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▶ **To cite this version:**

Didier Dupont, Remy Grappin. ELISA for differential quantitation of plasmin and plasminogen in cheese. Journal of Dairy Research, 1998, 65 (4), pp.643-651. 10.1017/S0022029998003173 . hal-04301381

HAL Id: hal-04301381
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Submitted on 23 Nov 2023

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ELISA for differential titration of plasmin and plasminogen in cheese. Concentration of the enzyme and its precursor varies according to cheese making technology

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Short title : Plasmin and plasminogen titration in cheese

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SUMMARY

Bovine plasmin (PLM) and its precursor, plasminogen (PLG), are present in cheese in which the protease is thought to play a significant role in proteolysis during cheese ripening. PLM in cheese is usually titrated using enzymatic techniques and concentrations are mostly expressed as activities. In order to determine PLM and PLG absolute concentrations in cheese, we have applied an Enzyme-Linked ImmunoSorbent Assay (ELISA), previously developed for PLM and PLG differential titration in milk, to cheese. The assay used two monoclonal antibodies, one specific for PLG and the other cross-reacting with PLM and PLG. PLM concentration was obtained by subtracting the PLG concentration from the PLM+PLG.

The evaluation of the assay in cheese was assessed by determining the total amount of PLG and PLM in milk used in the manufacture of 8 semi-hard cheese on a pilot plant scale, and comparing it to the total amount of PLG and PLM present in the 8 corresponding wheys and unripened cheeses. Concentrations of PLG and PLM of 19 commercial cheeses were also determined.

It was thus demonstrated that ELISA allows PLM and PLG titration in milk and cheese and that PLM and PLG concentrations in cheese are highly dependent on the technology used for cheese making.

Plasminogen (PLG) is the precursor of a serine-protease, plasmin (PLM) (E.C. 3.4.21.7), which displays an activity similar to that of trypsin (Wallen & Iwanaga, 1968). The PLM-PLG system is exported from blood through the mammary gland to the milk and affects milk properties. In milk, the PLM-PLG system is bound to the casein micelle. PLM hydrolyses β and αS2-casein and to a lesser extent αS1-casein. PLM activity in milk is considered to be significant from a technological viewpoint.

PLM is present in cheese (Richardson & Pearce, 1981 ; Lawrence *et al*. 1983) and is not, or only partially, eliminated in the whey at draining (Lawrence *et al*. 1987 ; Farkye & Fox, 1992). The enzyme is considered to play a significant role in the ripening of hard cheese (Collin *et al.* 1987 ; Ollikainen & Kivela, 1989 ; Farkye & Fox, 1990). It has been proposed that PLM activity in hard cheese depends on cooking temperature used during manufacture (Farkye & Fox, 1990). PLG could be converted to PLM during cooking at high temperatures possibly by inactivation of the inhibitors of plasminogen activators. Moreover, the high cooking temperature used during hard cheese manufacture leads to chymosin inactivation (Garnot & Molle, 1987 ; Boudjellab *et al.* 1994) and reinforces the importance of plasmin during ripening. The activity of PLM towards αS1-casein is relatively low, which presumably accounts in part for the typically firm texture of hard cheese (Ollikainen & Kivela, 1989).

PLM is maximally active at slightly alkaline pH and 37°C (Dulley, 1972) and is usually titrated in cheese by determination of its activity. In the traditional procedures (Richardson & Pearce, 1981 ; Korycka-Dahl *et al.* 1983), cheese is dispersed in 2% tri-sodium citrate solution pH 8.3 or in 0.05M Tris-HCl buffer, pH 7.4, containing 0.1M EDTA and the rate of degradation of a synthetic substrate by the enzyme is quantified. However, whether or not the intensity of PLM activity found using these techniques corresponds to the amount of PLM actually active inside the cheese is

questionable, because PLM activity in cheese is highly dependent on pH and water activity of cheese (Delacroix-Buchet & Trossat, 1991). PLM is usually expressed as an activity rather than as an absolute concentration and because the expression depends on the technique used for PLM titration, data between studies are not comparable. Thus, a sensitive, accurate and rapid technique that gives absolute concentrations for PLG and PLM, would be an interesting tool for a better understanding of the role of PLM in cheese ripening.

We have recently developed an ELISA for differential titration of PLM and PLG in milk (Dupont *et al.* 1997) based on the use of PLG specific and PLM+PLG cross-reacting monoclonal antibodies (Dupont *et al.* 1994). This technique was shown to be particularly suitable for PLM and PLG titration in late lactation and mastitic milks where accurate determination of PLM activity is not possible due to the presence of protease inhibitors. However, ELISA did not permit complete titration of milk plasminogen and plasmin. This lack of accuracy was due to casein interference in the PLM-PLG titration by ELISA.

The objective of this study was to improve the accuracy of the method for milk and cheese analysis and to apply ELISA to PLM and PLG titration in experimental and commercial cheeses.

MATERIALS AND METHODS

Method for measurement of plasmin and plasminogen concentrations

Sample Preparation. To improve the accuracy of the method, 50 mM ε-amino caproic acid (EACA) was used to allow dissociation of PLM and PLG from the casein micelles.

Milk and whey. Milk or whey samples (10 ml) were incubated for 2 h at room temperature with 50 mM EACA. Subsequently, serum containing PLG and PLM was freed of casein micelles by ultracentrifugation at 100000 x g for 1h at 4°C (Sorvall Ultra Pro 80, Du Pont Company, Newtown CT 06470, USA) and was submitted to PLG-PLM titration by ELISA.

Cheese. Cheeses (5 g) were finely grated and suspended in 20 ml 0.4 M trisodium citrate pH 8.3 (TCS). The suspensions were homogenised 1 min in an ice bath using an ultra-turrax T25 (IKA-Labortechnik, D-7813 Staufen i. Br., Germany) and blended (Stomacher 400, Lab-Blender, Seward medical UAC house, London SE19UG, UK) for 5 min at room temperature to allow total dissolution of the casein. Then, the suspensions were treated as described for the milk or whey samples and submitted to PLG-PLM titration by ELISA.

PLM/PLG determination. ELISA was carried out using a modification of the method previously published (Dupont *et al.* 1997). Briefly, flat-bottomed ELISA plates were coated with monoclonal antibody (Mab) 41(37) specific for PLG or Mab 1245(37) PLG and PLM cross-reacting diluted 1/500 in 0.1 M bicarbonate buffer pH 9.6 (100 µl per well) and incubated for 2 h at 37°C. Blocking of the remaining binding

sites was performed with 10 g/l gelatin (Sigma, St Louis MO 63178 USA) in 0.05 M phosphate buffer pH 7.2, 0.15 M NaCl /0.05 ml/l Tween 20 (PBS-T). Serial dilutions of purified PLG and PLM solutions (0-100 ng/ml) in PBS-T were used as standards. Milk, whey and cheese were diluted in PBS-T (1/50 to 1/125, 1/15 to 1/50 and 1/75 to 1/200 respectively) for PLG and PLM+PLG titration. Standards, milks, wheys and cheeses were added to each ELISA plate (100 µl/well) and the plates were incubated for 1.5 h at 37°C.

Then, a rabbit polyclonal PLG and PLM cross-reacting antibody (Dupont *et al.* 1997) diluted 1/250 in PBS-T was added to each ELISA plate well and further incubated for 1.5 h at 37°C. After washing, the reaction was revealed by a Goat anti-Rabbit Immunoglobulins-alkaline phosphatase conjugate (Sigma) diluted 1/3000 in PBS-T. P-Nitrophenyl phosphate (PNPP, 100 µl) at 1 mg/ml was used as substrate. Absorbance was measured at 405 nm using a Titertek Multiskan Autoreader (Life Science International, F-95610 Eragny-sur-Oise, France). PLG concentration was obtained directly whereas PLM concentration was determined by subtracting PLG from PLM+PLG concentration.

Assessment of the ELISA. Evaluation of the ELISA for titration of PLM and PLG in milk and cheese was performed using repeatability and recovery studies.

Repeatability of PLG and PLM titration in milk and cheese was assessed by determining the repeatability relative standard deviation (RSDr) after titration of PLM and PLG in 84 milk and 16 cheese samples in triplicate.

The analytical recovery of PLG was performed by measuring PLG and PLG+PLM in milk to which different amounts of purified PLG (0.05, 0.1, 0.25, 0.5, 0.75 and 1 µg/ml) had been added. Addition of exogenous PLM to a milk sample was not carried out because Mab 1245(37) has been previously shown to have the

same affinity for PLM and PLG (Dupont, unpublished data). PLG and PLM+PLG concentrations were determined in duplicate before and after each addition as described above, and results were expressed as a percentage of recovery of the amount of PLG added.

Evaluation of the ELISA for titration of PLM and PLG in cheese was studied by determining the total amount of PLG and PLM in the milk used for cheese making and comparing it to the total amount of PLG and PLM present in the corresponding whey and unripened cheese. Results were determined in triplicate and expressed as the percentage of the amount of PLG and PLM found in the whey+cheese compared to the amount found in milk.

Efficiency of the release of PLG and PLM from casein micelles by EACA was assessed by measuring PLM and PLG on 16 milks and 8 hard cheeses from commercial sources in the supernatant and the pellet obtained after ultracentrifugation. For this purpose, casein pellets were redisolved in 10 ml TCS prior to PLM and PLG titration by ELISA.

Measurement of plasmin and plasminogen in cheese

Cheese making. Eight Saint-Paulin-type cheeses were made in a pilot plant from raw milks (10 l) chosen for their differences in PLG and PLM concentrations. During cheese making, samples of the whey were removed prior to draining for PLM and PLG titration. The 8 cheeses were sampled 24 h after manufacture for titration of PLG and PLM. The other cheeses used for this study were from commercial sources.

Activation of plasminogen into plasmin in milk due to cooking. Assuming that the influence of heating was similar between milk and the mixture of curd grains and whey, we investigated whether or not cooking during hard cheese manufacture was responsible for activation of PLG into PLM by submitting 3 flasks of a raw milk to a heat treatment cycle in a waterbath comparable to the one traditionally used in the hard cheese plants. The temperature curve used for this experiment is presented in Fig.2. PLG and PLM were then titrated in these 3 milk samples in triplicate before heat-treatment (t=0), at t=3h (55.3°C), t=4h (49.7°C), t=8h (45°C) and t=24h (32°C).

Plasmin and plasminogen retention in curd during rennet and acid coagulation. PLM and PLG retention in curd according to the type of milk coagulation was measured by submitting the same milk to an acid and a rennet coagulation and quantifying PLM and PLG in the serum and curd obtained. Briefly, raw skim milk was divided in four 100 ml flasks. Two flasks were inoculated with a mixture of *Lactococcus lactis* + *Streptococcus salivarius* (EZAL MA 400 0.2 u/10l, Texel, F-86220 Dange Saint Romain) and *Streptococcus salivarius* + *Lactobacillus delbrueckii* (EZAL MY 800 0.2 u/10l, Texel) and incubated 10h at 42°C for coagulation. At the same time, the 2 other flasks were set at 42°C with 70 µl of calf rennet at 520 mg/l chymosin and kept at 42°C for 10h. After 10h, the serum and curd were separated by centrifugation for 15 min at 5000g and 4°C (J2-21 M/E; Beckman Instruments, F-93220 Gagny) and submitted to PLM and PLG titration by ELISA as described above.

RESULTS

Assessment of the ELISA for PLM and PLG titration in milk and cheese

Repeatability relative standard deviation (RSDr) for PLG and PLM titration in milk and cheese were respectively 13 and 15% and 10 and 12%.

For PLG titration, the percentage of PLG measured after addition of 0.05, 0.1, 0.25, 0.5, 0.75 and 1 µg/ml to a milk sample compared to the PLG theoretically present in this sample was 99, 101, 103, 108, 100 and 99% respectively with an average recovery of 102% (Fig.1a). For PLM+PLG titration, the percentage of PLM+PLG measured after addition of 0.05, 0.1, 0.25, 0.5, 0.75 and 1 µg/ml of PLG to a milk sample compared to the PLM+PLG theoretically present in this milk was 99, 100, 103, 106, 101 and 101% respectively with an average recovery of 102% (Fig.1b). Correlation coefficients (r) between theoretical and measured concentrations of PLG and PLM+PLG were 0.983 and 0.990 respectively. In the same time, the calculated amount of PLM remained unchanged between the PLG overloaded samples and the reference milk (data not shown).

Results of PLG and PLM concentrations determination by ELISA in milk, whey and cheese for 8 independent cheese fabrications are presented in Table 1. Percentage of the amount of PLG and PLM found in cheese+whey compared to the amount present in milk, varies between 97 and 105% for PLG with a mean value of 100%, and between 105 and 115% for PLM with a mean value of 109%.

Results presented in Table 1 also show that PLM and PLG are present in experimental semi-hard cheese at concentrations ranging from 2.90 to 6.20 µg/g and from 5.98 to 8.67 µg/g respectively. These differences in concentration were mostly due to differences in the amount of PLM and PLG present in milk. It also appears

that 25% of the PLG and 40% of the PLM present in milk is eliminated in whey at draining during semi-hard cheese production.

Assessment of PLG and PLM release from the casein in milk after EACA treatment showed that 84%±2 of PLG and 88%±8 of PLM were released from the micelle and located in the supernatant obtained through ultracentrifugation. In cheese, 84%±8 of PLG and 83%±7 of PLM were released from the micelle after EACA treatment. These results are in agreement with those of Korycka-Dahl *et al*. (1983) who found that 80% of the PLM activity was recovered in the supernatant after treatment of milk by EACA and centrifugation.

Plasmin and plasminogen in cheese

Activation of plasminogen into plasmin in milk due to cooking. The effect of the cooking temperature used during hard cheese manufacture on the concentration of PLG and PLM is illustrated on Fig.2. It shows that the total concentration of PLM+PLG remains unchanged after cooking. However, cooking causes significant activation of PLG into PLM with PLG and PLM concentrations of 1.80 and 0.74 µg/ml before cooking and 1.38 and 1.10 µg/ml after cooking respectively. The ratio PLM/PLG varies from 0.411 before cooking to 0.797 after cooking. Activation of PLG into PLM continues during the period of cooling giving at 24 h a concentration of PLM and PLG of 0.96 and 1.59 respectively with a ratio PLM/PLG of 1.656. Activation of PLG into PLM is probably due to the action of milk PLG activators.

Plasmin and plasminogen retention in curd varies between rennet and acid coagulation. PLG and PLM concentrations in serum and curd obtained after an acid

and a rennet coagulation are shown in Table 2. Final pH reached for acid and rennet coagulation were 4.52 ± 0.01 and 6.20 ± 0.01 respectively. It appears that only 7% and 16% of milk PLG and PLM are retained in the curd after acid coagulation, whereas 55% and 59% of milk PLG and PLM are retained after rennet coagulation. Table 2 also shows that the ratio PLM/PLM+PLG is higher in serum and cheese than in milk showing that PLG activation into PLM during rennet and acid coagulation has occurred.

PLG and PLM titration in commercial cheeses. Results of titration of PLG and PLM in 19 commercial cheeses are shown in Table 3. Fresh cheese was characterised by low concentrations or even absence of PLM. These low levels of PLM were probably the result of the intense decrease in pH during fresh cheese manufacture that could lead to dissociation of PLM from the casein micelle and elimination of PLM in the whey at draining as has been previously described (Grufferty and Fox, 1988). Soft cheeses as well as Blue cheeses were characterised by higher concentrations of PLM than PLG. For these samples, PLM/PLG ratio ranges from 1.408 for Munster to 5.152 for Camembert.

Semi-hard cheeses were characterised by higher concentrations of PLG than PLM. Indeed, PLM/PLG ratio ranges from 0.144 for Cantal to 0.568 for Raclette made from pasteurised milk.

In contrast, hard cheese showed higher percentages of PLM than PLG suggesting that activation of PLG into PLM has occurred during cheese making. PLM/PLG ratio ranges from 2.742 for Comté to 6.899 for Emmental. Furthermore, PLM+PLG concentrations of Emmental, Comté and Beaufort were 25.1, 22.3 ± 0.4 and 18.2 respectively. It is interesting to note that the ripening period is 2-3 mo. for Emmental, 4-6 mo. for Comté and 7-12 mo. for Beaufort. Thus, PLG and PLM concentrations

seem to decrease during ripening as has been previously suggested (Song *et al.* 1993).

DISCUSSION

In this study, we have demonstrated that ELISA allows determination of PLM and PLG concentrations in milk as well as in cheese.

For different varieties of cheese, we have found PLM concentrations ranging from 14.1 to 21.9 µg/g for Swiss-type cheese, from 2.7 to 10.3 µg/g for semi-hard cheese, from 8.2 to 11.4 µg/g for soft cheese and from 0 to 0.09 µg/g for fresh cheese. PLM/PLG mean ratio was 0.167, 2.791, 0.312, 4.093 for fresh, soft, semi-hard and hard cheese respectively. Thus, titration of PLM and PLG in 19 commercial cheeses allowed discrimination of the cheeses according to their cheese making technology

Values obtained for PLM and PLG concentrations in cheese are not comparable with data found in the literature, because, in most of the studies, PLM and PLG are expressed as activities. Only Richardson & Pearce (1981) by transforming results expressed as activities to concentrations found that Swiss-type and Cheddar cheese contain 6-13 and 3-4.5 µg/g PLM respectively. The differences between cheese varieties in the concentration of PLM+PLG is certainly due to differences in pH at draining. Grufferty and Fox (1988) showed that PLM and PLG are released from the micelles under pH 4.6, stipulating that PLM might be completely removed in the whey during manufacture of acid cheese. Indeed, when we compared the PLM+PLG content for the different commercial cheeses tested, it appeared that PLM+PLG content decreased as the pH of curd after draining decreased. Fresh cheeses contained no or small amounts of PLM that correspond to the pH 4.3-4.5 traditionally reached after draining. In the recovery experiment on semi-hard cheese, we found that 25% PLG and 40% PLM were lost in the whey at draining. These results are in contradiction with those of Farkye and Fox (1992) who found no PLM activity in the whey during cheddar cheese manufacture. It must be kept in mind that PLM

inhibitors are located in the serum phase of milk (Reimerdes *et al.* 1976 ; Korycka-Dahl *et al.* 1983) and therefore are eliminated in the whey at draining during cheese making. It is thus probable that these inhibitors strongly interfere with determination of PLM activity in the whey at draining and not with the concentration of PLM assessed by ELISA.

Activation of PLG into PLM occurs during scalding of milk during hard cheese manufacture. Thus, we have demonstrated that heating is responsible for intense activation of PLG into PLM confirming the hypothesis stipulated by others (Fox, 1989 ; Farkye & Fox, 1990). This activation is probably the consequence of an inactivation of the PLG activator inhibitor. For soft cheese made from pasteurised milk, PLG activation into PLM might occur before cheese making due to milk pasteurisation.

According to the PLM concentration found in the different commercial cheeses, it is probable that role of PLM in the proteolysis during cheese ripening varies greatly with the cheese making technology. In fresh cheese, PLM was not detected or present in extremely low concentrations. PLM is present in soft cheese but its activity is probably limited because cheese pH is not favourable to this alkaline enzyme and also because the ripening period is short. Nevertheless, Trieu-Cuot & Gripon (1982) who studied proteolysis in Camembert made from pasteurised milk during ripening found that plasmin activity was important at the cheese surface after 21 days of ripening and after 35 days in the centre of the cheese, corresponding to an increase of the pH at the surface and the centre of the cheese. They concluded that PLM is a major contributor to the ripening of Camembert. Our study showed that PLG predominated in semi-hard cheeses and that the ratio of PLM/PLG is close to the one found in milk. The low concentrations of PLM found in semi-hard cheeses suggest that PLM does not play a crucial role in semi-hard cheese ripening compared to chymosin that is probably the enzyme mostly responsible for primary

proteolysis. However, significant increase in PLM concentration caused by addition of exogenous amounts of PLM or activation of PLG leads to acceleration of the ripening and improvement of flavour development of semi-hard cheese (Farkye & Fox, 1991, 1992 ; Farkye & Landkammer, 1992). In contrast, hard cheeses showed higher concentrations of PLM than of PLG. The long period used for hard cheese ripening, together with the high PLM levels found in these cheeses leads to the conclusion that PLM is a key enzyme for proteolysis of hard cheese (Ollikainen & Nyberg, 1988 ; Ollikainen & Kivela, 1989) and its role is probably reinforced because of chymosin inactivation by the cooking temperature (Garnot & Molle, 1987 ; Boudjellab *et al.* 1994). Moreover, it was shown that an increase in PLM activity in hard cheese improves cheese flavour (Bastian *et al.* 1997) without concomitant increase in bitterness (Scherze *et al.* 1994).

We have shown that ELISA is a repeatable and sensitive assay for PLM and PLG differential titration in cheese. However, the dissociation of PLM and PLG from casein by EACA is not complete and leads to values that are not perfectly accurate. The results obtained in this study tend to prove that techniques for PLM and PLG titration that use EACA treatment of the samples prior to titration are not accurate. On the other hand, immunological or enzymatic techniques directly applied on milk are also susceptible to interference by casein in the assay and thus inaccurate results (Bastian *et al.* 1991 ; Dupont *et al.* 1997). Furthermore, ELISA allows quantification of the antigenic presence of a molecule, and does not give any information on the actual activity of the molecule in the cheese. Determination of PLM activity in cheese using enzymatic techniques is also questionable because PLM activity in cheese depends on pH and water activity of cheese (Delacroix-Buchet & Trossat, 1991), parameters that are not taken into account in activity determination by these enzymatic techniques. In conclusion, none of the existing

techniques for the determination of PLM and PLG activity or concentration seems to be sufficiently accurate when used alone. However, enzymatic and immunological techniques can be complementary and when used together could greatly contribute to a better understanding of the action of PLM in dairy products.

The authors are grateful to G. DUBOZ (INRA Poligny) for cheese making and to Sister N. MARCELLINO (INRA Poligny) for correcting the manuscript.

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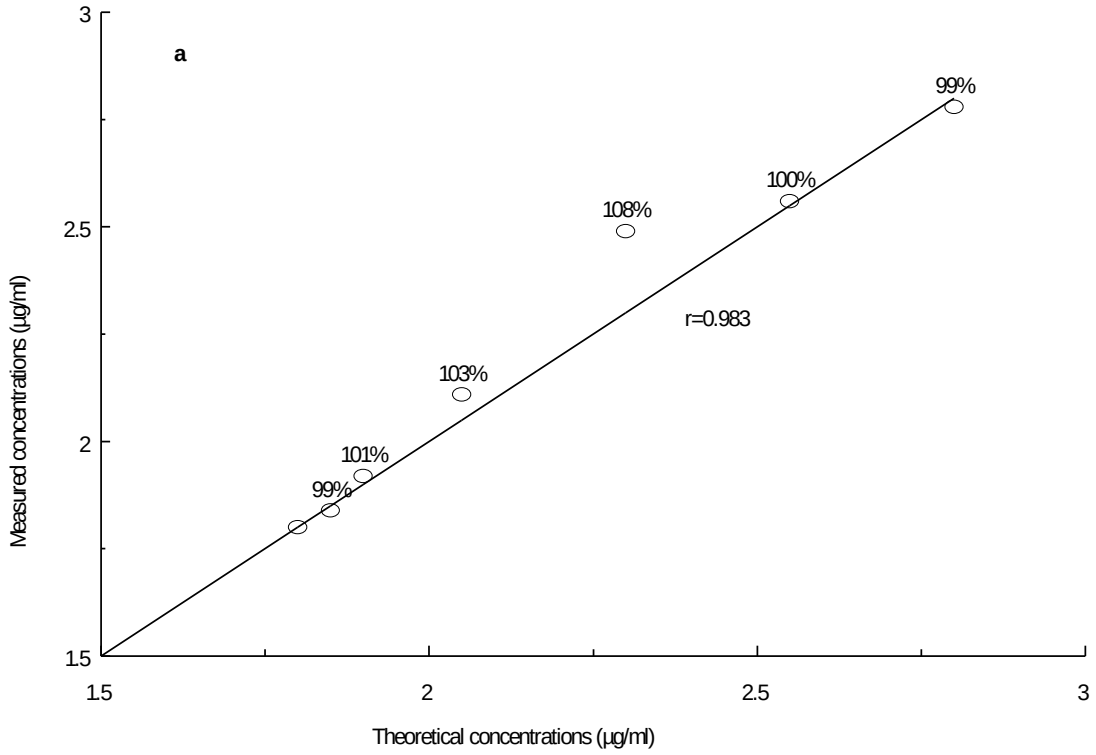

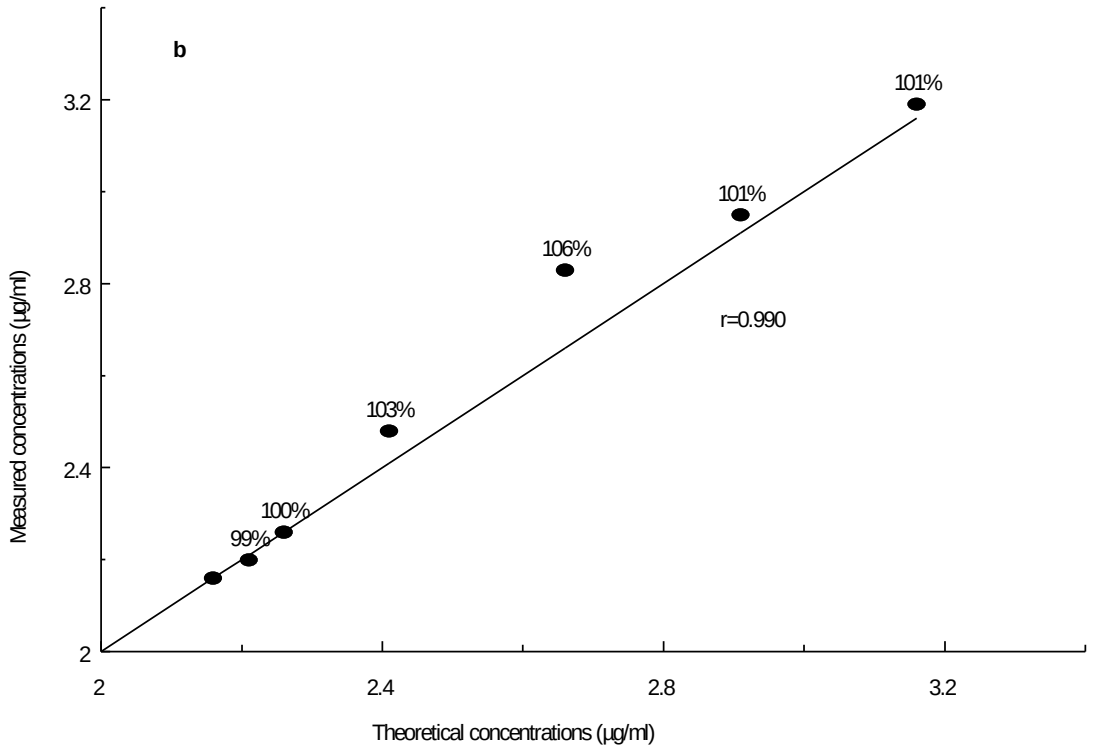

DUPONT & GRAPPIN
Fig.1

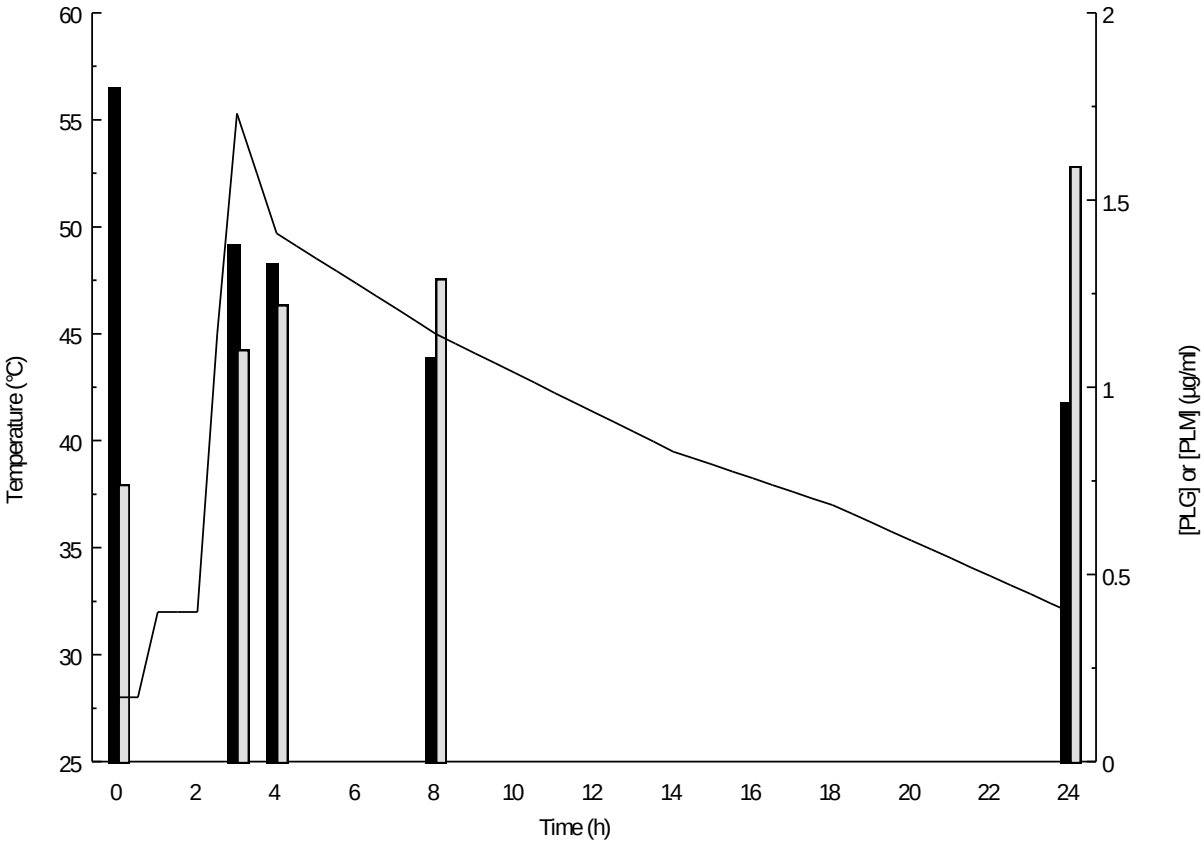

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Fig.2

Fig.1. Analytical recovery of plasminogen in milk by ELISA for plasminogen (a) and plasminogen+plasmin (b) titration. Evaluation was assessed by adding known amounts of purified plasminogen (0.05, 0.1, 0.25, 0.5, 0.75 and 1 µg/ml) to commercial milk and determining plasminogen and plasmin+plasminogen before and after this addition. Results are expressed as percentages of recovery of exogenous purified plasminogen added to the milk. Coefficient of correlation (r) between theoretical and measured values was determined by linear regression.

Fig.2. Evolution during 24h of plasmin (🖫) and plasminogen (🖴) concentrations (in µg/ml) in milk, determined by ELISA, during a heat-treatment traditionally used for hard cheese manufacture. Temperature measured during the heat-treatment is represented as a dark line.

Table 1. Total amounts of plasmin (PLM) and plasminogen (PLG) found by ELISA in milk and the corresponding cheese and serum obtained in 8 independant semi-hard cheese productions. Accuracy of the ELISA was assessed by calculation of the percentage recovery of PLM or PLG in cheese+serum compared to the amount previously present in milk.

(Values are mg, means±SD for n=3)

CHEESE PRODUCTION

		1	2	3	4	5	6	7	8	**Mean**
	Milk	9.54 ± 1.78	9.83 ± 2.27	9.20 ± 1.21	10.57 ± 0.95	9.34 ± 2.43	13.84 ± 2.59	11.09 ± 1.81	11.61 ± 1.04	**10.63**
	Serum	4.05 ± 0.32	5.28 ± 1.83	2.17 ± 0.68	3.51 ± 1.04	2.67 ± 1.08	4.47 ± 0.50	5.23 ± 0.19	6.95 ± 1.65	**4.29**
PLM	Cheese	6.12 ± 1.22	5.04 ± 1.63	7.79 ± 3.10	8.22 ± 3.10	7.58 ± 1.71	10.60 ± 2.30	6.98 ± 1.54	6.45 ± 2.06	**7.34**
	% recovery	107%	105%	108%	111%	110%	109%	110%	115%	**109%**
	Milk	14.68 ± 0.81	14.81 ± 0.51	18.94 ± 1.04	18.57 ± 0.84	19.37 ± 0.13	19.18 ± 3.44	14.39 ± 0.46	13.92 ± 0.21	**16.73**
	Serum	3.72 ± 0.49	4.29 ± 0.71	4.72 ± 0.18	4.40 ± 0.16	4.41 ± 0.80	5.66 ± 0.82	3.70 ± 0.36	3.15 ± 0.14	**4.26**
PLG	Cheese	10.94 ± 0.63	10.37 ± 0.34	14.63 ± 1.13	15.13 ± 0.27	14.75 ± 0.61	13.37 ± 0.79	10.25 ± 0.83	10.39 ± 2.05	**12.48**
	% recovery	100%	99%	102%	105%	99%	99%	97%	97%	**100%**

Table 2. Partition of milk plasmin (PLM) and plasminogen (PLG) between whey and curd, in µg/100g, according to acid or rennet coagulation

	PLASMIN				PLASMINOGEN				PLM/PLM+PLG	
	acid		rennet		acid		rennet		acid	rennet
Milk	63.29				189.87				0.25	
Serum	127.56	(116.91)	79.84	(74.81)	117.82	(107.98)	38.95	(36.49)	0.52	0.67
Curd	247	(23.19)	1236	(106.33)	81	(7.61)	522	(44.91)	0.75	0.70

( ) in µg/100g of milk

Table 3. Concentrations in plasmin and plasminogen of commercial cheeses (in µg/g of cheese) determined by ELISA

Cheese type	Commercial Name	Milk☜	Plasmin (µg/g)	Plasminogen (µg/g)	Ratio PLM/PLG
Fresh	Petit Suisse	p	0	0	0
Cheese	Boursin	p	0.09	0.27	0.333
	Brie	p	10.41	3.96	2.629
Soft	Camembert	r	11.36	5.75	1.976
Cheese	Camembert	p	10.20	1.98	5.152
	Munster	p	8.18	5.81	1.408
Blue Cheese	Bleu de Bresse	p	8.45	2.70	3.130
	St Nectaire	r	8.40	16.68	0.504
	Tome de Savoie	r	2.99	17.01	0.176
Semi-hard	Reblochon	r	4.47	11.24	0.398
Cheese	Raclette	p	10.29	18.12	0.568
	Cantal	r	2.67	18.53	0.144
	Morbier	r	2.81	12.00	0.234
	Gouda	p	3.65	22.73	0.161
	Emmental	r	21.94	3.18	6.899
Hard	Comté a	r	17.97	4.52	3.976
Cheese	Comté b	r	16.59	6.05	2.742
	Comté c	r	16.89	4.88	3.461
	Beaufort	r	14.08	4.16	3.385

☜p=pasteurised, r=raw