Development of a device for online monitoring of the milk coagulation process

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ABSTRACT

In this investigation we studied a device for monitoring the enzymatic or acid coagulation of milk. The instrument consists of a platinum heater/sensor, a Platinum Resistance Temperature Detector (PRTD) temperature sensor, a pH electrode, a direct constant current source, a digital acquisition system that, with the developed software runs on a Personal Computer. The heater/sensor was built with a 0.025 mm diameter platinum wire coiled around a ceramic structure and immersed in a small protective thermowell, which allowed monitoring the physical changes of milk. Under conditions of quiet air and room temperature (25°C), tests showed that this probe was able to withstand a DC current of at least 190 mA. Different tests performed with different values of DC constant current allowed to select the appropriate current (130 mA). Using this DC constant current, this experiment proved to be highly reproducible, with low signal to noise ratio. Heat transfer analysis also showed temperatures of <50°C on the heater/sensor thermowell surface, with no risk of soluble protein denaturation. The effects of temperature, pH, enzyme concentration and CaCl₂ addition, on the responses CT, Tmax and firmness, were similar to those obtained with other methods. The small size of this sensor makes it a non-invasive, though robust enough device, easy to clean in place or to sterilize using steam in place, which does not need to be removed during cheesemaking operations. In addition, the instrument meets the sanitary requirements of the 3-A and EHEDG standard, which allows it to be used on cheese or yogurt production lines.

Keywords: instrument, sensor, behavior, cheese, yogurt.
INTRODUCTION

In recent decades there has been growing interest in the development of techniques, methods, devices and instruments to adequately monitoring the enzymatic coagulation process of milk. These advances have been used not only to perform continuous process controls and to study the influence of critical variables such as pH, temperature, concentration of enzymes, and the addition of CaCl2, but also to optimize the cutting time, avoid the loss of “fines”, improve yields, and homogenize cheese quality.

Bynum and Olson (1982) demonstrated that control of curd firmness and cutting time can improve the recovery of casein and fat. Later, Ustunol and Hicks (1990) revealed that the control of the cheesemaking process can contribute to improve the yield of the final product and its quality.

Taking into account the economic aspects, numerous methods have been developed and studied to measure coagulation time and firmness. Also, several studies were conducted to describe the advantages and disadvantages of the different devices and instruments used in the control of the coagulation process by chymosin. Some of them are Tomaszow and Voss (1977), Kowalchyk and Olson (1978), Hatfield (1981), Marshall et al. (1982), Mc Mahon and Brown (1982), Richardson et al. (1985), Hori (1985), Van Hooydonk and Van Den Berg (1988), Ustunol and Hicks (1990), Fox et al. (2000), O’Callaghan et al. (2000).

In Argentina, Sbodio et al. (2002) demonstrated that the use of an objective and non-destructive method (hot wire method) to control the coagulum formation, plus the use of the response surface methodology, can be recommended to optimize the enzymatic coagulation of milk.

In recent years there have also been advances in the control of the syneresis of the curd, an essential step in the production of cheese. Several authors, including Fagan et al. (2007a, 2007b) investigated a novel light backscatter sensor to continuously monitor coagulation and syneresis, in order to improve control of the moisture content of the curd.

This technology proved to have a good potential not only to control coagulation and syneresis, but it could also be used to control curd fat loss and its yield, thus improving the control of the cheese-making process (Fagan et al., 2008). Taifi et al. (2006) used a non-intrusive ultrasonic method to characterize the syneresis and firmness of milk gel. Mateo et al. (2009) reported the validation of a curd syneresis sensor in a range of milk composition and process parameters.

Lucey (2002) and O’Callaghan et al. (2002) analyzed different systems to monitor the firmness of the curd during cheese making and reviewed the literature on the development of a suitable device to monitor the coagulation process and predict the appropriate cutting time. In their analysis, they demonstrated that a device to track curd formation in commercial cheese making needs additional requirements in comparison with those used in laboratory experiments.

As a matter of fact, they should be installed in the cheese vat, they should not be intrusive to prevent their removal during cutting and shaking operations, they should be robust, and they need to be quick and safe in predicting the cutting time so that manufacturers can monitor the formation of the curd in the cheese vat. In addition, since it is an online device, it should be possible to wash it on site. The device to be developed should have high sensitivity in the response and a low signal-to-noise ratio.

The instruments that were found to work on line can be grouped as compression wave, torsion, hot wire or optical probe. However, only two types of probes (hot wire and fiber optic reflectance) were used in the commercial manufacture of cheese (O’Callaghan et al., 2002).

Our goal was to evaluate the potential of a device for on-line control of the coagulation of cow’s milk, designed with a platinum thermopiece as a heater/sensor to monitor coagulation, a platinum thermopiece to measure the temperature of the medium, and a sanitary pH electrode.

MATERIALS AND METHODS

Experimental installation

A sensor prototype was designed at the National University of the Litoral [Universidad Nacional del Litoral] (UNL) along with the INTEC-UNL-CONICET. Figure 1 shows the schematic design.

The sensor consists of a 100 Ω double platinum thermocouple (W. C. Heraeus 2Pt100 KN 3045), 4.5 mm in diameter and 30 mm in length, connected in parallel in a four-wire configuration with 1000 silver extension wires, and a length of 30 cm each.

We chose this type of thermoelement because it offers a better distribution of the thermal energy to be transmitted, because the wire wound helically is distributed through four holes at 90° in the circumference of the ceramic structure, two holes for each element that, in turn, in the construction stage leaves each winding in contraphase, which creates a minimum inductance.

Considering the high excitation current of the thermoelement, the four wires configuration (Figure 2) avoids the potential drop in the extension cables, and consequently, the erroneous measurement. Two of the cables carry the direct current (DC) excitation to the thermoelement, while the other two cables allow the measurement of the voltage difference through a very high impedance instrument, thus rendering negligible the resistance of the extension wires.

The thermoelement shown in Figure 2 was installed in a sanitary well model TW810-C-DF-SA-045-015-T20-EDSF-N (Intemcpo Controls Ltd., Montreal, Canada) made of stainless steel 316 L, with a reduced tip for fast response time, approved by the 3-A sanitary standards (Sanitary Standards, Inc. 7403 Sensor), (Figure 3).

This allows both cleaning in place (CIP) and sterilization in place using steam (SIP). To fill the space between the thermoelement and the well to ensure the best heat transfer, we fabricated an electrolytic copper sheath with
the same length of the thermoelement. It presented with six longitudinal grooves 0.2 mm deep on its outer circumference, with an inner diameter of 4.5 mm and an external diameter of 6.3 mm, since the stainless steel (SS 316 L), of the sheath has a similar coefficient of expansion.

The grooves facilitate the drainage of the thermal compound used to fill the spaces between the components when the heater/sensor is inserted. The copper sheath has a bayonet-shaped end that allows the removal and separation of the thermoelement from the well. For this, a special tool was designed and manufactured.

The conductive material used to fill the space between the thermoelement and the copper sheath, and between the sheath and the well is a special thermal compound (Arctic Silver 5- Arctic Silver Incorporated - Visalia, CA, USA), with a conductivity thermally close to ~9 W m⁻¹ K⁻¹, very close to that of stainless steel 316 L. It endures a constant temperature of 130 °C and peaks greater than 180 °C for a short period without losing its properties or “bleeding”. Therefore, we called coagulation sensor “CS" (*patent pending) the device developed using a PRTD as a heater/sensor.

The CS must be located half of the height of the cheese vat, where holes are drilled in the double wall to place and weld connections of 316 L stainless steel with Tri-clamp flanges, depending on each of the sensors and whose dimensions allow that the sensitive part is level with the inside of the tub.

The temperature of the process was measured using a RTD (Pt100), 100 Ω at 0 °C, α= 0.00385 Ω/Ω °C according to DIN EN 60751, class AA, tolerance of ± 0.04% (± 0.1 °C) in a four-wire configuration, encased in a 316 L stainless steel sanitary sheath, 115 mm long, 1/4 “diameter, with a 2” Tri-Clamp flange and a reduced tip ¼” for a high response velocity (3-4 seconds), specially designed to control milk pasteurization according to the HTST requirements, Grade “A” Pasteurized Milk Ordinance Revision 2001, certified by 3-A Sanitary Standards, Inc. (sensor 74-03) from Intemco Controls Ltd., Montreal, Canada (3-A Sanitary RTD – RS25-L-P-3-S4-DB-S-045-N10-0-T20-MC-B2-SF).

To control pH we used an Orion Research EA 940 pH meter (Orion Research, Inc., Beverly, MA, USA), equipped with a combined sanitary electrode, with an incorporated Hamilton temperature sensor, model Easyferm Food VP 120 N° 243632. The potential was measured against the reference electrode, and the integrated temperature sensor Pt100 for pH correction and pressurized filling electrolytes to prevent diffusion of the sample to the sensor. The electrode was built to be in contact with food, innocuous for the fermenting microorganisms and has a certificate of aptitude in accordance with the standards of the EHEDG (European Hygienic Engineering Design Group).

These properties allow it to be used in the pharmaceutical, food, brewing and biotechnology industries. The electrode has a pH range of 0 to 14, sensitivity 57 to 59 mV/pH at 25 °C, cleaning in place (CIP), steam sterilization in place (SIP), working temperature from 0 to 135 °C, pressure range up to 6 bar with Variopin connector and PG 13.5 mounting thread. This special electrode, prepared for protein-fat solutions (electrochemical sensors Hamilton Hamilton Bo-
Naduz AG, Bonaduz, Switzerland) was calibrated with three buffer solutions (pH: 4.01, 7.00 and 10.00) (Hamilton Bonaduz, Switzerland).

The pH electrode is mounted on 316L stainless steel electrode holder for in-line mounting (Unifit H, Endress+Hauser model CPA442-E1A2A11), with 2” Tri-Clamp flange, female thread for PG13.5 electrode, certified by EHEDG and 3-A Sanitary Standards, Inc. (74-03), working temperature range between -15 and 140 °C, working pressure of up to 10 bar with a replaceable o-ring Viton electrode sealant, approved by the FDA, and protective sheet for the electrode.

Direct current (DC) was supplied by a constant current source model 6177C DC (Hewlett Packard, Santa Clara, CA, USA), with adjustable voltage range from 0 to 50 V and three current ranges (0 to 5 mA, 0 to 50 mA and 0 to 500 mA), controlled by a 10-lap Helipot potentiometer.

The output voltages of the CS signals, the temperature sensor and of the pH electrode were selected by a switch box controlled using an application program on a personal computer (PC), which switches the input of a 6 1/2 digit digital multimeter (Keithley model 2000, Test Instrumentation Group, Keithley Instruments, Inc. of Cleveland, Ohio, USA).
USA), directly connected to the PC. The electronic switch, the data acquisition and the control application program on the PC were developed by the research team.

The coagulation sensor, and the temperature and pH probes are assembled on a stainless steel plate, and can simultaneously monitor the milk coagulation process, either by chymosin or acidification, by recording parameters such as “viscosity”, temperature and pH evolution.

**Behaviour of the CD in the air**

Since the thermoelement, which is the primary component of the coagulation sensor, is made of a thin (0.0254 mm) platinum wire (Sensor Magazine, 2003), it was crucial to determine its behavior against high current flows. For that, tests were carried out in the range of 70-190 mA, in still air conditions, at 25 °C and the CS in a horizontal position. As the CS was built from a double PRTD of 100 Ω in parallel and with four wire configurations (two for the DC power supply and two for measurements) its nominal value at 0 °C is 50 Ω.

In order to determine the temperature of the CS and compare it with the tables ITS-90 (DIN 43 760) we used the theoretical resistance value, which was obtained by dividing the potential difference between its ends by the constant current CC and multiplying the factor by two. Exposed to still air the temperature of the thermocouple PRTD of the CS reaches values of 100 °C with currents above 170 mA. However, no physical changes or alterations in electrical properties were observed in the PRTD.

**Experimental preparation of skim (non fat) milk**

For acid coagulation we used skimmed milk powder (WPNI) <1.4; protein 35.1% (WW); fat 1.2% (WW) and moisture 3.2% (WW) obtained from Milkaut S.A. (Franck, Argentina), reconstituted at 10.45% (W/W) using distilled water (GT Lab., Rosario, Argentina). And, with the addition of sodium azide (0.02%) as preservative, we used it for enzymatic coagulation assays. Rapid dissolution of the powder was obtained by vigorously stirring the reconstitution vessel for 20 seconds. After the dissolution of the powder we added CaCl$_2$. The samples were agitated for two hours with a magnetic stirrer and left overnight to reconstitute, thus completing a total of 22 hours at room temperature. This is the time necessary for the reconstitution taking into account the firmness tests of the gel, according to Martin (2008).

**Selection and behavior of the appropriate current of the CS**

In previous experiments, enzymatic coagulation was monitored using bare platinum wires (diameter 0.1 mm, cross section 7.85398 x 10-9 m$^2$, length 110 mm) according to Sbodio et al. (2002), purity 99.95%, commercial grade Hard, resistivity p = 13.335 [Ω/m] at 0 °C, with a temperature coefficient $TC = 0.00375$ [Ω Ω$^{-1}$ C$^{-1}$] in the range of 0-100 °C (Sigmund Cohn Corporation, Mont Vernon, NY, USA). The device could monitor the coagulation process promoted by renin when excited with a constant current of 500 mA.

Considering the voltage drops in seven tests (performed in duplicate) at the beginning of the coagulation (fluid milk), the average total resistance of the platinum wire was 1.959666 Ω and the total power transferred to the milk was 0.4889 Watt. When the coagulum reaches its maximum firmness, the average resistance of the platinum wire is 1.961796 Ω and the total power transferred to the coagulum is 0.4904 Watt.

The power transferred by the wire per unit area at the beginning of the coagulation (fluid milk) and at the time of maximum firmness corresponds to 14,147.42 Watt/m$^2$ and 14,190.83 Watt/m$^2$, respectively. We used the resistivity value of the wire to calculate its resistance at 0 °C (1.466850 Ω), for which the temperature coefficient between 0 and 100 °C is 0.005501 Ω°C. This level of resistance allows us to determine 88.92 °C as the temperature of the wire submerged in fluid milk, and 89.98 °C for the coagulum in its maximum firmness, with a variation of 1.06 °C. At the end of each experiment, a thin layer of seemingly soluble proteins was seen to precipitate on the surface of the wire. The aforementioned temperatures, calculated on the surface of the wire in both milk and coagulums above 88 °C, can lead to denaturation of soluble proteins on the surface of the wire, which could modify the signal response.

To evaluate the initial performance of the new CS excited with different constant currents, seven tests (in duplicate) of the enzymatic coagulation by chymosin were performed. Reconstituted powdered skim milk was used as substrate.

At the beginning of each test the variables were adjusted to the standard conditions used by the cheese industry: pH 6.6, T= 35 °C, 0.0015 RU/ml milk (Chymax 950 IMCU), without adding CaCl$_2$. The applied DC levels were 30, 70, 90, 115, 130, 160 and 190 mA, (Figure 4). These firmness profiles (in mV) versus time evidence that when the CS is fed by currents above 130 mA results in shorter CT than when working at 130 mA. These results could also be attributed to the development of high temperatures on the surface of the sensor, which would induce denaturation of the soluble protein.

These denatured proteins would interfere and therefore modify the signal by adhering to the bare platinum wire or the tip of the CS. The signals’ responses to currents below 130 mA did not show an adequate sensitivity to the determination of firmness. A constant current of 130 mA results in a CT and a firmness response with very good repeatability (‘r’), expressed as SD (standard deviation). In two measurements, r values of 0.022 min and 0.0056 volt were obtained for CT and maximum firmness, respectively. The signal-to-noise ratio it is in the range of 250-500, calculated as the quotient between the maximum value of the signal (maximum firmness of the curd) and the standard error for estimating the flat line of the signal when the substrate is in the fluid or liquid state.
The subjective evaluation of the coagulation time (CT) was performed by observing the formation of small flocs on the walls of a manually rotated test tube. The average of the three simultaneously replicated assays shows that the CT is very close to the coagulation time measured by the sensor (<1 second).

From these observations we concluded that the excitation current of 130 mA was the appropriate value for the coagulation tests. In this condition, the temperature created inside by the CS’s wire in the coagulum corresponded to <51 °C (Table 2). This temperature would prevent the denaturation of proteins that could adhere to the sensor. However, more experiments in the range close to 130 mA are necessary to optimize the value of the excitation current of the CS.

Tables 1 and 2 show the measured parameters: constant direct current to excite the CS, voltages, resistances, temperatures according to tables ITS-90 (DIN 43 760), temperature difference (ΔT) - equivalent minus the temperature of the milk (35 °C) - and the power dissipated at the beginning of coagulation and when the coagulum reaches its maximum firmness, respectively.

**Acid and enzymatic coagulation assays**

After 22 hours of storage at room temperature, two liters of the milk prepared for the tests were placed in a thermostatic bath (Haake F2-C, Karlsruhe, Germany, accuracy ± 0.1 °C) at 30, 33, 35, 38 and 40 °C for each experiment. After adding CaCl₂, we adjusted the pH with 85% concentrated lactic acid (Mallinckrodt Chemical Work, Buenos Aires, Argentina) or with 0.1N NaOH (Cicarelli, Santa Fe, Argentina) to reach the different pH levels proposed for the experience. After verifying that the coagulation sensor reached a level of stationary heat dissipation, the enzyme was added (Chymax 950 IMCU, Chr. Hansen S.A.I.C., Quilmes, Argentina) and the milk was stirred for 1 min. Data acquisition began after the addition of chymosin.

With this device it is possible to create the voltage profile as a function of time. The coagulation time (CT) was defined as the tangent to the inflection point in the graph of the voltage output versus time, according to Kopelman and Cogan (1975), where tmax is the time of the maximum slope of the voltage profile. V30 was defined as the “firmness” obtained 30 minutes after adding the enzyme and Vmax was the maximum firmness at 60 minutes.

To monitor the acid coagulation process, which is characteristic of yoghurt processing, we inoculated the reconstituted substrate (skimmed milk 10.45%, W/W) without the addition of preservatives, with 7 ml of a stock preparation with 0.2 grams of starter Lyofast SLH (Sacco srl, Cadorago, CO, Italy), and incubated at 42 °C for 5 hours. The inoculum concentration was 1 x 10⁵ CFU/ml.

**Experimental design to evaluate the enzymatic coagulation process**

Considering that the temperature, the curd concentration, pH and the calcium chloride concentration are the most important factors that affect the enzymatic coagulation of milk (Hori, 1985; Tarodo de la Fuente et al., 1999; Benguigui et al., 1994; Sbodio et al., 2002) five levels of these factors were considered for the experiments.

Figure 4. Test profiles to monitor the detection of optimal direct current: firmness of the curd vs. time. CT5, CT6 and CT7 show the coagulation time at 130, 160 and 190 mA, respectively. (1), (2), (3), (4), (5), (6) and (7) are characteristic profiles of current sources of 30, 70, 90, 115, 130, 160 and 190 mA, respectively.
RESULTS AND DISCUSSION

Heat transfer analysis

The heat transfer analysis involved five experiments of enzymatic coagulation using the same substrate at 34.8°C, 34.5°C and 35°C. These tests allowed to determine the averages of dissipated power of the sensor both in the fluid milk as in the coagulum. Table 3 shows the average values of ΔV (voltage measured in the CS) for the fluid reconstituted milk powder and the coagulum, the temperature of the milk and of the coagulum, the resistance of the sensor, T of the sensor according to the table ITS-90 DIN 43760:1980, the difference between T of the table and milk and coagulum temperatures from a theoretical limit layer (ΔT1 + ΔT2) and the dissipated power of the sensor.

The average powers developed by the sensor for liquid milk and coagulum were 0.988376 Watt and 1.00246 Watt, respectively.

Statistical analysis

The data were analyzed by simple linear regression using the Statgraphics Package, version 7.1 (1994).

<table>
<thead>
<tr>
<th>Run</th>
<th>Current (A)</th>
<th>Voltage (Volts)</th>
<th>Calculated electrical resistance (Ω)</th>
<th>Equivalent resistance (Ω) (**)</th>
<th>Equivalent temperature of the PRTD wire (°C)(**)</th>
<th>ΔT (°C) (**)</th>
<th>Dissipated energy (Watt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.030</td>
<td>1.7103</td>
<td>57.0094</td>
<td>114.0188</td>
<td>36.07</td>
<td>1.07</td>
<td>0.051</td>
</tr>
<tr>
<td>2</td>
<td>0.070</td>
<td>3.9969</td>
<td>57.0994</td>
<td>114.1989</td>
<td>36.54</td>
<td>1.54</td>
<td>0.279</td>
</tr>
<tr>
<td>3</td>
<td>0.090</td>
<td>5.1318</td>
<td>57.0199</td>
<td>114.0398</td>
<td>36.13</td>
<td>1.13</td>
<td>0.462</td>
</tr>
<tr>
<td>4</td>
<td>0.115</td>
<td>6.7084</td>
<td>58.3342</td>
<td>116.6683</td>
<td>42.92</td>
<td>7.92</td>
<td>0.772</td>
</tr>
<tr>
<td>5</td>
<td>0.130</td>
<td>7.5916</td>
<td>58.3966</td>
<td>116.7932</td>
<td>43.25</td>
<td>8.25</td>
<td>0.987</td>
</tr>
<tr>
<td>6</td>
<td>0.160</td>
<td>9.5055</td>
<td>59.4096</td>
<td>118.8192</td>
<td>48.51</td>
<td>13.51</td>
<td>1.521</td>
</tr>
<tr>
<td>7</td>
<td>0.190</td>
<td>11.4488</td>
<td>60.2568</td>
<td>120.5136</td>
<td>52.91</td>
<td>17.91</td>
<td>2.175</td>
</tr>
</tbody>
</table>

Table 1. Parameters at the beginning of the coagulation.

(*) ITS-90 (DIN 43 760). (**) ΔT= T (Table ITS-90, DIN 43 760) – T milk (35 °C).

Table 2. Parameters at the moment of maximum firmness of the coagulum.

(*) ITS-90 (DIN 43 760). (**) ΔT= T (Table ITS-90, DIN 43 760) – T milk (35 °C).
in order to calculate the temperature difference \((\Delta T1)\) from the surface of the PRTD wire to the outer surface of the sheath.

The basic equation of heat conduction through a solid plate in a state of balance (Scott, 1974), for each layer can be expressed as:

\[
Q = -\frac{\Delta T}{\lambda} \times \frac{\xi}{\alpha}
\]

Where:

\(Q\): heat to be conducted (Watt).
\(\lambda\): distance through which the heat must be conducted. (m) for this model. \(\lambda = e\) (tube thickness). \(\kappa\): thermal conductivity of the material (W m\(^{-1}\) °C\(^{-1}\)).
\(\alpha\): cross section of the material through which heat is conducted (m\(^2\)). For this model, \(\alpha = \pi \times d \times l\), \(d\): diameter, \(l\): length.

Table 4 shows \(\lambda\), \(\alpha\), \(\kappa\), \(\Delta T\) for fluid milk and coagulum, corresponding to the different layers of the sensor materials.

Figures 6a and 6b show the diagrams of the different sensor layers, and Figure 6c shows the temperature differences between the wire and the sensor surface \((\Delta T1)\), and between the sensor surface and the so-called limit layer \((\Delta T2)\).

To calculate the temperature profile of the outer surface of the well to the (theoretical) limit layer of heat transfer in the milk and in the coagulum, the sensor was considered as a cylinder that has to dissipate the constant heat generated by the wire PRTD to the medium (milk/coagulum).

This development mainly focuses on the instant in which the milk begins to coagulate and to acquire the properties of a structured fluid in transition to a viscoelastic solid. Therefore, for practical purposes, we consider a simplified heat transfer model in which heat is transferred mainly by conduction (Figure 7). With equation (2), which corresponds to the conduction heat flow in a radial geometry in a stationary state, the outer boundary diameter \((d_o)\) can be calculated. From this distance, the temperature profile of the milk surrounding the sensor tip reaches a constant value in a theoretical radial limit layer. This radial layer moves during coagulation.

From the transfer equation (Scott, 1974).

\[
\Delta T = \frac{Q \times \lambda}{\kappa \times \alpha}
\]

Where:

\(\Delta T\): temperature difference between the platinum wire and the outer surface of the CS: 7.16 °C (fluid milk) and 12.91 °C (coagulum), respectively (Table 5).

\(Q\): heat transferred by conduction: 0.988376 Watt (fluid milk) and 1.00246 Watt (coagulum).

\(d_i\): internal diameter of the model (outer diameter of the well): 7.925 x 10\(^{-3}\) m.

\(l\): axial length of the well: 3.21 x 10\(^{-2}\) m.

\(\kappa\): thermal conductivity: 0.573 W m\(^{-1}\) °C\(^{-1}\) at 35 °C for fluid milk (Spells, 1961) and 0.530 W m\(^{-1}\) °C\(^{-1}\) at 32 °C for the coagulum (Passos, 1999).

<table>
<thead>
<tr>
<th>Experience</th>
<th>(\Delta V) Volts</th>
<th>(T_{milk}) °C</th>
<th>(R) (Ω)</th>
<th>(R_{50\times2})</th>
<th>(T_{(Table,,°C)})</th>
<th>(\Delta T_{1}+\Delta T_{2}) (°C)</th>
<th>(P_{sensor}) (Watt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluid milk</td>
<td>Average</td>
<td>7.5675</td>
<td>34.8</td>
<td>58.210</td>
<td>116.422</td>
<td>42.28</td>
<td>7.48</td>
</tr>
<tr>
<td></td>
<td>Coefficient of</td>
<td>0.0097</td>
<td>0.078</td>
<td>0.0977</td>
<td>0.099</td>
<td>0.774</td>
<td>1.743</td>
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<tr>
<td></td>
<td>variation (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coagulum</td>
<td>Average</td>
<td>7.7112</td>
<td>34.8</td>
<td>59.317</td>
<td>118.634</td>
<td>48.03</td>
<td>13.23</td>
</tr>
<tr>
<td></td>
<td>Coefficient of</td>
<td>0.0878</td>
<td>0.078</td>
<td>0.0878</td>
<td>0.088</td>
<td>0.561</td>
<td>3.057</td>
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<tr>
<td></td>
<td>variation (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3. \(\Delta V\) for fluid milk and coagulum, temperature of the milk and of the coagulum, temperature of the sensor according to the tables (1), resistance, \(\Delta T1 + \Delta T2\) and \(P\), power of the sensor.

(1) PRTD IEC 751-1995 – Class A and ITS 90 – DIN 43760.

\(\Delta T1\): temperature difference between the coiled platinum wire and the outer surface of the sensor.

\(\Delta T2\): temperature difference between the outer surface of the sensor and the estimated external surface (called limit layer).

\(\Delta V\) Volts: measured voltage for fluid milk and value of maximum firmness of the coagulum.
In (do / di): natural logarithm of the ratio of diameters.

After applying this equation we obtained do = 18.4 mm, ΔBL = 5.2 mm for fluid milk and do = 31.4 mm, ΔBL = 11.6 mm for the coagulum. The variations in energy dissipation and resistance variations were 0.019 Watt and 1.107 Ω, respectively. The power dissipation of the well, with an external surface of 8.0 cm², can be expressed as 0.122 Watt/cm² for the liquid milk at the beginning of the coagulation and as 0.124 Watt/cm² for the coagulum at its maximum strength, with a variation of 0.0024 Watt/cm².

The heat transfer analysis of the new sensor shows that the passage of a 130 mA direct current dissipates a mini-

<table>
<thead>
<tr>
<th>Layer of material</th>
<th>λ: (m)</th>
<th>α: (m²)</th>
<th>κ: W m⁻¹ °C⁻¹</th>
<th>ΔT (°C) Fluid milk</th>
<th>ΔT (°C) Coagulum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ceramic powder</td>
<td>0.00119</td>
<td>4.48 x 10⁻⁴</td>
<td>15</td>
<td>40 °C</td>
<td>0.03866</td>
</tr>
<tr>
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<td>4.241 x 10⁻⁴</td>
<td>30</td>
<td>40 °C</td>
<td>0.17420</td>
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<tr>
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<td>4.382 x 10⁻⁴</td>
<td>8.7</td>
<td>25 °C</td>
<td>0.01290</td>
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<tr>
<td>Copper</td>
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<td>6.032 x 10⁻⁴</td>
<td>400</td>
<td>40 °C</td>
<td>0.00387</td>
</tr>
<tr>
<td>Artic Silver 5 (Copper Stainless Steel)</td>
<td>0.00010</td>
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<td>8.7</td>
<td>25 °C</td>
<td>0.01818</td>
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<tr>
<td>Stainless steel</td>
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<td>6.22 x 10⁻⁴</td>
<td>14.6</td>
<td>20-100 °C</td>
<td>0.07149</td>
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<tr>
<td><strong>Suma (∆T₁)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>∑ = 0.31931</td>
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</table>

Table 4. λ, α, κ, ΔT for fluid milk and coagulum, corresponding to the different layers of the materials of the sensor. Equation (1) and the data in Table 4 lead to the parameters summarized in Table 5.

Figure 6. View of the cross sections of the CS (a and b) and temperature profiles (c).
mum impact power (0.122 Watt/cm²) and a wire temperature of 42.28 °C in the liquid state. The 10% reduction in the thermal conductivity of the milk during the transition process from fluid to gel, from 0.573 W m⁻¹ °C⁻¹ ( Spells, 1961) to 0.530 W m⁻¹ °C⁻¹ (Passos, 1999), respectively, produces an increase in power dissipation of only 0.0024 Watt/cm², which is attributed to self-heating and to an increase of 5.75 °C in the temperature of the platinum wire. This temperature increase corresponds to a resistance difference of 1.107 Ω, which is a key parameter in the sensitivity of the sensor.

With this new device, the temperature in the sensor surface is not enough to cause the denaturation of soluble proteins that could adhere to the surface and interfere with the signal. As the denaturation of soluble proteins of milk occurs at T> 70 °C, without other interferences the responses of this sensor are very repetitive. Conversely, when using higher values of DC, for example 190 mA, there are higher temperatures on the surface of the sensor (Tables 1 and 2), although they are significantly lower than those calculated in the bare platinum wire excited with 500 mA (around 89-90 °C).

Figure 8 shows the typical voltage profile as a function of time for a chymosin coagulation assay (Chymax, 950 IMCU/ml, ChR Hansen Argentina SAIC) under the standard conditions used in the cheesemaking industry (pH: 6.6, T: 37 °C, 0.0015 RU/ml of milk and 1.8 mM of added CaCl₂), conducted using fluid milk (raw milk, fat 3.5% (W/W); true protein 3.25% (W/W) and lactose 4.8% (W/W), supplied by Cooperativa Tambera and Agropecuaria Nueva Alpina Ltda, (Ceres, Santa Fe, Argentina).

This profile corresponds to an average of three repetitions. In the same figure the coagulation time (CT), the time of the maximum slope of the voltage profile (tmax), the voltage 30 min after the addition of enzymes (V30) and the maximum voltage (Vmax) values of the coagulation are represented. Thanks to the low noise level of the signal shown by the profile, the first derivative can be calculated quite easily.

Figure 9 shows the voltage profile as a function of time during the acid coagulation process under standard yoghurt processing conditions, with reconstituted skimmed milk powder (10.45% w/v), according to what was explained in the Materials and Methods section. The Profile (1) corresponds to reconstituted milk without heat treatment, while the Profile (2) represents the process with reconstituted milk heated at 85 °C for 30 minutes. The simultaneous control of temperature, pH and “viscosity” with the three sensors allows to measure and record the gel time, the pH of the gelification and the obtained firmness (voltage), and small oscillations in the temperature of about ± 0.1 °C.

On the one hand, this information could be used by the industry to monitor and optimize the fermentation time in the manufacture of each type of yogurt. The subsequent reduction of the viscosity signal in the profile (1) would indicate the presence of micro syneresis (draining of the curd). On the other hand, more experiences are needed to determine the relationship between the spontaneous expulsion of whey and the reduction of the signal. A potential limitation could be the difference in heat dissipation through a mixture of serum and coagulum compared to that produced in a homogeneous gel. Finally, the responses of the sensors are adjusted appropriately to the acid and enzymatic coagulation processes.

Table 6 shows four responses: CT, tmax, V30 and Vmax for five pH levels (6.2, 6.4, 6.5, 6.6 and 6.8), five levels of enzyme concentration (0.002, 0.003, 0.004, 0.005 and 0.008 RU/ml of milk), five temperature levels (30, 33, 35, 38 and 40 °C) and five levels of added CaCl₂ (0; 0.9; 1.8; 3.6 and 5.4 mM). Each value is the average of two repetitions.

Figure 10a shows the evolution of the voltage for different pH and the standard concentration of rennet used in the cheese industry (0.0025 RU/ml of milk, temperature 35 °C and addition of 1.8 mM CaCl₂).
The analysis of variance (ANOVA) and the linear regression that describes the relation between CT and pH had a level of significance of $p<0.05$ ($p: 0.0392$), which indicates that there is a statistically representative relationship at a confidence level of 95%.

The adjusted equation that associates CT and pH is $CT = -131.663 + 21.8086 \times pH$. The coefficient of determination $R^2 (0.9227)$ shows that the adjusted model accounts for 92.27% of the CT variability.

The adjusted model corresponding to $t = -150.673 + \max 25.0114 \times \text{pH}$, with $p<0.05$ ($p: 0.0309$) indicates that there is a significant relationship at a confidence level of 95%. The coefficient of determination $R^2$ represents 93.91% of the variability of $t_{\max}$.

Table 6 shows that the lowest CT (4.2 min) and $t_{\max}$ (5.1 min) are obtained at pH 6.2, while the highest CT (13.4 MIN) and $t_{\max}$ (15.6 min) are obtained at pH 6.6. The positive slopes for both responses indicate that the increase...
in pH also increase the values of CT and tmax. While the temperature, the enzymatic concentration and the added CaCl\textsubscript{2} concentration are maintained constant, the maximum value of V30 and Vmax is obtained at a pH 6.2. CS did not detect coagulation at a pH of 6.8 (there are no data on CT, Tmax and V30). Simple regression of the V30 and Vmax indicators versus pH did not result in a statistically significant relationship.

Changes in pH that show a significant relationship with the CT are detected by the sensor and are in agreement with the results reported by Storry and Ford (1982) and Imafidon and Farkye (1993). The pH of the milk would be the most important factor that should be strictly controlled during the curd elaboration process (Hori, 1985). Van Hooijdonk et al. (1986) found that a pH reduction leads to an increase in enzymatic and aggregation actions, as observed with the CS sensor.

Figure 10b shows the remarkable influence of enzyme concentration on the voltage versus time profiles. Table 6 shows the average values of the four responses (CT, tmax, V30 and Vmax). The CT and tmax responses decrease as the enzyme concentration increases. Plotting CT against the inverse of the enzyme concentration (ml of milk/RU) shows that the Holter Law is applied, as described by the equation CT= k x 1/C (k: constant and C: enzyme concentration). The linear regression that describes the relationship between CT and tmax and enzyme concentration (ANOVA) shows a level of significance p> 0.05, which indicates that there is no statistically significant relationship with a 95% confidence level.

For this reason, the experimental results were mathematically modified, adopting log CT and log tmax instead of CT and tmax, as the best fit values for both responses. The fitted model equation is for log CT = 3.66741-222.11 x enzyme concentration. The p-value: 0.0181 (ANOVA) shows statistically significant relationships for a confidence level of 95%, where R2 indicates that the model accounts for 88.0982% of the log CT variability. These observations coincide with Hori (1985), who reported that the measurement of the voltage showed that the CT of the skim milk decreased exponentially with the increase of the concentration of rennet.

The adjusted model for log tmax= 3.755558-204.929 x enzyme concentration, and p value: 0.0210 show that there is a statistically significant relationship between the log tmax and enzyme concentration. The R2 for the adjusted model accounts for 86.88% of the variability of the log tmax, although the levels of V30 and Vmax in Table 6 and its evolution in Figure 10b show that both responses increase as the enzyme concentration increases. ANOVA, however, shows that the levels of significance are p>0.05, which indicates that there is no statistically different relationship between the enzymatic concentration and V30 and the enzymatic concentration and Vmax.

On the one hand, the linear regression analysis of CT, tmax and V30 at different temperatures (30, 33, 35, 38 and 40 °C) presented in Table 6 shows that, under conditions for the set of variables (enzyme concentration, pH and addition of CaCl\textsubscript{2}), p values in the ANOVA table are >0.05, which leads to the conclusion that there is no statistically significant relationship between the CT, tmax and V30 responses and the temperature variable, with a confidence level of 95% or higher.

On the other hand, the fitted equation model that describes the relationship between Vmax and temperature (Vmax= 0.2666493-0.00390077 x Temperature), results in a p value= 0.0192, which means a statistically significant relationship to a 95% confidence level. The statistical R2 indicates that the adjusted model represents 87.6378% of the variability in Vmax. The highest and lowest viscosity indicators (volta)ge are obtained at 30 °C and 40 °C, respectively, where the percentage increase of V 30 ° C and that at 40 °C is 30.5%.

Figure 10c shows that at 30 °C the voltage increases indefinitely during the 60 min of the test, while at 33 and 35 °C the voltage reaches a maximum and then remains constant. Regarding its behavior at 40 °C, the voltage reaches a maximum and then drops, possibly due to the occurrence of micro syneresis.

It has been reported that the temperature affects both the primary (hydrolysis) and the secondary phase (aggregation), although the effect on the enzymatic hydrolysis is substantially lower. Ramet and Weber (1980), Storry and Ford (1982) and Van Hooydonk and Van Den Berg (1988) showed that the strength of the coagulum was significantly reduced by the increase in temperature, in agreement with the results obtained in our experience. Recently, Sbodio et al. (2013) using a similar device, a rotational composite design and response surface methodology, demonstrated that it is possible to study the effects of critical variables on chymosin coagulation in reconstituted milk powder.

The second-order models obtained using linear regression analysis effectively predicted the effects of temperature, enzyme concentration and pH on CT, tmax and V30. The study of all the enzymatic coagulation of milk and with the same sensor showed that temperature is the main factor that affects the development of firmness.

Regarding the effect of different concentrations of fixed pH (6.6), temperature (35 °C) and enzyme concentration (0.004 RU/ml of milk), the four responses (TC, tmax, V30 and Vmax) are shown in Table 6. The linear regression analyzes corresponding to the four responses show statistically non-significant relationships when the addition of CaCl\textsubscript{2} changes.

However, it is interesting to evidence that the time required to reach the maximum voltage is significantly related (p: 0.0262) to the CaCl\textsubscript{2} concentration. The linear regression Vmax Time= 60.5021-0.016531 x CaCl\textsubscript{2}, which shows that as the concentration of CaCl\textsubscript{2} increases, the time it takes to reach Vmax decreases. Figure 10d shows the evolution of voltage for different CaCl\textsubscript{2} concentrations. The comparison between these evolutions shows that the highest TC and tmax, and the lowest V30, are observed without addition of CaCl\textsubscript{2}.
However, the highest level of Vmax is achieved with the addition of 0.9 mM \( \text{CaCl}_2 \), and the lowest when using higher concentrations (3.6 and 5.4 mM \( \text{CaCl}_2 \)). The results obtained with the new device are in agreement with those reported by Okigbo et al. (1985) that evidenced that the addition of \( \text{CaCl}_2 \) in the chymosin coagulation is unnecessary if other factors (pH, temperature and concentration of chymosin) can be adequately adjusted. Conversely, Taifi et al. (2006) show that the increase in \( \text{CaCl}_2 \) concentration produces an increase in the firmness of the milk gel and also accelerates the appearance of syneresis.

The device developed and patented by Hori operates under the same principle. However, as it is presented, it is not suit to be used in a cheese vat. As for device developed and patented by INRA, it is a differential thermal analyzer. It is worth mentioning that in its final phase, this device has been designed and developed for industrial applications.

However, the difference between the device developed and patented by INRA according to FR2626371 (A1) y FR2626371 (B1) also published as WO8906794 (A1) NZ227709 (A) JPH03503449 (A) FI91330 (B) FI91330 (C) EP0389568 (A1) EP0389568 (B1) DK175990 (a) CA1297178 (C) AU2940489 (a) United States patent US5064294 (a) is that the device is based on a differential thermal measurement, similar to a differential calorimeter.

It has two probes constructed with platinum wire, one to determine the temperature of the medium and the second separated from the first, heated by a constant current, to measure the heat dissipation in gelatin solutions and cold curdled milk at 10 °C, in order to measure the evolution of milk coagulation as it is heated to 30 °C.

In our case, a single probe built with platinum wire is heated using a constant current to determine the thermal dissipation with milk temperature within an experimental range of 30 °C to 40 °C. To this, the rennet is added for enzymatic coagulation. Thus, in our case the heat dissipation per unit area is 1220W/m² versus 1600W/m² of the INRA device, with a significant increase in sensitivity.

To conclude, the measurement of enzymatic coagulation with our device shows that the sensor is sensitive to changes in the critical variables that characterize the process. The effect of pH, enzyme concentration, temperature, and \( \text{CaCl}_2 \) on the CT, tmax, V30 and Vmax responses were consistent with the results obtained using other methods. The signals emitted by the sensor have a low noise level and the CT and Vmax responses have very good repeatability. When the sensor is fed with 130 mA, the heat transfer analysis shows that the surface temperature development is close to 49-50 °C, with no risk of denaturation of the soluble proteins.

**Figure 10.** Comparative profiles of milk coagulation. A. pH variation. b. Variation of the concentration of the enzyme. c. Variation of temperature and d. Variation of added \( \text{CaCl}_2 \).
Considering its dimensions, the device is non-intrusive, which means that it does not interfere with gel cutting and stirring operations. It is robust enough and can be cleaned (CIP) and sterilized using steam (SIP) in situ. The temperature sensor CS and RTD comply with the sanitary requirements (sanitary regulations 3A, Inc. 74-03). The pH electrode and the pH electrode holder also comply with the EHEDG standards (European Hygienic Engineering Design Group). Accordingly, it can be inferred that the device may be suitable for online use in cheese making.

Finally, it should be mentioned that the use of this device in the development process of “set-type” yogurts, has allowed us to study the influence of temperature and concentration variables of different serum additives on gel time and consistency. The obtained model allows us to accurately predict gel time within a certain range of variables (unpublished data).

**ACKNOWLEDGEMENTS**

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**REFERENCES**


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**Table 6.** Effect of pH, enzyme concentration, temperature and addition of CaCl2 to the CT, tmax, V30 and Vmax of the reconstituted skim milk powder during the enzymatic coagulation.

<table>
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<th>Variables</th>
<th>CT (min)</th>
<th>t&lt;sub&gt;max&lt;/sub&gt; (min)</th>
<th>V&lt;sub&gt;30&lt;/sub&gt; (mV)</th>
<th>V&lt;sub&gt;max&lt;/sub&gt; (mV)</th>
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<td>pH</td>
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<td>6,2</td>
<td>4.2 ± 0.020</td>
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<td>6,6</td>
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<td>15.6 ± 0.027</td>
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<tr>
<td>6,8</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>33.5 ± 5.4</td>
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<td>C.E. (R.U./ml leche)</td>
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<td></td>
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<tr>
<td>0.002</td>
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<td>127.0 ± 3.8</td>
<td>137.2 ± 5.0</td>
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<td>0.003</td>
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<td>16.6 ± 0.030</td>
<td>110.5 ± 3.3</td>
<td>121.1 ± 6.5</td>
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