Using acoustic wave sensors to follow milk coagulation and to separate the cheeses according to the milk origin

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Abstract:

Optimum coagulation temperature and cutting time are usually empirically defined. The use of inexpensive acoustic wave sensors to monitor milk coagulation allows monitoring the process in real time, since its early stages. Besides, data are collected with minimum disturbance, which is an advantage over other methodologies, namely rheology.

Coagulation of milk was followed by measuring both the frequency and equivalent circuit resistance of a piezoelectric quartz crystal. Three types of milk, ewe, goat and cow, were coagulated using different rennets at three different temperatures. Maximum observed coagulation rate, either with plant or animal rennet, increased with temperature both for ewe and goat milk. Maximum coagulation rate was higher with plant rennet than with animal rennet at 25 °C and 32 °C, while the opposite was found at 50 °C. The time needed to attain maximum coagulation rate was always smaller with ewe's milk than with goat's milk, and maximum coagulation rate was higher with the former. Cow's milk was much more difficult to coagulate and needed twice the rennet quantity used for ewe and goat milk.

Cheese obtained with the mentioned types of milk and different rennets shows different flavours, usually perceived by experts and connoisseurs, and that an electronic nose built in our laboratory was also able to distinguish. One sensor was enough to separate cheeses made from cow, ewe and goat milk. Besides, the addition of a second sensor allowed to separate the cheeses by rennet among the ones produced with the same milk.

KEYWORDS: Piezoelectric quartz crystal, Acoustic wave sensor, Milk coagulation, Rennet, *C. cardunculus L.*, Electronic nose.

1. Introduction

Cheese manufacture begins with milk coagulation. Calf rennet is usually added to milk, although a few Portuguese cheeses, among which are Serra da Estrela, Serpa and Azeitão are produced with the plant coagulant extracted from the flowers of *C. cardunculus* L. This variety of thistle grows wild and abundant in dry, stony, uncultivated areas in the southern and northeastern parts of Portugal and has been used as a coagulant from ancient times.

Milk proteins are commonly classified as caseins, which are the large majority, and whey proteins. Milk from different animals has different proportions of the four major caseins (α s1, α s2, β and κ -CN) [1] in combination with micellar or colloidal calcium phosphate nanoclusters. Regardless of the milk origin, these aggregates, the casein micelles, are the building blocks of milk gels. Rennet coagulation involves two steps that normally overlap to some extent: enzymatic hydrolysis and aggregation. κ -CN does not bind much Ca, as it lacks phosphoserine residues, and can only hydrophobically bond with other caseins, acting like a chain terminator. Rennet cleaves κ -CN, to form para κ -CN, resulting in a reduction in the net negative charge and steric repulsion, which turns the altered micelles susceptible to aggregation. Calcium bridges, van der Waals forces, and hydrophobic interactions, all contribute to aggregate micelles into a spanning network [2].

All used coagulants are able to attack other peptide bonds besides the Phe₁₀₅-Met₁₀₆ bond of κ -casein. Animal coagulants, such as the ones obtained from the stomach of calfs, are compounds with the enzymes chymosin A and chymosin B and pepsin at different levels. Chymosin is a coagulant with high specific milk clotting activity and low non-specific activity, while pepsin has a higher general proteolytic activity than chymosin. The stigmas of *C. cardunculus* L. contain aspartic proteinases, cardosin A and cardosin B, and cyprosins [3]. Cardosin shows similar specificity (same site of attack and identical catalytic coefficients) against κ -CN than chymosin, but a stronger preference for bulky hydrophobic sequences and for bonds between hydrophobic residues than chymosin [4]. Sousa and Malcata reported a broader specificity of the proteinases of *C. cardunculus* L. than those from animal rennet [5] and also that animal rennet was more proteolytic on ewe β and α s caseins than plant rennet [6]. Salguero and Sanjuán [7] reported a stronger proteolytic activity of cyprosins of *C. cardunculus* L. relative to chymosin. Both cardosins attack the same type of bonds, but cardosin B has a broader specificity and is more proteolytic than cardosin A [3].

Besides the type of milk and rennet, the indigenous flora in milk, added starter cultures, concentration of calcium salts, pH, and temperature influence proteolysis and ripening, and therefore, final physical properties of cheese, as well as flavour.

Gelation temperature is usually optimized based on cheesemaking experience, and cutting is performed at previous defined times. However, there are a list of devices available to evaluate coagulum firmness [2,8,9], some of them suitable for on-line usage. Among them, hot wire probes proved to be capable of detecting the gel point, but are incapable of monitoring the rate of curd firming, which varies with milk composition. Optical systems are only sensitive to the geometry of the structure, although similar geometries are possible with chemical differences and consequently with different gel strengths. In fact, optical techniques need to be calibrated using a rheometer [8]. Rotational viscometry disrupt bonds during gel formation [8], and contribute to microsyneresis by the amplitude of motion. Therefore, it is mandatory that the rheological measurements are performed at very small deformations. The utilization of piezoelectric quartz crystals vibrating at 9MHz assure wave penetration and wavelength of several times the micelle diameter [10], meaning that frequency of oscillation and quartz crystal equivalent electric parameters change with micro-viscoelastic properties. In fact, the frequency of oscillation of a piezoelectric quartz crystal in contact with a liquid decreases with the square root of the product between density and viscosity, according to the Bruckenstein and Shay equation [11].

$$\Delta F = -2,26 \times 10^{-6} n f^{3/2} \sqrt{d\eta}$$

where f is the frequency of the crystal, ΔF is the observed frequency shift, d , the density, η the viscosity and n is the number of crystal surfaces contacting with the solution. After gel formation, the elastic, or solid component, is larger than the viscous, or liquid one, which is why viscosity measuring techniques are no longer the best option [9]. Motional resistance (R), which represents the dissipation of the energy in the quartz crystal, is a better parameter in determining bulk properties of the liquid than resonance frequencies [12]. The resistance is a helpful indicator of viscoelastic contributions, and changes in R can be the result of changes in G' and/or G'' , the storage and loss moduli, respectively [13]. Instead of motional resistance, energy dissipation factor (D) could be measured. Aung et al. [14] found that R and D values correlated well with each other when studying DNA film properties. While frequency measurements require the use of a simple oscillator, as the one employed by Bruckenstein and Shay [11], obtaining motional resistance demands expensive network analyzers, which have the advantage of allowing the device to be measured in isolation, in the absence of external circuitry influences, under the passive operation of the sensor [15]. G' and G'' can be

calculated from the measured values of the real and the imaginary part of the impedance, R and X , respectively, after calibrating the crystal with liquids of known viscosity and density [16]. The measurement of D reduces the cost of instrumentation in comparison with the network analysis, but the requirements for the quality of the instrumentation remains high, as well as its dimensions [16].

Acoustic wave sensors have already shown to be able to detect blood coagulation [17] and reversible thermal gelation of hydroxypropyl methylcellulose [18]. Although the potential of acoustic sensors to follow milk coagulation is known for some time [8,10], and they have been used to study casein micelles interaction after acidification in yoghurt production [19], applications are still scarce. In this paper, a piezoelectric quartz crystal was used to follow cow, ewe and goat milk coagulation since the very early stages, by monitoring the series frequency and the equivalent circuit resistance. For the first time, the practical usefulness of this technique is demonstrated, and the results obtained here are discussed and compared with conclusions from other works, available in the literature, drawn from data obtained by conventional methodologies. At this point, it must be said that many of the reports found in the literature result from studies of the proteolytic action of isolated proteases on pure caseins and not experiments where the rennet was added to the whole milk.

Certified Portuguese cheese Serra da Estrela has a characteristic flavour and must be produced from ewe milk and plant rennet. Frauds need to be detected, and an electronic nose able to distinguish cheese produced from different types of milk, but also to distinguish them by the rennet used, could be of great assistance to food inspectors. There has been a few attempts to use electronic noses, most of them using commercial instruments with MOS sensors, to monitor cheese ripening [20,21], flavour variations with fat and salt content of Cheddar cheese [22,23], and to identify cheeses with known percentages of cow's and goat's milk [24]. Electronic noses based on piezoelectric quartz crystals coated with sensitive compounds were also used to determine ripening stage of Emmental cheese by monitoring 2-heptanone [25], and to discriminate cheese variety [26]. An home-made electronic nose assembled with sensors that presented different sensitivities to compounds evolved from the cheeses produced with cow, goat and ewe milk, obtained with different rennets, was used in order to show that the bouquet of those cheeses can be recognised by electronic devices.

2. Materials and Methods

2.1 Materials

In order to guarantee the uniform properties of milk in different experiments, all milk samples were reconstructed from fat milk powders. Cow's milk powder (C) was from Insulac, Produtos lácteos Açoreanos, S.A, and ewe (E) and goat milk (G) powders were from Vetagri Humana. The extract of coagulant from *C. cardunculus L.* (P) was obtained from the dried style and stigma part of the flower, animal rennet (A) from HERCO was bought at a local pharmacy, microbiological rennet (M) and another plant rennet, here designated by industrial plant rennet (I), were obtained from a cheese industry. Calcium chloride (Labsolve 50075) was also used in cheese manufacture.

The quartz crystals used in the electronic nose were coated with Carbowax® 20M (Supelco 21032), polyvinylpyrrolidone (Fluka 81420), triethanolamine (Merck 8377), tetramethylammonium fluoride tetrahydrate (Aldrich 10,721-2), and with copper phthalocyanine (Aldrich 42,316-5). Chloroform (AnalaR Normapur 09860) or ethanol (Panreac 121086.1212) were used as solvents for those compounds. Nitrogen was Alphagaz from ArLíquido.

A solid phase microextraction (SPME) fibre of Carboxen-PDMS (Supelco) was used to extract the compounds from the headspace of the vials containing 2.0 g of the cheese sample, which were afterwards desorbed and carried by a nitrogen stream to the sensors.

2.2 Preparation of milk samples

Milk from ewe, goat and cow was prepared from milk powder and distilled water (137 g L⁻¹). Milk powder was reconstituted with gentle agitation during 2 h.

2.3 Preparation of plant and animal coagulant

The aqueous extract from *C. cardunculus L.* was obtained from 28 g of stylets and stigmatae of dry flowers by soaking in distilled water (28 g L⁻¹), with magnetic stirring, for 24 h. The suspension was filtered.

The animal rennet was obtained by dissolution of the commercial powder in distilled water (56 g L⁻¹).

2.4 Cheese preparation

40 mL of the reconstituted milk from cow, ewe or goat, were heated in a water-bath at 32 °C. Then, the rennet (0.56 g L⁻¹ milk, for animal rennet, and 0.28 g L⁻¹ milk, for all other rennet's) and CaCl₂ (0.1 g L⁻¹ milk) were added and the milk was left to rest for 16 h. The curd was cut and left to rest for 1 hour. Then, the curd was squeezed and the whey was drained through a fine mesh cloth. Cheese was left to drain for another 2 hours.

Immediately after cheese preparation, 2.0 g of cheese were weighed into a 17 mL vial, which was then closed with a silicon septum coated with Teflon, and a removable centre crimp seal. The sample was stored at -19 °C, until analysis. Two to three replicates for each cheese were stored.

2.5 Apparatus

Set-up used for coagulation studies

The piezoelectric crystals were 9 MHz, polished, AT-cut, HC-6/U with gold electrodes. (ICM- International Crystal Manufacturing Co, Inc.).

Fig. 1.a shows the experimental layout. The piezoelectric quartz crystal was inserted between two o-rings into a home made teflon cell. The grooves into the upper part of the Teflon cell allowed to tightly connecting a glass cup where milk was poured into. This glass cup has an open bottom through which the milk contacted with the upper face of the piezoelectric quartz crystal, while the other face of the piezoelectric quartz crystal remained dry.

Oscillators were laboratory-made and the frequency of oscillation of the crystal used to follow coagulation was monitored by a frequency counter, Leader LF-827, interfaced to a computer through a GPIB. To measure the electrical parameters of the equivalent circuit, the quartz crystal was disconnected from the oscillator and connected to an HP4395A network/spectrum analyzer coupled to an HP43961A impedance test kit.

The experiments were performed at controlled temperatures, and, for this purpose, the piezoelectric crystal, inserted into a Teflon cell connected to the glass cup containing the milk, was mounted inside an oven (Friocell 55, *Medcenter Einrichtungen Group, MMM*).

Set-up used with the electronic nose

The piezoelectric crystals were 9 MHz, AT-cut, HC-45 with silver electrodes. Crystals were coated by depositing a drop of solutions of the selected compounds. Solvents were then allowed to dry. Compounds were selected among the ones that responded to the most abundant compounds extracted by SPME from the cheeses headspace and detected by

GC/MS, namely acetic acid, 2,3-butanediol, 3-methyl-1-butanol, butanoic acid, 3-methylbutyl acetate, ethanol, butanol, ethyl hexanoate, and ethyl acetate. Coating amounts can be inferred by the frequency decrease observed after coating has dried ($-\Delta F$). Carbowax® 20M $\Delta F=-44$ kHz (sensor 1), polyvinylpyrrolidone $\Delta F=-44$ kHz (sensor 2), triethanolamine $\Delta F=-19$ kHz (sensor 3), tetramethylammonium fluoride tetrahydrate $\Delta F=-55$ kHz (sensor 4), and with copper phthalocyanine $\Delta F=-20$ kHz (sensor 5), respectively.

Fig. 1.b shows the experimental layout. Each one of the five coated piezoelectric quartz crystals was housed inside a PVC cell previously described [27].

A small oven allows to desorbing the compounds from the SPME fibre. A nitrogen flow, controlled by a flowmeter (Cole Parmer), carry the compounds till a distribution valve (OMNIFIT 1103), that divided the main stream by the five sensors. Each sensor was driven by a home-made oscillator. The frequencies of oscillation of the sensors were simultaneously monitored with a Counter/Timer Device PXI 6608, from National Instruments, and stored in a PC.

2.6 Procedure

Procedure used in coagulation studies

The crystal was rinsed with detergent, and Milli-Q water before each experiment. The crystal was only considered to be clean when the reading of its frequency shows the initial value and resistance was zero. The crystal was dried and inserted between the two o-rings of the Teflon cell. Then the glass cup was attached to the cell and the quartz crystal connected to the oscillator inside the oven. A glass cup with the milk was also placed inside the oven set to the desired temperature (25 °C, 32 °C or 50 °C). No experiment was run before a period of temperature stabilization of 30 minutes. Then, the coagulant was added to the milk in the proportion of 10 μ L of coagulant solution per 2 mL of milk and quickly mixed. Concentrations of both coagulants were doubled when used with cow's milk.

A volume of 2 mL of the mixture was poured into the glass cup attached to the crystal cell, which was then closed with parafilm to prevent evaporation. The frequency of the quartz crystal, or the resistance of the equivalent circuit, began to be recorded. The series frequency was stored in a PC at intervals of 3s, while the resistance was measured at intervals of 5 min. Experiments were run at several temperatures with animal or plant coagulant and with cow, goat and ewe milk.

Cheese analysis by SPME

The SPME fibre was cleaned in a homemade oven, at $\sim 230\text{ }^{\circ}\text{C}$, and the complete desorption of compounds was confirmed by analysing the frequency of oscillation of the quartz crystals.

Before the analysis, one vial with a frozen cheese sample was placed at $4\text{ }^{\circ}\text{C}$ for 24 h. The vial was then immersed into a water bath at $30.0\text{ }^{\circ}\text{C}$, and, after 30 minutes, the SPME fibre was introduced through the septum, into the vial headspace and left there for exactly 60 minutes.

Meanwhile, nitrogen was continuously flowing through all the sensors (total flow of 30 mL/min), and baseline frequencies were recorded. The SPME fibre was afterwards inserted into the oven, and the desorbed compounds were carried to the five crystals by the nitrogen stream. The frequencies of the five sensors were simultaneously displayed on the PC monitor, and saved to disc at 1 s intervals. The minimum frequency value was recorded for each sensor, and the difference to the corresponding baseline frequency computed.

The frequencies of the piezoelectric crystals decreased due to the interaction of the desorbed compounds with the coatings, after which they began to increase again till they reach the baseline values, as soon as desorption from the fibre, as well as from the coatings of the piezoelectric quartz crystals, was completed. The electronic nose and fibre were then ready for a new analysis. The reported frequency shift value for each cheese and each sensor was the mean of two replicate analyses.

3. Results and Discussion

The measurements on the network analyzer were made using the Butterworth-van-Dyke equivalent circuit. Fig. 2 shows the equivalent circuit motional resistance shift along goat's milk coagulation at $32\text{ }^{\circ}\text{C}$. The mentioned concentrations of coagulant solutions, 28 g L^{-1} of dried flowers and 56 g L^{-1} of commercial animal rennet were the necessary to obtain visible coagulation in one hour, and a similar increase in resistance with both coagulants. These quantities became the first choice for all the following studies, regardless the coagulation temperature and the type of milk.

Fig. 2 shows that after an initial time lag of ≈ 20 minutes, the resistance increases exponentially with time, after which it starts to flatten. As resistance is affected by changes in the viscoelastic properties, its increase can be correlated with milk coagulation. During the lag period only hydrolysis of the k-CN is taking place, but no micellar aggregation.

Fig. 3 shows the resistance shifts obtained for ewe and goat milk kept at 25 °C, 32 °C and 50 °C. As can be seen, independently of the rennet used, time lag decreased with temperature, and resistance shift due to coagulation increases with it. Those curves were fitted by a sigmoid function, and inflection point and slope of the tangent to the curve at the inflection point are displayed on Table1. Table1 shows the coagulation rate and the time needed to attain it, for goat and ewe milk, both with animal and plant rennet, at 25 °C, 32 °C and 50 °C. The rate at which resistance increases at the exponential part of the graph agrees with the previously reported increase of the aggregation rate of rennet altered micelles with temperature [2]. The maximum coagulation rate was attained sooner at the highest temperature, for all milks and for animal or plant rennet. For ewe's milk, and with the quantities of rennet mentioned before, the time lag with the plant rennet was longer than the one observed with the animal rennet, and it was necessary to wait longer to achieve the maximum coagulation rate, although this rate was higher with plant rennet than with animal rennet at the lower temperatures (25 °C and 32 °C). At 50 °C, the maximum coagulation rate was higher with animal rennet than with plant rennet. Coagulation of goat's milk needs longer times than ewe's milk and the maximum coagulation rates are lower at any of the three tested temperatures, and with both plant and animal rennet. It can also be seen that it is almost impossible to make cheese from goat's milk and animal rennet at 25 °C. Coagulation rate at 32 °C is still very small, and a temperature of 50 °C is necessary to achieve significant, although moderate, coagulation rates. These results agree with empirical knowledge, as we are aware of goat cheeses produced at 50 °C.

Fig. 4 a, b and c shows both frequency and resistance shifts measured along milk coagulation with animal and plant rennet, for ewe's milk at 32 °C, goat's milk at 50 °C, and for cow's milk at 32 °C, respectively. Frequency measurements can be made with inexpensive instrumentation, as all it is needed is a very simple oscillator, which was assembled in the lab, a power supply, the crystal cell, which was made at the workshop of the University, a quartz crystal, which is widely used in electronics, and a frequencymeter, which only needs to be connected to a PC if storage of data is necessary. Measuring impedance needs an impedance/network analyzer which is much more expensive than the frequencymeter and oscillator. As can be seen from Fig. 4, coagulation can also be followed if frequency is monitored instead of resistance. Therefore, being inexpensive, frequency measuring technique is well suited to be used in cheese making industries with limited resources.

Comparing the results for the three types of milk, it can be seen that ewe's milk clots more rapidly than cow or goat milks, due to the highest casein levels [28].

Ewe's cheese Serra da Estrela, one of the Portuguese cheeses holding the Protected Designation of Origin status, must be made from raw milk and *C Cardunculus L* rennet, which gives it the well known particular taste and flavour. The milk used in these experiments is also from ewe, but it was prepared from powder milk, and does not have the same characteristics and especially not the microflora. However, Fig. 4a shows that *C Cardunculus* rennet was adequate to coagulate ewe's milk, and while frequency decrease obtained with this plant rennet shows a much slower decrease along time than the one obtained with animal rennet, the resistance plot shows a variation that, although starting a bit later than when animal rennet was used, has a larger magnitude. This is a consequence of resistance dependence on viscoelastic properties. An explanation to this larger resistance shift can be found in Macedo et al. [29] who reported a higher coagulation activity of cardoon flower extract relative to animal rennet for ewe's milk. Lucey [2] suggested a possible reason for this coagulation activity, by saying that as this plant rennet hydrolyzes several additional sites on caseins compared with chymosin, the rate of gel firming may increase due to the removal of peptides that otherwise may act as a barrier to particle diffusion.

Fig.4b shows very similar resistance profiles, when goat's milk was coagulated with animal or plant rennet at 50 °C. Similar profiles had already been observed in Fig.2 for 32 °C. Coagulation of goat's milk showed to be different from the coagulation of ewe's milk, as there is no difference in time lag when using animal or plant rennet, and this was observed either at 32 °C or at 50 °C.

Cow's milk is more difficult to coagulate and the quantity of the rennet used needed to be doubled. Besides, it can be seen from Fig. 4c that lag time is much larger than with ewe or goat milk. In spite of the fact that *C. cardunculus L.* is not used in the production of cow's milk cheese, it can be seen that coagulation with the plant rennet is very efficient, with larger R shifts due to gelation than those obtained with animal rennet. These findings are corroborated by the work of Esteves et al. [30] who reported higher values of G', and of its increasing rate, with *C. cardunculus* than with chymosin. The non use of the plant rennet is therefore not linked to low rennet activity, but must be a consequence of the bitter taste obtained with it.

Fig. 5 shows the responses, in terms of frequency shift, observed with each sensor for all the samples. Besides the animal rennet and the plant rennet (*C. cardunculus*) used in the coagulation studies, two other rennet samples obtained from a cheese producing industry, another plant rennet (I) and a microbiological rennet (B) were also tested, with the milk samples previous used. The most evident conclusion is that the highest responses were

obtained with sensor 4. This is a consequence of the sensitivity of the coating (tetramethylammonium fluoride tetrahydrate) to the compounds desorbed from the fibre, but also of the amount of this compound on the piezoelectric crystal (frequency decrease due to coating was 55 kHz). In fact, irrespectively of the magnitude of the responses, the fingerprints observed in each sensor with the different cheeses are very similar. The goat cheeses produced the highest responses in all sensors, except for the goat cheese produced from animal rennet. With animal rennet, the highest responses were obtained with ewe milk in all sensors except for sensor 5. For all cheeses, the highest responses were obtained with the industrial plant rennet, which could be due to the rennet composition, but could also be explained by its concentration, which was unknown.

Fig. 6 shows a plot of responses of sensor 5 vs. sensor 1 for all samples. It can be seen that just one sensor is enough to clear distinguish the goat cheeses, which show the highest responses on sensor 5, followed by the ewe cheeses and by the cow cheeses that exhibit the smaller responses. On the plot with the responses from two sensors, 5 and 1, the cheeses produced from animal rennet are far away from the centroid of the responses of cheeses made from the same milk but other rennets. Adding the responses of sensor 1 allowed having some separation among cheeses produced from the same milk but from different rennets. The other sensors are irrelevant for the work, as their responses are correlated with the ones obtained with sensor 1 or sensor 5.

CONCLUSION

The use of this inexpensive technique based on 9 MHz piezoelectric quartz crystals, allowed to obtain information related to milk gelation in real time with a minimum disturbance of the studied system. Therefore, this work opens the doors to the introduction of acoustic wave sensors in the cheese making industry, and showed its value for optimization of coagulation processes. As was demonstrated, the results allow inferring about coagulation kinetics, which renders the technique particularly useful when adjustments of CaCl_2 content, rennet, pH or milk solid content in the industry are being performed.

A very simple electronic nose with just two sensors is enough to clear discriminate cheeses made from cow, ewe and goat milk, and different responses have also been obtained by changing the rennet, with samples obtained from animal rennet away from the samples produced from plant and microbiological rennet. These results can open a real opportunity to develop a dedicate instrument devoted to detect frauds in a particular cheese with certified

denomination of origin, which must be produced from a specific type of milk (frauds of mixing cow milk in ewe milk are known), and with a particular rennet.

ACKNOWLEDMENTS

This project was financed by FCT, POCTI and FEDER, through the project PTDC/QUI/74312/2006. We thank Insulac, Produtos Lácteos Açoreanos, S.A. and Vetagri Humana for offering the milk powders.

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Fig. 1 a) Experimental layout to follow milk coagulation by monitoring the series frequency: A- frequency counter, B- power supply, C - oven, D- PC, E- oscillator, F- glass cup with milk, G – Teflon crystal cell, H – piezoelectric quartz crystal.

Fig. 1 b) Electronic nose: B – power supply, C – oven, D- PC, E- oscillator, I – PVC crystal cell, J – distribution valve, K – counter/timer PXI-6608, L – BNC 2121 box, M – flowmeter.

Fig. 2 Goat's milk coagulation at 32 °C, using animal rennet (56g L⁻¹) and plant rennet (28g L⁻¹).

Fig. 3 Equivalent circuit resistance obtained along ewe and goat milk coagulation, using animal and plant rennet, at three different temperatures.

Fig. 4 Series frequency and resistance obtained along a) ewe's milk coagulation, with animal and plant rennet, at 32 °C, b) goat's milk coagulation, with animal and plant rennet, at 50 °C, c) cow's milk coagulation, with animal and plant rennet, at 32°C (rennet quantities were twice the originals).

Fig. 5 Responses of the five sensors to the bouquet of cheeses produced from ewe, goat and cow milk and four different rennets: microbial, animal, industrial plant and plant (*C. cardunculus*).

Fig. 6 Plot of the responses of sensor 5 vs. sensor 1 to the bouquet of cheeses produced from ewe, goat and cow milk and four different rennets: microbiological (B), animal (A), industrial plant (I) and *C. cardunculus* plant (P).

Table 1

Table 1 Maximum coagulation rate and time necessary to attain it, for ewe’s milk and goat’s milk, using plant (*C. Cardunculus*) and animal rennet.

milk	rennet	time to attain maximum coagulation rate (min)			maximum coagulation rate ($\Omega \text{ min}^{-1}$)		
		25 ⁰ C	32 ⁰ C	50 ⁰ C	25 ⁰ C	32 ⁰ C	50 ⁰ C
ewe	plant	56.6	31.3	13.5	0.23	0.53	1.5
	animal	26.2	9.9	2.1	0.12	0.44	1.7
goat	plant	67.8	33.2	20.5	0.23	0.40	0.57
	animal	39.3	37.6	17.1	0.13	0.25	0.78

Figure 1a
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