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Optimizing moisture testing by Karl Fischer method (AOAC 991.02)

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Previous Work

- Performed comparison study between modified AOAC 991.02 Karl Fischer method and two difference air oven methods.
  - 135°C, 2 h
  - 104 °C, 3 h
- Samples covered two different finished product matrices (wet & dry) and three different vendors
- Objective of the study was to understand how different air oven conditions compared to the current Karl Fischer method for soft moist and semi-moist pet foods.

OMA Online
Official Methods of Analysis
AOAC INTERNATIONAL

4.1.09

AOAC Official Method 991.02
Moisture in Soft-Moist and Semi-Moist Pet Foods

Karl Fischer Method
First Action 1991
Final Action 1995

(Applicable to products containing 20–30% moisture and other volatile materials.)

Results of the interlaboratory study supporting acceptance of the method:

$s_r = 0.3$; $s_R = 0.4$; $RSD_r = 1.1\%$; $RSD_R = 1.7\%$

A. Principle

Water is extracted with methanol from pet food that contains other volatile components, and aliquot is titrated with Karl Fischer reagent.

B. Apparatus and Reagents

(**a**) *Karl Fischer titration assembly.*—Manual or automatic, with stirrer.

(**b**) *Karl Fischer reagent.*—Stabilized, single solution (Fisher Scientific Co., SK3, or equivalent). (*Caution:* If solution contains pyridine, *see* Appendix B, safety note on pyridine.) To standardize reagent, add 100 mg H_2O from weighing pipet, or other suitable device, to 30–50 mL pretitrated CH_3OH, and titrate with Karl Fischer reagent. C = mg H_2O/mL reagent.

(**c**) *Methanol.*—Reagent grade, ≥99.8% CH_3OH. ≤0.1% H_2O, ≤0.001% acetone.

C. Preparation of Test Sample

Reduce test sample particle size to as fine a particle size as possible. Household-style blender may be used, shaking blender back and forth to move product into blades.

D. Determination

Accurately weigh 8–10 g test portion into Erlenmeyer that contains magnetic stirring bar. Add 200 mL CH_3OH, and stopper flask. Stir magnetically 15 min. Let solids settle. Transfer 10 mL aliquot to titration vessel containing pretitrated methanol and titrate with Karl Fischer reagent. Determine blank on 10 mL CH_3OH as above and subtract from test portion determination.

$$\text{Water, \% (w/w)} = \frac{2 \times (\text{mL reagent} \times C)}{\text{g test portion}}$$

Reference: *JAOAC* **73**, 399(1990).

Summary/background information:

Table 1. Comparison of loss-on-drying (LOD) oven methods with Karl Fischer method in pet foods

			Moisture, %							
			Karl Fischer		Oven Methods					
						135°C, 2 h			104°C, 3 h	
Type of Food	Vendor	N	Mean		Mean	% Recovery[c]	Bias[b]	Mean	% Recovery[c]	Bias[b]
	A	3	6.87[a]		7.08[a]	103.1	0.21	6.84[a]	99.6	-0.03
Dry Dog Food	B	3	8.47		8.99	106.2	0.52	8.84	104.4	0.37
	C	3	10.32[a]		10.43	101.1	0.11	10.30[a]	99.8	-0.02
	A	3	5.58		6.44	115.4	0.86	5.98	107.2	0.40
Dry Cat Food	B	3	7.06		7.36	104.2	0.30	7.21	102.1	0.15
	C	3	8.83[a]		9.06	102.6	0.23	8.77[a]	99.3	-0.06
	A	3	76.40		76.13	99.7	-0.27	76.17	99.7	-0.23
Wet Dog Food	B	3	81.10		80.47	99.2	-0.63	80.43	99.2	-0.67
	C	3	74.37[a]		74.33[a]	100.0	-0.03	74.27[a]	99.9	-0.10
	A	3	81.43[a]		81.33[a]	99.9	-0.10	81.30[a]	99.8	-0.13
Wet Cat Food	B	3	79.53[a]		79.47[a]	99.9	-0.06	79.23[a]	99.6	-0.30
	C	3	76.50[a]		76.37[a]	99.8	-0.13	76.30[a]	99.7	-0.20

[a] Means within a sample with the same superscript letter are not statistically different (P > 0.05) from the Karl Fischer mean

[b] Bias is each LOD moisture method minus Karl Fischer

[c] Recovery as a percent of Karl Fischer Moisture

Major findings

- Results generated by the oven method (104 °C, 3 h) are more similar to the results generated by the Karl Fischer method than the results obtained by the method (135 °C, 2 h).

- There is a good agreement between the two oven methods for wet pet food.

- There are slight differences detected between the oven methods and Karl Fischer method for two of the six wet pet foods analyzed.

- Additional studies needed to better understand AOAC 991.02 Karl Fischer method applied to all pet food matrices (dry, wet, & semi moist)

Karl Fischer Optimization Study

- Objective of the study the study was to better understand impact of method conditions of AOAC 991.02 Karl Fischer method and find optimized conditions that are applicable to all pet food matrices (dry, wet, & semi moist)

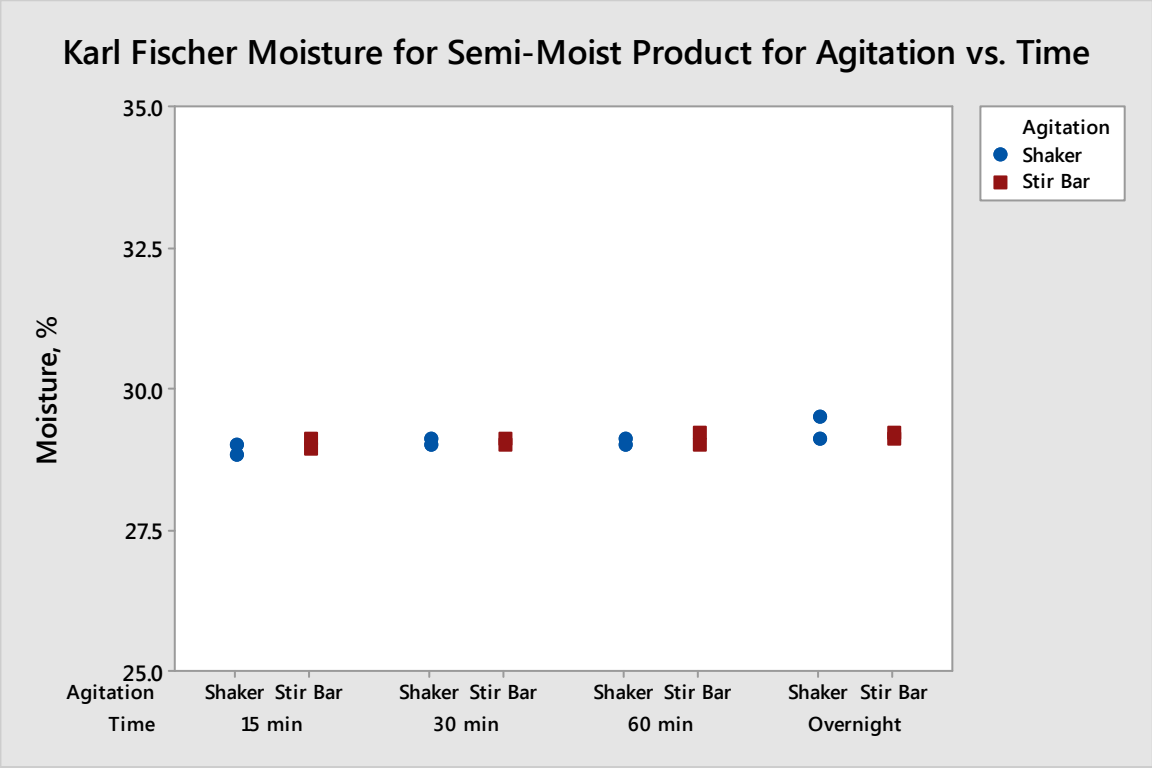
- Two experiments were conducted:
  - Experiment 1: Extraction Time and Agitation Optimization
  - Experiment 2: Sample Size and Extraction Volume Optimization

- These single laboratory optimization experiments were exploratory in nature, to better understand trends and patterns in method behavior.

Experiment 1: Time of extraction and shaking conditions

Sample	Replicate	15 min	30 min	1 hour	overnight
Semi-Moist (Shaker Table)	1	29.0	29.1	29.1	29.5
	2	28.8	29.0	29.0	29.1
Semi-Moist (Stir Bar)	1	29.1	29.0	29.2	29.2
	2	28.9	29.1	29.0	29.1
Wet Pet (Shaker Table)	1	72.1	74.6	76.7	76.8
	2	71.3	73.0	76.3	76.6
Wet Pet (Stir Bar)	1	76.3	76.4	76.2	76.9
	2	76.3	76.9	76.3	76.6
Dry Pet (Shaker Table)	1	4.92	5.15	5.76	7.00
	2	4.86	5.19	5.74	7.00
Dry Pet (Stir Bar)	1	4.96	5.46	5.96	7.00
	2	5.00	5.38	6.00	7.00

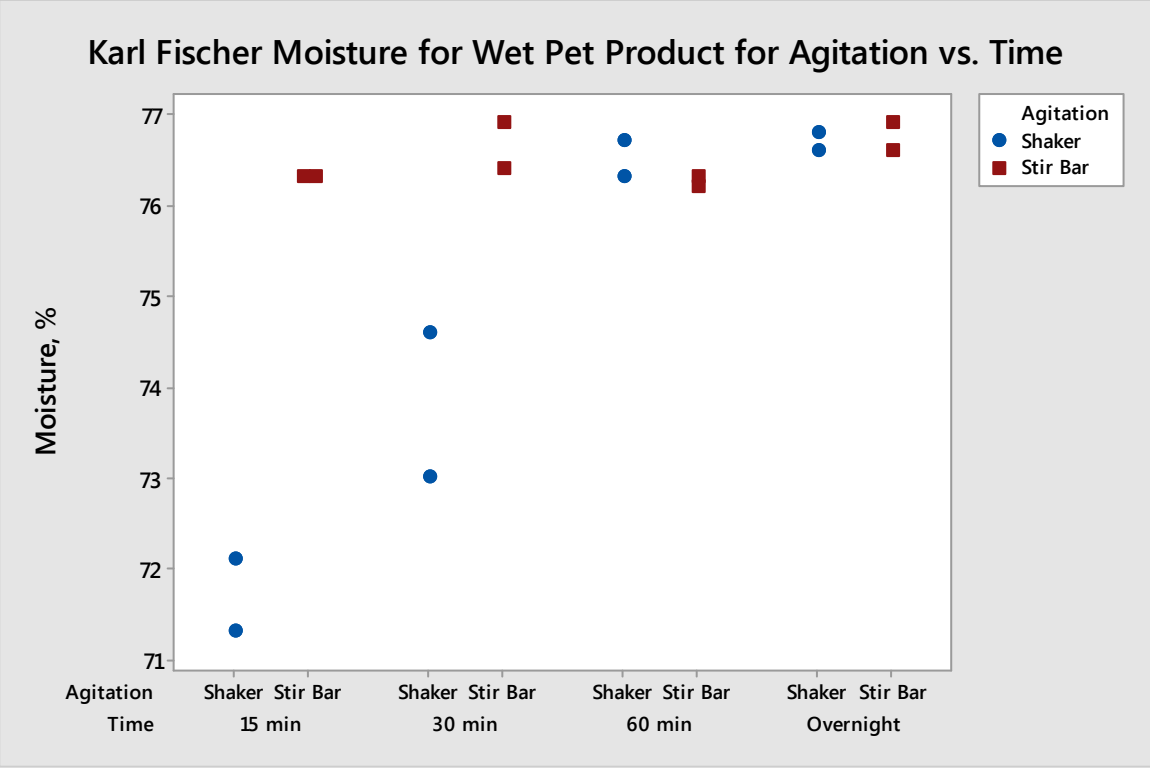
Results



Analysis of Variance for KF method for semi-moist product

Source	P value	Comments
Time	0.126	
Agitation	1.000	
Time and Agitation	0.642	P>0.5

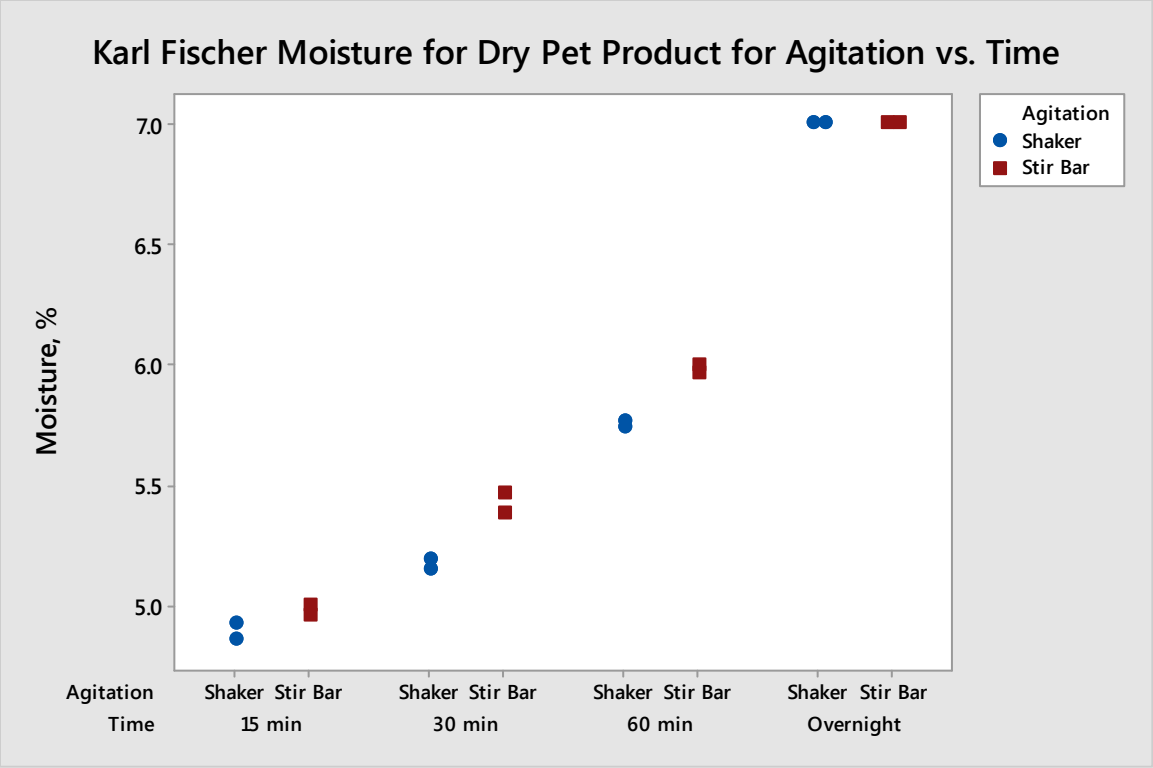
Results



Analysis of Variance for KF method for wet pet product

Source	P value	Comments
Time	0.00	
Agitation	0.00	
Time and Agitation	0.00	P = 0

Results



Analysis of Variance for KF method for dry pet product

Source	P value	Comments
Time	0.00	
Agitation	0.00	
Time and Agitation	0.00	P = 0

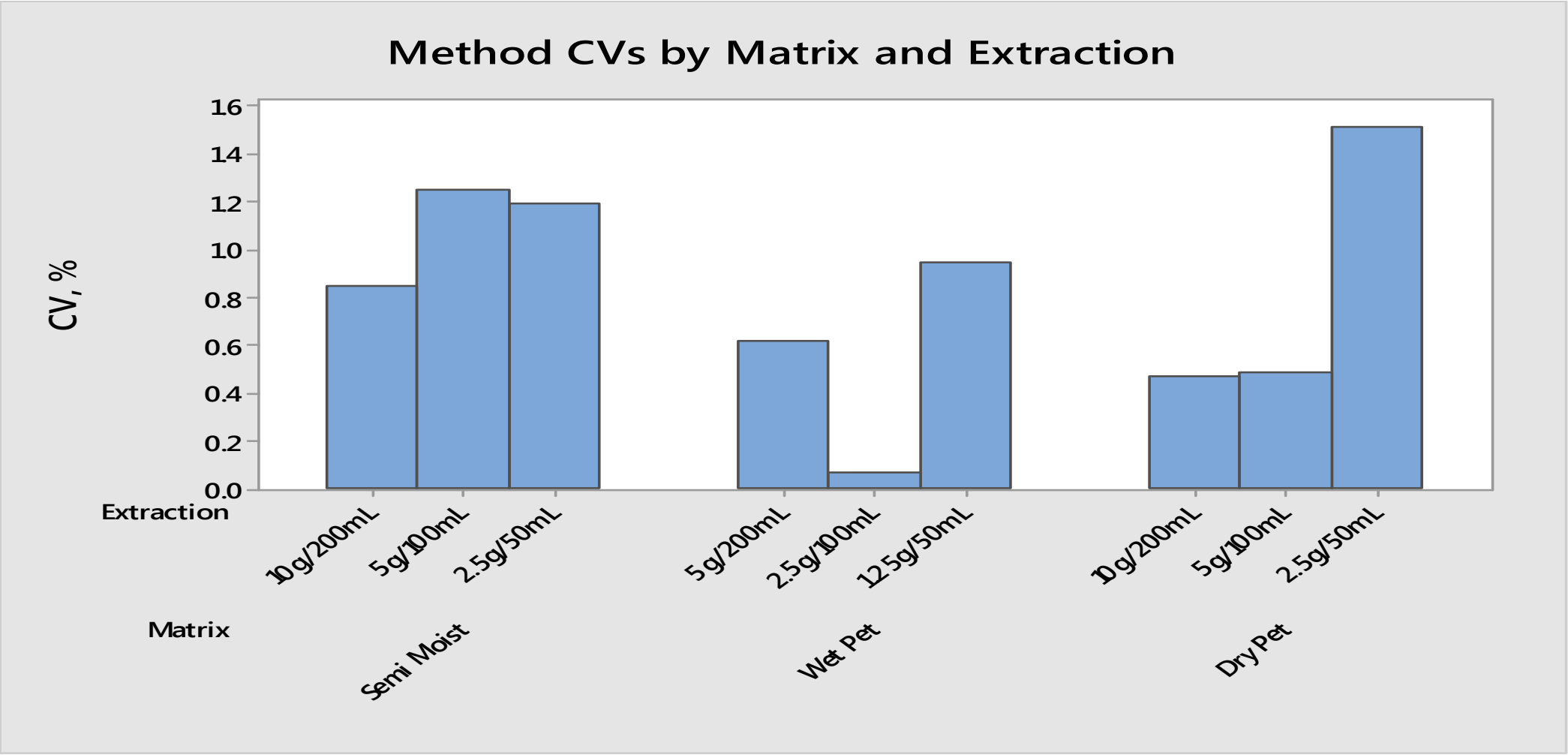
Results

- Semi-moist product: no statistical differences in using shaker/stirring bar and time of extraction,
- Wet product: statistical difference in using shaker/stirring bar and time of extraction (overnight extraction with shaker delivers the best results),
- Dry product: statistical difference in using shaker/stirring bar and time of extraction (overnight extraction delivers the best results).
- ***The most robust conditions: shaker and overnight extraction***

Experiment 2: Sample size and extraction solvent volume

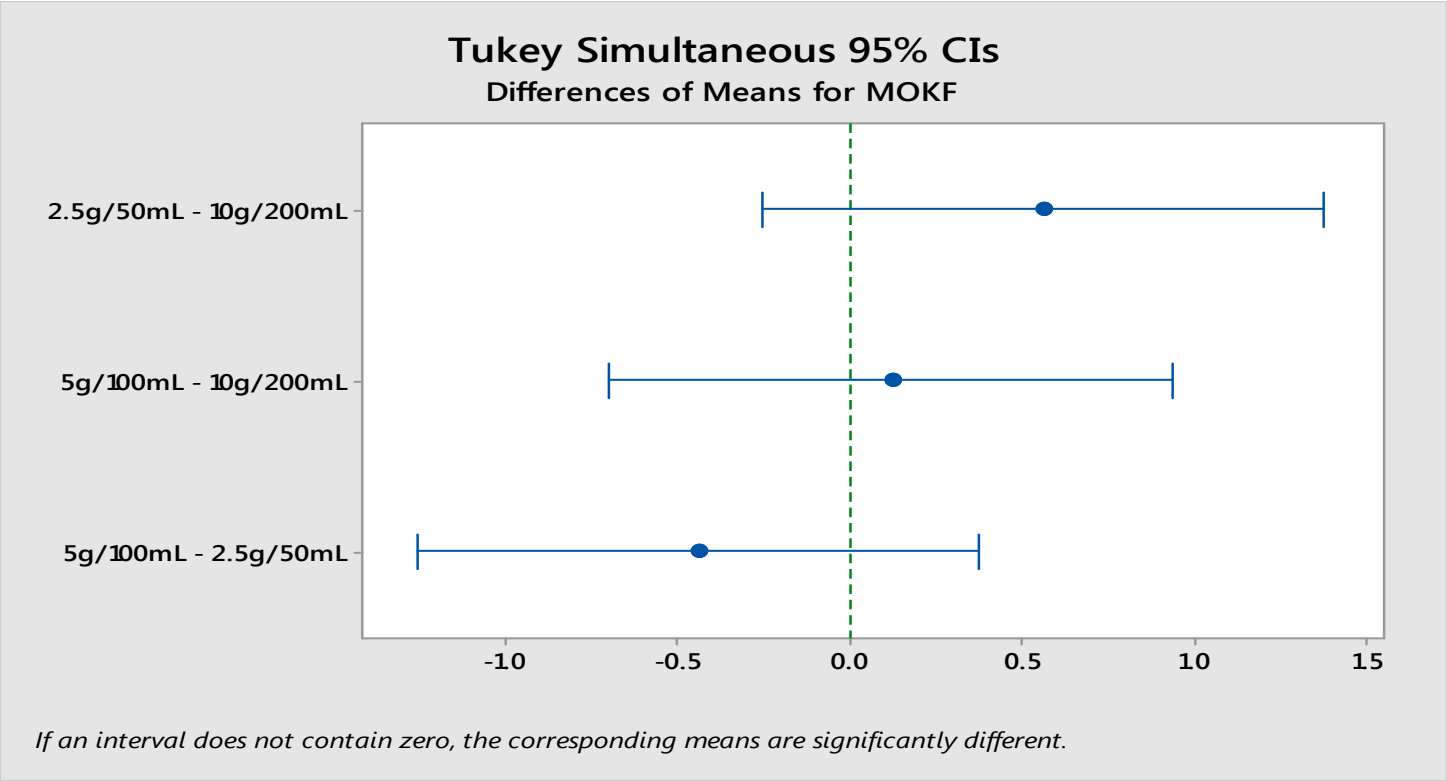
Sample	Replicate	Sample size (g)/ MeOH volume (mL)					
		10g/200 mL	5g/200 mL	5g/100 mL	2.5g/100 mL	2.5g/50 mL	1.25g/50 mL
Semi-Moist	1	28.80		29.16		29.52	
	2	29.16		28.84		29.35	
	3	29.26		29.57		30.03	
Wet Pet Product	1		76.40		76.40		76.64
	2		75.48		76.44		75.84
	3		76.06		76.50		77.29
Dry Pet Product	1	7.24		7.31		7.45	
	2	7.17		7.31		7.45	
	3	7.21		7.25		7.26	

Results


Method CVs by Matrix and Extraction
CV, %
16
14
12
10
0.8
0.6
0.4
0.2
0.0
Extraction
10g/200mL
5g/100mL
2.5g/50mL
5g/200mL
2.5g/100mL
12.5g/50mL
10g/200mL
5g/100mL
2.5g/50mL
Matrix
Semi Moist
Wet Pet
Dry Pet

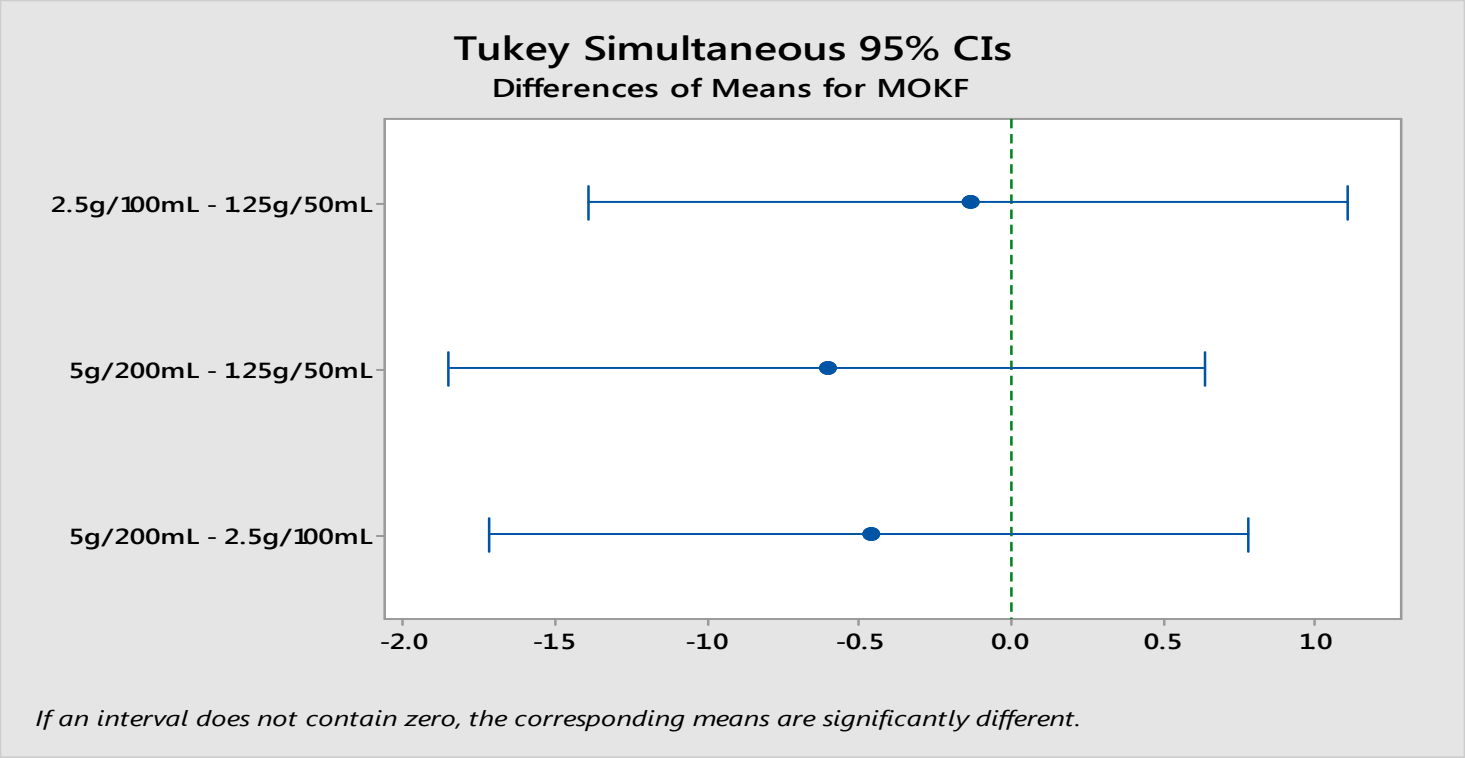
Results: Semi-Moist product

Sample	Extraction	Mean (n=3)	SD	P-Value (ANOVA)
Semi -Moist	10 g /200 mL	29.08	0.25	0.17
	5 g /100 mL	29.19	0.37	
	2.5 g /50 mL	29.63	0.35	



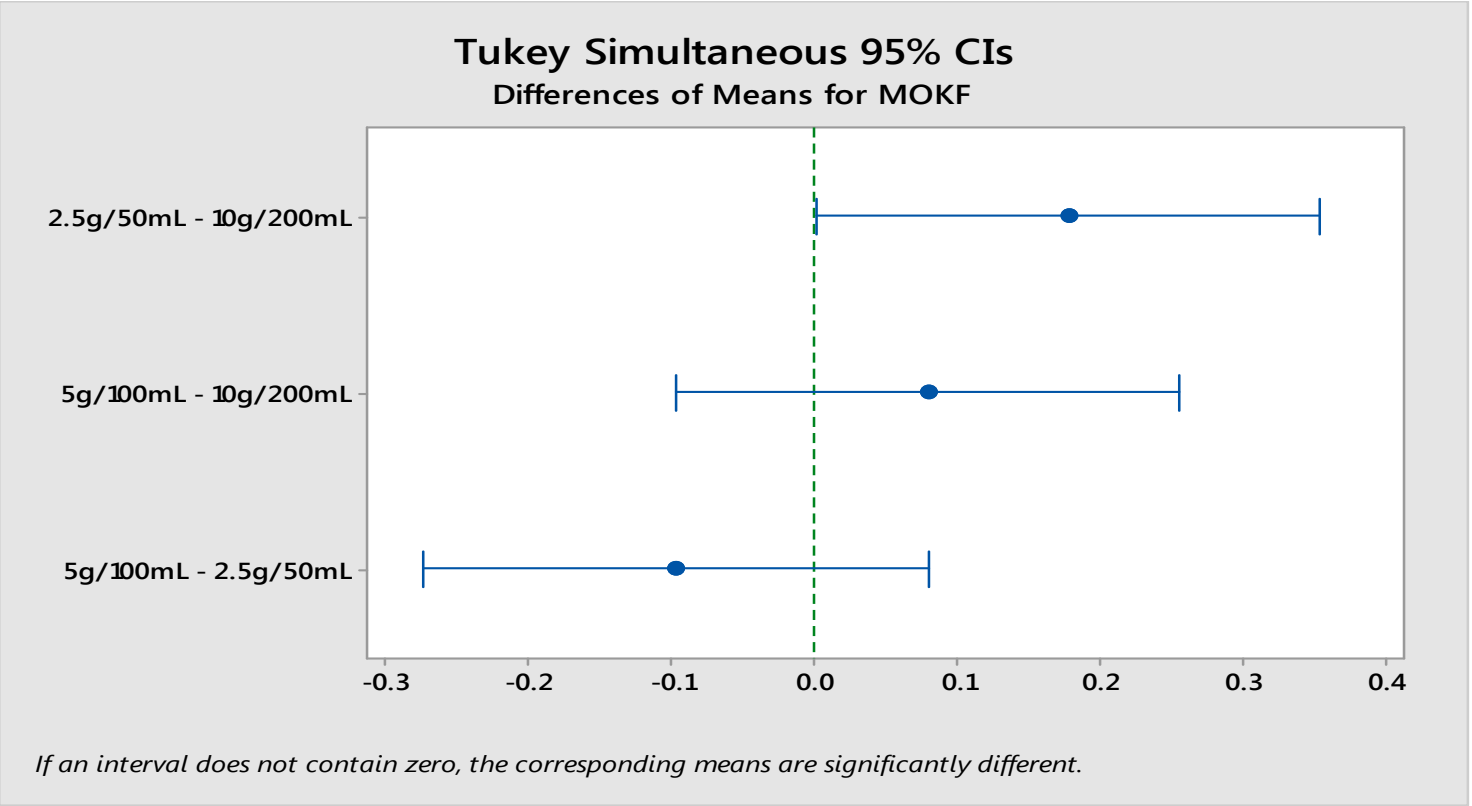
Results: Wet product

Sample	Extraction	Mean (n=3)	SD	P-Value (ANOVA)
Wet product	1.25 g /50mL	76.59	0.72	0.36
	2.5 g /100 mL	76.45	0.05	
	5g /200 mL	75.98	0.47	



Results: Dry product

Sample	Extraction	Mean (n=3)	SD	P-Value (ANOVA)
Dry product	10g/200 mL	7.21	0.03	0.06
	5g/100 mL	7.29	0.04	
	2.5g/50 mL	7.38	0.11	



Major findings

- Optimal sample mass and solvent volume used for testing:
  - Semi-moist product: 2.5 g /50 mL
  - Wet product : 2.5 g/100 mL
  - Dry product: 5 g/100 mL

Conclusions

- Current method conditions for AOAC 991.02 are not applicable to pet food matrices beyond semi-moist products
- Optimization experiments demonstrate that one set of method conditions can be used across all pet food matrices (dry, wet, & semi-moist)
- Optimization experiments also demonstrated that a reduction sample mass and extraction volume can be achieved, resulting in cost saving and decreased chemical waste
- Additional studies needed across multiple laboratories to better characterize method performance and to fully validate optimized method conditions

Thank you !