

CHEMICAL ASSAYS
– Tests; Theory
(Vitamin A, Folic acid, Iron)

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Chemical Analysis

- Is only as good as the sample itself
- Requires skilled analysts
- For fortification - requires relatively expensive to very expensive equipment and consumables
- Is time consuming and
- Most importantly its expensive

Validity of Analysis

- Inspectors frequently take a grab sample - as they are overworked as well – so the sample is not representative but it is considered legal
- Mills are not pharmaceutical level processors and fortified foods are not like vitamin tablets (every single one the same) – we can get mills to that level of homogeneity but not economically

- The general public isn't like an astronaut taking pills and pastes they eat bulk quantities of a food vehicle i.e. Bread which has undergone a further mixing process
- The analyst takes 0.5g of sample and tries to find the micronutrients – the consumer eats 200g of sample and lets the body find the micronutrients

A Case in Point

- 2 internationally accredited (for vitamin and mineral analysis) laboratories plus 5 pre-mix supplier laboratories participate in a ring trail to assess how much reliance can the RSA Department of Health place on an external analysis for prosecution purposes.
- The 2 accredited laboratories had already been verified against the Canadian accredited reference laboratory for such analysis.

Method

- Laboratories are provided with freshly prepared pre-mixes which are then adulterated to be below the legal limit.
- Each lab receives 2 original, but different, pre-mix formulations, 2 adulterated by 10% and 2 adulterated by 20%
- Each of the above is provided to the laboratory on 2 or 3 different occasions i.e. Blind duplicate or triplicate samples

Results

- Each laboratory is requested to analyse the pre-mixes for Vitamin A, Riboflavin, Thiamine, Niacin, Pyridoxin, Folic acid, Iron and Zinc
- Each laboratory correctly identifies the 100%, 90% and 80% samples.
- The coefficient of variation (CV) within anyone laboratory was <5%
- The CV between laboratories was typically 10-12% depending on micronutrient

Conclusion

- If you fool around with fortification pre-mix any reasonably competent laboratory will catch you out.

On Fortified Product??

- Same experimental design using pre-mixes designed to be used at 200g/MT i.e. 1:5000
- Samples prepared in laboratory using the same food vehicle (wheat flour) but the 2 different pre-mixes (avoids variability in intrinsic value issues) and made thoroughly homogenous.

Results

- Each laboratory is requested to analyse the pre-mixes for Vitamin A, Riboflavin, Thiamine, Niacin, Pyridoxin, Folic acid, Iron and Zinc
- Individual laboratory CV's >10% so even within a laboratory compliance verification questionable.
- Between laboratory CV's >40%

Conclusions

- Group could definitely not distinguish even at 20% adulteration level so disputes are inevitable.
- Compliancy or not would depend on luck

So we scrap chemical assays?

- No – vital role to play in fortification programme.
- Ensure pre-mix is “fit for purpose” – note this is different to “conforms to specification” (concrete life jacket)
- Recognise the limitations of wet chemistry and use it not abuse it.

Rapid Tests

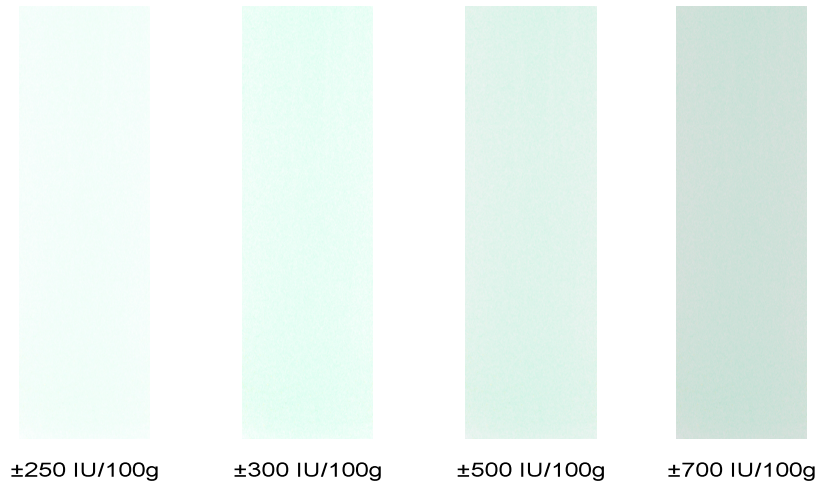
- Iron
- Vitamin A

- iCheck
- Semi quantitative i.e. BASF; Akzo Nobel
- Spot Test

Iron

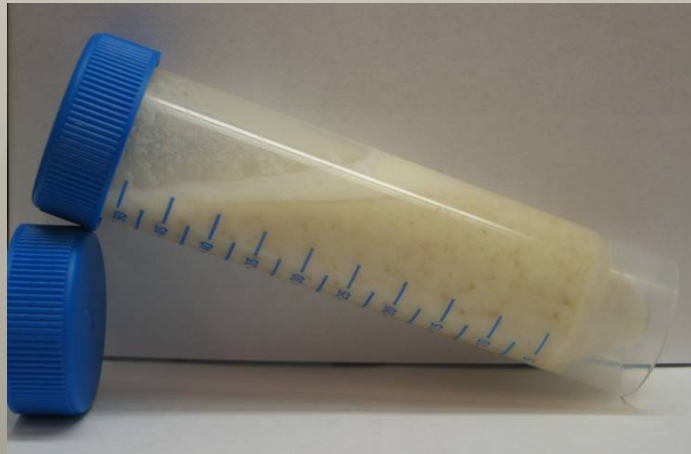


Vitamin A



Mix with Water

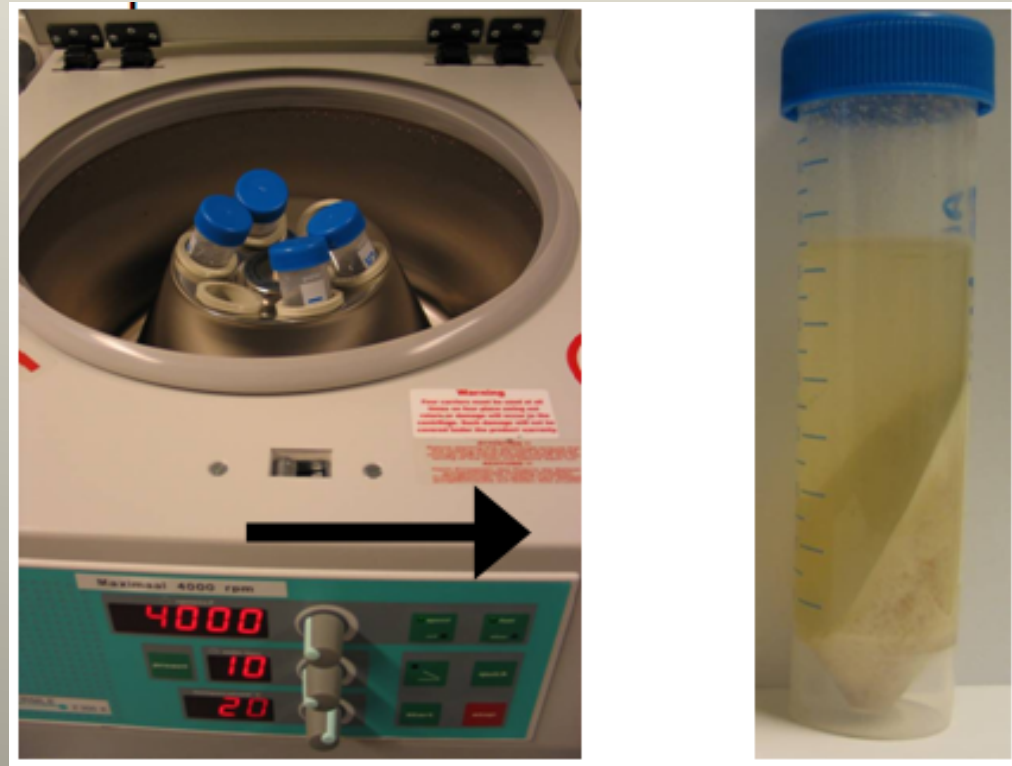
- Transfer 10 g of fortified flour into a 50-mL test tube
- Add 30 mL water and mix thoroughly to suspend all flour
- Shake resulting suspension vigorously for 1 minute



Centrifuge

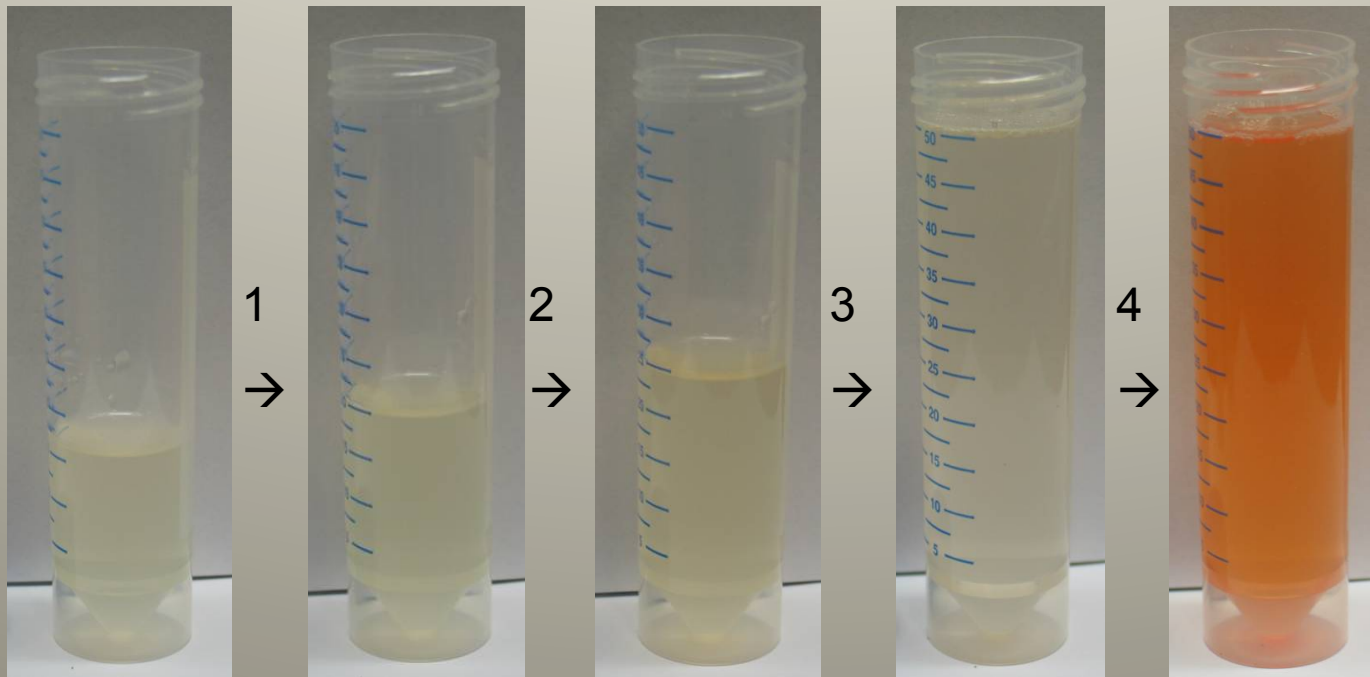
Centrifuge at 4000 rpm for e.g. 10 minutes until all flour has been precipitated at the bottom of the tube.

Then take out 15 ml of the supernatant liquor and transfer this to a 50 ml tube.



Add Reagents

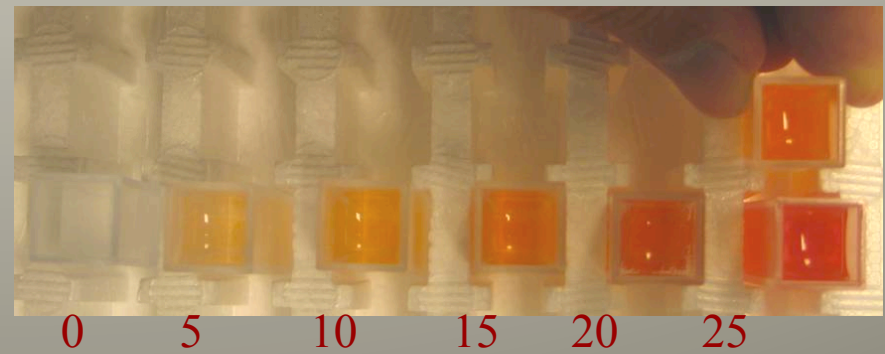
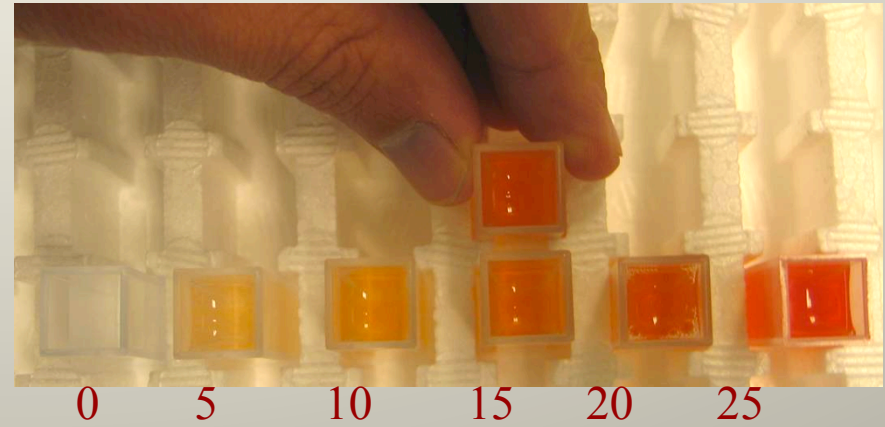
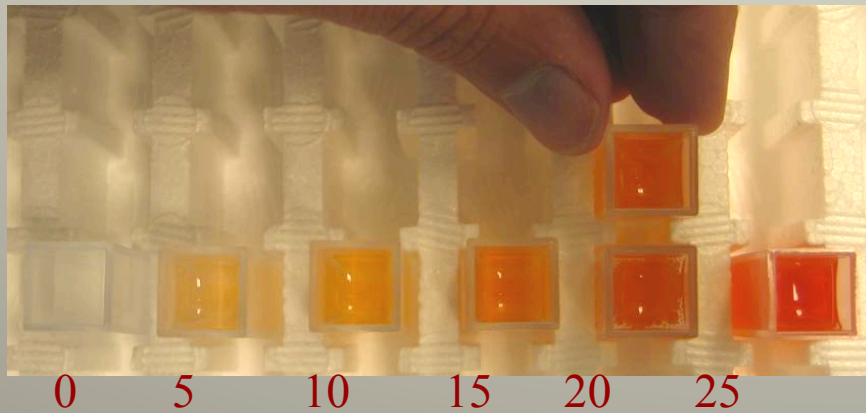
1. 5.0 ml of vitamin C solution (1 g/L)
2. 5.0 ml of phenanthroline solution (1 g/L)
3. Fill up to 50 mL and mix
4. After 10 minutes the color reaction has completed



Transfer to Cuvette



and Compare Visually



and Measure with Spectrophotometer



Measure absorbance at 510 nm against sample from unfortified flour

Determination [Fe] in Flour

Assume in flour:

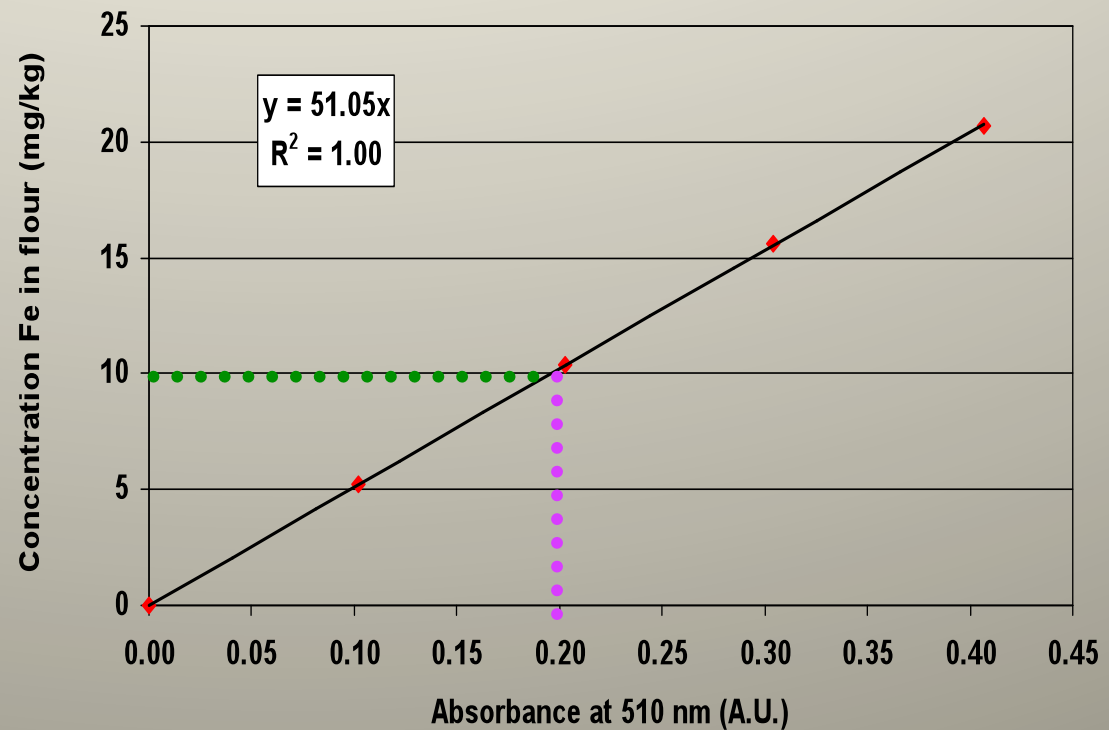
10 ppm Fe as Ferrazone



→ cuvette: 1 mg/L Fe

Absorbance: ~ 0.196

[Fe] in flour vs. Absorbance



Three different Methods to assess Vitamin A in fortified Oil

HPLC, BASF test kit and iCheck

CHROMA

1 HPLC



- Lab equipment needed
- Technically highly trained staff
- High-Priced
- Quantitative
- Golden Standard

2 BASF Test kit



- Applicable in the field
- Technically trained staff
- Low-Priced
- Semi-quantitative
- Screening tool

3 iCheck test kit



- Applicable in the field
- Low trained staff
- Middle-Priced
- Quantitative
- Precise easy-to-use test kit

Vitamin A

Field Test Kits Towards Cost-Effectiveness in Quality Control/Monitoring

Company Level

Producers:
In-house **Quality Check**

External QC

FDA/Bureau of Standards:
Screening

Laboratories:
Control

Screening with Test Kit

Screening with Test Kit

HPLC
(High performance liquid chromatography)

Number of Samples



\$ 0.02 -0.05



\$ 8-15



\$ 50-100