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APPENDIX I
DUNCAN MULTIPLE RESPONSE TEST

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The SAS System22:31 Wednesday, July

The GLM Procedure

Class Level Information

Class	Levels	Values
Treatment	3	BT CF LD
Rep	2	1 2

Number of Observations Read96
Number of Observations Used66

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The SAS System22:31 Wednesday, July

The GLM Procedure

Dependent Variable: C40

	Source	DF	Sum of Squares	Mean Square	F Value	Pr
> F	Model	3	70.57751700	23.52583900	6632.29	
0.0002	Error	2	0.00709433	0.00354717		
	Corrected Total	5	70.58461133			

R-Square0.999899
Coeff Var1.667049
Root MSE0.059558
C40 Mean3.572667

	Source	DF	Type I SS	Mean Square	F Value	Pr
> F	Rep	1	0.00851267	0.00851267	2.40	
0.2615	Treatment	2	70.56900433	35.28450217	9947.24	
0.0001						

	Source	DF	Type III SS	Mean Square	F Value	Pr
> F	Rep	1	0.00851267	0.00851267	2.40	
0.2615	Treatment	2	70.56900433	35.28450217	9947.24	
0.0001						

The GLM Procedure

Dependent Variable: C60

	Source	DF	Sum of Squares	Mean Square	F Value	Pr
> F						
<.0001	Model	3	0.00644050	0.00214683	12881.0	
	Error	2	0.00000033	0.00000017		
	Corrected Total	5	0.00644083			
	R-Square	Coeff Var	Root MSE	C60 Mean		
	0.999948	1.762223	0.000408	0.023167		

	Source	DF	Type I SS	Mean Square	F Value	Pr
> F						
0.4226	Rep	1	0.00000017	0.00000017	1.00	
<.0001	Treatment	2	0.00644033	0.00322017	19321.0	

	Source	DF	Type III SS	Mean Square	F Value	Pr
> F						
0.4226	Rep	1	0.00000017	0.00000017	1.00	
<.0001	Treatment	2	0.00644033	0.00322017	19321.0	

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The GLM Procedure

Dependent Variable: C100

	Source	DF	Sum of Squares	Mean Square	F Value	Pr
> F						
0.0188	Model	3	0.01487383	0.00495794	52.47	
	Error	2	0.00018900	0.00009450		
	Corrected Total	5	0.01506283			
	R-Square	Coeff Var	Root MSE	C100 Mean		
	0.987453	5.933537	0.009721	0.163833		

	Source	DF	Type I SS	Mean Square	F Value	Pr
> F						
0.7418	Rep	1	0.00001350	0.00001350	0.14	
0.0126	Treatment	2	0.01486033	0.00743017	78.63	

	Source	DF	Type III SS	Mean Square	F Value	Pr
> F						
0.7418	Rep	1	0.00001350	0.00001350	0.14	
0.0126	Treatment	2	0.01486033	0.00743017	78.63	
24, 2013 52			The SAS System		22:31 Wednesday, July	

The GLM Procedure

Dependent Variable: C120

	Source	DF	Sum of Squares	Mean Square	F Value	Pr
> F						
0.0468	Model	3	0.12533450	0.04177817	20.52	
	Error	2	0.00407233	0.00203617		
	Corrected Total	5	0.12940683			
	R-Square	Coeff Var	Root MSE	C120 Mean		
	0.968531	17.77698	0.045124	0.253833		

	Source	DF	Type I SS	Mean Square	F Value	Pr
> F						
0.4498	Rep	1	0.00176817	0.00176817	0.87	
0.0319	Treatment	2	0.12356633	0.06178317	30.34	
> F						
0.4498	Rep	1	0.00176817	0.00176817	0.87	
0.0319	Treatment	2	0.12356633	0.06178317	30.34	

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The GLM Procedure

Dependent Variable: C140

	Source	DF	Sum of Squares	Mean Square	F Value	Pr
> F						
<.0001	Model	3	35.88837617	11.96279206	13461.5	
	Error	2	0.00177733	0.00088867		
	Corrected Total	5	35.89015350			
	R-Square	Coeff Var	Root MSE	C140 Mean		

0.999950 0.758441 0.029811 3.930500

	Source	DF	Type I SS	Mean Square	F Value	Pr
> F						
	Rep	1	0.00046817	0.00046817	0.53	
0.5434	Treatment	2	35.88790800	17.94395400	20192.0	
<.0001						

	Source	DF	Type III SS	Mean Square	F Value	Pr
> F						
	Rep	1	0.00046817	0.00046817	0.53	
0.5434	Treatment	2	35.88790800	17.94395400	20192.0	
<.0001						

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24, 2013 54

The GLM Procedure

Dependent Variable: C150

	Source	DF	Sum of Squares	Mean Square	F Value	Pr
> F						
	Model	3	0.70270783	0.23423594	604.48	
0.0017	Error	2	0.00077500	0.00038750		
	Corrected Total	5	0.70348283			

R-Square Coeff Var Root MSE C150 Mean
0.998898 5.168933 0.019685 0.380833

	Source	DF	Type I SS	Mean Square	F Value	Pr
> F						
	Rep	1	0.00093750	0.00093750	2.42	
0.2601	Treatment	2	0.70177033	0.35088517	905.51	
0.0011						

	Source	DF	Type III SS	Mean Square	F Value	Pr
> F						
	Rep	1	0.00093750	0.00093750	2.42	
0.2601	Treatment	2	0.70177033	0.35088517	905.51	
0.0011						

The SAS System 22:31 Wednesday, July
24, 2013 55

The GLM Procedure

Dependent Variable: C160

	Source	DF	Sum of Squares	Mean Square	F Value	Pr
> F						

0.0003	Model	3	1309.532725	436.510908	3510.68
	Error	2	0.248676	0.124338	
	Corrected Total	5	1309.781401		
	R-Square		Coeff Var	Root MSE	C160 Mean
	0.999810		0.650513	0.352616	54.20583

> F 0.6141 0.0002	Source	DF	Type I SS	Mean Square	F Value	Pr
	Rep	1	0.043520	0.043520	0.35	
	Treatment	2	1309.489204	654.744602	5265.84	

> F 0.6141 0.0002	Source	DF	Type III SS	Mean Square	F Value	Pr
	Rep	1	0.043520	0.043520	0.35	
	Treatment	2	1309.489204	654.744602	5265.84	

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The GLM Procedure
Dependent Variable: C170

> F 0.0811	Source	DF	Sum of Squares	Mean Square	F Value	Pr
	Model	3	0.18419833	0.06139944	11.49	
	Error	2	0.01058700	0.00534350		
	Corrected Total	5	0.19488533			
	R-Square		Coeff Var	Root MSE	C170 Mean	
	0.945163		18.53743	0.073099	0.394333	

> F 0.5895 0.0555	Source	DF	Type I SS	Mean Square	F Value	Pr
	Rep	1	0.00216600	0.00216600	0.41	
	Treatment	2	0.18203233	0.09101617	17.03	

> F 0.5895 0.0555	Source	DF	Type III SS	Mean Square	F Value	Pr
	Rep	1	0.00216600	0.00216600	0.41	
	Treatment	2	0.18203233	0.09101617	17.03	

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The GLM Procedure

Dependent Variable: C180

	Source	DF	Sum of Squares	Mean Square	F Value	Pr
> F						
0.0011	Model	3	31.06698250	10.35566083	875.63	
	Error	2	0.02365300	0.01182650		
	Corrected Total	5	31.09063550			

R-Square	Coeff Var	Root MSE	C180 Mean
0.999239	0.577887	0.108750	18.81850

	Source	DF	Type I SS	Mean Square	F Value	Pr
> F						
0.9285	Rep	1	0.00012150	0.00012150	0.01	
0.0008	Treatment	2	31.06686100	15.53343050	1313.44	

	Source	DF	Type III SS	Mean Square	F Value	Pr
> F						
0.9285	Rep	1	0.00012150	0.00012150	0.01	
0.0008	Treatment	2	31.06686100	15.53343050	1313.44	

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The GLM Procedure

Dependent Variable: C200

	Source	DF	Sum of Squares	Mean Square	F Value	Pr
> F						
0.0461	Model	3	0.10199650	0.03399883	20.86	
	Error	2	0.00325900	0.00162950		
	Corrected Total	5	0.10525550			

R-Square	Coeff Var	Root MSE	C200 Mean
0.969037	23.95672	0.040367	0.168500

	Source	DF	Type I SS	Mean Square	F Value	Pr
> F						
0.2259	Rep	1	0.00487350	0.00487350	2.99	

0.0325	Treatment	2	0.09712300	0.04856150	29.80	
	Source	DF	Type III SS	Mean Square	F Value	Pr
> F						
	Rep	1	0.00487350	0.00487350	2.99	
0.2259						
	Rep	1	0.00487350	0.00487350	2.99	
0.2259	Treatment	2	0.09712300	0.04856150	29.80	
0.0325						
	The SAS System			22:31 Wednesday, July		
24, 2013	59					

Dependent Variable: C210

Source	DF	Sum of Squares	Mean Square	F Value	Pr
> F					
Model	3	0.90985800	0.30328600	5758.59	
0.0002					
Error	2	0.00010533	0.00005267		
Corrected Total	5	0.90996333			
R-Square	Coeff Var		Root MSE	C210: Mean	
0.999884	2.051983		0.007257	0.353667	
Source	DF	Type I SS	Mean Square	F Value	Pr
> F					
Rep	1	0.00041667	0.00041667	7.91	
0.1066					
Treatment	2	0.90944133	0.45472067	8633.94	
0.0001					
Source	DF	Type III SS	Mean Square	F Value	Pr
> F					
Rep	1	0.00041667	0.00041667	7.91	
0.1066					
Treatment	2	0.90944133	0.45472067	8633.94	
0.0001					
The SAS System				22:31 Wednesday, Ju	
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Dependent Variable: C220

	Source	DF	Sum of Squares	Mean Square	F Value	Pr
> F						
	Model	3	0.00320400	0.00106800	400.50	
0.0025	Error	2	0.00000533	0.00000267		
	Corrected Total	5	0.00320933			

R-Square Coeff Var Root MSE C220 Mean
0.998338 9.997917 0.001633 0.016333

	Source	DF	Type I SS	Mean Square	F Value	Pr
> F						
	Rep	1	0.00000267	0.00000267	1.00	
0.4226	Treatment	2	0.00320133	0.00160067	600.25	
0.0017						

	Source	DF	Type III SS	Mean Square	F Value	Pr
> F						
	Rep	1	0.00000267	0.00000267	1.00	
0.4226	Treatment	2	0.00320133	0.00160067	600.25	
0.0017						

The SAS System 22:31 Wednesday, July
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The GLM Procedure

Dependent Variable: C230

	Source	DF	Sum of Squares	Mean Square	F Value	Pr
> F						
	Model	3	0.00348333	0.00116111	7.74	
0.1166	Error	2	0.00630000	0.00015000		
	Corrected Total	5	0.00378333			

R-Square Coeff Var Root MSE C230 Mean
0.920705 73.48469 0.012247 0.016667

	Source	DF	Type I SS	Mean Square	F Value	Pr
> F						
	Rep	1	0.00015000	0.00015000	1.00	
0.4226	Treatment	2	0.00333333	0.00166667	11.11	
0.0826						

	Source	DF	Type III SS	Mean Square	F Value	Pr
> F						
	Rep	1	0.00015000	0.00015000	1.00	
0.4226	Treatment	2	0.00333333	0.00166667	11.11	
0.0826						

The SAS System 22:31 Wednesday, July
24, 2013 62

The GLM Procedure

Dependent Variable: TotSAF

	Source	DF	Sum of Squares	Mean Square	F Value	Pr
> F						
<.0001	Model	3	1409.274720	469.758240	342848	
	Error	2	0.002740	0.001370		
	Corrected Total	5	1409.277461			
	R-Square	Coeff Var	Root MSE	TotSAF Mean		
	0.999998	0.044987	0.037016	82.28117		

	Source	DF	Type I SS	Mean Square	F Value	Pr
> F						
0.0512	Rep	1	0.024704	0.024704	18.03	
<.0001	Treatment	2	1409.250016	704.625008	514262	

	Source	DF	Type III SS	Mean Square	F Value	Pr
> F						
0.0512	Rep	1	0.024704	0.024704	18.03	
<.0001	Treatment	2	1409.250016	704.625008	514262	

The SAS System 22:31 Wednesday, July 24, 2013 63

The GLM Procedure

Duncan's Multiple Range Test for C40

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha 0.05
Error Degrees of Freedom 2
Error Mean Square 0.003547

Number of Means 2 3
Critical Range .2563 .2448

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	Treatment
A	8.36150	2	BT
B	1.84350	2	LD
C	0.51300	2	CF

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The GLM Procedure

Duncan's Multiple Range Test for C60

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha 0.05
Error Degrees of Freedom 2
Error Mean Square 1.667E-7

Number of Means 2 3
Critical Range .001757 .001678

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	Treatment
A	0.0695000	2	CF
B	0.0000000	2	BT
B	0.0000000	2	LD

The SAS System 22:31 Wednesday, July

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The GLM Procedure

Duncan's Multiple Range Test for C100

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha 0.05
Error Degrees of Freedom 2
Error Mean Square 0.000095

Number of Means 2 3
Critical Range .04183 .03996

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	Treatment
A	0.234000	2	CF
B	0.133500	2	BT
B	0.124000	2	LD

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The GLM Procedure

Duncan's Multiple Range Test for C120

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha 0.05
Error Degrees of Freedom 2
Error Mean Square 0.002036

Number of Means	2	3
Critical Range	.1942	.1855

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	Treatment
A	0.45500	2	CF
B	0.17650	2	BT
B	0.13000	2	LD

The SAS System 22:31 Wednesday, July

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The GLM Procedure

Duncan's Multiple Range Test for C140

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	2
Error Mean Square	0.000389

Number of Means	2	3
Critical Range	.1283	.1226

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	Treatment
A	7.36350	2	BT
B	2.57850	2	CF
C	1.84950	2	LD

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The GLM Procedure

Duncan's Multiple Range Test for C150

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	2
Error Mean Square	0.000388

Number of Means	2	3
Critical Range	.08470	.08092

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	Treatment
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A	0.86150	2	BT
B	0.18700	2	CF
C	0.09400	2	LD

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The GLM Procedure

Duncan's Multiple Range Test for C160

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha		0.05
Error Degrees of Freedom	2	
Error Mean Square	0.124338	
Number of Means	2	3
Critical Range	1.517	1.450

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	Treatment
A	71.5255	2	CF
B	55.6650	2	BT
C	35.4270	2	LD

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The GLM Procedure

Duncan's Multiple Range Test for C170

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha		0.05
Error Degrees of Freedom	2	
Error Mean Square	0.005344	
Number of Means	2	3
Critical Range	.3145	.3005

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	Treatment
A	0.58600	2	LD
A			
B	0.43250	2	CF
B			
B	0.16450	2	BT

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The GLM Procedure

Duncan's Multiple Range Test for C180

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	2
Error Mean Square	0.011827

Number of Means	2	3
Critical Range	.4679	.4471

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	Treatment
A	20.4360	2	LD
A			
A	20.4190	2	BT
B	15.6005	2	CF

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The GLM Procedure

Duncan's Multiple Range Test for C200

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	2
Error Mean Square	0.00163

Number of Means	2	3
Critical Range	.1737	.1659

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	Treatment
A	0.34550	2	CF
B	0.10800	2	BT
B			
B	0.05200	2	LD

The SAS System 22:31 Wednesday, July

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The GLM Procedure

Duncan's Multiple Range Test for C210

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha 0.05
 Error Degrees of Freedom 2
 Error Mean Square 0.000053

Number of Means 2 3
 Critical Range .03123 .02983

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	Treatment
A	0.904000	2	CF
B	0.093000	2	BT
B	0.064000	2	LD

The SAS System 22:31 Wednesday, July

24, 2013 74

The GLM Procedure

Duncan's Multiple Range Test for C220

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha 0.05
 Error Degrees of Freedom 2
 Error Mean Square 2.667E-16

Number of Means 2 3
 Critical Range .007026 .006713

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	Treatment
A	0.049000	2	CF
B	0.000000	2	BT
B	0.000000	2	LD

The SAS System 22:31 Wednesday, July

24, 2013 75

The GLM Procedure

Duncan's Multiple Range Test for C230

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha 0.05
 Error Degrees of Freedom 2
 Error Mean Square 0.00015

Number of Means 2 3
 Critical Range .05270 .05035

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	Treatment
A	0.05000	2	BT
A			
A	0.00000	2	CF
A			
A	0.00000	2	LD

The SAS System 22:31 Wednesday, July

24, 2013 76

The GLM Procedure

Duncan's Multiple Range Test for TotSAF

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	2
Error Mean Square	0.00137

Number of Means	2	3
Critical Range	.1593	.1522

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	Treatment
A	93.25150	2	BT
B	92.98400	2	CF
C	60.60800	2	LD

The SAS System 22:31 Wednesday, July

24, 2013 77

The GLM Procedure

Class Level Information

Class	Levels	Values
Treatment	3	BT CF LD
Rep	2	1 2

Number of Observations Read	6
Number of Observations Used	6

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24, 2013 78

The GLM Procedure

Dependent Variable: C141

	Source	DF	Sum of Squares	Mean Square	F Value	Pr
> F						
0.0091	Model	3	0.23667867	0.07889289	108.87	
	Error	2	0.00144933	0.00072467		
	Corrected Total	5	0.23812800			
	R-Square		Coeff Var	Root MSE	C141 Mean	
	0.993914		10.08226	0.026920	0.267000	

	Source	DF	Type I SS	Mean Square	F Value	Pr
> F						
0.9145	Rep	1	0.00001067	0.00001067	0.01	
0.0061	Treatment	2	0.23666800	0.11833400	163.29	
	Source	DF	Type III SS	Mean Square	F Value	Pr
> F						
0.9145	Rep	1	0.00001067	0.00001067	0.01	
0.0061	Treatment	2	0.23666800	0.11833400	163.29	

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The GLM Procedure

Dependent Variable: C151

	Source	DF	Sum of Squares	Mean Square	F Value	Pr
> F						
0.0017	Model	3	0.18910717	0.06303572	605.14	
	Error	2	0.00020833	0.00010417		
	Corrected Total	5	0.18931550			
	R-Square		Coeff Var	Root MSE	C151 Mean	
	0.998900		8.132436	0.010206	0.125500	

	Source	DF	Type I SS	Mean Square	F Value	Pr
> F						
0.4226	Rep	1	0.00010417	0.00010417	1.00	
0.0011	Treatment	2	0.18900300	0.09450150	907.21	

	Source	DF	Type III SS	Mean Square	F Value	Pr
> F						
0.4226	Rep	1	0.00010417	0.00010417	1.00	
0.0011	Treatment	2	0.18900300	0.09450150	907.21	
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The GLM Procedure						
Dependent Variable: C171						
	Source	DF	Sum of Squares	Mean Square	F Value	Pr
> F						
0.1021	Model	3	0.03747900	0.01249300	8.95	
	Error	2	0.00279100	0.00139550		
	Corrected Total	5	0.04027000			
	R-Square	Coeff Var	Root MSE	C171 Mean		
	0.930693	18.49326	0.037356	0.202000		

	Source	DF	Type I SS	Mean Square	F Value	Pr
> F						
0.7320	Rep	1	0.00021600	0.00021600	0.15	
0.0697	Treatment	2	0.03726300	0.01863150	13.35	
	Source	DF	Type III SS	Mean Square	F Value	Pr
> F						
0.7320	Rep	1	0.00021600	0.00021600	0.15	
0.0697	Treatment	2	0.03726300	0.01863150	13.35	
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The GLM Procedure						
Dependent Variable: C161						
	Source	DF	Sum of Squares	Mean Square	F Value	Pr
> F						
0.0226	Model	3	0.64256050	0.21418683	43.36	
	Error	2	0.00988033	0.00494017		
	Corrected Total	5	0.65244083			
	R-Square	Coeff Var	Root MSE	C161 Mean		

0.984856 9.873985 0.070286 0.711833

	Source	DF	Type I SS	Mean Square	F Value	Pr
> F						
0.8261	Rep	1	0.00030817	0.00030817	0.06	
0.0152	Treatment	2	0.64225233	0.32112617	65.00	

	Source	DF	Type III SS	Mean Square	F Value	Pr
> F						
0.8261	Rep	1	0.00030817	0.00030817	0.06	
0.0152	Treatment	2	0.64225233	0.32112617	65.00	

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The GLM Procedure

Dependent Variable: C181cis

	Source	DF	Sum of Squares	Mean Square	F Value	Pr
> F						
0.0076	Model	3	13.16032050	4.38677350	131.29	
	Error	2	0.06682438	0.03341217		
	Corrected Total	5	13.22714483			

R-Square Coeff Var Root MSE C181cis Mean
0.994948 13.29543 0.182790 1.374833

	Source	DF	Type I SS	Mean Square	F Value	Pr
> F						
0.4766	Rep	1	0.02522017	0.02522017	0.75	
0.0051	Treatment	2	13.13510033	6.56755017	196.56	

	Source	DF	Type III SS	Mean Square	F Value	Pr
> F						
0.4766	Rep	1	0.02522017	0.02522017	0.75	
0.0051	Treatment	2	13.13510033	6.56755017	196.56	

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The GLM Procedure

Dependent Variable: C201

	Source	DF	Sum of Squares	Mean Square	F Value	Pr
> F						

0.0333	Model	3	1.61474650	0.53824883	29.23
	Error	2	0.03682233	0.01841117	
	Corrected Total	5	1.65156883		

R-Square	Coeff Var	Root MSE	C201 Mean
0.977705	8.758758	0.135688	1.549167

> F	Source	DF	Type I SS	Mean Square	F Value	Pr
0.6235	Rep	1	0.00608017	0.00608017	0.33	
0.0224	Treatment	2	1.60866633	0.80433317	43.69	

> F	Source	DF	Type III SS	Mean Square	F Value	Pr
0.6235	Rep	1	0.00608017	0.00608017	0.33	
0.0224	Treatment	2	1.60866633	0.80433317	43.69	

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Dependent Variable: C241

> F	Source	DF	Sum of Squares	Mean Square	F Value	Pr
0.0010	Model	3	0.03163200	0.01054400	988.50	
	Error	2	0.00002133	0.00001067		
	Corrected Total	5	0.03165333			

R-Square	Coeff Var	Root MSE	C241 Mean
0.999326	8.362311	0.003266	0.051333

> F	Source	DF	Type I SS	Mean Square	F Value	Pr
0.4226	Rep	1	0.00001067	0.00001067	1.00	
0.0007	Treatment	2	0.03162133	0.01581067	1482.25	

> F	Source	DF	Type III SS	Mean Square	F Value	Pr
0.4226	Rep	1	0.00001067	0.00001067	1.00	
0.0007	Treatment	2	0.03162133	0.01581067	1482.25	

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Dependent Variable: C221n9

	Source	DF	Sum of Squares	Mean Square	F Value	Pr
> F						
0.0010	Model	3	0.22039650	0.07346550	999.53	
	Error	2	0.00014700	0.00007350		
	Corrected Total	5	0.22054350			
	R-Square	Coeff Var	Root MSE	C221n9 Mean		
	0.999333	6.327095	0.008573	0.135500		

	Source	DF	Type I SS	Mean Square	F Value	Pr
> F						
0.4226	Rep	1	0.00007350	0.00007350	1.00	
0.0007	Treatment	2	0.22032300	0.11016150	1498.80	

	Source	DF	Type III SS	Mean Square	F Value	Pr
> F						
0.4226	Rep	1	0.00007350	0.00007350	1.00	
0.0007	Treatment	2	0.22032300	0.11016150	1498.80	

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Dependent Variable: TMUFA

	Source	DF	Sum of Squares	Mean Square	F Value	Pr
> F						
0.0725	Model	3	6.25207450	2.08402483	12.96	
	Error	2	0.32150633	0.16075317		
	Corrected Total	5	6.57358083			
	R-Square	Coeff Var	Root MSE	TMUFA Mean		
	0.951091	9.066604	0.400940	4.422167		

	Source	DF	Type I SS	Mean Square	F Value	Pr
> F						
0.5713	Rep	1	0.07238017	0.07238017	0.45	

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B
B 0.00000 2 LD

The GLM Procedure

Duncan's Multiple Range Test for C171

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha 0.05
Error Degrees of Freedom 2
Error Mean Square 0.001396

Number of Means 2 3
Critical Range .1607 .1586

Means with the same letter are not significantly different.

Duncan Grouping		Mean	N	Treatment
	A	0.28250	2	LD
	A			
B	A	0.22850	2	CF
B				
B		0.09500	2	BT

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The GLM Procedure

Duncan's Multiple Range Test for C161

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha 0.05
Error Degrees of Freedom 2
Error Mean Square 0.00494

Number of Means 2 3
Critical Range .3024 .2889

Means with the same letter are not significantly different.

Duncan Grouping		Mean	N	Treatment
	A	1.15600	2	CF
B		0.60200	2	LD
B				
B		0.37750	2	BT

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The GLM Procedure

Duncan's Multiple Range Test for C181cis

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha 0.05
Error Degrees of Freedom 2
Error Mean Square 0.033412

Number of Means 2 3
Critical Range .7865 .7514

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	Treatment
A	3.4620	2	BT
B	0.4600	2	CF
B	0.2025	2	LD

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The GLM Procedure

Duncan's Multiple Range Test for C201

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha 0.05
Error Degrees of Freedom 2
Error Mean Square 0.018411

Number of Means 2 3
Critical Range .5838 .5578

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	Treatment
A	2.2230	2	CF
B	1.4605	2	LD
B	0.9640	2	BT

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The GLM Procedure

Duncan's Multiple Range Test for C241

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha 0.05
Error Degrees of Freedom 2
Error Mean Square 0.000011

	A	5.6000	2	BT
	A			
B	A	4.5435	2	CF
B				
B		3.1230	2	LD

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The GLM Procedure

Class Level Information

Class	Levels	Values
Treatment	3	BT CF LD
Rep	2	1 2

Number of Observations Read 6
Number of Observations Used 6

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The GLM Procedure

Dependent Variable: C182trans

	Source	DF	Sum of Squares	Mean Square	F Value	Pr
> F						
0.0295	Model	3	0.00873300	0.00291100	33.02	
	Error	2	0.00017633	0.00008817		
	Corrected Total	5	0.00890933			

R-Square 0.980208
Coeff Var 8.260743
Root MSE 0.009390
C182trans Mean 0.113667

	Source	DF	Type I SS	Mean Square	F Value	Pr
> F						
0.1835	Rep	1	0.00035267	0.00035267	4.00	
0.0206	Treatment	2	0.00838033	0.00419017	47.53	

	Source	DF	Type III SS	Mean Square	F Value	Pr
> F						
0.1835	Rep	1	0.00035267	0.00035267	4.00	
0.0206	Treatment	2	0.00838033	0.00419017	47.53	

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	Source	DF	Type III SS	Mean Square	F Value
> F					
	Rep	1	0.00952017	0.00952017	3.90
0.1870	Treatment	2	0.07000833	0.03500417	14.33
0.0652					

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Dependent Variable: C183n3

	Source	DF	Sum of Squares	Mean Square	F Value
> F					
	Model	3	2.08357200	0.69452400	433.90
0.0023	Error	2	0.00320133	0.00160067	
	Corrected Total	5	2.08677333		

R-Square	Coeff Var	Root MSE	C183n3 Mean
0.998466	6.363998	0.040008	0.628667

	Source	DF	Type I SS	Mean Square	F Value
> F					
	Rep	1	0.00004267	0.00004267	0.00
0.8853	Treatment	2	2.08352933	1.04176467	650.8
0.0015					

	Source	DF	Type III SS	Mean Square	F Value
> F					
	Rep	1	0.00004267	0.00004267	0.00
0.8853	Treatment	2	2.08352933	1.04176467	650.8
0.0015					

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The GLM Procedure

Dependent Variable: C222

	Source	DF	Sum of Squares	Mean Square	F Value
> F					
	Model	3	0.02764400	0.00921467	265.0
0.0038	Error	2	0.00006933	0.00003467	

	Source	DF	Type III SS	Mean Square	F Value	Pr
> F						
	Rep	1	0.00952017	0.00952017	3.90	
0.1870	Treatment	2	0.07000833	0.03500417	14.33	
0.0652						
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Dependent Variable: C183n3

	Source	DF	Sum of Squares	Mean Square	F Value	Pr
> F						
	Model	3	2.08357200	0.69452400	433.90	
0.0023	Error	2	0.00320133	0.00160067		
	Corrected Total	5	2.08677333			
R-Square		Coeff Var	Root MSE	C183n3 Mean		
0.998466		6.363998	0.040008	0.628667		

	Source	DF	Type I SS	Mean Square	F Value	Pr
> F						
	Rep	1	0.00004267	0.00004267	0.03	
0.8853	Treatment	2	2.08352933	1.04176467	650.83	
0.0015						
	Source	DF	Type III SS	Mean Square	F Value	Pr
> F						
	Rep	1	0.00004267	0.00004267	0.03	
0.8853	Treatment	2	2.08352933	1.04176467	650.83	
0.0015						
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Dependent Variable: C222

	Source	DF	Sum of Squares	Mean Square	F Value	Pr
> F						
	Model	3	0.02764400	0.00921467	265.81	
0.0038	Error	2	0.00006933	0.00003467		
	Corrected Total	5	0.02771333			
R-Square		Coeff Var	Root MSE	C222 Mean		
0.997498		7.679792	0.005888	0.076667		

	Source	DF	Type I SS	Mean Square	F Value	Pr
> F						
0.6349	Rep	1	0.00001067	0.00001067	0.31	
0.0025	Treatment	2	0.02763333	0.01381667	398.56	

	Source	DF	Type III SS	Mean Square	F Value	Pr
> F						
0.6349	Rep	1	0.00001067	0.00001067	0.31	
0.0025	Treatment	2	0.02763333	0.01381667	398.56	

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The GLM Procedure
Dependent Variable: C203n6

	Source	DF	Sum of Squares	Mean Square	F Value	Pr
> F						
<.0001	Model	3	0.13397733	0.04465911	Infty	
	Error	2	0.00000000	0.00000000		
	Corrected Total	5	0.13397733			

R-Square Coeff Var Root MSE C203n6 Mean
1.000000 0 0 0.181667

	Source	DF	Type I SS	Mean Square	F Value	Pr
> F						
	Rep	1	0.00000000	0.00000000	.	.
<.0001	Treatment	2	0.13397733	0.06698867	Infty	

	Source	DF	Type III SS	Mean Square	F Value	Pr
> F						
	Rep	1	0.00000000	0.00000000	.	.
<.0001	Treatment	2	0.13397733	0.06698867	Infty	

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The GLM Procedure
Dependent Variable: C226

	Source	DF	Sum of Squares	Mean Square	F Value	Pr
> F						
0.0471	Model	3	0.09524850	0.03174950	20.39	

Error	2	0.00311433	0.00155717
Corrected Total	5	0.09836283	

R-Square	Coeff Var	Root MSE	C226 Mean
0.968338	22.39978	0.039461	0.176167

Source	DF	Type I SS	Mean Square	F Value	Pr
Rep	1	0.00050417	0.00050417	0.32	
Treatment	2	0.09474433	0.04737217	30.42	

Source	DF	Type III SS	Mean Square	F Value	Pr
Rep	1	0.00050417	0.00050417	0.32	
Treatment	2	0.09474433	0.04737217	30.42	

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The GLM Procedure

Dependent Variable: C204n6

Source	DF	Sum of Squares	Mean Square	F Value	Pr
Model	3	0.28612717	0.09537572	67.16	
Error	2	0.00284033	0.00142017		
Corrected Total	5	0.28896750			

R-Square	Coeff Var	Root MSE	C204n6 Mean
0.990171	14.98413	0.037685	0.251500

Source	DF	Type I SS	Mean Square	F Value	Pr
Rep	1	0.00058017	0.00058017	0.41	
Treatment	2	0.28554700	0.14277350	100.53	

Source	DF	Type III SS	Mean Square	F Value	Pr
Rep	1	0.00058017	0.00058017	0.41	
Treatment	2	0.28554700	0.14277350	100.53	

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The GLM Procedure

Dependent Variable: C202

	Source	DF	Sum of Squares	Mean Square	F Value	Pr
> F	Model	3	2.39770850	0.79923617	97865.7	
<.0001	Error	2	0.00001633	0.00000817		
	Corrected Total	5	2.39772483			
	R-Square		Coeff Var	Root MSE	C202 Mean	
			0.492855	0.002858	0.579833	

	Source	DF	Type I SS	Mean Square	F Value	Pr
> F	Rep	1	0.00070417	0.00070417	86.22	
0.0114	Treatment	2	2.39700433	1.19850217	146755	
<.0001						

	Source	DF	Type III SS	Mean Square	F Value	Pr
> F	Rep	1	0.00070417	0.00070417	86.22	
0.0114	Treatment	2	2.39700433	1.19850217	146755	
<.0001						

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The GLM Procedure

Dependent Variable: TPUFA

	Source	DF	Sum of Squares	Mean Square	F Value	Pr
> F	Model	3	1571.523707	523.841236	19725.0	
<.0001	Error	2	0.053114	0.026557		
	Corrected Total	5	1571.576821			
	R-Square		Coeff Var	Root MSE	TPUFA Mean	
			1.228864	0.162964	13.26133	

	Source	DF	Type I SS	Mean Square	F Value	Pr
> F	Rep	1	0.000451	0.000451	0.02	
0.9083	Treatment	2	1571.523256	785.761628	29587.6	
<.0001						

	Source	DF	Type III SS	Mean Square	F Value	Pr
> F						
0.9083	Rep	1	0.000451	0.000451	0.02	
<.0001	Treatment	2	1571.523256	785.761628	29597.6	
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The GLM Procedure

Duncan's Multiple Range Test for C182trans

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	2
Error Mean Square	0.000088
Number of Means	2 3
Critical Range	.04040 .03860

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	Treatment
A	0.163500	2	CF
B	0.104000	2	LD
B	0.073500	2	BT

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The GLM Procedure

Duncan's Multiple Range Test for C182cis

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	2
Error Mean Square	0.003037
Number of Means	2 3
Critical Range	.2371 .2266

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	Treatment
A	31.90900	2	LD
B	0.57650	2	BT
C	0.13700	2	CF

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The GLM Procedure

Duncan's Multiple Range Test for C183n6

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	2
Error Mean Square	0.002442
Number of Means	2
Critical Range	.2126

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	Treatment
A	0.51950	2	LD
A			
B	0.41700	2	CF
B			
B	0.25700	2	BT

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The GLM Procedure

Duncan's Multiple Range Test for C183n3

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	2
Error Mean Square	0.001601
Number of Means	2
Critical Range	.1721

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	Treatment
A	1.46200	2	LD
B	0.21900	2	BT
B			
B	0.20500	2	CF

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The GLM Procedure

Duncan's Multiple Range Test for C222

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha 0.05
Error Degrees of Freedom 2
Error Mean Square 0.000035

Number of Means 2 3
Critical Range .02533 .02420

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	Treatment
A	0.165000	2	CF
B	0.065000	2	LD
C	0.000000	2	BT

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The GLM Procedure

Duncan's Multiple Range Test for C203n6

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha 0.05
Error Degrees of Freedom 2
Error Mean Square 0

Number of Means 2 3
Critical Range 0 0

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	Treatment
A	0.3660	2	CF
B	0.1790	2	LD
C	0.0000	2	BT

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The GLM Procedure

Duncan's Multiple Range Test for C226

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha 0.05
Error Degrees of Freedom 2
Error Mean Square 0.001557

Number of Means 2 3
Critical Range .1698 .1622

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	Treatment
A	0.28450	2	CF
A			
A	0.24400	2	LD
B	0.00000	2	BT

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The GLM Procedure

Duncan's Multiple Range Test for C204n6

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	2
Error Mean Square	0.00142

Number of Means	2	3
Critical Range	.1621	.1549

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	Treatment
A	0.53200	2	CF
B	0.22250	2	LD
C	0.00000	2	BT

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The GLM Procedure

Duncan's Multiple Range Test for C202

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	2
Error Mean Square	8.167E-6

Number of Means	2	3
Critical Range	.01230	.01175

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	Treatment
A	1.472000	2	LD

B	0.181500	2	CF
C	0.086000	2	BT

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The GLM Procedure

Duncan's Multiple Range Test for TPUFA

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	2
Error Mean Square	0.026557
Number of Means	2 3
Critical Range	.7012 .6699

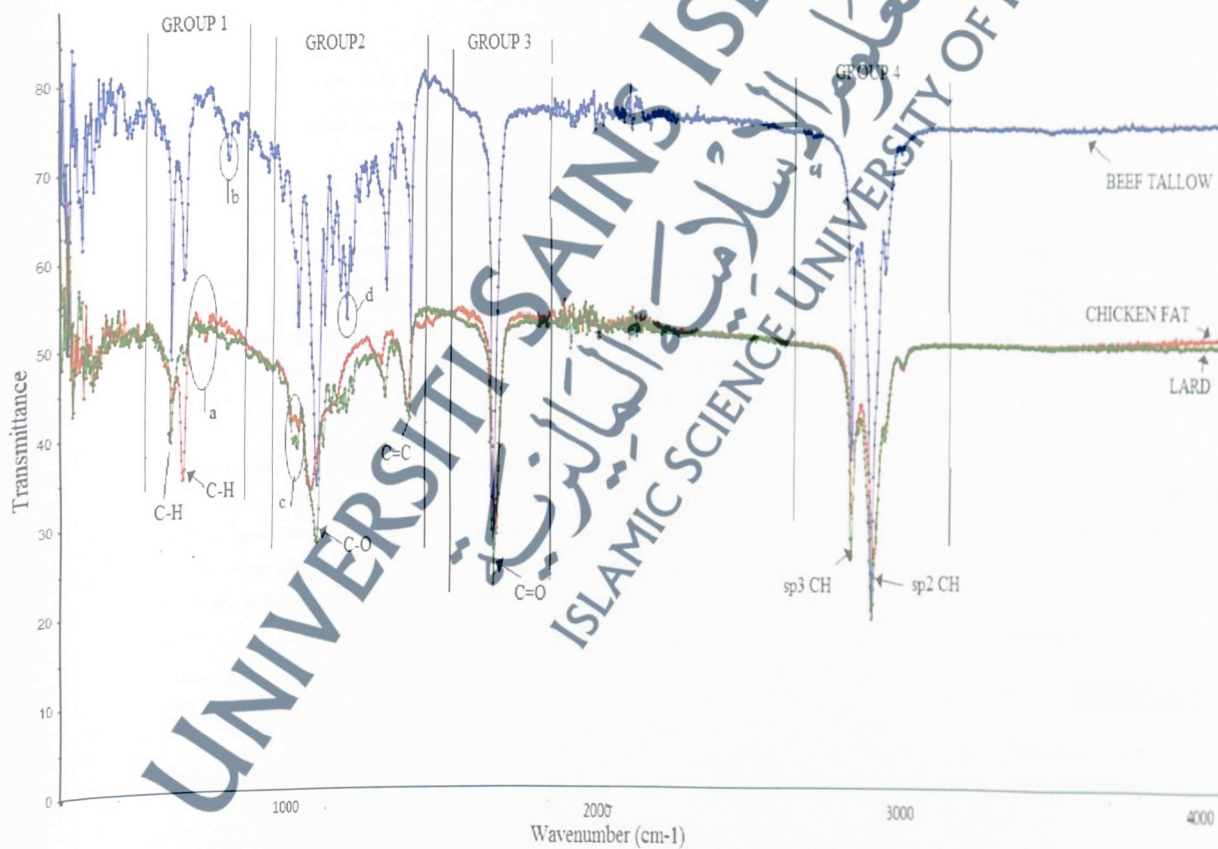
Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	Treatment
A	36.1375	2	LB
B	2.4490	2	CF
C	1.1975	2	BT

APPENDIX II

FTIR SPECTRA OF PURE BEEF TALLOW (BT), CHICKEN FAT (CF) AND LARD (LD)

Fig. 16 FTIR spectra of pure beef tallow (BT), chicken fat (CF), and lard (LD)



APPENDIX III

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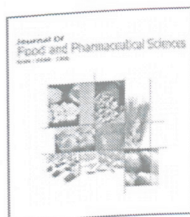
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Research Article

Differentiation of Lard from Other Edible Fats by Gas Chromatography-Flame Ionisation Detector (GC-FID) and Chemometrics

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ABSTRACT

The presence of lard or its derivatives in any food products is a serious religious issue among Muslim and Judaism. Thus, the objective of this study was to investigate the use of gas chromatography with flame ionisation detector (GC-FID) coupled with chemometrics techniques such as Principle Components Analysis (PCA) and K-mean cluster analysis to differentiate lard adulteration at very low concentrations in beef and chicken fats. The measurements were made from the pure lard, beef tallow, pure chicken fat; and beef tallow (BT), chicken fat (CF) adulterated with different concentrations of lard (0.5%-10% in BT and CF). The data were first scaled into standardisation before PCA is performed to each of the scaled data using Unscrambler software. The Scores plots and loadings plots of each scaled data were compared and studied. The results showed that lard contains higher fatty acid (FA) of C18:2cis and low C16:0 FA, but oppositely for beef tallow and chicken fat. The amount of C4:0, C14:0, and C18:0 FAs are approximately similar for all fats. Others FAs are small in amount and nearly similar for both. Additionally, PCA was able to significantly identify lard, beef fat, chicken fat and the mixtures of lard and beef tallow, lard and chicken fat, even at lower concentration level (0.5 % lard-99.5% beef tallow / chicken fat (w/w). K-mean cluster only able to classify the pure lard (LD), pure chicken fat (CF) and pure beef tallow (BT)

Keywords: adulteration, lard, beef tallow, chicken fat, GC-FID, chemometrics

1. Introduction

Edible fats and oils are complex mixtures containing a wide range of compounds. They are principally composed of free fatty acids (FFAs), diacylglycerols (DGs), triacylglycerols (TAGs), phospholipids, and other minor components. (Janssen et al., 2003). Since a long time, the trade of fats and oils has been facing a serious adulteration problem, which is usually motivated in a bid for maximizing profit by replacing an expensive ingredient with cheaper ones (Cserháti, et al., 2005); and

versatile in providing the functional properties of the product. Lard in its original or its industrially modified fats might be mixed with other animals or vegetable oils to produce shortenings, margarine and other food oils and its detection as an adulterant in food systems is a major concern for many countries due to religious prohibitions and health reasons (Marikkar et al., 2005). Therefore, the detection of lard in foods is a major concern for many countries due to religious prohibitions and health reasons. The presence of lard in food is a serious issue for some religions like Islam and Judaism

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where consuming any foods containing porcine and its derivatives is forbidden even at trace level Regenstein et al. (2003). However, detection of lard when it exists as a minor component in other oils and fats, is difficult. Hence, there is a great need for robust and trustworthy techniques for lard differentiation and detection for the practice of Halal authentication analysis by the relevant Halal authorities. The use of gas chromatography coupled with chemometrics may provide a solution to this low concentration detection problem.

Haswell (1992) has defined chemometrics as the combination of mathematical, statistical, and other logic-based methods for managing and interpreting efficiently the chemically-derived data. Principal components analysis (PCA) is an unsupervised pattern recognition technique used in multivariate analysis (Yaakob B. Che Man et al, 2011). It projects the original data to reduced dimensions in matrices called scores and loadings. Shin et al. (2010) has reported that PCA is a method of identifying patterns in data and expressing the data in such a way as to emphasize their similarities and differences. The authors also reported that PCA can designate interactions between groups of variables in a data set and show relationships that might exist between objects. PCA has been successfully used to define relationships that exist in fatty acid classification studies of food lipids, due to the capability to manage and interpret large data sets (Kadegowda et al, 2008; Matos et al, 2007). K-mean cluster analysis (CA) is calculated based on Euclidean distance from the object of each cluster to its centre by which pre-determined the number of cluster is K (Jianyong Chen and Changsheng Zhang, 2011). The object belongs to its cluster when the distance is near to the centre compared with others centers. CA has an advantage over PCA where CA information is more objective and provides numerical results. In addition, CA is able to reduce dimensionality, while retaining the required information (Che Man et al, 2011).

Thus, the objective of this study was to investigate the use of gas chromatography-flame ionization detector coupled with chemometrics techniques, as a means for distinguishing lard adulteration in very low concentrations from other animal fats such as beef tallow and chicken fats.

2. Materials and Methods

2.1. Materials

Adipose tissues of pig (lard), chicken and beef were obtained from different local supermarkets. The analytical solvents used for fats extraction and GC analysis were: methanol 99.9 % (analytical, GC grade); acetone 99.9% (analytical, GC grade); n-hexane 99.0% (analytical, GC grade), chloroform 99.8% (analytical grade) and sodium methoxide 1% solution. All mentioned solvents were from Sigma- Aldrich, UAS.

2.2. Standards

The 37 Component FAME Mix from Supelco, Sigma-Aldrich, USA was used to identify the fatty acid composition of the pure and adulterated samples.

2.3. Samples preparation

The fats were extracted according to the Bligh-Dyer method (1959). Accurately, 300 to 400 mg of sample was weighed; then, 4 ml methanol, 2 ml chloroform and 0.4 ml water were added; homogenized and vortexed for 30 sec. After that, 2 ml of chloroform and 2 ml of water were added; vortexed for 30 sec and centrifuged at $3300 \times g$ for 15 min, at 5° to 25°C . Using a Pasteur pipette the upper layer (methanol/water) was then removed, and the lower layer (methanol/chloroform) was transferred into a clear tube. Finally, the solvents were removed using a rotary evaporator under reduced pressure, at 40°C .

The extracted animal fat, lard, beef fat (BF), chicken fat (CF) and a series of 14 samples containing (0.5 - 10 % w/w) of lard in BF, and CF were analyzed. Samples containing lard were assigned as adulterated; while pure beef fat, pure chicken fat and pure lard were marked beef tallow (BT), chicken fat (CF) and lard (LD), and running theme using GC-FID.

2.4. Analysis of fatty acid methyl esters (FAME) using GC-FID

The fatty acid compositions were determined by conversion of the oil to fatty acid methyl esters (FAME) according to the method of Cocks and Van Rede (1966) with a slight modification. FAME was prepared by adding 950 μl of n-hexane into 50 mg of fat, followed by 50 μl of sodium methoxide. The mixtures were vortex for 5 sec and allowed to settle for 30–60 min. The top layer (1 μl) was injected into a gas chromatograph (Agilent 7890) equipped with a flame-ionization detector and a polar capillary column (HP88-Agilent Technologies, USA), 100 m, 0.25mm, and 0.25 μm film, internal diameter and film thickness, respectively, to obtain individual peaks of FAME. The temperature of the column was 90°C , programmed to increase to 220°C at $15^{\circ}\text{C}/\text{min}$ (for 5 min), $2^{\circ}\text{C}/\text{min}$ and $15^{\circ}\text{C}/\text{min}$ (for 1 min). The temperature of the injector and detector was maintained at 240°C . The run time was 40min. The FAME peaks were identified by comparing their retention time with certified reference standards of FAME (Supelco, Sigma-Aldrich, USA). Percentage relative of fatty acid was calculate based on the peak area of a fatty acid species to the total peak area of all the fatty acids in the oil samples.

2.5. Chemometrics Software

The percentage of FAME of all samples was transformed to a multivariates data set. Then, the data was standardized using Microsoft Excel software 2007. Principal component analysis (PCA) and K-mean cluster were performed by using an Unscrambler software (X10).

3. Results and Discussion

3.1. Fatty Acid Methyl Esters (FAME)

Table 1 shows the fatty acid compositions of lipid extracts from beef, lard and their mixtures. Butyric acid (C4:0) ranged from 8.29% to 1.85%; Capric acid (C10:0) 0.129% to 0.12%; Lauric acid (C12:0), 0.175% to 0.12%; Myristic acid (C14:0), 7.34% to 1.86%; (C14:1), 0% to 0.30%; Pentadecanoic (C15:0), 0.09% to 0.83%; Palmitic acid (C16:0), 35.37% to 56.03%; Palmitoleic Acid (C16:1), 0.31% to 0.60%; Margaric acid (C17:0), 0.18% to 0.58%; Stearic acid (C18:0), 20.33% to 20.49%; Oleic acid (C18:1cis), 0.20% to 3.24%; Linoleic Acid (C18:2trans),

0.10% to 0.08%; Linoleic acid (C18:2cis), 0.64% to 31.9%; linoleic acid (C18: 2, n6), 2.85% to 33.92%; Alpha-linolenic acid (C18:3n3), 0.24% to 1.46%; Arachidic acid (C20:0), 0.87% to 2.18%; Eicosenoic acid (C20:1n9), 0.82% to 1.46%; Dihomo-gamma-linolenic acid (C20:3n6), 0% to 0.17%; Erucic acid (C22:1n9), 0% to 0.39%; Nervonic acid (C24:1), 0% to 0.15%; Docosahexaenoic acid (C22:6), 0% to 0.25%; Arachidonic acid (C20:4n6), 0% to 0.23%; Eicosadienoic acid (C20:2), 0.1% to 1.48%. These results correspond with those from other studies (Enser et al, 1996).

Table 1. FAME results of the pure and the adulterated samples.

FA/%	C4:0	C6:0	C10:0	C12:0	C14:0	C14:1	C15:0	C15:1	C16:0	C16:1	C17:0	C17:1	C18:0	C18:1cis	C18:2trans
BT	8.290	ND	0.129	0.176	7.349	0.308	0.834	0.364	56.038	0.316	0.185	0.104	20.335	3.248	0.085
CF	0.462	0.069	0.243	0.509	2.605	0.497	0.177	ND	71.461	1.192	0.355	0.192	15.643	0.474	0.175
LD	1.853	ND	0.124	0.128	1.864	0.000	0.094	0.000	35.374	0.606	0.586	0.292	20.491	0.208	0.104
S1	6.618	ND	0.141	0.177	6.481	0.247	0.700	0.293	55.833	0.442	0.153	0.089	22.609	2.715	0.067
S2	5.700	ND	0.144	0.182	6.473	0.274	0.755	0.290	56.401	0.461	0.152	0.086	22.831	2.692	0.067
S3	6.737	ND	0.139	0.177	6.370	0.242	0.687	0.091	55.780	0.456	0.156	0.085	22.828	2.681	0.067
S4	6.732	ND	0.097	0.120	6.186	0.790	0.661	0.275	54.915	0.456	0.589	0.403	22.608	2.367	0.127
S5	6.630	ND	0.144	0.176	6.333	0.237	0.678	0.183	55.670	0.467	0.153	0.084	22.485	2.608	0.067
S6	7.102	ND	0.141	0.182	6.347	0.239	0.680	0.284	55.106	0.445	0.598	0.150	22.213	2.432	0.128
S1*	1.010	0.041	0.235	0.426	2.538	0.379	0.203	ND	66.777	1.132	0.495	0.260	19.644	0.460	0.157
S2*	1.061	0.038	0.228	0.423	2.538	0.362	0.198	ND	66.745	1.127	0.504	0.271	20.102	0.443	0.157
S3*	1.017	0.019	0.156	0.300	2.146	0.311	0.171	ND	58.939	0.997	0.459	0.258	18.274	0.386	0.141
S4*	1.181	0.053	0.226	0.411	2.557	0.345	0.194	ND	66.245	1.116	0.535	0.276	21.026	0.452	0.155
S5*	0.418	0.034	0.136	0.248	1.899	0.174	0.064	ND	49.629	4.772	0.318	0.182	15.181	15.627	0.739
S6*	1.073	0.040	0.228	0.400	2.580	0.338	0.193	ND	65.564	1.114	0.545	0.292	21.035	0.439	0.157

3.2. Principal component analysis (PCA)

In this study, the multivariate data matrix of 30 FAME (i.e., the 30 FAME in BF, LA, and CF as well as their mixtures) was subjected to PCA in order to reduce the number of descriptors associated with the data set without losing the information of the original data. Scores and loadings matrices were generated using 7 principal components (PCs). An eigenvalue of about 98% was achieved using seven PCs where PC1 accounted for 1% of the variation, while PC2 described 97% of the variation; therefore, the remaining five ($\leq 2\%$ total) did not explain significant variability in the data.

Figure 1 demonstrates the scores plot of PCA of 30 fatty acids methyl esters (FAME) representing the projection of samples defined by the first (PC1) and second (PC2) components. It explains the variation characteristics of the 30 FAME's in the samples; there are some fatty acids are significantly different which medium chain and polyunsaturated fatty acids. These fatty acids are distributed in the upper left region in the figure for example C15:1, oleic acid (C18:1cis), and linoleic acid (C18:2cis) specifically in lard. This may be due to the unchanging of this fatty acids inside pig stomach; oppositely to the ruminants with several stomachs have microbes that change fatty acids structure into saturated

fats. Therefore, they can pass into the blood stream in the small intestine, and from there into tissues (Teye et al., 2006a; Teye, Wood, Whittington, Stewart, & Sheard, 2006b). In addition, the Figure 1 shows that there are some saturated fatty acids with significant presence and they located in the lower left region in the figure, for instance: butyric acid (C4:0), myristic acid (C14:0), and stearic acid (C18:0). Furthermore, Figure 1 illustrates that palmitic acid (C16:0) which is sited on the right region and far from others acids. This means that it is significantly higher from other fatty acids and this especially in BT; and that may be due to the microbial biohydrogenation of fatty acids inside the ruminants stomach; where the fatty acids are changed from poly and mono-unsaturated to saturated fatty acids (J. D. Wood et al., 2008).

Figure 2 shows the loading plot for the different concentrations of the samples (BT+ L, and CF+L) as well as the pure samples BT, CF and LA. The PCA loading plot describes the different distribution of variables (concentrations) in two main groups. The first group (1, 4, 5, 6, 7, 8, 9, and 10) which refers to BT and BT+LD; and it was located under the X-axis. The second group (3, 11, 12, 13, 14, 15, 16, and 17) refers to CF and CF+LD; and it is located up of X-axis. However, for the sample 2, which

refers to lard sample, is very isolated from the other two groups. This grouping of samples is maybe because of the differentiation in their physic - chemical

characteristics. Therefore, Figure 2 helps to differentiate BT and CF from LA, and it aids to distinguish very low adulterated concentration (0.5%) of lard in BT and CF.

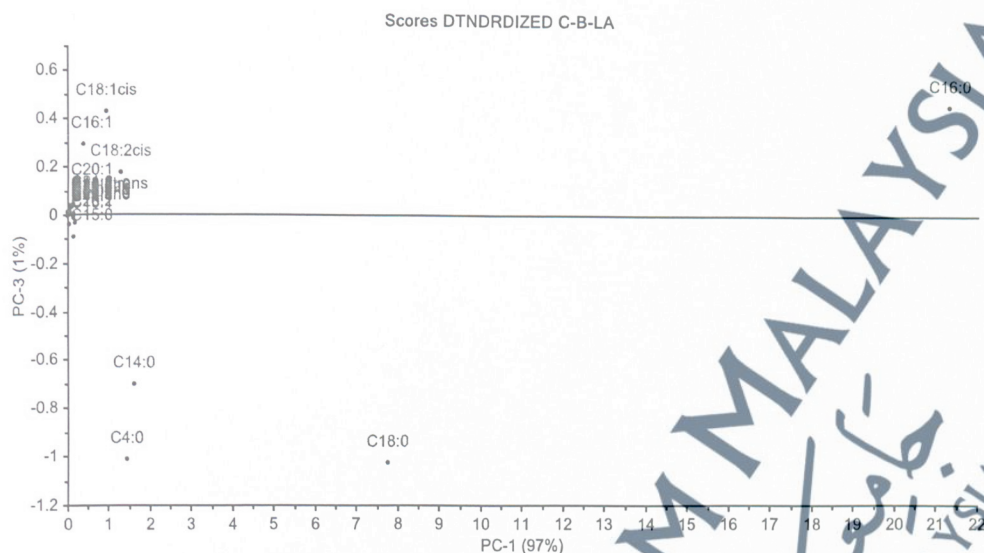


Fig 1. The scores plot for the first two principal components (PC) for 30 FAME from the samples studied



Fig 2. Principal component analysis (PCA) loadings plot of PC1 versus PC2 showing the pure and the adulterated samples, where: 1 refers to beef tallow (BT); 2 lard (LD); 3 chicken fat (CF); 4 BT+0.5 %LD; 5 BT+1 %LD; 6 BT+2 %LD; 7 BT+3 %LD; 8 BT+4 %LD; 9 BT+5 %LD; 10 BT+10 %LD; 11 CF+0.5 %LD; 12 CF+1 %LD; 13 CF+2 %LD; 14 CF+3 %LD; 15 CF+4 %LD; 16 CF+ 5 %LD and 17 CF+ 10 %LD.

3.3. Cluster analysis

The original data set (Table 2), X (17, 30) consisted of 30 variables measured in 17 samples (3 samples are pure animal fats: BT, LD and CF and other 14 are BT+LD, CF+LD respectively), where each entry matrix was an average value of three replications. The data was standardized prior to the analysis, and then they were subjected to cluster analysis (CA). The K-mean cluster analysis results are given in (Table 2 (a), (b) and (c)) that grouped all samples into three different classes class 1

for LD, class 2 for BF and BF+LD (Table 2 (b); while, class 3 for CF and CF+LD (Table 2 (c)). The mixtures were classified according to their main component of fat; CF+LD in class 1 (CF) and BF+LD in class 2(BF). Nevertheless, this k-mean cluster results could not distinguish lard in small concentration (0.5%) from other concentrations (more than 0.5%), but it may help to differentiate lard from other animal fats if it (lard) is dominant in the mixtures.

Table 2 (a):

Sample	Concentration of lard %	Class
LD	100	1
BT	0	2
CF	0	3

k-mean cluster results for the pure samples BT is pure beef tallow; CF is pure chicken fat and LD is pure lard

Table 2 (b):

Sample	Concentration of lard %	Class
S1	0.5	2
S2	1	2
S3	2	2
S4	3	2
S5	4	2
S6	5	2
S7	10	2

k-mean cluster results for BT+ LD where: S1 is BT+ 0.5% LD; S2 is BT + 1% LD; S3 is BT+ 2% LD; S4 is 3% LD; S5 is BT + 4 % LD; S6 is BT+ 5% LD; S7 is BT + 10% LD

Table 2 (c):

Sample	Concentration of lard %	Class
S1*	0.5	3
S2*	1	3
S3*	2	3
S4*	3	3
S5*	4	3
S6*	5	3
S7*	10	3

k-mean cluster results for CF+ LD where: S1* is CF+ 0.5% LD; S2* is CF + 1% LD; S3* is CF + 2% LD; S4* is 3% LD; S5* is CF + 4 % LD; S6* is CF + 5% LD and S7* is CF + 10% LD

4. Conclusion

GC-FID combined with the chemometric technique such as PCA and K-mean cluster can be employed to differentiate lard from beef tallow and chicken fat. PCA has showed the ability of detection of lard within the mixtures of beef tallow- lard and chicken fat-lard as low as 0.5 % of lard. Meanwhile, K-mean cluster was only able to differentiate between pure beef tallow, pure chicken fat and pure lard. In future continuation from this study will look into more replications to elaborate more information about k-mean cluster analysis in intention of detection of lard in food matrix in very small possible amounts.

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