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Confirm or contrast the behaviour of active ingredients involved in homogenisation, segregation and carry-over

When developing an active ingredient, it may be useful for producers (licensing application) or users to characterise the various ways the active ingredient behaves in one or more foodstuffs. Licensing applications often include a description of both the homogeneous dispersion of an active ingredient in the targeted foodstuffs and the stability of this dispersion (absence of segregation). When having to choose between ingredients, the client may base their decision on the potential for limiting the carry-over of a given active ingredient at the production plant.
These three behaviours are studied according to the following protocol, as part of the application process for the Tecaliman T3P Product Performance "label".

1. Principle

The sponsor selects one or more foodstuffs in which these behaviours will be characterised. The same foodstuff is used to make up mix batches, tracer batches or collector batches.
The behaviour of the targeted active ingredient is systematically compared against a reference ingredient incorporated at a concentration of 250 ppm (RF-blue lake microtracer). This reference ingredient provides for:

- in relation to homogenisation - validating mixer performance, thereby confirming that any heterogeneity observed in the active ingredient is not due to a mixer malfunction.
- testing the repeatability of the tests when these are repeated in relation to segregation or carry-over.

2. Equipment and apparatus

2.1. Mixer

The equipment consists of a 100-litre Théaudin-brand blade mixer, designed under a Buhler license, with a shape similar to that of the "speed mix" mixer (Figure 1).

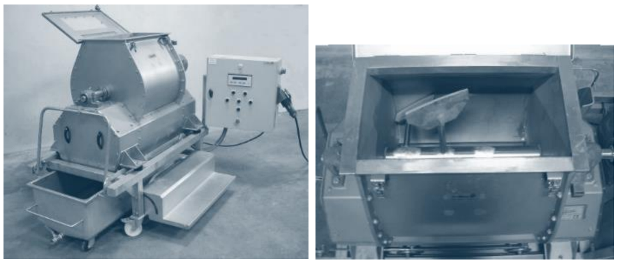

Figure 1: photographs of the mixer's exterior and interior

2.2. Elutriation column

This consists of a 5.5 m PVC tube with diameter 80 mm - in three sections.
The top section is fitted with a funnel; a pot may be placed at its lower end.

2.3. Handling machine

A pilot machine is used (Figure 2). In the direction of product travel, this machine comprises:

- One feed hopper
- One feed screw
- One elevator (height 4m)
- One bi-directional junction box:
  - Orientation 1 to an upper hopper that opens onto the feed hopper via a butterfly valve
  - Orientation 2 to the pilot machine's output

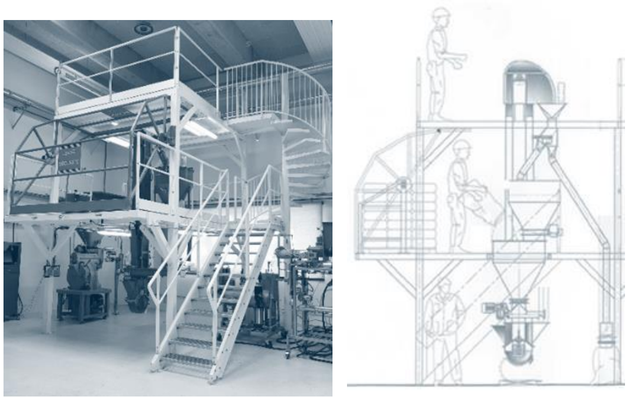

Figure 2: Photograph and drawing of the machine

During a test, the flow rate is approx. 2.5 t/h for a standard feedstuff.

3. Methods

3.1. Homogeneity

These tests are generally performed on 50-kg batches of feedstuff mix:

- Introduction of 25.0 kg of feedstuff
- Introduction of the desired quantity of test product and 12.5 g of reference tracer
- Introduction of the necessary amount of feedstuff to provide a batch size of 50 kg
- Mixing time - 300 s. (5 minutes) at 60 rpm
- Emptying

The mixing capability of the mixer used in the test is regularly checked under these conditions, while the reference tracer provides an additional control under the exact same test conditions.
Sampling: 20 samples are taken directly from the tray located under the mixer (Figure 3): 10 samples from the top layer and 10 from the bottom layer.
Ten of these 20 samples are used for the analyses:

- 5 samples from the top: 1, 3, 5, 7 and 9
- 5 samples from the bottom: 12, 14, 16, 18 and 20

The remaining ten samples are set aside and, where necessary, used for additional analyses.
Each of the 10 analysis samples are split in half:

- One fraction is sent to an external laboratory for analysis of the test product
- One fraction is used in-house to analyse the reference tracer

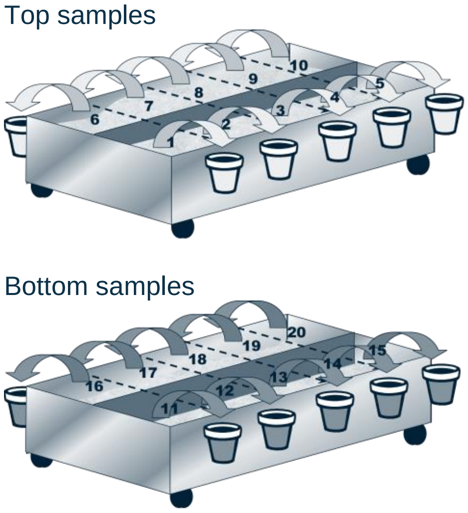

Figure 3: Sampling procedure for homogeneity tests

The results of the analyses on the 10 selected samples are used to compute the coefficient of variation (CV_{total}) by calculating the variance (V) and the mean (m). This coefficient of variation is said to be "total" as it covers all possible sources of variation.

$$CV_{total} = \frac{\sqrt{V_{total}}}{m}$$

The result is interpreted by:

- Comparing it against the dispersion of the reference tracer under the same conditions
- Professional benchmarks

3.2. Segregation by elutriation

While this test is not linked to other tests, at the laboratory, a mix of a minimum of 1 kg is prepared using a lab mixer. Each mix contains the target matrix or feedstuff, the reference tracer at the expected concentration of 250 ppm and the test product at the concentration chosen by the sponsor. The mix is stirred for 2 minutes at the lowest rotation speed.
If the test is combined with a homogeneity test, a sample of at least 1 kg is prepared using multiple increments sampled from the mix used for the homogeneity test.
In both cases, the resultant kilogramme of product is split into four equal fractions. Three fractions are used for the elutriation tests, while the last fraction is set aside. Each sample must have a volume of approx. 500 ml, i.e. approx. 300 g of mix used to fill the start tank.

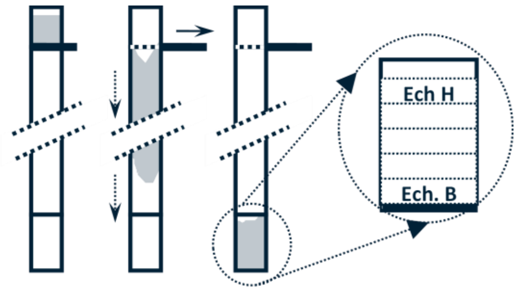

Figure 4: Diagram of the test and sampling protocol used for the elutriation

The following protocol is used for each test based on the samples (Figure 4):

- The column is cleaned thoroughly
- The collector pot is placed at the base of the column, making sure that it is perfectly leaktight
- The "shutter" is placed on top of the start tank and the whole equipment is turned over.
- The whole equipment is positioned on the funnel at the top of the column
- The shutter is removed to allow the abrupt fall of the mix into the collector pot.
- The collector pot is removed

This operation is repeated three times in order to take account of its variability. The following is performed on each sample collected after the drop:

- Samples are taken, each one corresponding to one layer of the mix in the collector tank (1/5 of the initial volume): Sub-sample H at the top and Sub-Sample B at the bottom.
- Each sub-sample is split into two fractions
- One 25-g aliquot fraction of each of the sub-samples is sent to an external laboratory for analysis of the test product
- Each sub-sample is again split into two fractions in order to analyse the reference tracer

The analysis results for the test product and the reference tracer are expressed as an elutriation ratio (IE):

$$\mathbf{IE} = \frac{C_H - C_B}{\left[\frac{C_H + C_B}{2}\right]} .100$$

Where C_H: concentration of the active ingredient in the top sample
C_B: concentration of the active ingredient in the bottom sample

This calculation gives:

- limit values: + 200 and – 200. If the entire target product is transferred to a sample (top or bottom), its concentration would be 100% as opposed to 0% for the other ingredient. This would give an IE of +/-10000 divided by 50, i.e. +/- 200 (see equation).
- a negative ratio (from 0 to -200) when the product is mainly found at the bottom and therefore dropped at a faster rate. This gives it a tendency to migrate along with the coarsest fraction.
- a positive ratio (from 0 to +200) when the product is mainly found at the top and therefore dropped at a slower rate. This gives it a tendency to migrate along with the finest fraction.
- a ratio close to zero indicates that the product's distribution has remained stable during the drop.

As the migration of active ingredients into the sample is not proportional, with most of them likely to end up in the centre, it is not always possible to calculate the mean concentration of the source feedstuff by calculating the mean between the two extreme values.
The results analysis is performed on the following basis:

- test repeatability as evaluated using the two tracers (test product and reference tracer)
- elutriation ratio of the test product and its comparison with the ratios of other products under the same conditions, together with that of the reference tracer.

3.3. Carry-over

Three mixes are prepared under the same conditions as the homogeneity test (see § 3.1) in order to perform the test 3 times and account for a certain behavioural variability. The reference tracer is used to test this variability. Each mix serves as a “tracer” batch. Equivalent loads (50 kg) of tracer-free feedstuff are prepared to serve as collector batches.
Each test is performed as follows:

- The whole machine is cleaned thoroughly
- The 50-kg tracer batch is introduced into the feed hopper
- The tracer batch is transferred to the top hopper (screw speed: 35 Hz – elevator speed: 40 Hz)
- The transfer is stopped once the whole batch has been fed through
- The top hopper is emptied and cleaned to ensure maximum transfer of the batch to the bottom hopper
- The direction of the junction box is changed
- The tracer batch is transferred to an external collector tray (screw speed: 35 Hz – elevator speed: 40 Hz)
- The transfer is stopped once the whole batch has been fed through
- The 50-kg collector batch is introduced into the feed hopper (bottom)
- The batch is fed through the machine once (the elevator, followed by the feed screw, is stopped 30 seconds after the screw is emptied)
- The whole collector batch is recovered in a second tray and placed in bags
- The whole machine is cleaned using a suction hose

Each tracer and collector batch is then:

- Entirely split in half seven times to obtain 2 sub-samples of approx. 400 g each
- These 400-g sub-samples are then split again and sent to the laboratory for analysis of the traced product in 100 g, together with an analysis of the reference tracer

For each test, the carry-over rate is calculated as a percentage of the concentration in the collector batch compared against the initial concentration in the tracer batch. These values are then compared and evaluated in relation to professional benchmark values.

4. Example of results

The following results serve as an example and are taken from anonymised services carried out on this basis. There is no link between the results given for each section.

4.1. Homogeneity

In this example (Table 1), while the reference tracer appears to be evenly distributed, on the basis of a target of 5%, the two active ingredients show dissimilar distribution patterns, with one of them appearing to have a more even distribution. The test provides an effective means of determining the behaviour of the two active ingredients tested.

	Mix 1		Mix 2	
Products	Reference tracer	Active ingredient 1	Reference tracer	Active ingredient 2
$CV_{total\ (\%)}$	**3.7**	**6.8**	**4.0**	**5.3**

Table 1: Example showing the result of the homogeneity test on 2 tested active ingredients

4.2. Segregation by elutriation

An active ingredient manufacturer wished to test the mix stability of 3 of these active ingredients in two feedstuff matrices. Under the same conditions (but out of 9 tests per matrix), the reference tracer illustrates a variation in elutriation ratios with coefficients of variation of 5.3 and 5.7% in the 2 matrices (Table 2).

	Matrix feedstuff M1	Matrix feedstuff M2
Elutriation ratio	123.8	95.4
Standard deviation	6.6	5.5
Coefficient of variation	5.3%	5.7%

Table 2: Example showing the results repeatability tests

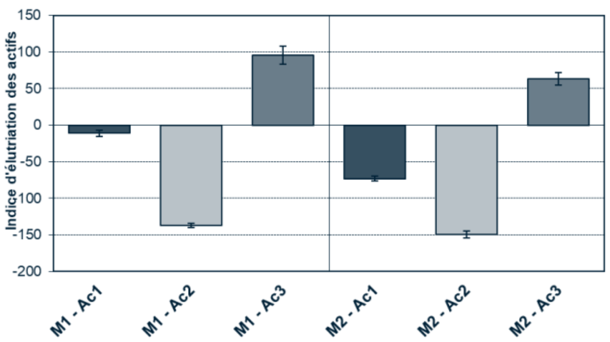

Figure 5: Example showing elutriation results for 2 matrix foods (M1 and M2) for 3 active ingredients

The results for the 3 active ingredients (Figure 5) demonstrate that their behaviours are globally similar in the 2 matrices: relative stability of active ingredient Ac1 in mix 1 but not in feedstuff 2, migration towards the bottom for active ingredient Ac2 in both matrices, while the last active ingredient (Ac3) migrated towards the top.
This test makes it possible to compare and contrast products or product/matrix relationships while providing a statistical validation of these behaviours.

4.3. Carry-over

In the last example, the client wishes to compare the behaviour of two active ingredients found in the same feedstuff. The behaviour of the reference tracer is therefore tested 6 times under the same conditions, and each active ingredient 3 times. While these repetitions can be used to calculate the coefficient of variation, the reference tracer CV carries greater weight as it is based on 6 measurements. These conditions make it possible to identify a significant difference in the behaviour of the two active ingredients.

Products	Reference tracer	Active ingredient 1	Active ingredient 2
Percentage of carry-over	**4.6%**	**1.1% a**	**3.7% b**
CV Carry-over (%)	8.2	9.1	12.1

Table 3: Example showing carry-over test results for two tested active ingredients (*the letters indicate the existence of a significant difference with a 95% confidence interval*)

5. Conclusion

These tests that compare and contrast the behaviour of active ingredients within a selected environment can be used to clearly demonstrate potential behavioural differences combined with a statistical validation. These protocols form one of the bases for validating product behaviour under the Tecaliman T3P Product Performance “label”.

6. Bibliography

i’Tec_H8: Methods used to assess the segregation of additives during laboratory testing at Tecaliman.
i’Tec_H11: Segregating feedstuffs at industrial sites
i’Tec_H14: Results of pilot tests on particle segregation by elutriation
i’Tec_T12: Pilot study on the carry-over of micro-ingredients in bucket elevators: