

ThermoFisher SCIENTIFIC

Organic Elemental Analyzer for Food Analysis

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The world leader in serving science

- Cereals, beans and seeds
- Milk and dairy products
- Meat and meat products
- Animal feed
- Beverages
- Beer (wort, malt and barley)
- Food supplements





FLASH 2000 N/Protein Users















KERRY

INGREDIENTS



ALcontrol Laboratories







Animal Feed





 Cargill, international colossus leader in animal feed field, has chosen Thermo Scientific FLASH instruments: more than 15 instruments for N/Protein determination.
 The Flash N/Protein becomes therefore, for Thermo Scientific, an international reference in order to evaluate the protein content.



FlashSmart Analyzer – Introduction

Organic Elemental Analysis (OEA): Determination of Carbon, Hydrogen, Nitrogen, Sulfur and Oxygen in <u>every</u> type of materials (organic and inorganic).

| Quantification of the sample | Weighing |
|--------------------------------------|----------------|
| Quantitative oxidation of the sample | Combustion |
| Reduction of combustion gases | Reduction |
| Separation of the oxidation gases | Chromatography |
| Generation of signal | Detection |

Micro Elemental Analysis: Simultaneous analysis of CHNS/ with small sample weights (low mg)

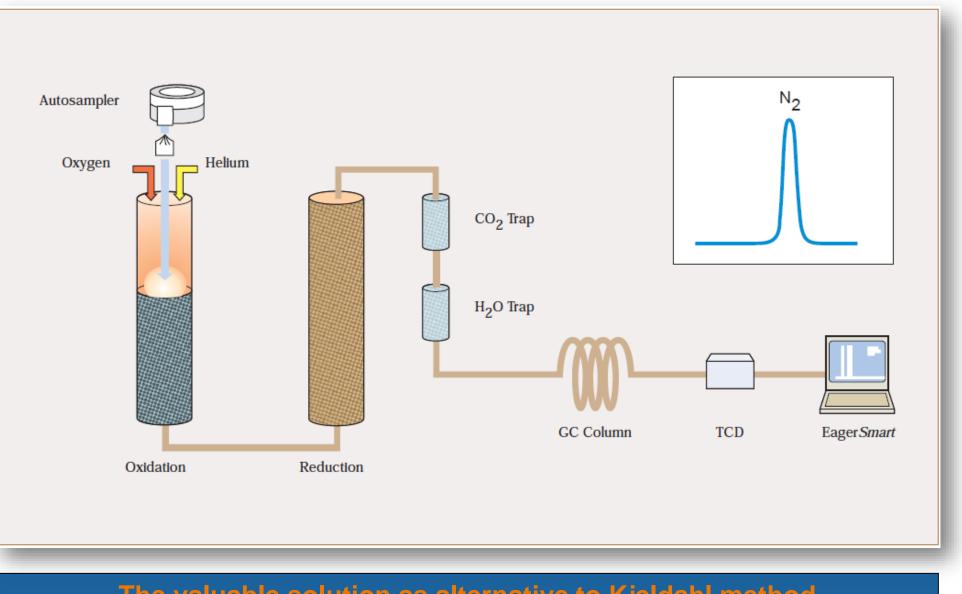
Macro Elemental Analysis: Analysis of NC and N with large sample weights (high mg)

> Fully Automatic Technology Based on Dumas Method





FLASH 2000 Analyzer – Nitrogen / Protein Configuration



The valuable solution as alternative to Kjeldahl method



Flexibility of the FlashSmart Analyzer

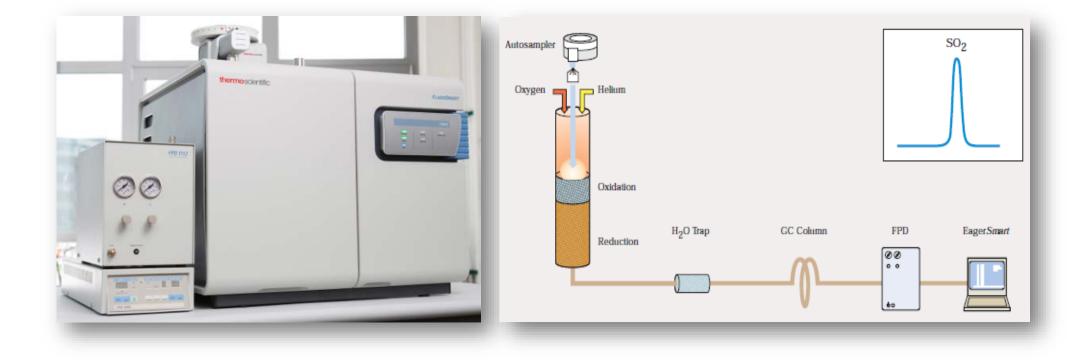
- Conversion from N/Protein to NC configuration
- Conversion from N/Protein to CHNS / CHN / NCS configuration
- Conversion from N/Protein to Oxygen configuration
- Conversion form N/Protein to Sulfur determination by FPD detector

- Analysis of solids, liquids and viscous samples
- MAS Plus and AI/AS 1310 Liquid Autosampler installed on the same system





FlashSmart+ FPD Detector: lower S det. 5 – 10 ppm



FPD : Flame Photometric Detector



OEA / FPD key features

- Total Sulfur determination
- For every type of materials
- Wide application range
- High and Low Conc. of S in the same instrument
- Constant FPD conditions with different samples
- OEA / FPD can be coupled to FlashSmart and previous OEA models
- Eager Smart Data Handling Software
- Sulfur Reference Materials included in the OEA / FPD system





Sulfur analysis by FPD detector

| Sample | S % | Av. S% | RDS % |
|--------------|--------|--------|-------|
| | 0.3565 | | |
| | 0.3648 | | |
| Chocolate | 0.3551 | 0.3585 | 1.14 |
| | 0.3551 | | |
| | 0.3589 | | |
| | 0.0065 | | |
| | 0.0063 | | |
| Maize Starch | 0.0065 | 0.0065 | 2.28 |
| | 0.0064 | | |
| | 0.0067 | | |
| | 0.0549 | | |
| | 0.0524 | | |
| Dried fruit | 0.0548 | 0.0538 | 2.39 |
| | 0.0547 | | |
| | 0.0525 | | |
| | 0.1535 | | |
| Diet food | 0.1535 | 0.1520 | 1.67 |
| | 0.1491 | | |
| | 0.3449 | | |
| Spiked diet | 0.3362 | 0.3409 | 1.29 |
| | 0.3417 | | |







N / Protein determination of Corn Gluten and Soya

| Sample | N % | RSD % | Protein % | RSD % |
|--------|---------|--------|-----------|--------|
| | 10.0489 | | 62.8055 | |
| 1 | 10.0461 | 0.0274 | 62.7881 | 0.0271 |
| | 10.0516 | | 62.8222 | |
| | 9.4731 | | 59.2069 | |
| 2 | 9.4775 | 0.0235 | 59.2345 | 0.0235 |
| | 9.4759 | | 59.2241 | |
| | 10.4201 | | 65.1256 | |
| 3 | 10.4356 | 0.0925 | 65.2225 | 0.0925 |
| | 10.4378 | | 65.2362 | |
| | 10.7510 | | 67.1940 | |
| 4 | 10.7734 | 0.1170 | 67.3337 | 0.1169 |
| | 10.7722 | | 67.3265 | |
| | 10.3815 | | 64.8846 | |
| 5 | 10.3800 | 0.0159 | 64.8752 | 0.0161 |
| | 10.3782 | | 64.8637 | |

| Sample | | | N % | | | | | Protein % | | |
|----------|------|------|------|------|------|-------|-------|-----------|-------|-------|
| Soya 50% | 7.05 | 7.06 | 7.00 | 6.98 | 7.04 | 44.07 | 48.11 | 43.74 | 43.66 | 44.00 |
| Soya 55% | 7.86 | 7.81 | 7.83 | 7.78 | 7.84 | 49.14 | 48.80 | 48.93 | 48.64 | 48.99 |
| Soya CR | 5.51 | 5.61 | 5.58 | | | 34.47 | 35.07 | 34.86 | | |
| Soya M | 5.92 | 5.85 | 5.88 | | | 36.98 | 36.57 | 36.76 | | |





N /Protein determination of Cured Meats

| Sample | N % | Protein % | RSD % |
|--------|-------|-----------|-------|
| | 2.744 | 17.153 | |
| | 2.725 | 17.032 | |
| | 2.710 | 16.939 | |
| | 2.755 | 17.224 | |
| Bacon | 2.734 | 17.090 | 1.867 |
| Dacon | 2.834 | 17.716 | |
| | 2.664 | 16.654 | |
| | 2.752 | 17.199 | |
| | 2.814 | 17.590 | |
| | 2.772 | 17.325 | |
| | 4.649 | 29.057 | |
| | 4.512 | 28.201 | |
| | 4.808 | 30.051 | |
| | 4.550 | 28.439 | |
| Salami | 4.530 | 28.315 | 2.206 |
| Salami | 4.682 | 29.265 | |
| | 4.708 | 29.424 | |
| | 4.677 | 29.233 | |
| | 4.771 | 29.821 | |
| | 4.569 | 28.556 | |



| Sample | No. of runs | N % | RSD % |
|------------------|-------------|--------|--------|
| Beef sausage | 34 | 2.9972 | 1.6697 |
| Pepperone salame | 36 | 3.2163 | 1.7932 |
| Turkey salame | 27 | 5.0592 | 1.6987 |
| Genoa salame | 40 | 3.3069 | 1.4353 |



| W (mg) | N % | Prot. % |
|--------|-------|---------|
| 214.8 | 2.393 | 14.956 |
| 221.0 | 2.415 | 15.092 |
| 271.6 | 2.442 | 15.263 |
| 297.6 | 2.404 | 15.026 |
| 175.0 | 2.441 | 15.259 |
| 209.5 | 2.401 | 15.007 |
| 247.0 | 2.450 | 15.310 |
| 205.3 | 2.427 | 15.166 |
| 230.3 | 2.444 | 15.274 |
| 258.0 | 2.418 | 15.115 |

Statistical Data:

Number of Analyses: 10

Average N%: 2.423 Std. Dev.: 0.020 RSD %: 0.834

Average Protein %: 15.147 Std. Dev.: 0.126 RSD %: 0.834



N / Protein determination in Cheese

| Sample | N % | Protein % |
|----------------------|-------|-----------|
| "Caprine-" | 2.283 | 14.564 |
| Caprine- | 2.298 | 14.662 |
| "Massarpono" | 0.642 | 4.099 |
| "Mascarpone" | 0.629 | 4.013 |
| | 4.335 | 27.657 |
| | 4.394 | 28.031 |
| | 4.364 | 27.843 |
| | 4.293 | 27.391 |
| Seasoned "Provolone" | 4.350 | 27.756 |
| Seasoned Provolone | 4.366 | 27.852 |
| - | 4.358 | 27.806 |
| | 4.382 | 27.960 |
| | 4.324 | 27.589 |
| | 4.359 | 27.811 |
| | 5.190 | 33.115 |
| | 5.164 | 32.944 |
| | 5.172 | 32.999 |
| | 5.181 | 33.054 |
| "Parmesan" | 5.261 | 33.567 |
| Pannesan | 5.267 | 33.604 |
| | 5.229 | 33.360 |
| | 5.260 | 33.558 |
| | 5.170 | 32.984 |
| | 5.230 | 33.370 |





| Sample | N % | Protein % | RSD % |
|-----------------|-------|-----------|-------|
| | 1.018 | 6.365 | |
| White chocolate | 1.019 | 6.370 | 0.190 |
| | 1.016 | 6.347 | |
| | 0.969 | 6.057 | |
| Black chocolate | 0.969 | 6.057 | 0.114 |
| | 0.971 | 6.069 | |
| Plain chocolate | 1.530 | 9.540 | |
| Fiam chocolate | 1.512 | 9.442 | 0.745 |





N / Protein determination of Starch

| Sample | N % | Protein % | RSD % |
|--------|--------|-----------|--------|
| | 0.0546 | 0.3409 | |
| 1 | 0.0551 | 0.3445 | 0.5256 |
| | 0.0548 | 0.3426 | |
| | 0.0371 | 0.2322 | |
| 2 | 0.0366 | 0.2285 | 0.8299 |
| | 0.0370 | 0.2312 | |
| | 0.0129 | 0.0804 | |
| 3 | 0.0126 | 0.0789 | 2.2977 |
| | 0.0123 | 0.0768 | |
| | 0.0476 | 0.2975 | |
| 4 | 0.0474 | 0.2962 | 0.2530 |
| | 0.0474 | 0.2962 | |





N / Protein determination in Brewery Industry

MALT

| BEER | |
|------|--|
|------|--|

WORT

| N % | Protein % | | | |
|--|-----------|--|--|--|
| 1.588 | 9.925 | | | |
| 1.580 | 9.875 | | | |
| 1.560 | 9.750 | | | |
| 1.575 | 9.844 | | | |
| 1.553 | 9.706 | | | |
| 1.560 | 9.750 | | | |
| 1.555 | 9.719 | | | |
| 1.530 | 9.562 | | | |
| 1.547 | 9.669 | | | |
| 1.574 | 9.837 | | | |
| 1.563 | 9.769 | | | |
| 1.537 | 9.606 | | | |
| 1.576 | 9.850 | | | |
| 1.562 | 9.762 | | | |
| 1.549 | 9.681 | | | |
| 1.565 | 9.781 | | | |
| 1.562 | 9.762 | | | |
| 1.558 | 9.737 | | | |
| 1.546 | 9.662 | | | |
| 1.575 | 9.844 | | | |
| <i>Statistical Data:</i> Number of analysis: 20 Av. N %: 1.561 Av. Protein %: 9.754 | | | | |
| RSD %: 0.941 | | | | |

| | Day 1 (ppm N) | Day 2 (ppm N) | | | | | |
|-------------------|--|---------------|--|--|--|--|--|
| | 691 | 677 | | | | | |
| | 679 | 691 | | | | | |
| | 701 | 698 | | | | | |
| | 685 | 698 | | | | | |
| | 689 | 706 | | | | | |
| | 688 | 696 | | | | | |
| | 692 | 678 | | | | | |
| | 693 | 706 | | | | | |
| | 699 | 698 | | | | | |
| | 689 | 675 | | | | | |
| | 708 | 691 | | | | | |
| | 724 | 710 | | | | | |
| | 731 | 722 | | | | | |
| | 701 | 709 | | | | | |
| | 708 | 699 | | | | | |
| | 705 | 697 | | | | | |
| | 724 | 685 | | | | | |
| | 693 | 698 | | | | | |
| | 699 | 692 | | | | | |
| | 704 | 684 | | | | | |
| _ | | | | | | | |
| | <i>Statistical Data</i> Number of analysis: 20 Day 1: Av. N: 700 ppm | | | | | | |
| RSD %: 1.961 | | | | | | | |
| Day 2: Av. N: 696 | | | | | | | |
| | RSD %: 1 | | | | | | |
| L | | | | | | | |

| litrogen % |
|------------|
| 0.1232 |
| 0.1229 |
| 0.1243 |
| 0.1235 |
| 0.1248 |
| 0.1259 |
| 0.1247 |
| 0.1253 |
| 0.1263 |
| 0.1245 |
| 0.1215 |
| 0.1253 |
| 0.1222 |
| 0.1234 |
| 0.1256 |
| 0.1246 |
| 0.1249 |
| |



Statistical Data: Number of analysis: 17 Average N%: 0.1243 RSD %: 1.0701

| Sample Name | Nitrogen % | Protein % | RSD % |
|-------------|------------|-----------|--------|
| | 0.3674 | 2.2964 | 0.3459 |
| 1 | 0.3658 | 2.2864 | |
| | 0.3649 | 2.2804 | |
| | 0.0534 | 0.3340 | 1.0884 |
| 2 | 0.0536 | 0.3347 | |
| | 0.0545 | 0.3407 | |





| Sample | N % | Protein % | RSD % |
|--------|--------|-----------|-------|
| | 0.6135 | 3.8346 | |
| 1 | 0.6134 | 3.8339 | 0.384 |
| | 0.6176 | 3.8598 | |
| 2 | 0.8181 | 5.1132 | |
| | 0.8207 | 5.1293 | |
| | 0.8186 | 5.1161 | 0.144 |
| | 0.8190 | 5.1188 | |
| | 0.8206 | 5.1287 | |





Food Supplements – NC Determination



| Sample | N % | RSD % | C % | RSD % |
|--------|---------|--------|---------|--------|
| 1 | 5.7388 | 0.0713 | 14.5980 | 0.0845 |
| | 5.7468 | | 14.6226 | |
| | 5.7443 | | 14.6122 | |
| 2 | 11.3650 | 0.1232 | 45.6246 | 0.3854 |
| | 11.3911 | | 45.3035 | |
| | 11.3692 | | 45.3432 | |
| 3 | 5.8447 | 0.2686 | 17.1273 | 0.1376 |
| | 5.8313 | | 17.1149 | |
| | 5.8626 | | 17.1605 | |
| 4 | 3.6302 | 0.3101 | 37.0935 | 0.2010 |
| | 3.6131 | | 36.9753 | |
| | 3.6343 | | 36.9562 | |



CHNS determination of Food Related Products

| Sample | N % | RSD % | C % | RSD % | H % | RSD % | S % | RSD % |
|-------------------|--------|-------|--------|-------|-------|-------|-------|-------|
| | 16.249 | | 43.023 | | 6.902 | | 0.394 | |
| Fish gelatine | 16.212 | 0.185 | 43.099 | 0.089 | 6.608 | 2.632 | 0.408 | 2.004 |
| | 16.189 | | 43.051 | | 6.586 | | 0.408 | |
| | 15.796 | | 44.615 | | 6.623 | | 0.531 | |
| Bovine gelatine | 15.835 | 0.148 | 44.647 | 0.037 | 6.658 | 0.309 | 0.536 | 0.601 |
| | 15.838 | | 44.624 | | 6.622 | | 0.537 | |
| | 16.088 | | 44.460 | | 6.631 | | 0.531 | |
| Porcine gelatine | 16.016 | 0.226 | 44.397 | 0.096 | 6.659 | 0.585 | 0.536 | 0.970 |
| | 16.043 | | 44.379 | | 6.582 | | 0.537 | |
| | 2.530 | | 31.008 | | 5.396 | | 0.399 | |
| | 2.516 | | 30.850 | | 5.456 | | 0.396 | |
| Starch | 2.537 | 0.329 | 31.000 | 0.204 | 5.415 | 1.004 | 0.391 | 0.902 |
| | 2.520 | | 30.956 | | 5.373 | | 0.392 | |
| | 2.528 | | 30.967 | | 5.310 | | 0.398 | |
| | 13.168 | | 52.179 | | 6.665 | | | |
| Food supplement A | 13.160 | 0.137 | 52.084 | 0.104 | 6.626 | 0.311 | | |
| | 13.194 | | 52.178 | | 6.626 | | | |
| | 0.071 | | 8.0197 | | 2.207 | | | |
| Food supplement B | 0.071 | 1.109 | 8.0251 | 0.036 | 2.199 | 0.254 | | |
| | 0.073 | | 8.0241 | | 2.209 | | | |
| | 0.330 | | 40.615 | | 6.274 | | 0.366 | |
| Food supplement C | 0.332 | 0.533 | 40.396 | 0.323 | 6.331 | 0.488 | 0.368 | 1.581 |
| | 0.329 | | 40.630 | | 6.323 | | 0.357 | |

N / Protein determination of Dietary Fiber (celite)

| Sample 1 | N % | RSD % | Protein % | RSD % |
|----------|-------|--------|-----------|--------|
| | 0.123 | | 0.771 | |
| 1 | 0.125 | 0.9337 | 0.782 | 0.8198 |
| | 0.123 | | 0.771 | |
| | 0.175 | | 1.094 | |
| 2 | 0.174 | 0.3305 | 1.089 | 0.2423 |
| | 0.175 | | 1.093 | |
| | 0.358 | | 2.240 | |
| 3 | 0.352 | 0.9786 | 2.200 | 1.0061 |
| | 0.352 | | 2.203 | |
| | 0.281 | | 1.755 | |
| 4 | 0.285 | 0.9316 | 1.782 | 1.0328 |
| | 0.286 | | 1.790 | |



N / Protein determination in Animal Feed

Cargill produces and distributes crop nutrients and feed ingredients to farmers, <u>beef</u>, <u>dairy</u>, <u>pork</u> and <u>poultry</u> producers and animal feeders. They originate and process grain, oilseeds and other agricultural commodities for distribution to makers of food, feed and other products.

Cargill also collaborates with food manufacturers, food service, distributors and retailers with a focus on customer and consumer benefits. Cargill offers insights in food and beverage ingredients, meat and poultry products, and food applications that help customers succeed.





| Sample | N % | Protein% |
|----------|------|----------|
| Petfood | 4.65 | 29.07 |
| | 4.61 | 28.81 |
| | 4.70 | 29.40 |
| | 4.69 | 29.32 |
| | 4.69 | 29.34 |
| Av. % | 4.67 | 29.19 |
| RSD % | 0.81 | 0.84 |
| Fishfood | 7.89 | 49.32 |
| | 7.94 | 49.64 |
| | 8.02 | 50.11 |
| | 7.84 | 49.01 |
| | 8.09 | 50.57 |
| Av. % | 7.96 | 49.73 |
| RSD % | 1.24 | 1.24 |

N/Protein determination in fish meals

| Day | Day 1 | | Day 2 | | Day 3 | |
|-----------|---|--|---|---|-------------------------|-------------------------|
| Data | N % | Prot % | N % | Prot % | N % | Prot % |
| | 11.20 11.21 11.21 11.19 11.17 11.15 11.13 11.13 11.17 11.14 11.21 | 69.99 70.07 70.04 69.95 69.84 69.71 69.57 69.84 69.59 70.09 | 11.19 11.19 11.20 11.21 11.20 11.14 11.25 11.19 11.19 | 69.92 69.91 69.92 70.02 70.07 70.00 69.60 70.33 69.93 | 11.20 11.18 11.19 | 69.99 69.90 69.97 |
| Average % | 11.18 | 69.87 | 11.19 | 69.96 | 11.19 | 69.95 |
| RSD % | 0.27 | 0.27 | 0.26 | 0.26 | 0.07 | 0.07 |

N / Protein determination in Animal Feed

| Sample | N % | RSD % |
|-------------------|-------|-------|
| Fish Meal | 10.29 | 1.221 |
| Gluten Meal | 10.52 | 0.854 |
| Maize | 7.95 | 0.784 |
| Starch | 0.046 | 2.031 |
| Chicken Feed | 3.01 | 1.546 |
| Straw | 0.45 | 0.623 |
| Green Grass | 3.28 | 0.954 |
| Meat and Bone Mix | 8.20 | 1.422 |

Samples analyzed in triplicate





Flash Smart vs. Kjeldahl Method – Technical Comparison

| Information | Kjeldahl Method | FLASH 2000 Analyzer (Dumas Meth | od) | | |
|--|--|---|--|---|---|
| Range of sample weight | 500 – 1000 mg (2000 mg) | 100 - 1000 mg (solid, viscous and liquid | | | |
| Procedure steps | Sample preparation- weighing-digestion-distillation-titration- calculations-results. | Sample preparation-weighing-analysis-r | | | |
| Timing | | | | | |
| Warming up | 10 - 45 minutes (once a day) | 30 minutes (from stand-by condition). Warming up is not needed using the Wa automatic function. | ke-up | | |
| Preparation of the sample (Homogenization) | 5 – 20 minutes | 5 - 20 minutes This can be done when the system is analyzing samples (not possible with Kje | ldhal) | | |
| Preparation of reagents | Manual preparation: 10 - 20 minutes | Filling of two reactors and two traps: 20 minutes | Safety | High cost | Low cost |
| Digestion | 2 – 6 hours | No | | Concentrated acids at boiling temperature Toxic catalyst and chemicals | No furnes, acids and toxic reagents No atmospheric pollution Special stainless steel tubes Consumables: EDTA, CuO-Pt on alumina, copper, soda lime, molecular sieve, silicag quartz wool The separation column is not a consumat |
| Cool Down | 20 mins – 2 hours | No |] | Glass tubes | |
| Nitrogen analysis time | 10 minutes | 4 – 6 minutes | 1 | Consumables: H,SO, 96-98%, NaOH 40 %, | |
| Other steps | 5 – 10 minutes (distillation/tritation, washing of distillation system) | No | - | H_BO_ 3%, HCI Ô.1N, CuSO_5H_O, ZnSO_ KSO4, H_SO_ 0.1N, NaOH 0.1N, Acetanilide 10N NaOH in a 25 litre vessel | |
| Total time for one N determination (excluding warming up) | 3 – 10 hours | 4 - 6 minutes/sample (samples can be weighed during a sequence of analyses) | Waste disposal | Alkali waste High cost Large amount Note: destruction of 1 batch of 20 tubes takes almost 3 hours | Ashes in the crucible (the amount depends of the sample nature). Exhausted catalyst, copper and so (CO ₂ adsorber) |
| | | | Equipment | | |
| | | | Lifetime of instrument | Moderate lifetime due to acidic environment Frequent servicing every 3-4 months | Long lifetime of instrument |
| | | | Leakage Problems | Often rubber tubing breaks, leading to leaking | None. Automatic Leak Test (Eager software function) is performed af maintenance |
| | | | Damage to safety cabinet | Slight damage due to accident spillage of corrosive chemicals | No |
| | | | Laboratory requirements Anti acid table Need of chimney Gases | Cluttered workplace Yes Yes No | Clean workplace No No Yes, helium and oxygen, optional a alternative to helium) |
| | | | User knowledge | High Basic laboratory and chemistry knowledge. Knowledge of tritrimetric methods Lab technician, analytical training needed Labor intensive | Low Basic laboratory and chemistry kn Basic knowledge in gas chromato Lab technician, analytical training instrument operation and mainten training needed. |



Flash*Smart* vs. Kjeldahl Method

Technical Comparison

| Information | Kjeldahl Method | FLASH 2000 Analyzer (Dumas Method) |
|--|--|---|
| Maintenance | High | Low |
| | Regular maintenance of seals, reagent pumps, glass parts of the system. Distillated system, washing of tubes and filters every 3 months. Frequent replacement of rubber tubing and modules (hot block digestion). Manual cleaning of glass tubes Daily care of instrument | Lifetime: Catalyst ≥1000 runs, copper ≥500 runs, traps about 120 runs. Ash removal: 80 – 120 runs. Maintenance is scheduled in the Eager Xperience software, automatic signal when needed. Large number of runs with same reactors Easy to maintain: special stainless reactors and crucible for faster ash removal. |
| Capacity (number of analyses in continuous cycle) | 6 – 20 per day The capacity is limited by the digestion time | 1 drum for 32 samples With 3 extra drums, capacity is up to 125 samples |
| Automation and unattended analysis (number of analyses) | Not automated There is no automation in digestion/distillation No automatic sample loading | Automatic MAS 200R autosampler Truly unattended operation Ability to add extra samples during the analys |
| Quantitative recovery of N | Matrix problems Incomplete N recovery from some samples even after hours of digestion | Total conversion of organic and inorganic material to elemental gases. Results unbaised by sample matrix due to th flash combustion method. |
| Manual and/or automatic protein calculation | Manual (Excel file) In house spreadsheet for keying in data and final protein calculation | Dedicated Eager Xperience Software, automatically calculates the protein content. It is possible to use different protein factors according to sample nature. |
| Software | No dedicated software | Eager Xperience dedicated software with th following features: Stand-by, Wake-up and Auto-Start automat functions Maintenance program OxyTune option automatically calculates the amount of oxygen necessary for complete combustion of the sample Ability to insert the humidity of the sample fi proper protein calculation Automatic transfer of the weight from the balance Standard and personal reports Automatic Leak Test CFR 21 part 11 compliance |
| Validation | - | Ability to validate/qualify the FLASH 2000 0 |
| Modularity | No | FLASH 2000 N/Protein analyzer can be eas modified to NC, CHN, CHNS, NCS, 0xygen determination without changing hardware ar software The FLASH 2000 0EA can be upgraded wit a dedicated liquid autosampler Al/AS 1310. The Eager Xperience software is able to control the liquid autosampler without any updated version and without extra cost. |



Method:

- Weigh approx. 2 g of sample
- Add catalyst: 7 g K_2SO_4 , 0.25 g HgO
- Add 15 ml H_2SO_4
- Add 3 ml H_2O_2
- Digestion at 410 ° C for 45 min
- Cool for 10 min
- Add NaOH/ Na₂SO₃ solution
- Steam distillation
- Collect in H₃BO₃
- Titrate with HCI



Kjeldahl user's nightmares !!

SAFETY

Concentrated acids at boiling temp Toxic catalyst and chemicals

WASTE DISPOSAL

TIME CONSUMPTION

RELIABILITY OF RESULTS





FlashSmart vs. Kjeldahl Method – Analytical Comparison

Analysis of BIPEA (Bureau InterProfessionnel d'Etrudes Analytiques, France) Reference Material.

The first table shows the average and range indicated in the relative Reference Materials Certificates.

The second table shows the N/ Protein data of the BIPEA samples analyzed in duplicate by the Flash *Smart* using a sample weight of about 200 – 300 mg.

| Sample | Moisture | Fat | Carbohydrate | Kjeldahl Protein | | Combustion Protein | |
|-----------------------------|----------|-----|--------------|------------------|-----------|--------------------|-----------|
| | % | % | % | Av.% | Tolerance | Av.% | Tolerance |
| Bipea - Feed for Sow | 9.8 | 2.8 | 48.7 | 16.0 | 0.6 | 16.2 | 0.6 |
| Bipea - Dehydrated Alfalfa | 7.7 | | 29.3 | 14.8 | 0.6 | 15.1 | 0.6 |
| Bipea - Hyperproteic Powder | | 0.8 | | 85.4 | 3.4 | 86.4 | 3.5 |

BIPEA sample information available

Reproducibility of Nitrogen / Protein determination in BIPEA Reference Materials

| Sample | Bipea - Feed for Sow | | Bipea - Dehydrated Alfalfa | | Bipea - Hyperproteic Powder | |
|-----------|----------------------|-----------|----------------------------|-----------|-----------------------------|-----------|
| % | N % | Protein % | N % | Protein % | N % | Protein % |
| | 2.60 | 16.25 | 2.45 | 15.31 | 13.65 | 85.31 |
| | 2.58 | 16.12 | 2.44 | 15.25 | 13.63 | 85.19 |
| Average % | 2.59 | 16.185 | 2.445 | 15.28 | 13.64 | 85.25 |
| RSD % | 0.546 | 0.568 | 0.289 | 0.278 | 0.104 | 0.099 |

Nitrogen / Protein determination in Milk Reference Material

Reference Material from Cetre d'Étude et de Controle des Analyses en Industrie Laitiére, France

Kjeldahl Method - Mean from the results of 5 laboratories: 0.5284 % N



Flash Smart data

| N % | Average N % | RSD % | Protein % | Average Protein % | RSD % | | |
|--------|---------------|--------|-----------|-------------------|--------|--------|--|
| 0.5312 | | | 3.3891 | | | | |
| 0.5286 | * | | 3.3722 | | | | |
| 0.5321 | * | | 3.3948 | | | | |
| 0.5339 | * | | 3.4065 | | | | |
| 0.5306 | 0.5298 0.5604 | 0.5004 | 0.5004 | 3.3854 | 3.3800 | 0.5605 | |
| 0.5251 | | 3.3504 | 3.3000 | 0.0000 | | | |
| 0.5335 | | | 3.4035 | | | | |
| 0.5264 | | | | | 3.3584 | | |
| 0.5288 | | | 3.3735 | | | | |
| 0.5276 | | | 3.3659 | | | | |

Nitrogen / Protein determination in Fish Meal

| Fish meal sample | FlashSmart - Protein % | Kjeldahl Method - Protein % | Difference |
|------------------|------------------------|-----------------------------|------------|
| 1 | 63.7 | 63.5 | 0.2 |
| 2 | 65.4 | 65.4 | 0.0 |
| 3 | 65.5 | 65.2 | 0.3 |
| 4 | 69.7 | 70.2 | -0.5 |
| 5 | 69.8 | 70.0 | -0.2 |
| 6 | 71.6 | 72.0 | -0.4 |
| 7 | 69.7 | 69.5 | 0.2 |
| 8 | 67.9 | 68.5 | -0.6 |
| 9 | 69.6 | 69.4 | 0.2 |
| 10 | 70.4 | 70.0 | 0.4 |
| 11 | 69.9 | 69.6 | 0.3 |
| 12 | 67.5 | 67.3 | 0.2 |
| 13 | 67.8 | 67.5 | 0.3 |
| 14 | 65.3 | 64.8 | 0.5 |
| 15 | 69.7 | 69.7 | 0.0 |
| 16 | 65.4 | 65.3 | 0.1 |
| 17 | 70.5 | 70.0 | 0.5 |
| 18 | 70.7 | 70.2 | 0.5 |
| 19 | 71.9 | 71.9 | 0.0 |
| 20 | 69.1 | 69.5 | -0.4 |
| 21 | 69.9 | 70.0 | -0.1 |
| 22 | 65.4 | 65.6 | -0.2 |
| 23 | 67.3 | 67.6 | -0.2 |
| 24 | 65.2 | 64.8 | 0.4 |





Nitrogen / Protein determination in Brewery industry

| Sample Name | Kjeldahl Method | | Flash <i>Smart</i> | | | |
|-------------|-----------------|-----------|--------------------|-----------|-------|--|
| | N % | Protein % | N % | Protein % | RSD % | |
| Malt 1 | 1.66 | 10.38 | 1.67 | 10.44 | 0.25 | |
| Malt 2 | 1.75 | 10.94 | 1.78 | 11.12 | 0.67 | |
| Malt 3 | 1.54 | 9.62 | 1.53 | 9.56 | 0.51 | |
| Malt 4 | 1.43 | 8.94 | 1.40 | 8.75 | 0.66 | |
| Barley 1 | 1.39 | 8.69 | 1.42 | 8.88 | 0.46 | |
| Barley 2 | 1.35 | 8.44 | 1.34 | 8.38 | 0.87 | |
| Barley 3 | 1.56 | 9.75 | 1.57 | 9.81 | 0.56 | |
| Barley 4 | 1.47 | 9.19 | 1.45 | 9.06 | 1.01 | |



| Samula Namo | Kjeldahl Method | Flash | Smart |
|-------------|-----------------|--------|-------|
| Sample Name | N % | N % | RSD % |
| Beer 1 | 0.0587 - 0.0592 | 0.0594 | 1.133 |
| Beer 2 | 0.0641 - 0.0644 | 0.0647 | 1.023 |
| Beer 3 | 0.0650 - 0.0666 | 0.0659 | 0.956 |
| Beer 4 | 0.0614 - 0.0619 | 0.0618 | 1.011 |
| Beer 5 | 0.0628 - 0.0630 | 0.0630 | 0.892 |
| Beer 6 | 0.0640 - 0.0645 | 0.0637 | 0.912 |
| Wort 1 | 0.0885 - 0.0890 | 0.0892 | 1.232 |
| Wort 2 | 0.1140 - 0.1150 | 0.1170 | 0.874 |
| Wort 3 | 0.1300 - 0.1310 | 0.1320 | 0.912 |
| Wort 4 | 0.0995 - 0.0993 | 0.0993 | 1.112 |
| Wort 5 | 0.0825 - 0.0827 | 0.0821 | 1.098 |
| Wort 6 | 0.0889 - 0.0893 | 0.0899 | 1.210 |

Flash Smart vs. Kjeldahl Method – Analytical Comparison

| Sample | Flash | Smart | Kjeldahl Method | | |
|--------------------|-------|-----------|-----------------|-----------|--|
| | N % | Protein % | N % | Protein % | |
| Soya | 6.27 | 39.20 | 6.27 | 39.18 | |
| Lentils | 4.35 | 27.17 | 4.35 | 27.19 | |
| Rice | 1.13 | 7.08 | 1.12 | 7.00 | |
| Wheat | 1.75 | 10.91 | 1.74 | 10.89 | |
| Beans | 3.74 | 23.35 | 3.74 | 23.38 | |
| UHT milk 1 | 0.53 | 3.38 | 0.53 | 3.37 | |
| UHT milk 2 | 0.50 | 3.19 | 0.49 | 3.17 | |
| Crude milk 1 | 0.57 | 3.65 | 0.57 | 3.66 | |
| Crude milk 2 | 0.47 | 3.03 | 0.47 | 3.02 | |
| Crude milk 3 | 0.41 | 2.65 | 0.42 | 2.66 | |
| Pasteurized milk 1 | 0.50 | 3.21 | 0.50 | 3.19 | |
| Pasteurized milk 2 | 0.46 | 2.96 | 0.47 | 2.99 | |
| Milk powder 1 | 4.32 | 27.56 | 4.30 | 27.43 | |
| Milk powder 2 | 4.18 | 26.64 | 4.19 | 26.73 | |
| Milk powder 3 | 5.46 | 34.83 | 5.43 | 34.64 | |
| Yoghurt | 0.080 | 0.51 | 0.078 | 0.50 | |
| Mascarpone cheese | 0.635 | 4.05 | 0.638 | 4.07 | |
| Grapes | 0.52 | 3.25 | 0.51 | 3.19 | |
| Bacon (low fat) | 2.73 | 17.06 | 2.70 | 16.86 | |
| Meat loaf | 2.01 | 12.57 | 1.97 | 12.31 | |
| Ham | 2.56 | 16.00 | 2.54 | 15.87 | |
| Biscuits 1 | 1.40 | 8.80 | 1.39 | 8.72 | |
| Biscuits 2 | 1.36 | 8.51 | 1.34 | 8.37 | |
| Flour | 1.34 | 8.40 | 1.32 | 8.24 | |

AACC (American Association of Cereal Chemists)

Crude Protein in Cereals 46-30, 1999

AOAC (Association of Official Analytical Chemists)

Protein (crude) in Animal Feed, official Method 990.03, 4.2.08 Crude Protein in Meat and Meat Products including Pet Foods, Official Method 992.15, 39.1.16 Crude Protein in Cereal, Grains and Oilseeds, Official Method 992.23, 32.2.02 Nitrogen (Total) in Fertilizers, Official Method 993.13, 2.4.02

AOCS (American Oil Chemists Society)

Combustion Method for determination of Crude Protein Official Method Ba 4e-93 (revised 1995)

ASBC (American Society of Brewing Chemists)

Nitrogen determination in Barley, official Method, 1996

ASBC (American Society of Brewing Chemists)

Total Nitrogen in Wort and Beer by combustion method. Report of subcommittee, 1994

IDF (International Dairy Federation)

Nitrogen determination in Dairy Products by combustion method, 14891 – FIL 185

IFFO (International Fishmeal and Fish Oil organization Ltd.)

Nitrogen determination in Fish Meal by combustion method

ISO (International Organization for Standarization)

Food Products – Determination of the Total Nitrogen content by combustion according to the Dumas principle and calculation of the crude protein content. Part 1: Oil seeds and Animal Feeding Stuffs, 16634-1, 2008

Office International de la Vigne et du Vin

Quantification of Total Nitrogen by Dumas method (Musts and Wines)

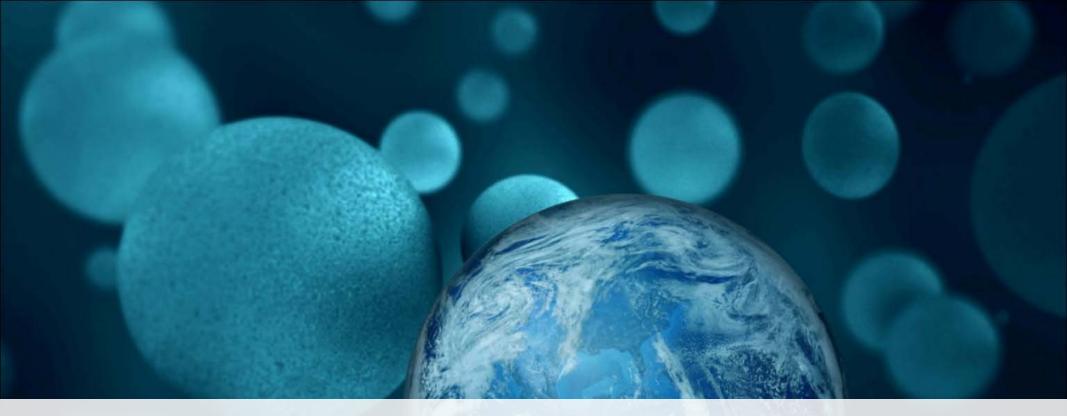
AOAC 990.03 Official Method

Requirements:

- The analysis of Nicotinic acid, Lysine chloride and a mixture of corn grain and soybean according to the AOAC 990.03 Performance Requirements (Association of Official Analytical Chemists) in which is indicated that the system must meet or exceed following minimum performance specification:
- System must be capable of measuring Nitrogen in feed materials containing 0.2 20 % Nitrogen.
- Accuracy of system is demonstrated by making 10 successive determinations of Nitrogen in Nicotinic acid and Lysine chloride. Means
 of determinations must be within ± 0.15 of the respective theoretical values, with standard deviation ≤ 0.15.
- Suitable fineness of grind is that which gives relative standard deviation (RSD) ≤ 2.0 % for 10 successive determinations of Nitrogen in mixture of corn grain and soybean (2+1) that has been ground for analysis. RSD % = (SD / mean %N) x 100. Fineness (ca. 0.5 mm) required to achieve this precision must be used for all mixed feeds and other nonhomogeneous materials.

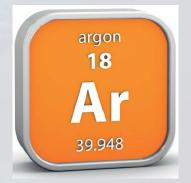
| Sample | Nicotinic acid | Lysine chloride | Mixture of corn g | rain and soybean |
|-----------|----------------|-----------------|-------------------|------------------|
| % | Nitrogen % | Nitrogen % | Nitrogen % | Protein % |
| Data | 11.30 | 15.31 | 3.27 | 20.44 |
| | 11.30 | 15.23 | 3.25 | 20.29 |
| | 11.35 | 15.16 | 3.26 | 20.38 |
| | 11.29 | 15.22 | 3.24 | 20.28 |
| | 11.37 | 15.27 | 3.28 | 20.52 |
| | 11.42 | 15.11 | 3.27 | 20.42 |
| | 11.42 | 15.22 | 3.25 | 20.36 |
| | 11.44 | 15.19 | 3.26 | 20.39 |
| | 11.48 | 15.19 | 3.26 | 20.37 |
| | 11.39 | 15.25 | 3.28 | 20.49 |
| Average % | 11.38 | 15.21 | 3.26 | 20.39 |
| RSD % | 0.576 | 0.372 | 0.404 | 0.379 |

Flash Smart Nitrogen determination according to AOAC 990.03



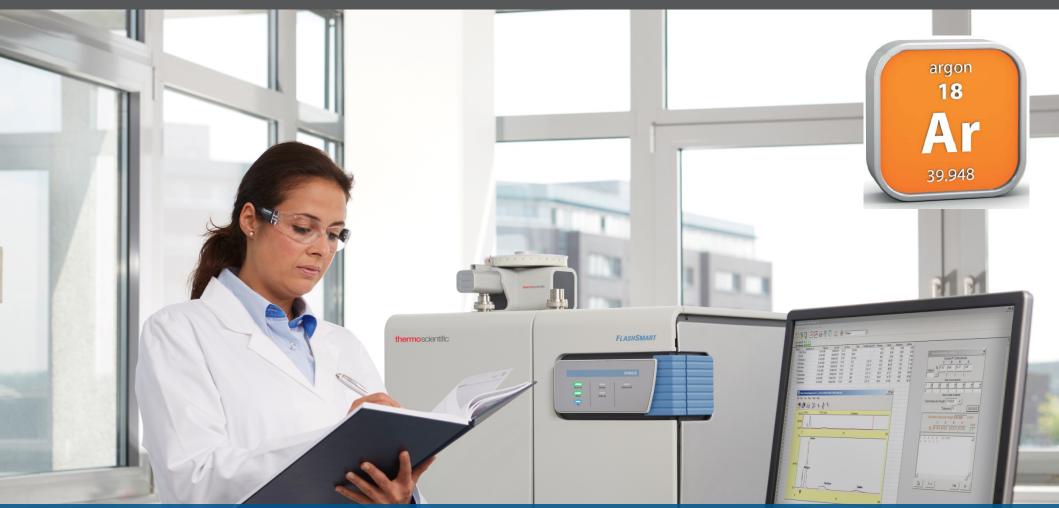
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FLASH 2000 Analyzer Argon Gas Option for N and NC analysis



The world leader in serving science

Flash Smart Analyzer Argon Gas Option for N and NC analysis

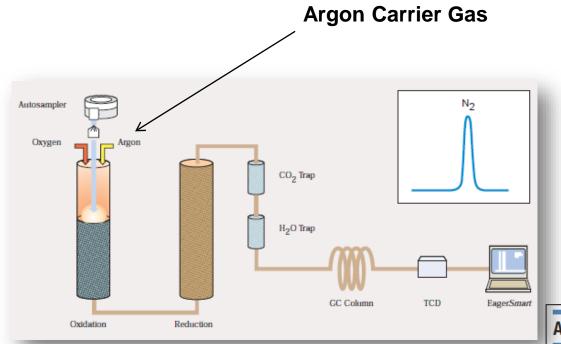


Eliminate the Risk \rightarrow Switch from Helium to Argon

- Argon is already available
- Comparable results with He carrier gas
- Up to 50% lower cost than Helium



Flash Smart Nitrogen Configuration





| Analytical conditions | |
|---------------------------------|--------------------------------------|
| Combustion Furnace Temperature: | 950 °C |
| Reduction Furnace Temperature: | 840 °C |
| Oven Temperature: | 50 °C (GC column inside the oven) |
| Argon Carrier Flow: | 60 ml/min |
| Argon reference Flow: | 60 ml/min |
| Oxygen Flow: | 300 ml/min |
| Sample Delay: | 10 sec |
| Run time: | 10 mins |



BIPEA Reference Materials

| Sample | Moisture | Fat | Carbohydrate | Kjeldahl Protein | | Combustion Protein | |
|---------------------------------------|----------|-----|--------------|------------------|-----------|--------------------|-----------|
| | % | % | % | Av. % | Tolerance | Av. % | Tolerance |
| Bipea - Feed for Sow 3/2009 | 9.8 | 2.8 | 48.7 | 16.0 | 0.6 | 16.2 | 0.6 |
| Bipea - Dehydrated Alfalfa 3/2009 | 7.7 | | 29.3 | 14.8 | 0.6 | 15.1 | 0.6 |
| Bipea - Hyperproteic Powder 1/2008 | | 0.8 | | 85.4 | 3.4 | 86.4 | 3.5 |

Data obtained with Flash Smart using Argon as carrier gas

| Sample | Bipea – Feed for Sow | | Bipea – Dehy | drated Alfalfa | Bipea - Hyperp | proteic Powder |
|-----------|----------------------|-----------|--------------|----------------|----------------|----------------|
| % | N % | Protein % | N % | Protein % | N % | Protein % |
| | 2.67 | 16.71 | 2.47 | 15.46 | 13.53 | 84.59 |
| | 2.67 | 16.67 | 2.49 | 15.57 | 13.56 | 84.74 |
| | 2.66 | 16.63 | 2.45 | 15.34 | 13.67 | 85.42 |
| | 2.60 | 16.22 | 2.41 | 15.04 | 13.62 | 85.10 |
| | 2.63 | 16.41 | 2.47 | 15.41 | 13.69 | 85.57 |
| | 2.67 | 16.72 | 2.48 | 15.49 | 13.66 | 85.36 |
| | 2.67 | 16.66 | 2.37 | 14.81 | 13.60 | 85.02 |
| | 2.67 | 16.71 | 2.44 | 15.27 | 13.63 | 85.21 |
| | 2.61 | 16.32 | 2.38 | 14.87 | 13.71 | 85.72 |
| | 2.65 | 16.55 | 2.37 | 14.79 | 13.67 | 85.43 |
| Average % | 2.65 | 16.56 | 2.43 | 15.21 | 13.63 | 85.22 |
| RSD % | 1.02 | 1.09 | 1.93 | 1.97 | 0.42 | 0.42 |

N/protein Data Comparison – Argon vs Helium

Weight Weight Protein Ν RSD RSD Ν RSD Protein RSD Name % % % (mg)% % % % % (mg)1.91 11.93 1.92 11.98 Wheat 130 - 140 1.93 0.79 12.05 0.87 160 - 200 1.93 0.79 12.06 0.75 flour 12.14 1.94 1.90 11.88 1.25 7.84 1.26 7.88 1.25 7.83 0.27 150 - 2001.26 7.93 Maize 1 130 - 140 0.46 0.46 0.32 1.26 7.87 1.27 7.91 1.48 9.24 1.50 9.41 Maize 2 130 - 140 1.49 0.399.29 0.31 150 - 200 1.50 0.38 9.39 0.38 1.49 9.29 1.49 9.34 4,79 29.92 4.79 29.92 DDGS 120 - 130 4.78 0.55 29.85 0.64 150 - 160 4.80 0.31 30.03 0.38 4.83 30.21 4.77 29.80 6.02 37.62 6.10 38.15 Sunflower 100 - 120 6.08 0.62 38.01 0.65 170 - 200 6.07 0.25 37.91 0.31 1 6.09 38.08 6.09 38.07 5.69 35.54 5.65 35.31 Sunflower 35.95 130 - 140 5.75 0.53 0.58 170 - 200 5.61 0.37 35.05 0.37 2 5.71 35.68 5.64 35.22 7.98 8.11 50.66 49.87 Soya 130 - 150 8.08 0.71 50.51 0.70 170 - 180 8.03 0.31 50.21 0.34 8.00 49.99 8.01 50.06 9.31 58.20 9.34 58.36 9.27 9.33 Gluten 1 130 - 140 0.22 57.92 0.24 170 - 180 0.16 58.33 0.14 58.02 58.21 9.28 9.31 9.32 9.31 58.18 58.28 9.37 130 - 140 9.35 0.38 58.44 0.40 170 - 180 58.55 0.25 Gluten 2 0.28 9.28 57.98 9.36 58.50 2.95 18.43 2.91 18.18 Poultry 120 - 140 2.98 1.22 18.62 1.22 150 - 160 2.961.09 18.53 1.13 feed 1 2.96 18.48 2.90 18.13 27.30 4.31 26.96 4.37 Poultry 27.17 130 - 140 4.39 1.14 27.43 1.11 150 - 160 4.35 0.82 0.91 feed 2 4.30 26.87 4.42 27.65

Argon Gas

Helium Gas

Animal Feed

Thermo Fisher SCIENTIFIC

Application Notes available

- AN 42157 Thermo Scientific FLASH 2000 Protein Analyzer for Cereals and Beans
- AN 42159 Reproducibility of Nitrogen / Protein determination with the Thermo Scientific FLASH 2000 Protein Analyzer
- AN 42186 Sulfur determination in Food by the Thermo Scientific FLASH 2000 Elemental Analyzer coupled with FPD detector
- AN 42196 Characterization of Food and Animal Feed Related Products by the Thermo Scientific FLASH 2000 Elemental Analyzer
- TN 42214 Analytical Comparison of the Thermo Scientific FLASH 2000 Nitrogen / Protein Analyzer with the traditional Kjeldahl Method
- TN 42215 Technical Comparison of the Thermo Scientific FLASH 2000 Nitrogen / Protein Analyzer with the traditional Kjeldahl Method
- AN 42200 Nitrogen / Protein determination in Animal Feed by the Thermo Scientific FLASH 2000 Analyzer using Argon as Carrier Gas
- AN 42201 Nitrogen / Protein determination in Flours by the Thermo Scientific FLASH 2000 Analyzer using Argon as Carrier Gas
- AN 42203 Thermo Scientific FLASH 2000 Nitrogen / protein Analyzer using Argon as Carrier Gas: Stability, Linearity, Repeatability and Accuracy
- AN 42262 Nitrogen/Protein Determination in Food and Animal Feed by Combustion Method (Dumas) using the Thermo Scientific Flash *Smart* Elemental Analyzer



OEA CookBook



OEA CookBook Organic Elemental Analysis

4th Edition Your Samples, Our Experience



The OEA CookBook includes a chapter on **OEA-IRMS applications**





OEA CookBook

| FLASH 2000 | | | OEA / FLASH 200 | 00 / Nitrogen/F | Protein Analy | sis / Alphabeti | cal Index |
|---|---------------------------------------|--------------------|-------------------------------------|-----------------|-----------------|-----------------|-----------------|
| | | | | | | | B / Beans |
| Classical Organic Elemental Analysis | | | | | ; | 0 0 N/Protei | n determination |
| Sulfur Analysis (FPD) | BARLEY • BEANS • | BRAN • C | CORN • LENTILS • MIL | | R • PEAS • RICE | • SOYA • WHEAT | (continued) |
| | Sample | | N % | F | Protein % | RSD | |
| Nitrogen/Protein Analysis | Corn | | 1.137 | | 7.104 | 0.73 | |
| | Soya | | 6.207 | | 38.796 | 0.54 | |
| Overview | Corn-soya (2:1) | | 2.545 | | 15.907 | 0.6 | |
| 010111011 | Barley | | 1.590 | | 9.939 | 1.3 | |
| Analytical Index | Bran | | 2.271 | | 14.192 | 0.8 | |
| / mary tear matex | Wheat | | 1.742 | | 10.889 | 0.30 | |
| Alphabetical Index | Oats-wheat mix | | 2.871 | | 16.361 | 0.70 | |
| , apriabolical matrix | Rice | | 1.101 | | 6.270 | 0.83 | |
| | Seeds of cartam | | 2.375 | | 14.845 | 1.53 | |
| | Lentils | | 3.840 | | 23.993 | 0.5 | |
| | Beans | | 3.544 | | 22.150 | 0.69 | |
| | Peas | | 3.873 | | 24.204 | 0.53 | |
| | Milk | | 0.510 | | 3.254 | 0.70 | |
| | Milk powder | | 1.840 | | 11.741 | 0.46 | 50 |
| | Sample information | | | | | | |
| | Standard: | Aspartio | c acid | 10.52 %N | | | |
| Search | Standard weight: N/Protein factor: | Mills and | | 50 - 100 mg | | | |
| Search | N/Protein factor: | Milk pro Others | oducts | 6.38 6.25 | | | |
| Main Menu | Sample weight: | Others | | 200 - 300 mg | | | |
| Print | OxyTune Category: | B cat, A | A cat. for milk powder | 200 300 mg | | | |
| Bibliography | Notes | | | | | | |
| Contact Us | Each value is the aver | | runs. ted in a rotary mill, 1 mm | granulomotov | | | |
| OEA Distributor Network | Crops and beans have | been trea | ted in a locary min, i min | granulometry. | | | |
| Thermo | | | | | | | 6.2 |





ThermoFisher SCIENTIFIC

Food Origin & Authenticity: Revealing the Truth using Isotope Fingerprints

Drs. Christopher Brodie, Lionnel Mounier & Lin-Tang GOH* Factory Product Manager (Germany) for IRMS, *Regional Senior Manager (SEA) for Mass Spectrometry

The world leader in serving science

Isotope Fingerprint for Food Authenticity and Food Integrity

- Food and beverage products have a fingerprint, a unique chemical signature that allows the product to be identified.
- To visualize this fingerprint, Isotope Ratio Mass Spectrometry (IRMS) can be used, identifying the isotope fingerprint of the product.
- The isotope fingerprint is region or process specific (Table 1), which means that products can be differentiated based on geographical region (cheese, coffee, sugar, fish and animal feeding areas), botanical processes (beans, seeds, olive oil, vanilla), soil and fertilization processes (fruits and vegetables) and fraudulent practices (sugar addition to honey, watering of wines and spirits).



Some Examples of Food Fraud

- Food and Beverages:
 - Fruit juices
 - Wine
 - Vinegar
 - Beers
 - Alcoholic beverages
 - Honey
 - Olive oils
 - Tea, Coffee
 - Dairy products
 - Meat
 - Fish
 - Fruit and vegetables

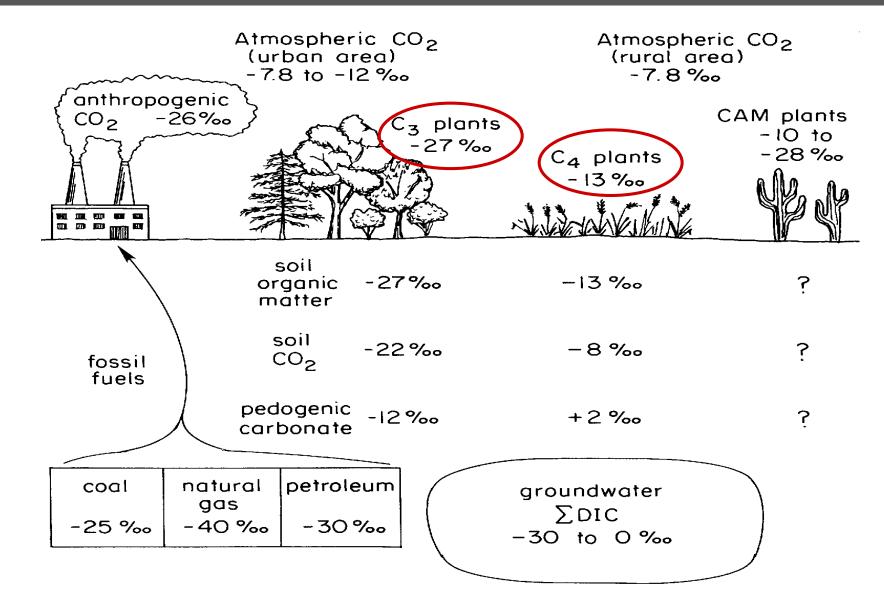
Potential Fraud:

Watering, sweetening Watering, chaptalization, label declaration Origin identification (maize, cider, grape, ...) Origin identification (grains other than malt) Mislabeling, origin identification Addition of inverted and cane sugars Addition of cheaper oils Mislabeling and origin Addition of undeclared milk, Mislabeling Mislabeling (origin) and feeding diet Mislabeling (wild \leftrightarrow farmed) Mislabeling (organic versus inorganic)

Summary of isotope fingerprints in Food Fraud

| Stable Isotope | What is the biogeochemical interpretation? | What is an example of food fraud interpretation? | What products can be affected? |
|----------------|--|---|---|
| Carbon | Photosynthesis (C3, C4 and CAM pathways) | Adulteration (e.g. sweetening with cheap sugar) | Honey; Liquor; Wine; Oliver oil; Butter |
| Nitrogen | Fertilizer assimilation by plants | Mislabeling (Differentiate organic and non-organic) | Vegetables; Animal meat |
| Sulfur | Local soil conditions; Proximity to shoreline | Origin of product | Vegetables; Animal meat; Honey |
| Oxygen | Local-regional rainfall; geographical area | Watering of beverages; Origin | Coffee; Wine; Liquor; Water; Sugar; Meat |
| Hydrogen | Local-regional rainfall; geographical area | Watering of beverages; Origin | Coffee; Wine; Liquor; Water; Sugar; Meat |

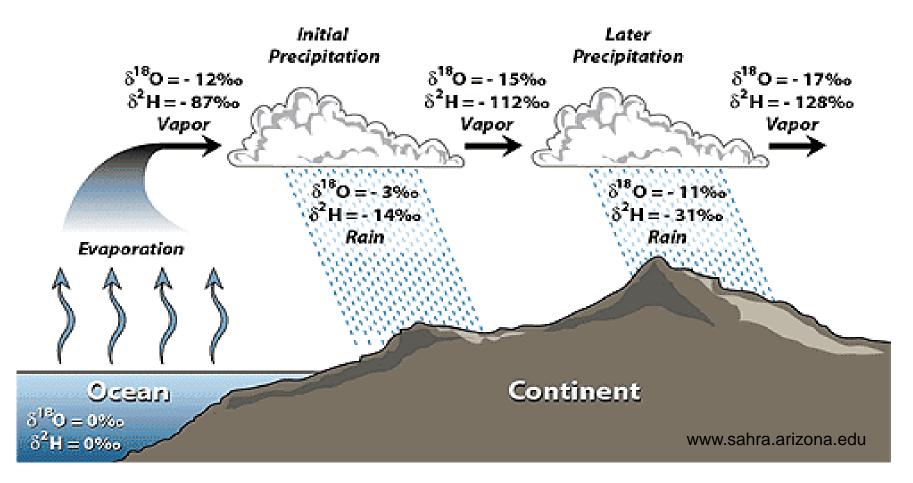
What can δ^{13} C tell us?



• δ^{13} C values change due to fractionation induced by photosynthesis

• For example, this can differentiate between products derived from C3 and C4 plants

What can δ^2 H and δ^{18} O tell us?



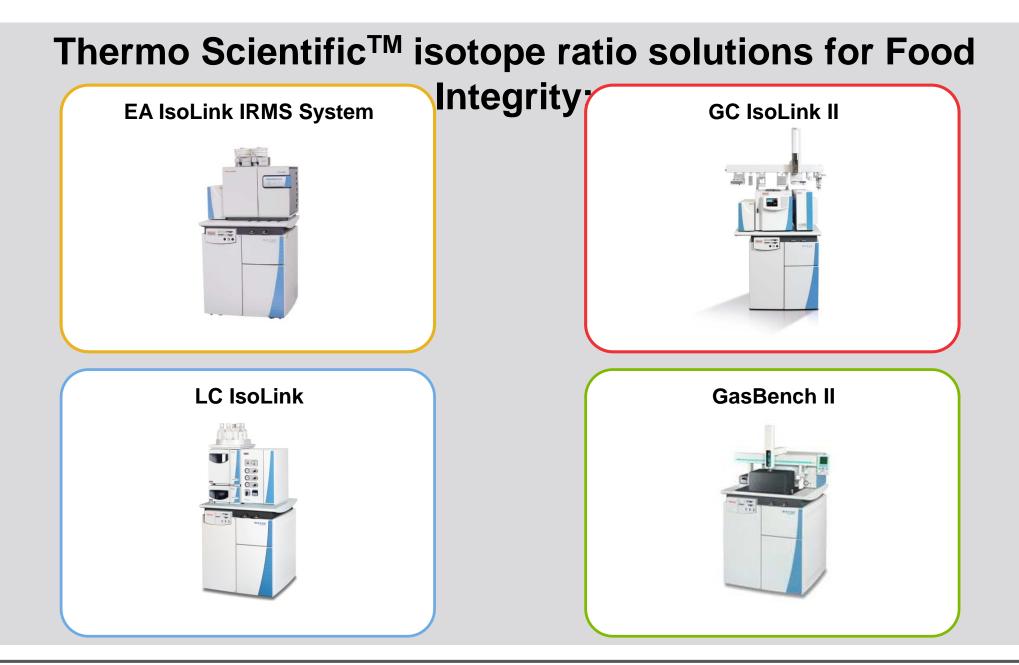
 Hydrogen and oxygen isotopes are fractionated in the water cycle through evaporation, transpiration, sublimation, condensation and precipitation processes across the latitudes, giving rise to unique local – regional signatures, which transfer to biological material during their growth period

What can $\delta^{15}N$ and $\delta^{34}S$ tell you?

- Nitrogen Isotopes in food and beverages can provide information on:
 - Nitrogen sources for plants (e.g. fertilization)
 - Sources for animal feed stuffs
- Sulfur Isotopes in food and beverages can provide information on:
 - Sulfur sources for plants (e.g. fertilization), complimenting Nitrogen
 - Coastal versus inland geography (sea-spray)
 - Sources for animal feed stuffs

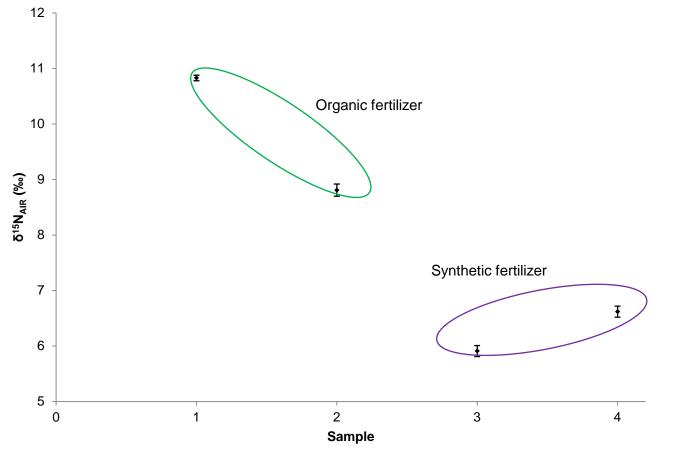


Thermo Scientific solutions for Food Industry



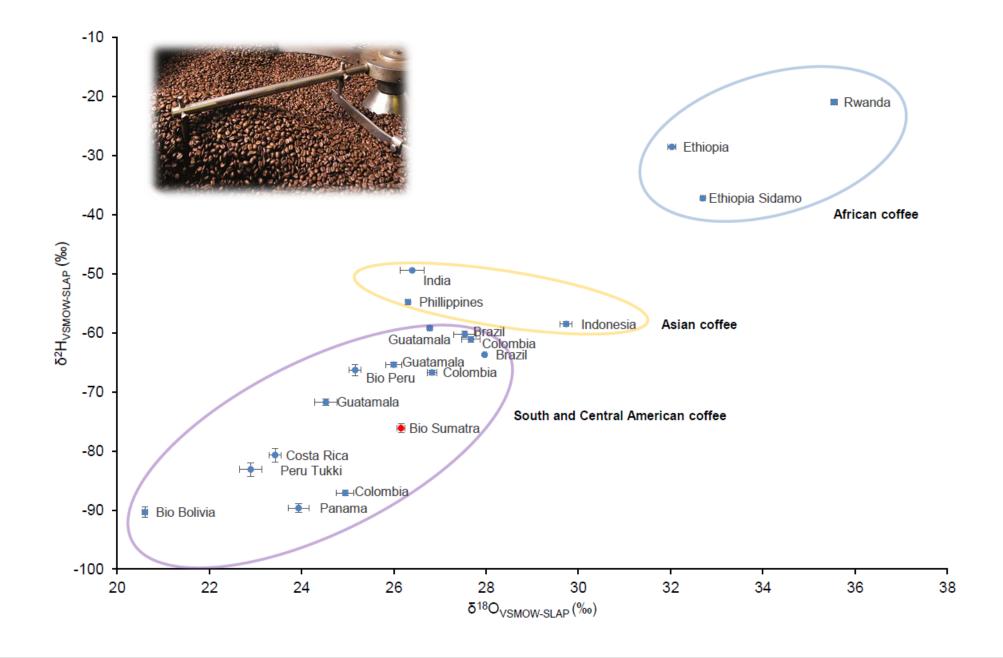


Mineral fertilizer show low N values while organic fertilization by compost reults in higher N values.





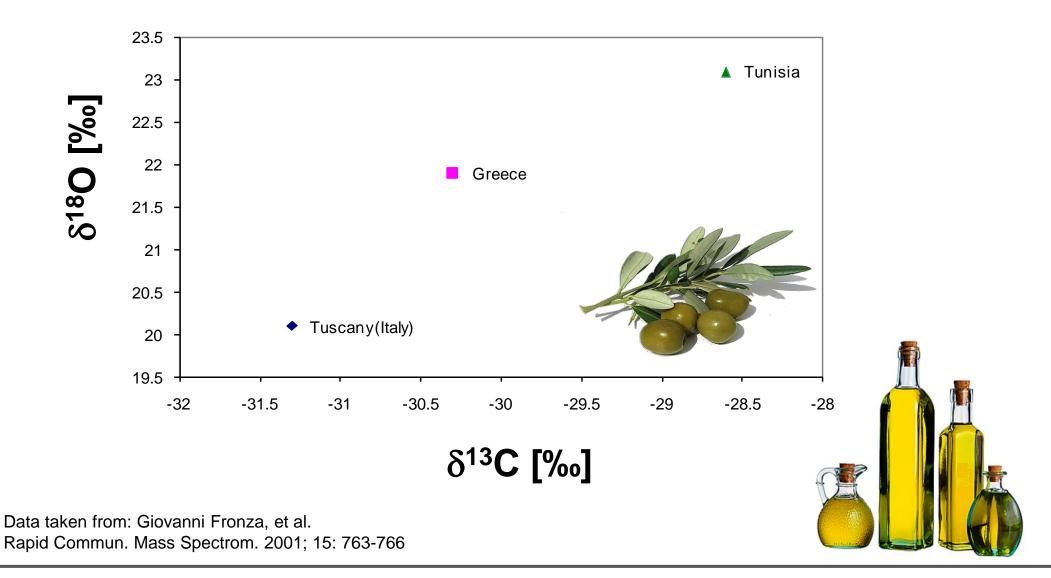
EA-IRMS: δ^2 H and δ^{18} O in Roasted Coffee Beans





EA-IRMS: δ^{13} C and δ^{18} O in Olive Oil

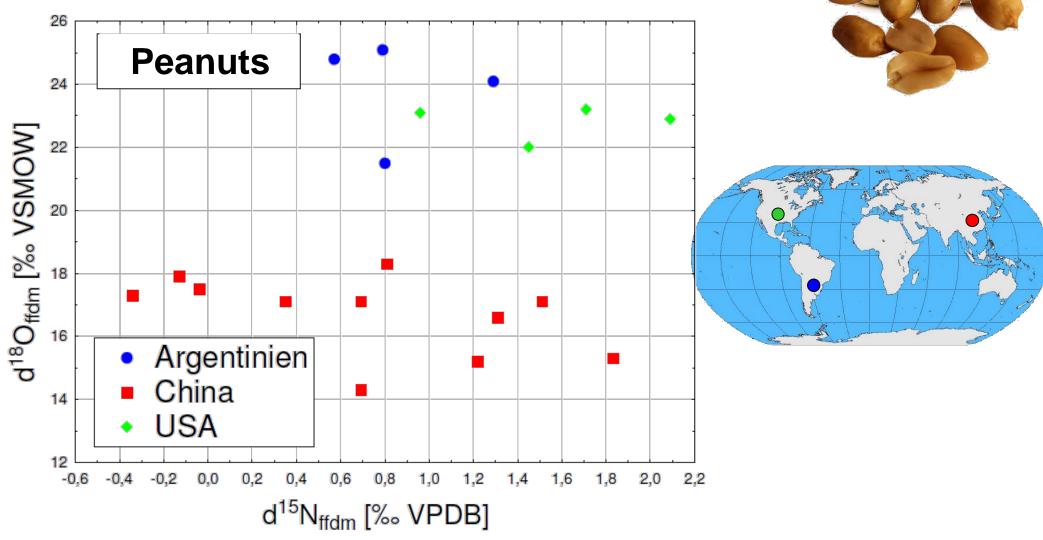
Where does your olive oil come from?



ThermoFisher SCIENTIFIC

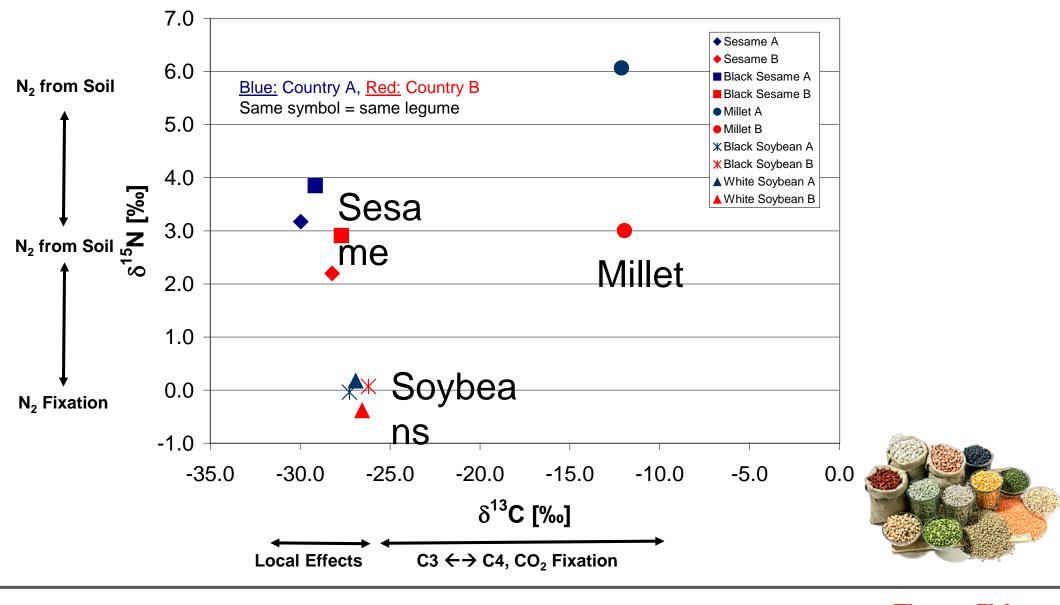
EA-IRMS: Origin of Nuts

δ^{18} O as indicator for origin δ^{15} N often related to fertilizers





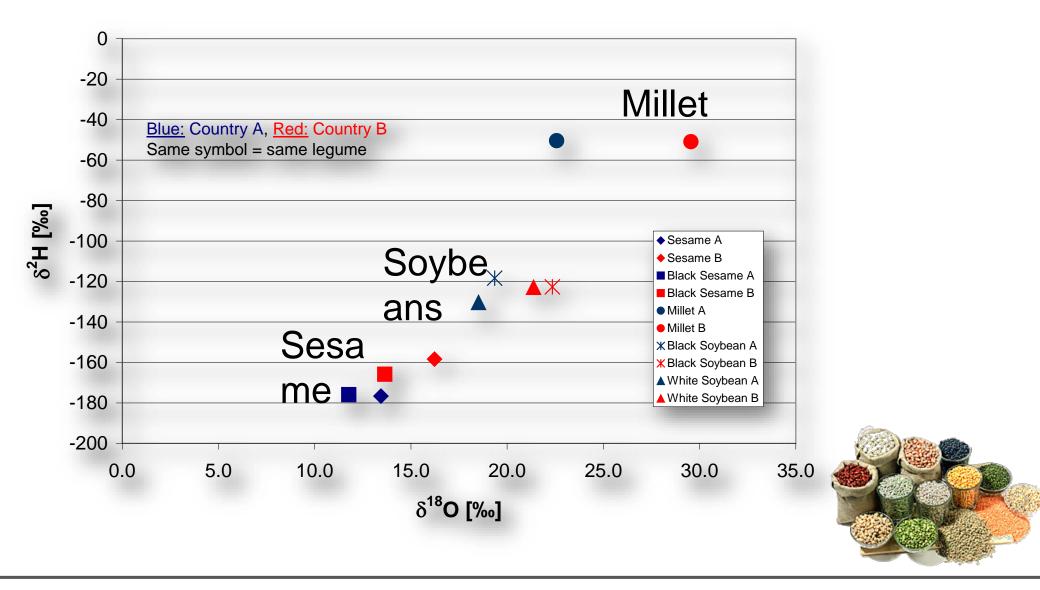
Agricultural products from Korea (Blue) and China (Red)



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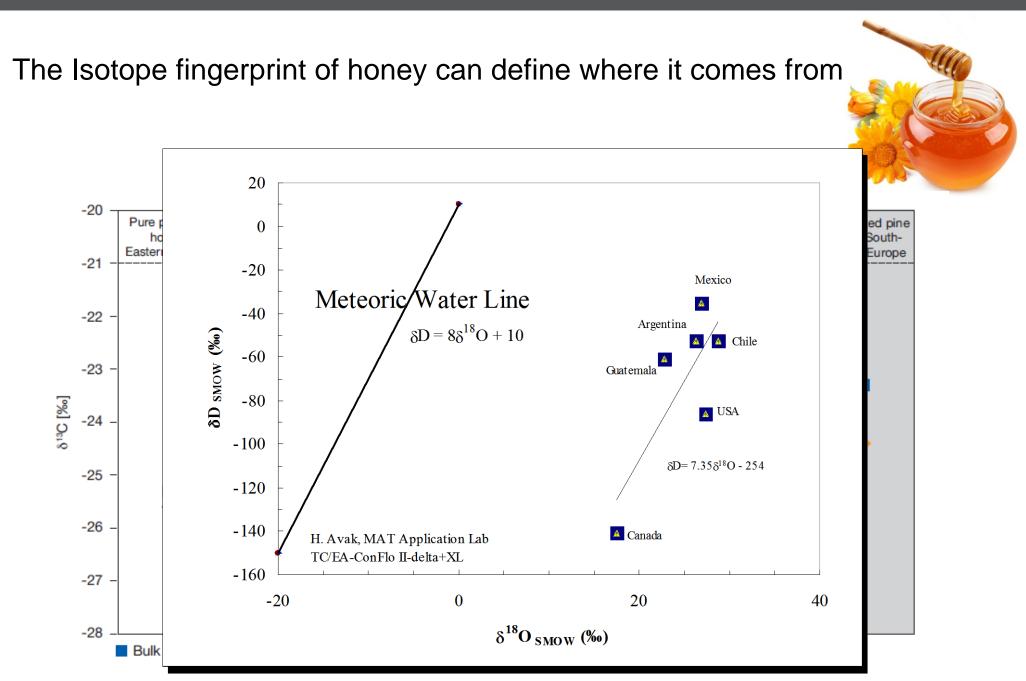
EA-IRMS: Origin of seeds, millet and soybean

Agricultural products from Korea (Blue) and China (Red)



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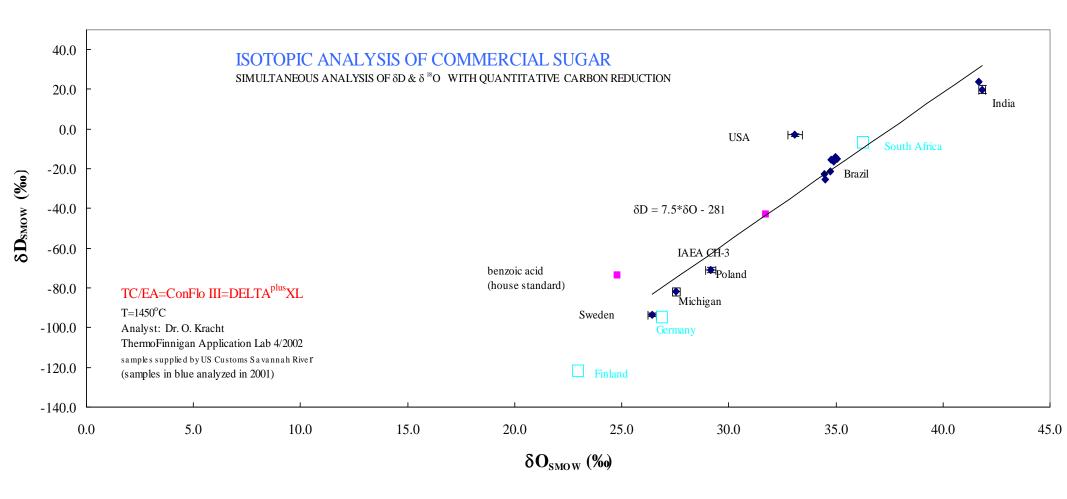
EA-IRMS: Isotopes in honey define origin





EA-IRMS: H and O Isotope fingerprints for sugar origin

The origin of Sugar as defined by Hydrogen and Oxygen isotopes





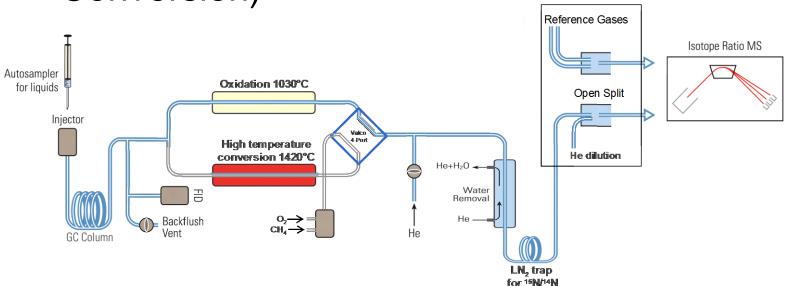
Carbon isotope fingerprints can determine if label claims are correct: Is your sugar really beet sugar?

| Sample | $\delta^{13}C_{VPDB}$ (mean ± 1 σ) | Label Claim | Do the δ ¹³ C fingerprints agree? |
|----------------|--|-------------|---|
| China | -12.61 ± 0.15 | Corn sugar | Corn sugar |
| France | -12.14 ± 0.12 | Cane sugar | Cane sugar |
| Hawaii (Brown) | -12.41 ± 0.13 | Cane sugar | Cane sugar |
| Italy (Brown) | -12.22 ± 0.05 | Cane sugar | Cane sugar |
| Ivory Coast | -12.24 ± 0.19 | Cane sugar | Cane sugar |
| Philippines | -12.95 ± 0.09 | Cane sugar | Cane sugar |
| San Francisco | -12.89 ± 0.04 | Cane sugar | Cane sugar |
| Senegal | -12.42 ± 0.25 | Cane sugar | Cane sugar |
| United Kingdom | -12.75 ± 0.04 | Cane sugar | Cane sugar |
| Dubai | -25.02 ± 0.02 | Not stated | Beet sugar |
| Germany | -26.69 ± 0.08 | Not stated | Beet sugar |

Thermo Scientific GC IsoLink II



Compound Specific Isotope Analysis for ¹³C/¹²C Isotope Ratios and ¹⁵N/¹⁴N Isotope Ratio (GC-Combustion) and ²H/¹H Isotope Ratios and ¹⁸O/¹⁶O Isotope Ratio (GC-High Temperature Conversion)







Plant

This liquor is made exclusively in Mexico from the agaves,

Agave tequilana weber.

Origin of Tequila Harvest: after 6-10 years

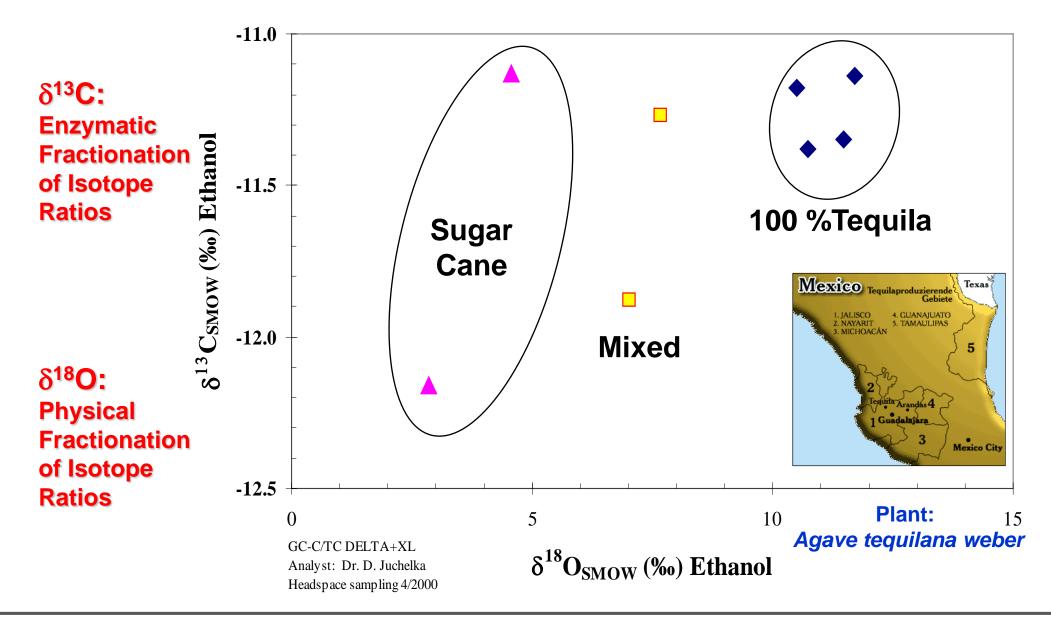
Tequila is produced **exclusively** in 5 regions

of Mexico:

- ➤ Jalisco,
- > Nayarit,
- > Michoacán,
- > Guanajuato,
- Tamaulipas



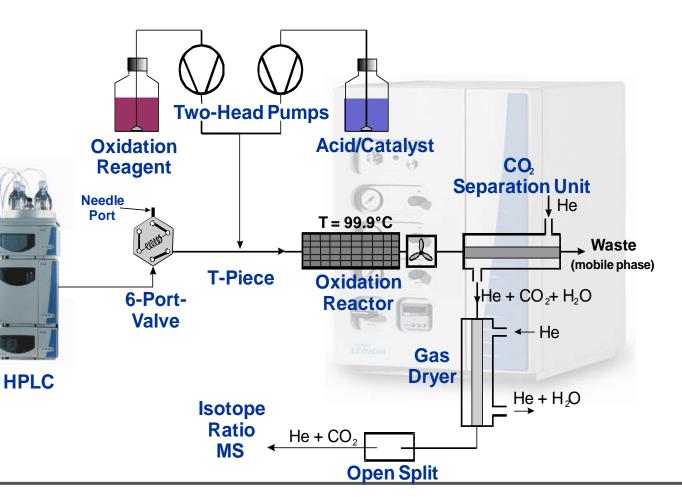
GC-IRMS: Isotopes can tell if Tequila is real or not



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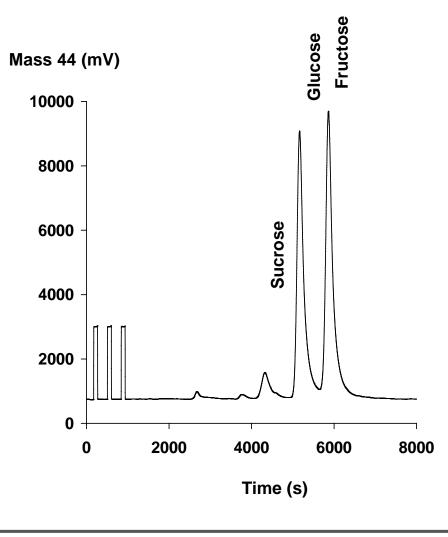
Thermo Scientific LC IsoLink







The carbon isotope value of fructose additives can identify adulterated honey

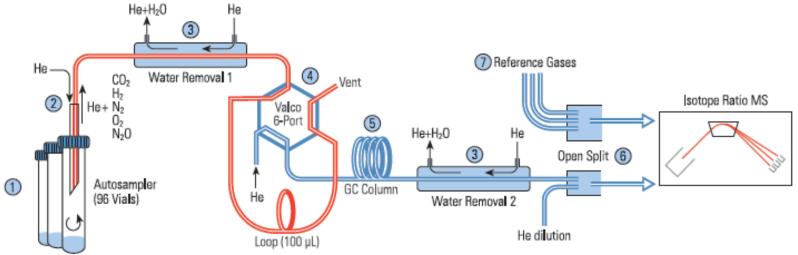


| Honey | Glucose δ^{13} C‰ | Fructose δ ¹³ C‰ | Area Fru/Glu | |
|-------|--------------------------|--------------------------------|-----------------|-------------|
| А | -27.9 | -27.8 | 1.13 | pure |
| В | -25.1 | -26.4 | 2.17 | adulterated |
| С | -26.5 | -26.5 | 1.35 | pure |
| D | -26.1 | -26.0 | 4.53 | adulterated |
| Е | -11.2 | -13.9 | 0.65 | adulterated |

- Absolute δ^{13} C value
- δ¹³C difference, Glu –
 Fru
- Ratio of area, Fru / Glu

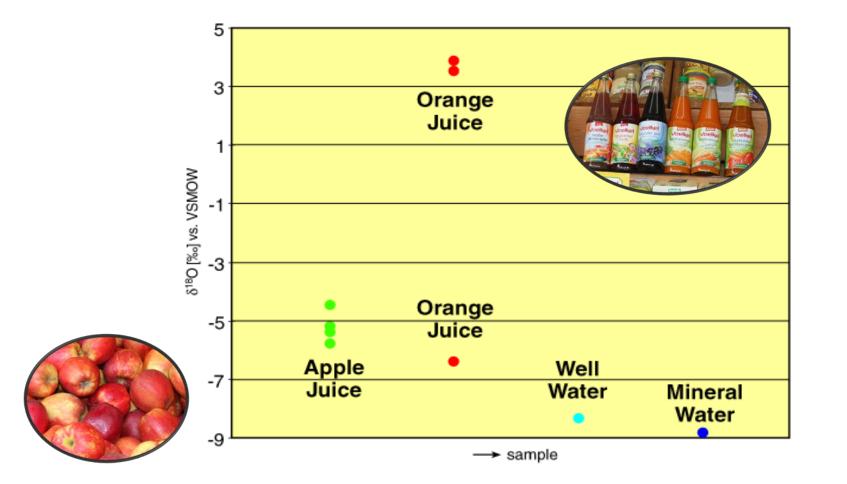
Thermo Scientific GasBench II







Analysis of fruit juices with the GasBench II







¹⁸O equilibration of water in wine from China, Australia & Europe

| Hoo-Roo_Austrialia QYTB1 HeBiNuo1999_ZhangYe-1 QYTB GreatWall2002_T-1 COTES DE CASTILION_France-1 GreatWall1995_ChangLi-2 QYTB2 | X 7.39 -0.92 5.27 -8.13 5.60 1.63 2.13 -20 | σ1 0.11 0.06 0.09 0.07 0.03 0.07 0.07 | n 3 3 3 3 3 3 3 3 3 3 | QYTB1assigned for SMOWQYTB2assigned for SLAPQYTBChinese QC (-8.14 permil δ18O) |
|---|--|---|---|---|
| Dragon Seal-ShaCheng-9 GreatWall_S-2 Dynasty_TianJin-6 Dynasty_TianJin-2 | -20 -0.04 4.96 2.33 2.32 | 0.10 0.15 0.10 0.24 | 3 3 2 3 | • QYTB (QC) = (-8.13 permil δ ¹⁸ Ο) |
| Dynasty_TianJin-1 | 5.28 | 0.08 | 3 | Average σ1 is 0.09 (n=31) Measurements showed |
| GreatWall_S-1 | 5.02 | 0.13 | 2 | |
| GreatWall_S-4 | 3.69 | 0.09 | 3 | |
| MouTai_ChangLl-3 | -0.70 | 0.10 | 3 | |
| GreatWall_S-3 | 4.25 | 0.07 | 2 | |
| Gynasty_TianJin-4 | 0.40 | 0.13 | 3 | perfect agreement what is expected for a normal pure |
| Gynasty_TianJin-3 | 5.37 | 0.12 | 2 | |
| MoGao_WuWei-2 | 4.69 | 0.04 | 3 | |
| Grand Dragon_YanTai-1 | -0.52 | 0.21 | 3 | |
| GreatWall_ShaCheng-3 | 3.96 | 0.04 | 3 | |
| Virtopia_YunNan-1 | 1.66 | 0.05 | 3 | water measurement |
| ChangYu_YanTai-4 | 1.00 | 0.08 | 3 | |
| GreatWall Longxi99_HuaiZhuo-1 | 2.28 | 0.11 | 3 | |
| YunnanHong_YunNan-5 Baroncini_Italy YunnanHong_YunNan-4 Ice Wine_TongHua Grand Dragon_YanTai-2 GreatWall1995_ShaCheng-2 Dragon Seal_ShaCheng-10 | -7.42 2.96 -3.47 -7.41 1.31 4.13 0.15 | 0.02 0.23 0.06 0.11 0.09 0.04 0.05 | 3 3 3 3 3 3 3 3 3 3 | Total n = 31 of which 27 are triplicates and 4 are duplets Delta V Advantage |

GasBench II and Flash HT data with Delta V

| ¹⁸ O/ ¹ analyte | ⁶ O by GB-IRMS IAEA accepted Five value $\delta^{18} O/^{16} O \delta^{18}$ vsmow [‰] vsmow | lash HT ³ O/ ¹⁶ O | GE | Dev ma | uracy between vices & Organic rich atrices ilk and coffee cream 1σ comment |
|--|---|--|------------|-----------------|--|
| V-SMOW 06 | 0 | 0.00 | 0.05 | 0.01 | 0.02 measured as sample |
| V-SMOW 03 | 0 | n.d. | n.d. | 0.03 | 0.23 measured as sample |
| SLAP | -55.50 | n.d. | n.d. | -55.50 | 0.04 measured as sample |
| GISP | -24.50 | -24.80 | 0.06 | -24.76 | 0.06 measured as sample |
| GISP 98 | | n.d. | n.d. | -24.25 | 0.08 measured as sample |
| GISP 06 | | n.d. | n.d. | -24.81 | 0.08 measured as sample |
| orange juice | | n.d. | n.d. | -7.04 | 0.04 measured as sample |
| coffee cream | | n.d. | n.d. | 1.40 | 0.001 measured as sample |
| HBW-1 | | n.d. | n.d. | -8.05 | 0.000 measured as sample |
| HBW-3 | | -7.91 | 0.04 | -7.86 | 0.01 measured as sample |
| Ethanol | | 24.26 | 0.08 | n.d. | n.d. measured as sample |
| Flt PTSW | | | | 4.13 | 0.58 wine as sample |
| analyzed by Ther | ma Electron (Brom | an) IDMC A | nnlightion | a Laboratory lu | Ing 2006 |

analyzed by Thermo Electron (Bremen) IRMS Applications Laboratory, June 2006 all ratios and σ 1 resultant from SMOW/SLAP correction

Flash HT with AS 3000

GB used with 4ml, 12ml vials and 100 $\mu l,$ 200 μl sample, respectively

Official methods and Isotope Fingerprints

| Product | Official method | Isotope fingerprint | Sample | What does it address? | Analytical solution |
|----------------------|------------------------|---|--|---|---|
| Wine | | | | | |
| | OIV-MA- AS2-12 | õ ¹⁸ O | Water | Adulteration, Geographical origin, Year of vintage | Thermo Scientific [™] GasBench II System, Thermo Scientific [™] Dual Inlet |
| | OIV-MA- AS312-06 | ô ¹³ C | Ethanol, Wine must, Grape sugar | Adulteration, origin | Thermo Scientific [™] EA IsoLink [™] IRMS System, Thermo Scientific [™] GC IsoLink II [™] Interface for GC-IRMS |
| | OIV-AS312-07 | ð ¹³ C | Glycerol in wines | Adulteration by addition of glycerol from C4 maize or Fossil sources | GC IsoLink II Interface for GC-IRMS, Thermo Scientific™ LC IsoLink [™] Interface for IRM-LC/MS |
| 18 - 14 | OIV-OENO 510-2013 | ð ^{t3} C | Acetic acid in wine, vinegar | | GC IsoLink II Interface for GC-IRMS, EA IsoLink IRMS System |
| | OIV-OENO 510-2013 | ô ¹⁸ O | Water in wine, vinegar | Adulteration, Geographical Origin, Year of Vintage | Thermo Scientific [™] GasBench II System, Dual Inlet |
| Sparkling wine | | | | | |
| | OIV-MA- AS314-03 | ô ^{t3} C | CO ₂ in sparkling wine | Origin and authenticity of sparkling wine | GasBench II System, EA IsoLink IRMS System, GC IsoLink, Dual Inlet |
| Spirits | | | | | |
| the state | OIV-AS312-07 | ð ¹³ C | Glycerol in spirits | Adulteration by addition of glycerol from C4 maize or Fossil sources | GC IsoLink II Interface for GC-IRMS, LC IsoLink Interface for IRM-LC/MS |
| Fruit Juice | | | | | |
| | EU - CEN 1995 | δ ^{t3} C | Sugars | Adulteration | GasBench II System, LC IsoLink Interface for IRM-LC/MS, GC IsoLink II Interface |
| | USA – AOAC 1981 | δ¹ ³ C | Sugars | Adulteration | GasBench II System, LC IsoLink Interface for IRM-LC/MS, GC IsoLink II Interface |
| | EU - CEN 1998 | δ ^{t3} C | Sugars and pulp | Adulteration | GasBench II System, LC IsoLink Interface for IRM-LC/MS, GC IsoLink II Interface |
| Vy By | EU - CEN 1995 | δ ² H and δ ¹⁸ O | Water | Adulteration | GasBench II System, LC IsoLink Interface for IRM-LC/MS, GC IsoLink II Interface |
| | AOAC method 2004.01 | δ ¹³ C | Ethanol (From Fermentation) | Adulteration | GasBench II System, LC IsoLink Interface for IRM-LC/MS, GC IsoLink II Interface |
| Fruit Juice (Concent | trate) | 6 6 2 5 | | | |
| Î. | AOAC 1992 | ô ¹⁸ O | Water | Adulteration | GasBench II System, LC IsoLink Interface for IRM-LC/MS, EA IsoLink IRMS System |
| Honey | 4040 11 1 | | | | |
| | AOAC method 991.41 | ð ¹³ C | C-4 plant sugars at concentration >7% | Adulteration of honey | EA IsoLink IRMS System |
| | AOAC method 998.12 | ð¹³C | C-4 plant sugars at concentration >7% | Adulteration of honey | EA IsoLink IRMS System |
| Cheese | | | | | |
| de la | EU Reg 548/2011 | ð¹3C | PDO | PDO Grana Padano | EA IsoLink IRMS System |
| | | | | | |



Thank You!



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