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Method used to analyse RF-blue lake microtracer following extraction in an aqueous medium

1. Focus

The method involves using a magnet to extract a metal microtracer from a powdered mix. Spectrophotometric analysis is then used to determine the quantity of microtracer initially present in this mix. When processing pellets, sample preparation will include a grinding phase.
This method is the latest development in Tecaliman's research (i'Doc_T9) into how to use microtracer methodology in the largest possible feedstuff manufacture configurations, particularly in difficult cases involving the use of large quantities of liquids, mineral-based premix tests or feed pelletisation.

2. Preparing samples

2.1. Powdered mixes

Powdered mix samples are prepared in steps such as individual premixing of samples, aggregates and/or divisions.

2.2. Grinding pellets or crumbs

Pellets and crumbs first have to be ground before the microtracer can be extracted from them. The ground pellets/crumbs are then put through the same microtracer extraction protocol as the powdered mixes.
If the feedstuff is not ground, there is no guarantee of being able to extract the total amount of microtracer present.
This grinding must be performed without raising the temperature.
Several grinding methods were tested; the methods that gave the best results are listed below:
Drum grinder with two throughputs or two rows
Coffee grinder
Kenwood grinder / crusher
Retsch grinder GM 200 or 300

Table 1 shows the operating modes, pros and cons for these 4 types of grinder.

Grinder type	Operating mode	Pros	Cons
Drum grinder	Run through 2 drums at least with gradual tightening	Continuous - Quick clean No limit on sample size	Long grinding time This type of grinder has limited commercial availability
Standard coffee grinder	Grinding time: 2 x 5 seconds	Fast Quick and easy to clean	Small sample size (max. 100 g) Hard to clean - May overheat
Kenwood grinder / crusher	Spacing: 2 – Speed: 3	Continuous - No limit on sample size	Long grinding time and long cleaning times
Retsch grinder GM 200 or 300	10 s. at 10 000 rpm	Fast – Quick and easy to clean	Sample of 500 g

Table 1: Conditions of use and pros/cons for each possible grinder

3. Extraction from a meal or ground pellets

3.1. Equipment

- 2 1.5-l pots with a predefined volume of 1.4 l marked inside them
- 1 3-l beaker with handle
- 1 vacuum pump
- 1 1000-ml vacuum flask
- Hoses
- 1 filter holder
- 1 filter funnel
- 1 forceps
- 1 stainless steel whisk
- 1 mixer
- 1 stand
- 1 base
- 1 gripper for holder the mixer
- 1 wash bottle for tap water

- 1 MSP 100 magnetic probe - SETEM brand
- 1 countdown stopwatch
- Precision scales accurate to 0.01g
- Blower

3.2. Consumables

- Tap water
- Tween 20
- Cellulose nitrate filters (Sartorius brand) 0.8 µm
- Small mini-grip bags
- Sample pots - volume 350 ml

3.3. Assembly

Figure 1 below illustrates the assembly.

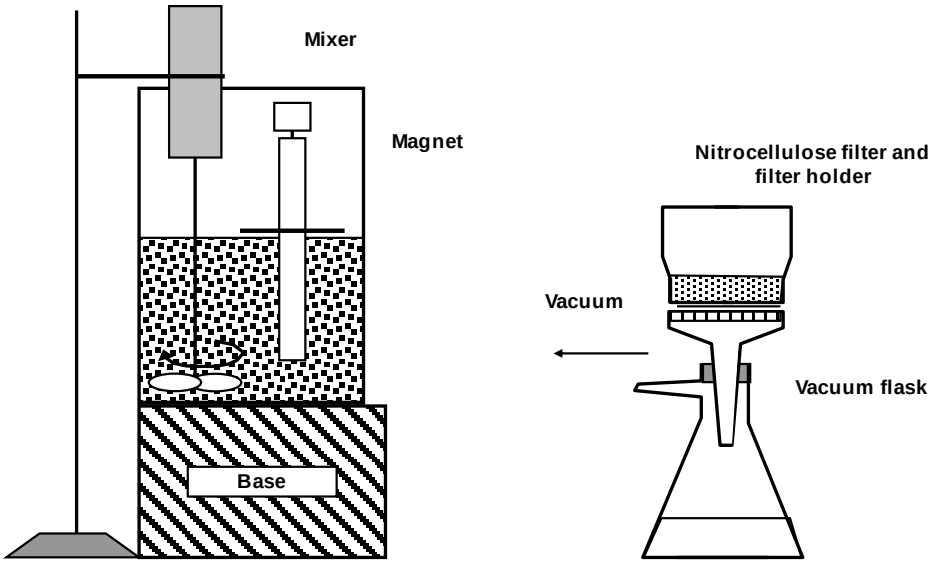

Figure 1: Assembly diagram

3.4. Method

1. Place the cellulose nitrate filter on the filter holder.
2. Fill the 2 pots of 1.5 l with water up to the predefined volume mark (1.4l)
3. Open the pot containing the sample with the incorporated microtracer.
4. Place the pot on the scales, then zero the scales.
5. Pour the sample into the 3-litre beaker.
6. Reweigh the empty pot and record the weight.
7. Clean the pot and its cover with the blower.
8. Empty a pot of water onto the sample.
9. Disperse the sample in the water using a stainless steel whisk, then rinse the whisk in the 2nd pot of water (rinsing pot).
10. Place the beaker on the base in order to lower the mixer blade into it.
11. Place the magnetic probe inside the beaker.
12. Mix for 30 seconds.
13. Remove the magnetic probe from the beaker.
14. Place the magnetic probe in the rinsing pot.
15. Free the particles by raising the magnet in the probe and shaking it lightly.
16. Lower the magnet back into the probe and stir to collect the particles.
17. Place the magnetic probe above the filter holder.
18. Free the particles by raising the magnet in the probe.
19. Use the wash bottle to delicately spray the particles on the probe towards the filter holder.
20. Switch the vacuum pump on to suck out the water on the filter.
21. Lower the magnet back into the probe.
22. Add a few drops of Tween 20 to the beaker.
23. Repeat steps 10 to 20 for the second extraction.
24. Rinse the magnetic probe for the next analysis.
25. Rinse the filter bowl so that all the particles fall onto the filter.
26. Wait for the filter to "dry out" using the vacuum.
27. Remove the filter holder bowl.
28. Clean the edge of the filter bowl with a finger.
29. Rinse your finger over the filter by spraying it with the wash bottle.
30. Switch off the vacuum pump.
31. Use the forceps to fold the filter in half, remove it from the filter holder and place it in the container used to make up the solution: the pot that had contained the feedstuff if the solvent volume is equal to or greater than 5 ml, or a mini-grip bag for volumes under 5 ml.

Clean the beaker with water to prepare it for the next analysis.

3.5. Equipment

- Automatic doser - 10 to 50 ml
- Graduated flask - 50 ml
- Manual micropipette
- Automatic pipette - 5 ml
- Stopwatch
- Plastic funnel
- Spectrophotometer cell holder
- Spectrophotometer operating in the visible region at the least.

3.6. Consumables

- 1.5-ml micro-cells for spectrophotometer
- Pipette cone tips - 1 and 5 ml
- Filter paper - Whatman code 2 - filter fineness 8 µm
- Adaptable pipette filters at tip of 1-ml silex cones (external filter tips 20 µm)
- Solvent (50% superfine alcohol at 96% / 45% distilled water / 5% soda 1 mol/litre)

3.7. Method

1. Switch on the spectrophotometer
2. Set the wavelength to 629 nm if using a blue microtracer.
3. "Zero" the spectrophotometer prior to any analysis:
   - Use the pipette to remove 1 ml of solution that will be used as a reference.
   - Place a pipette filter on the end of the silex cone.
   - Fit a cell in the spectrophotometer taking care not to soil the sides.
   - Filter 1 ml of the sampled solution into the cell.
   - "Zero" the spectrophotometer
   - Remove the cell, the spectrophotometer is now ready to do the analysis.
4. Use a 50-ml graduated flask to check and adjust the automatic doser, either at the beginning of the analysis day or after having filled the automatic doser tank.
5. Add the required quantity of solvent to the container with the filter containing the microtracer; this volume of solvent will have been determined previously based on the sample's forecast concentration:
   - Add 50 ml of solvent using the automatic doser for all homogeneity samples (approx. 100 g to 250 g/t)
   - Add 10 ml using the automatic pipette (2 x 5ml) for all carry-over samples (or low concentrations).
6. Start the stopwatch for 2 minutes
7. Shake vigorously by hand during this time.
8. For homogeneity tests:
   - Open the pot and sample approx. 1 ml of liquid using the manual micropipette.
   - Place a pipette filter on the end of the silex cone.
   - Filter the 1 ml into a spectrophotometer cell.
9. For low concentrations (carry-over):
   - Filter at least 5 ml of liquid through a Fisher filter paper using the cell support and the plastic funnels.
10. Place the cell in the spectrophotometer.
11. Read the optical density at 629 nm and record the value.
12. If the optical density is > 1.5, a twofold dilution may be made or an analysis run on a sample of a lower weight

NB: the spectrophotometer measurement must be made within 5 minutes of the microtracer being placed in the solvent solution.

3.8. Preparing and analysing the standard range

Standard curves are established under the extraction and analysis conditions described above.
The equipment required is identical to that used for extraction and analysis. Additional equipment includes:
Rotary detector
Precision scales accurate to 0.1 mg for weighing microtracer pure or in premix.
Numbered plastic wells for microtracer weighings

To ensure the same conditions for both the standard range and the analyses, the microtracer weighed pure or in premix should be added in increasing quantities to 100-g samples of "blank" powdered mix, i.e. free of microtracer.
This "blank" mix can be obtained directly from the industrial site, always the best option, or by dry microtracer extraction using a rotary detector. This operation is only effective with dry meal mixes. In all other cases, the "blank" mix will have to be sourced at the industrial site.
It is recommended to establish the standard range based on 5 mixes that contain increasing quantities of microtracer and a read blank made up using 100g of blank feedstuff free of microtracer.
Table 2 lists the targeted weights of microtracer or premix to be used in 50-ml solution. These provide optical densities ranging from 0.15 to 1.5 using 50 ml of solvent; these values may vary according to the microtracer's colorant content.

Pure RF-blue lake microtracer	Premix at 5% of RF-blue lake microtracer	Premix at 12.5% of RF-blue lake microtracer
0.00000 g	0.00000 g	0.00000 g
0.00500 g	0.10000 g	0.05000 g
0.01500 g	0.30000 g	0.15000 g
0.02500 g	0.50000 g	0.25000 g
0.03500 g	0.70000 g	0.35000 g
0.04500 g	0.90000 g	0.45000 g

Table 2: Weights usually targeted to establish standard curves

Weighings must be made in the wells using high-precision scales with a display accurate to 0.1mg.
If using a premix, the range should be established directly on the basis of this premix. This methodology provides for creating the same conditions as those used to make the mix and to indirectly check premix homogeneity with very small quantities.
The microtracer/premix and blank feedstuff is mixed briefly in each pot prior to extraction.
Making up a "read blank" under similar conditions to the samples using feedstuff with no added microtracer helps to correct for the presence of any residual microtracer. Failing this, the range will be read on a blank consisting of pure solvent. If the reference blank differs between the range and the analyses, any variance in optical density will have to be measured.
The quantity of solvent is always 50 ml, and is added via an automatic doser.

3.9. Processing the results

The standard curve is used to identify the linear relationship between a mass of microtracer (m) and an optical density (O.D.): m = a O.D. + b.

Coefficient b represents the gap that can exist between the solvent blank and the feedstuff blank. This relationship can be used to determine the mass of microtracer in the mass of a given sample.

Straight line quality can be assessed by using the equation to compute the quantity of microtracer that corresponds to the range's O.D., and then comparing these against the actual values. Deviations of up to 10% are tolerated at the two lower plots (5 and 15 mg), and then 5% above this.
The microtracer concentration is then identified by plotting the mass of microtracer against the mass of the corresponding sample.

4. Soluble fraction

For laboratories wishing to test the soluble colorant fraction in a microtracer batch in order to make it a quality criterion - this criterion is obtained as follows:

Take 2 fractions of approx. 0.025 g of microtracer and record their weights

Perform a liquid extraction on one fraction in order to carry out an analysis in 50-ml of solvent

The other fraction is placed directly in 50-ml of solvent

Compare the two optical densities with the same quantity of microtracer

Free fraction obtained using the following equation :

$$\text{Free fraction} = 100 \text{ x } \frac{\text{OD without extraction - OD with extraction}}{\text{OD without extraction}}$$

5. Equipment suppliers

Equipment	Reference / Supplier
Mixer	Mixer Bamix Gastro / La Bovida (18 - Bourges) Fax: 02.48.66.73.06
Sampling probe	MSP 100 / SETEM (80 - Lamotte) Fax: 03.22.42.37.33
Spectrophotometer	Bioblock Scientific (67 – Illkirch) Fax: 03.88.67.11.68
Vacuum pump	Mini diaphragm pump N735 KNF – Grosseron (44 – St Herblain) Fax: 02.40.92.07.10
Filter holder	Gravi-seal filtration funnel M26 999 / Bioblock Scientific (67 – Illkirch) Fax: 03.88.67.11.68
Cellulose nitrate filters	Sartorius filters 113 04 050N Retention limit 0.8µm – Diameter 50 mm / Sartorius Division filtration (91 - Palaiseau) Fax: 01.60.11.26.44
External filter tips	OFT 2002-20-MIC / Labsience, Inc - Mme Sorrentino www.labsience.com Fax : 001 775 747 9584

6. Bibliography

i'Doc_T9. 2003 – Elaboration d'une nouvelle technique d'extraction du microtraceur RF Bleu lake