

*Like any analysis, it has a specific methodology. The methodology must be understood by the trainers. There is always room for discussion in relation to this test*

## EQUIPMENT

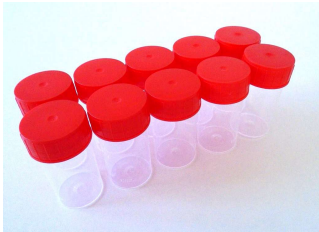
Kit available on <https://www.obsalim.com/materiel-outils.htm>



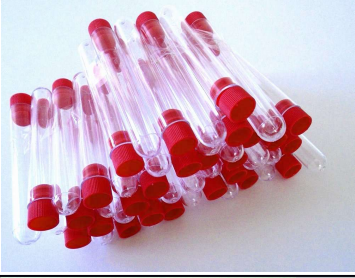

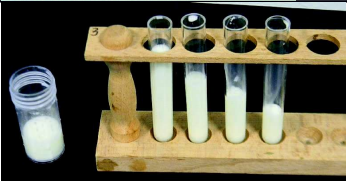


**SAMPLES**

MATERIAL		METHOD	OBJECTIF	PRECAUTIONS
Bulk tank milk		Collect at the end of milking before cooling.	Obtain an average value for the herd	Mix the milk in the tank before collecting (when collection takes place long after milking). Take into account the changes that may happen when samples are not kept cool before analysis.
On a group of animals		Take samples of 5 -10 animals, collect at half milking (or ideally using a special sampling devise that collects milk throughout the milking), mix properly	Obtain an average for this group to compare to other groups.	Decide which animals will belong to what group: depending on the characteristics of their production.
Individual follow-up or comparison between individuals.		Collect at half milking but ideally use a sampling devise that collects milk throughout the milking time.	Make a link with Obsalim symptoms, with feed batches or with the production level.	Keep all samples in a cool place







**SAMPLING**

MATERIAL		METHOD	OBJECTIF	PRECAUTIONS
Use appropriate sample pots of minimum 30 ml ideally 60 ml. (see Obsalim equipment)		Close quickly after sampling, avoid contamination during sampling.	Avoid bacterial contamination	Use clean pots, single use is preferred, make sure to identify properly.
Continuous collection during milking. (special sampling devise)		Homogenisation of variations in milk composition during milking	Reduce individual variation linked to animals and milk volume produced.	
Preservation by cold chain between 4 and 8°C unless analysed within 4 hrs.			Limite the acidification of the milk due to its own flora.	Start the analyset as soon as possible.
Before starting the analyse.		Mix by turning and returning softly the sample to make sure the cream part of the milk is incorporated.	Homogeneity of different coagulation tubes for the same sample.	Gentle agitation by turning the sampling pots upside down







MATERIAL		METHOD	OBJECTIF	PRECAUTIONS
5 to 6 tubes of 10ml for the micro coagulation, (See Obsalim equipment)		2 or 3 tubes with 10ml of whole milk, properly identified, 100% - to check morphology and conservation - other tubes are for the dilution	Possibility to perform several tests of micro-cheese. • comparison and conservation of samples. • 3rd tube, coagulate weighing and whey recovery	Make sure the sample is well homogenised. Wait for the temperature to rise before adding the rennet.
Syringes of 12 ml. (See Obsalim equipment)		Use to add the right amount of milk and water to the tubes.	Definition of the standard initial volume of 10ml	When preparing dilutions add water before the milk
Dilution tubes : 3 needed (same tubes as above, in addition to the 100% milk samples).		Prepare tubes with demineralised water, • 3 ml / 5 ml / 7 ml of water • add 7ml / 5ml/ 3 ml of milk To create 70% / 50% / 30% dilutions.	To test the effect of the dilution on the strength of the casein binding forces.	Mix the diluted milk samples well before adding the rennet. Mix gently!
Buy the rennet (pharmacy/specialised shop) and keep refrigerated.		Add 2 drops of rennet to each sample and immediately mix gently by turning the tubes upside down.	Create a coagulant using rennet.	Miw immediately to distribute the rennet before curdling. Be careful it can be very quickly. .
Put in a bain-marie at 38C exactly: add a thermometer to verify. (See Obsalim equipment.)		Leave for 24 hrs at 38C, then remove to allow to settle to at room temperature.	• Coagulation and retraction : of the micro cheese. • sedimentation of non-retracted coagulable proteins, • flotation of fats and other light particles Keeping non-coagulable proteins in solution	24 h without movement Tubes vertical Replace the caps if expelled in the first few hours Avoid temperature elevation over 42°C the coagulation will be disrupted. Maximum possible overrun of 6 hours.

EXTRACTION OF THE MICRO CHEESE AND RECOVERY OF THE WHEY


MATERIAL		METHODE	OBJECTIF	PRECAUTIONS
Precision scales. (see Obsalim equipment.)		Weigh tube 1 (with 100% milk, coagulated)	Reference weight.	Handle the tube gently without damaging the micro cheese
Fix flexible filter paper over the top of the tube. (see Obsalim equipment)		After fixing the soft filter over the tube, turn upside down to recuperate the whey.	Collect the cheese: the coagulated and retracted proteins. Collect the whey containing the soluble proteins and the proteins not captured in the retracted coagulant.	Tightness of the junction. Beware of micro cheese that block the top of the tube: gently peel them off. Percussion to bring the liquid down the walls Beware of liquid losses between tubes
				
Weigh the coagulant (cheese).		Determine the weight of the micro cheese (tube1) by deducting the weight of an empty tube (tube 1 after extraction – tube 2 dry). (See measurements)	Cheese yield: weight of the micro cheese/ weight of the milk. (tube 1 after extraction – tube 2 dry)	Respect the same time between the end of retraction at 24 hours and weighing. Retraction continues beyond 24 hours and reduces the yield.

**COAGULATION OF THE SOLUBLE PROTEINS**



MATERIAL		METHODE	OBJECTIF	PRECAUTIONS
Whey tube filled to 7mL.		Use tube nr 2 that was used to collect the whey out of tube 1 (100% milk)	To make a semi-quantitative assessment of the soluble proteins.	Handle tubes gently! Make sure to use exactly 7 ml of whey.
Add 3 ml of 15% chloric acid		Use commercially available chloric acid at 23%: add 1ml of distilled water to 2 ml of this chloric acid. Add this to the 7 ml of whey.	Obtaining the standard initial volume of 10mL and acidification.	Use the 10ml syringe to mix 2 ml of chloric acid, 1 ml of distilled water and add to the 7 ml of whey to make 10 ml.
Heat up in Bain-marie to 85C for at least 1 hr		Acid and high temperature coagulation followed by protein separation by density	Recovery of all non-shrinkable and non-coagulable proteins from the whey.	Care when handling the chloric acid.
Measure		decanting 6 hrs: Height of the 3 density layers: <ul style="list-style-type: none"> <li>• Low density = top</li> <li>• Medium density = middle</li> <li>• High density = bottom</li> </ul>	Evaluate the strength of the coagulation and retraction of the milk and the relative fraction of albumin and globulin as part of the milk protein.	Handle gently to avoid mixing!

## MEASUREMENTS AND THEIR MEANINGS

MATERIAL		METHODE	OBJECTIF	PRECAUTIONS
Examination of the morphology of the coagulant.		<ul style="list-style-type: none"> <li>- Thickness</li> <li>- Edge</li> <li>- Torsion</li> </ul>	Relative classification from 0 - 3	Measurements need to be made between 24 hrs after adding rennet and max 30hrs after, since shrinkage continues. Do not stir, handle carefully.
Thickness		Place on a dark surface and measure	Indicates mainly the calcium effect	Good temperature control is required. Cumulated effect of the casein activity.
Torsion		0 : straight 1 : slightly curved 2 : curved 3 : twisted at the bottom <i>Ideal coagulant : 2</i>	Mainly related to phosphor: 0 : Ca/P high 3 : Ca/P very low	The tubes need to be maintained vertically during the 24 hrs incubation!
Sharpness of the edges of the coagulant		0 : very neat, smooth 1 : neat and fluffy areas 2 : fluffy edges 3 : filaments <i>Ideal coagulant : 0</i>	Casein production	Be careful of temperature.
Aspect of the whey :				
Trouble		0 : transparent 1 : slightly trouble 2 : opalescent 3 : very cloudy	Presence of low density non shrinkable coagulable proteins remaining in suspension.	Control of immobility on the first few hours  To be followed by whey coagulation and measurement of non-coagulable proteins.
Deposits		0 : no deposits 1 : traces 2 : 2 mm deposits 3 : 4 mm deposits <i>Ideal coagulant : 0</i>	Presence of non-shrinkable coagulable proteins	
Resistance to the dilution :		Measure the strength of the retraction	Determined by the highest dilution which allows retraction	
Dilution		0 : dil° 30%coagulated 1 : dil° 30% not coagulated 2 : dil° 50% not coagulated 3 : dil° 70% coagulated <i>Ideal coagulant: 0</i>	Efficacy of the retraction of the casein proteins	
Cream		0 : no deposits 1 : traces 2 : 2 mm deposits 3 : 4 mm deposits <i>Ideal coagulant : 0</i>	Fat capture by casein.	

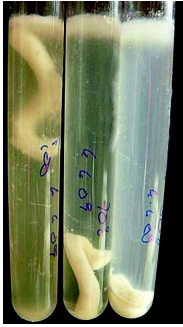
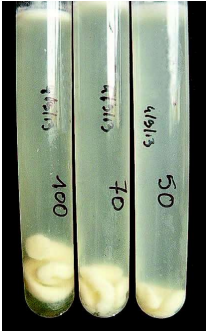

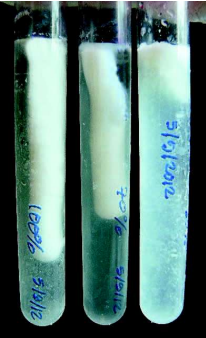
MATERIAL		METHODE	OBJECTIF	PRECAUTIONS
Weighing the micro cheese.		Precision scales Set the scales to zero with a dry tube and top, or deduct the weight of the empty dry tube and top.	Cheese yield related to this cheese making process: 24 hrs coagulation with rennet followed by immediate and fast draining	Weighing immediately. Express in % of the initial milk weigh (10mL)
Weighing the non-coagulated proteins.		Acid and high temperature curdling of whey.	Measure the height of the deposits: high, medium, low	Standardisation of the gesture and delay

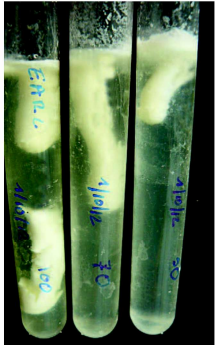
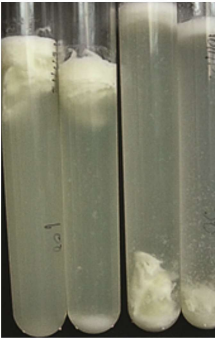
### CONSERVATION OF THE SAMPLES

MATERIAL	METHODE	OBJECTIF	PRECAUTIONS
On blotting paper.		Coagulant conservation	Only make comparisons between equally dried coagulants.
Pictures of a serie			Illuminate through a transparent screen from the back or hold a black sheet behind the tubes.
In the second tube.			Acidification allows a several months conservation at ambient temperature



EXAMPLES OF SCORING

TYPE	PHOTO	ANNOTATION	INTERPRETATION
Reference		<p>Thickness 4 mm</p> <p>Torsion 2 : curved</p> <p>Edge of coagulant 2 : fluffy edges</p> <p>Trouble 1 : slightly trouble</p> <p>Deposits 1 : traces</p> <p>Dilution 21: dilution 30% not coagulated</p>	<p>Ideal coagulant : strong contraction, good curving with clean edges, only a small amount of non coagulable or low density proteins</p> <p>The energy yield of the ration through digestion is efficient. The ration has a good Ca/P balance.</p>
Rubbery		<p>Thickness 4 mm</p> <p>Torsion 3 ; twisted at the bottom</p> <p>Edge of coagulant 0 : very neat, smooth t</p> <p>Trouble 2 : opalescent</p> <p>Deposits 0 : no deposits</p> <p>Dilution 1 : dilution at 30% not coagulated</p>	<p>Rubbery coagulant, Micro cheese with a very high contraction, curved with clear edges and few non-coagulated or small proteins. Important imbalance of minerals: either too much Phosphor or a relative deficit on Calcium.</p> <p>There is a risk of food remaining too long in the abomasum causing a cramp of the pylorus.</p>
Poor yield		<p>Thickness 2 mm</p> <p>Torsion 0 : straight</p> <p>Edge of coagulant 0 : filaments</p> <p>Trouble 1 slightly trouble</p> <p>Deposits 1 : traces</p> <p>Dilution 1 : dilution at 30% not coagulated</p>	<p>Very thin coagulant with poor quality edges but good resistance to dilution, Poor coagulant yield, no torsion, fluffy edges. Few non-coagulable and low density proteins</p> <p>Poor energy conversion of the digested ration: the caseins are of poor quality: they show little response to the Phosphor and calcium action.</p>
Globular coagulants with cloudy whey.		<p>Thickness 7 mm</p> <p>Torsion 0 : straight</p> <p>Edges of the coagulant 0 : filaments</p> <p>Trouble 1 slightly trouble</p> <p>Deposits 1 : traces</p> <p>Dilution 1: dilution at 30% not coagulated</p>	<p>Very thick coagulant with fluffy edges, and little resistance to dilution</p> <p>Good yield of micro cheese but no torsion, poorly defined edges, poor shrinkage and very difficult to dry.</p> <p>Poor energy performance of the ration. The caseins do not respond to the action of calcium and phosphor.</p>

TYPE	PHOTO	ANNOTATION	INTERPRETATION
Globular coagulant, clear whey, fracture of the coagulant		<p>Thickness 8 mm</p> <p>Torsion 0 : straight</p> <p>Edges of the coagulant 2 : Neat and fluffy areas</p> <p>Trouble 0 : transparent</p> <p>Deposits 1 : traces</p> <p>Dilution 1 : dilution at 30% not coagulated</p>	<p>Very thick coagulant with shaded edges and break down of the structure, poor resistance to dilution.</p> <p>High yield but no torsion, poorly defined edges, lack of structure and little retraction, very difficult to dry.</p> <p>Poor energetic performance of the ration, the caseins are not responsive to the action of calcium and phosphor.</p>
Lack of structure		<p>Thickness cannot be defined</p> <p>Torsion 0 : straight</p> <p>Edges of coagulant 0 : filaments</p> <p>Trouble 3 : very cloudy</p> <p>Deposits 2 : 2 mm</p> <p>Dilution 3 : dilution of 70% not coagulated</p>	<p>Coagulant with no structure, poor defined edges, no resistance to dilution.</p> <p>Poor quality casein. Not responding to the action of calcium and phosphor.</p> <p>Casein may have been damaged during transport or conservation of the sample.</p> <p>Low energetic performance of the ration, casein do not have any structure or reaction to Calcium/Phosphor</p>

## REFERENTIAL

MATERIAL	METHOD	OBJECTIF	PRECAUTIONS
Collective samples.	Make a link to Obsalim diagnostic.	Monitor the evolution of coagulants (cheese production) according to groups of animals or feeding periods (changes in feeding)	
Compare individual animals	comparison	Between different animals, breed, ages (in relation to production level, Obsalim symptoms, ...)	Can help in selecting the best individuals in relation to their digestive abilities.