessary for a double compression) and cohesiveness (Bourne, 1978). During the manufacture of Fresh cheese, the curd is finely milled and salted, producing a crumbly texture in the cheese (VanHekken and Farkye, 2003). Because of this, with a small load the cheese matrix can be fractured. Fresh cheeses do not melt when heated, because at the pH of this kind of cheese (6.1) almost all the colloidal calcium phosphate (CCP) is undissolved. When the pH is lowered the CCP dissolves, increasing salt bridges between caseins and swelling; modifying the texture properties of the cheese (Lucey et al., 2003; VanHekken and Farkye, 2003).

The miniature Chihuahua cheeses presented the same hardness and adhesiveness than three of the four brands of Chihuahua cheese analyzed (Table 2). The springiness of the miniature cheeses was similar to the brands four and one, but different to the brands two and three. In contrast, the values of chewiness of the miniature Chihuahua cheeses were similar to the brands two and three but different to the brands one and four. The Chihuahua cheese is described as being a cross between Cheddar and brick cheese, but some rheological similarities to Colby and Havarti cheese have been reported. Chihuahua cheese requires similar force for fracture as fresh Cheddar cheese, but tolerates deformation similar to Colby cheese and has shear rigidity like Havarti.
and brick cheeses (Tunick et al., 2007; Van Hekken et al., 2007; Tunick et al., 2008).

The texture of the cheese is produced by a complex interaction of different factors like cheese composition, manufacturing and ripening conditions (Lucey et al., 2003). For this reason, it is normal to observe coincidences and differences between the commercial cheese samples and the miniature cheeses. Nevertheless, to know if miniature cheeses were similar to their corresponding commercial versions, it was performed a multivariate analysis, considering all the responses of the TPA and the compositional analysis. From the PCA, the first principal component (PC1) had an eigenvalue of 5.45, which explain 54.5% of the total data variability. The second and third principal components (PC2 and PC3) had eigenvalues of 1.54 and 1.28. In Figure 2a, all the cheese samples, including the commercial Fresh and Chihuahua cheeses as well as the miniature Fresh-type and Chihuahua-type cheeses, were plotted using their scores from the PCA. Miniature Fresh-type cheeses have similar characteristics to the Fresh cheese of brand one. The Fresh cheeses of brand two were slightly different from the other Fresh cheeses; probably, because its higher protein content (Table 1) and acidic pH (6.3), but also due to differences in its cohesiveness and chewiness (Table 2). On the other hand, miniature Chihuahua-type cheeses were similar to Chihuahua cheeses of brand one, two and three. However, Chihuahua cheeses of brand four had different characteristics. The main differences observed in the cheeses of brand four were the high adhesiveness, and the low values of chewiness and cohesiveness. These results suggest that Chihuahua cheese of brand four perhaps were added with an additional ingredient like gum.

In Figure 2b, vectors represent a graphical display of the loading for the variables used in the PCA. The variables of cohesiveness, hardness and chewiness had similar loads than the variables of protein and fat content, indicating that changes in fat and protein content will affect these texture variables. This observation is related to the described by Gunasekaran and Ak (2003), that a reduction in fat content produces an increase in the hardness of the cheese. In contrast, the adhesiveness and springiness showed similar loads that the pH and the moisture.

### Table 2. Texture profile analysis (TPA) of commercial Fresh cheese and Chihuahua cheese samples, and miniature Fresh-type and Chihuahua-type cheeses.

<table>
<thead>
<tr>
<th>CHEESE TYPE</th>
<th>Hardness (N)</th>
<th>Fracturability (N)</th>
<th>Adhesiveness (Ns)</th>
<th>Springiness (mm)</th>
<th>Chewiness (mJ)</th>
<th>Cohesiveness</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh-type miniature</td>
<td>7.59 ± 0.59a</td>
<td>2.54 ± 0.41a</td>
<td>0.20 ± 0.08a</td>
<td>6.79 ± 0.80a</td>
<td>9.98 ± 0.56a</td>
<td>0.26 ± 0.04a</td>
</tr>
<tr>
<td>Fresh cheese (Brand 1)</td>
<td>6.84 ± 0.79a</td>
<td>4.07 ± 3.53a</td>
<td>0.10 ± 0.02a</td>
<td>7.61 ± 0.37a</td>
<td>12.53 ± 1.83b</td>
<td>0.24 ± 0.02a</td>
</tr>
<tr>
<td>Fresh cheese (Brand 2)</td>
<td>6.60 ± 0.51a</td>
<td>1.99 ± 3.44a</td>
<td>0.03 ± 0.01b</td>
<td>8.65 ± 0.44b</td>
<td>22.62 ± 2.17c</td>
<td>0.40 ± 0.02b</td>
</tr>
<tr>
<td>Fresh cheese (Brand 3)</td>
<td>4.50 ± 0.58b</td>
<td>3.81 ± 0.58a</td>
<td>0.27 ± 0.02a</td>
<td>6.76 ± 0.48a</td>
<td>7.19 ± 1.43a</td>
<td>0.23 ± 0.01b</td>
</tr>
<tr>
<td>Chihuahua-type miniature</td>
<td>14.81 ± 2.55a</td>
<td>5.48 ± 1.78a</td>
<td>0.26 ± 0.16a</td>
<td>4.54 ± 1.25a</td>
<td>34.69 ± 2.75a</td>
<td>0.62 ± 0.02d</td>
</tr>
<tr>
<td>Chihuahua cheese (Brand 1)</td>
<td>23.84 ± 2.36b</td>
<td>ND</td>
<td>0.19 ± 0.26a</td>
<td>6.71 ± 1.29a</td>
<td>64.28 ± 2.48c</td>
<td>0.38 ± 0.01c</td>
</tr>
<tr>
<td>Chihuahua cheese (Brand 2)</td>
<td>9.27 ± 0.96a</td>
<td>ND</td>
<td>0.18 ± 0.10a</td>
<td>7.04 ± 0.49b</td>
<td>27.14 ± 5.85b</td>
<td>0.52 ± 0.02b</td>
</tr>
<tr>
<td>Chihuahua cheese (Brand 3)</td>
<td>11.76 ± 2.30a</td>
<td>10.59 ± 3.26a</td>
<td>0.05 ± 0.07a</td>
<td>8.05 ± 0.36b</td>
<td>26.91 ± 7.93d</td>
<td>0.28 ± 0.02a</td>
</tr>
<tr>
<td>Chihuahua cheese (Brand 4)</td>
<td>9.29 ± 2.00a</td>
<td>6.39 ± 0.56a</td>
<td>0.67 ± 0.12a</td>
<td>5.78 ± 0.50a</td>
<td>13.09 ± 2.92a</td>
<td>0.24 ± 0.02a</td>
</tr>
</tbody>
</table>

Mean value ± standard deviation; n=3. ND = not detected.

a,b,c,d Superscripts with different letters in the same column, for the same type of cheese, indicate statistically significant differences (P<0.05).
Figure 2. (a) Principal component analysis obtained from the variables of composition and texture of Chihuahua cheeses, Fresh cheeses and mini-cheese versions of Fresh and Chihuahua cheeses; (b) Graphical display of the loadings of the variables used for the principal component analysis.
Conclusion

The composition and texture characteristics of the miniature cheeses were similar to the obtained in the samples of commercial Fresh and Chihuahua cheeses. For this reason, miniature Fresh-type and Chihuahua-type cheeses could be used for the assessment of texture or changes in texture by addition or modification of ingredients; saving space, time and costs.

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References


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Resúmenes curriculares de autor y coautores

NESTOR GUTIÉRREZ MENDEZ. Ingeniero Agroindustrial por la Universidad Autónoma del Estado de Hidalgo (UAEH), con maestría en Ciencia y Tecnología de Alimentos por la Universidad Autónoma de Chihuahua (UACH) y doctorado en Ciencia de los Alimentos por el Centro de Investigación en Alimentación y Desarrollo (CIAD). Actualmente se desempeña como profesor investigador en la Facultad de Ciencias Químicas de la Universidad Autónoma de Chihuahua. Participa en los programas de licenciatura y en el posgrado en Ciencia y Tecnología de Alimentos. Ha sido ganador en dos ocasiones del Premio Nacional en Ciencia y Tecnología de Alimentos. Actualmente miembro del Sistema Nacional de Investigadores Nivel I.

NALLELI TRANCOSO REYES. Terminó su licenciatura en 2009, año en que le fue otorgado el título de Ingeniero Bioquímico por el Instituto Tecnológico de Durango (ITD). Realizó su posgrado en la Facultad de Ciencias químicas de la Universidad Autónoma de Chihuahua, donde obtuvo el grado de Maestro en Ciencias en el área de Tecnología de alimentos. Ha participado en 3 ponencias en congresos y ha sido reconocida con el premio Nacional en Ciencia y Tecnología de Alimentos Coca-Cola 2011, en la categoría Profesional en Tecnología de Alimentos.

MARTHA YARELY LEAL RAMOS. Terminó su licenciatura en 2001, año en que le fue otorgado el título de Ingeniero Químico opción Alimentos por la Facultad de Ciencias Químicas de la Universidad Autónoma de Chihuahua (UACH). Realizó su posgrado en Chihuahua, México, donde obtuvo el grado de Maestro en Ciencias en Tecnología de Alimentos en 2005 por la Universidad Autónoma de Chihuahua, y el grado de Doctor en Filosofía en el área de alimentos cárnicos en 2012 por la Universidad Autónoma de Chihuahua. Desde 2010 labora en la Facultad de Ciencias Químicas de la UACH y posee la categoría de Académico titular B. Ha solicitado su ingreso al Sistema Nacional de Investigadores en 2013. Su área de especialización es sobre tecnologías emergentes para el procesamiento de alimentos, entre ellos la carne.