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Towards the identification of metabolite markers of nipple pain and inflammation in human milk

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Appendices

Appendix 2.1 Consent form provided to all participants prior to milk collection.



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Composition of human milk

Consent Form

This study aims to learn more about the composition of human milk and the changes in concentration during the day and throughout lactation, and possible contaminants.

I

FAMILY NAME

GIVEN NAMES

have read the information sheet about this study and any questions I have asked have been answered to my satisfaction. I agree to participate in this activity, realising that I may withdraw at any time without reason and without prejudice.

I understand that all information provided is treated as strictly confidential and will not be released by the investigator unless required to do so by law.

I have been advised as to what data is being collected, what the purpose is, and what will be done with the data upon completion of the research.

I agree that research data gathered for the study may be published provided my name or other identifying information is not used.

Participant Date

The Human Research Ethics Committee at the University of Western Australia requires that all participants are informed that, if they have any complaint regarding the manner, in which a research project is conducted, it may be given to the researcher or, alternatively to the Secretary, Human Research Ethics Committee, Registrar's Office, The University of Western Australia, 35 Stirling Highway, Crawley, WA 6009 (telephone number 6488-3703). All study participants will be provided with a copy of the Information Sheet and Consent Form for their personal records.

Appendix 2.2. General information sheet provided to all participants prior milk collection.

Have you previously participated in a study with the Hartmann Human Lactation Research Group?	
First Name	
Last Name	
DOB Baby (dd/mm/yy)	
DOB mother (dd/mm/yy)	
Email	
Phone/Mobile	
Address	
Number of Children	
Have you done a HIV and Hepatitis check?	
If you have other children, did you breastfeed any of them? If yes, for how long?	
General Healthy Status of mother/baby (presence of nipple pain trauma/no trauma)	
Current Medication	

Visual Analogue Scale (pain intensity during breastfeeding)

No pain

Worst Pain Imaginable



Appendix 2.3. Online questionnaire which participants were asked to fill out following milk ample collection.

Retrieved from: <https://breastfeeding.bcs.uwa.edu.au>

Section A

Date of Birth
Last year of completed education
Further completed education
Marital Status
Number of Children
Ethnic Group
Height and Weight
Left or Right handed

Section B

Bra Size before pregnancy
Current Bra Size
Breast Piercing
Did you smoke during pregnancy?
Do you smoke now?
List of experienced problems:
High Blood Pressure
Gestational Diabetes
Antenatal Bleeding
Postpartum Haemorrhage
Depression
Hospitalisation for any reason
Current medications
Allergies

Section C

Infant Gender
Infant Date of Birth
Gestational Weeks and Days
Birth Weight
Birth Length

Birth Apgar Scores
Mode of Delivery
Drugs during delivery
Was the baby admitted to special Care nursery?
Special Care Nursery Details?
Baby's allergies
Baby's current medications
Time before baby's first feed
Day milk came in
Baby feed method
Intended baby feed length
Baby's weight at 6 weeks
Baby's dummy usage
Breastfeeding relationship concerns

Appendix 3.1. Human sample demographic data.

Participant ID	Group ID	Right Breast	Left Breast	Mother Age (Years)	Infant Age (Weeks)	Parity	AB (Y/N)	Pain (1-10)
M01	C01	Control	N/A	23.65	11.9	1	N	0
M02	C02/NP05	NP-No Trauma	Control	29.15	5.9	1	N	8
M03	C03/NP10	NP-Trauma	Control	31.7	5.7	1	Y	6
M04	C04	Control	N/A	28.39	15.7	2	N	0
M05	C05	Control	N/A	25.78	8.3	2	N	0
M06	C06	N/A	Control	32.84	16.3	3	N	0
M07	C07	Control	N/A	30.87	17.7	2	N	0
M08	C08	Control	N/A	29.84	21.1	1	N	0
M09	C09	Control	N/A	30.72	25.6	2	N	0
M10	C10	N/A	Control	40.2	25.7	1	N	0
M11	C11	Control	N/A	34.35	26.1	2	N	0
M12	C12	Control	N/A	34.34	10.7	3	N	0
M13	C13	Control	N/A	22.59	19.7	2	N	0
M14	C14	N/A	Control	35.06	17.4	2	N	0
M15	C15	Control	N/A	32.8	22.7	1	N	0
M16	C16	Control	N/A	33.28	22.3	1	N	0

M17	C17	N/A	Control	40.8	14	3	N	0
M18	C18/NP02	NP-No Trauma	Control	28.06	10.4	1	N	10
M19	C19	Control	N/A	22.19	5	2	N	0
M20	C20	N/A	Control	31.26	7.6	2	N	0
M21	C21	Control	N/A	24.18	7.1	1	N	0
M22	C22/NP06	Control	NP-No Trauma	34.82	7.1	1	N	2
M23	NP01	NP-No Trauma	N/A	28.94	21.6	1	N	4
M24	NP03	N/A	NP-No Trauma	40.43	6.9	1	N	3
M25	NP04	NP-No Trauma	N/A	39.18	6.9	4	N	8
M26	NP07	NP-Trauma	N/A	37.11	5.1	2	N	5
M27	NP08	NP-Trauma	N/A	35.87	4.6	2	N	4
M28	NP09	NP-Trauma	N/A	29.5	7	2	N	7
M29	NP11	NP-Trauma	N/A	34.79	22.7	2	N	4

Highlighted rows indicate participants who were suffering from unilateral nipple pain and provided a control and nipple pain sample.

Appendix 3.3. CFU counts for blood agar cultures of human milk samples and individual and pooled vat bovine milk samples.

Participant ID	Sample ID	Group	CFU/ml
M01	C01	Control	500
M02*	C02	Control	2670
M03*	C03	Control	0
M04	C04	Control	1050
M05	C05	Control	300
M06	C06	Control	18680
M07	C07	Control	50
M08	C08	Control	10
M09	C09	Control	760
M10	C10	Control	80
M11	C11	Control	70
M12	C12	Control	10
M13	C13	Control	70
M14	C14	Control	50
M15	C15	Control	500
M16	C16	Control	400
M17	C17	Control	310
M18*	C18	Control	2500
M19	C19	Control	130
M20	C20	Control	6750
M21	C21	Control	500
M22*	C22	Control	320
M23	NP01	Pain- No Trauma	20
M18*	NP02	Pain- No Trauma	760
M24	NP03	Pain- No Trauma	580
M25	NP04	Pain- No Trauma	690
M02*	NP05	Pain- No Trauma	380
M22*	NP06	Pain- No Trauma	210
M26	NP07	Pain- Trauma	110
M27	NP08	Pain- Trauma	450

M28	NP09	Pain- Trauma	990
M03*	NP10	Pain- Trauma	12000
M29	NP11	Pain- Trauma	340
B01	BC01	Control	410
B01	BM01	Mastitis (Untreated)	330
B01	BM02	Mastitis (Untreated)	1010
B02	BM03	Mastitis (Treated)	1400
B02	BC02	Control	20
B03	BC03	Control (Colostrum)	30
B04	BM04	Mastitis (Untreated)	50
V01	BV01	VAT 1	4630
V02	BV02	VAT 2	4330

(*) Indicates paired samples for human participants who donated both a control and nipple pain sample.

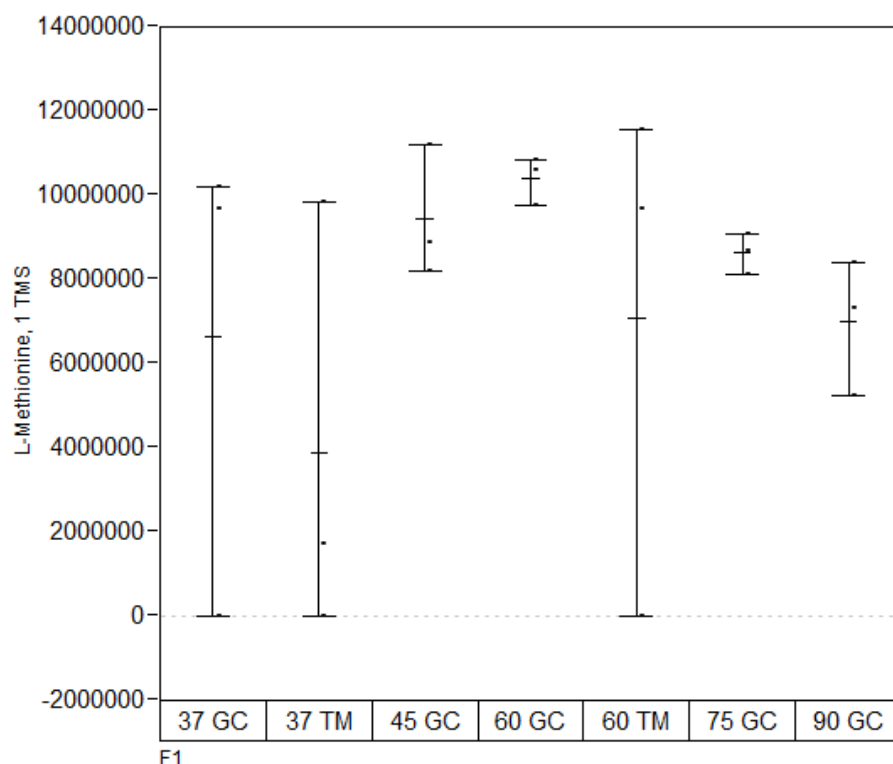
Appendix 3.4. Complete Na⁺/K⁺ data set.

Participant ID	Sample ID	Group	Na⁺ (mM)	K⁺ (mM)	Na⁺/K⁺ Ratio
M01	C01	Control	6.490	12.416	0.523
M02*	C02	Control	5.744	14.403	0.399
M03*	C03	Control	4.073	13.461	0.303
M04	C04	Control	4.555	11.757	0.387
M05	C05	Control	7.138	12.635	0.565
M06	C06	Control	5.126	13.431	0.382
M07	C07	Control	4.699	13.199	0.356
M08	C08	Control	4.407	12.553	0.351
M09	C09	Control	3.613	11.108	0.325
M10	C10	Control	3.948	13.379	0.295
M11	C11	Control	4.918	13.579	0.362
M12	C12	Control	4.490	13.608	0.330
M13	C13	Control	3.933	12.858	0.306
M14	C14	Control	4.593	11.886	0.386
M15	C15	Control	5.222	15.013	0.348
M16	C16	Control	2.541	10.047	0.253
M17	C17	Control	3.204	12.443	0.257
M18*	C18	Control	3.613	10.541	0.343
M19	C19	Control	3.990	13.274	0.301
M20	C20	Control	3.409	11.527	0.296
M21	C21	Control	2.698	14.275	0.189
M22*	C22	Control	2.621	12.447	0.211
M23	NP01	Pain- No Trauma	3.641	11.217	0.325
M18*	NP02	Pain- No Trauma	4.809	13.466	0.357
M24	NP03	Pain- No Trauma	6.068	14.179	0.428
M25	NP04	Pain- No Trauma	9.212	16.552	0.557
M02*	NP05	Pain- No Trauma	4.720	14.332	0.329
M22*	NP06	Pain- No Trauma	6.392	11.527	0.555
M26	NP07	Pain- Trauma	11.528	14.209	0.811
M27	NP08	Pain- Trauma	7.032	14.580	0.482

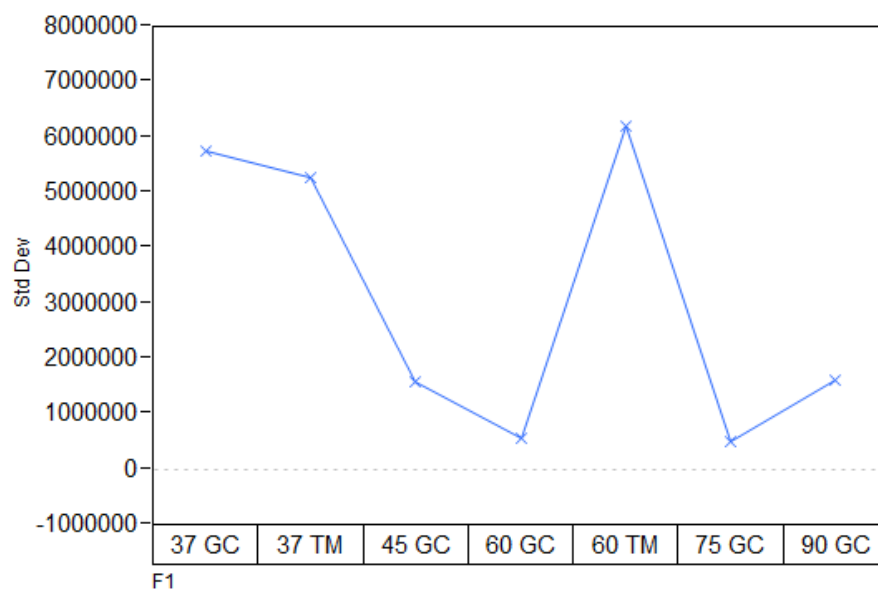
M28	NP09	Pain- Trauma	7.300	14.301	0.510
M03*	NP10	Pain- Trauma	9.136	16.875	0.541
M29	NP11	Pain- Trauma	5.204	12.277	0.424
B01	BC01	Control	19.779	21.011	0.941
B01	BM01	Mastitis (Untreated)	45.482	12.789	3.556
B01	BM02	Mastitis (Untreated)	29.776	17.654	1.687
B02	BM03	Mastitis (Treated)	26.562	20.432	1.300
B02	BC02	Control	8.442	25.495	0.331
B03	BC03	Control (Colostrum)	22.357	24.527	0.912
B04	BM04	Mastitis (Untreated)	19.213	25.440	0.755
V01	BV01	VAT 1	11.528	24.058	0.479
V02	BV02	VAT 2	11.552	24.370	0.474

** Indicate mothers that have given both a control and nipple pain sample*

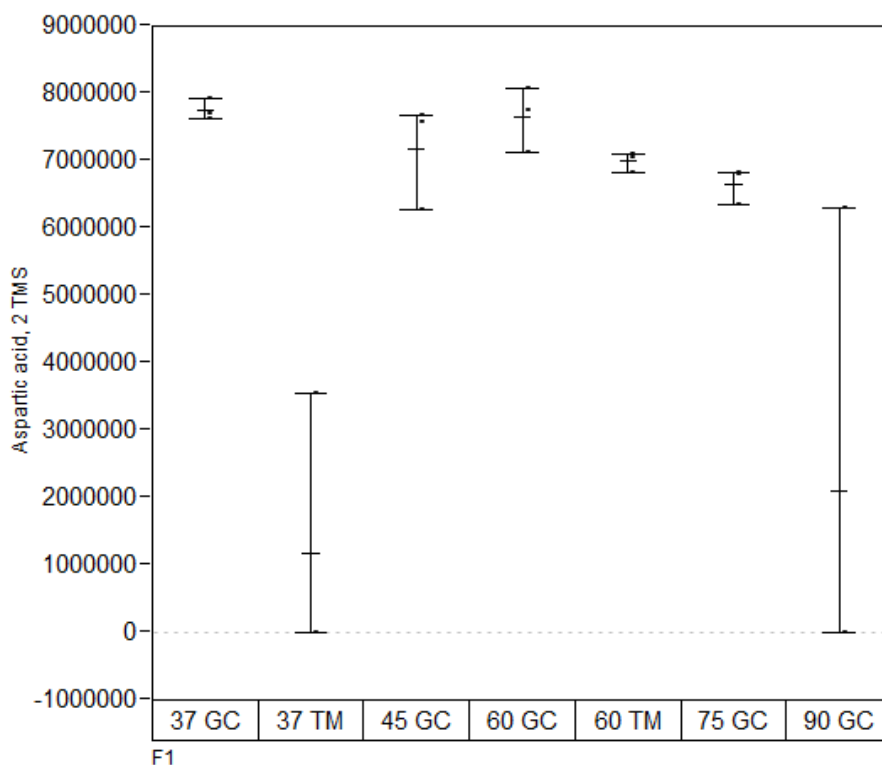
Appendix 3.5a Measured peak area of L-Methionine (1 TMS) MSTFA incubation temperatures of 37°C, 45°C, 60°C, 75°C, 90°C with the incubation performed in a GC oven (GC) or with agitation in a Thermomixer (TM).



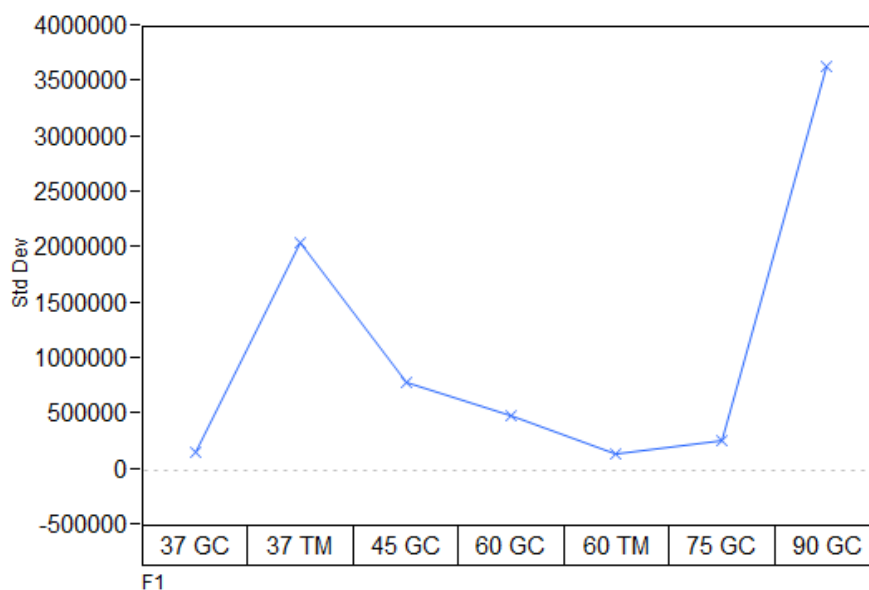
Appendix 3.5b Standard deviation of the measured peak area (n=3) L-Methionine (1 TMS) using MSTFA incubation temperatures of 37°C, 45°C, 60°C, 75°C, 90°C with the incubation performed in a GC oven (GC) or with agitation in a Thermomixer (TM).



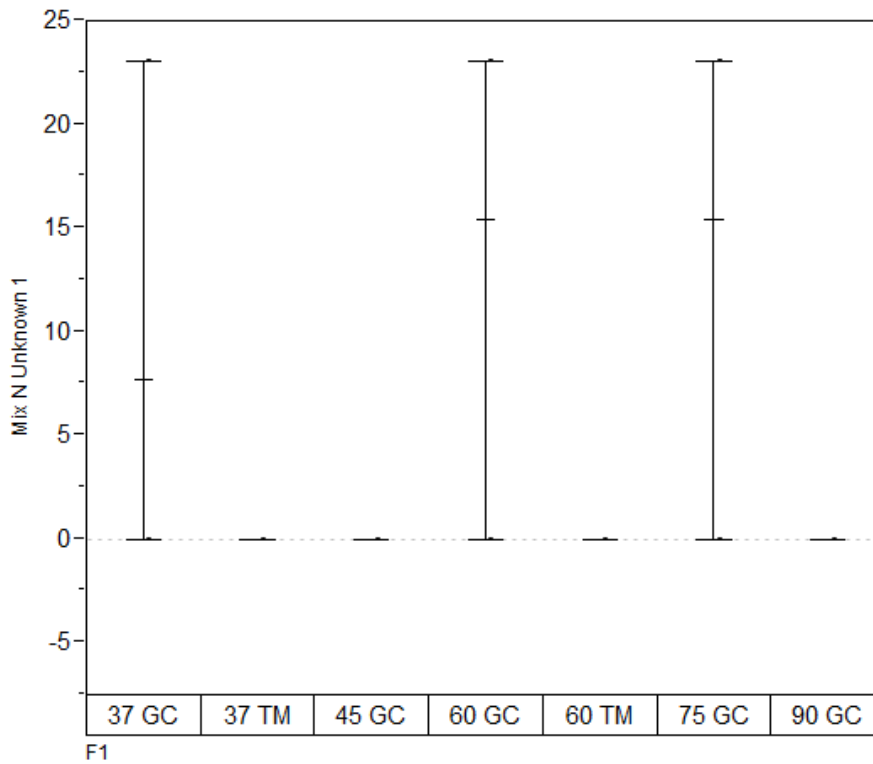
Appendix 3.5c Measured peak area of Aspartic acid (2 TMS) using MSTFA incubation temperatures of 37°C, 45°C, 60°C, 75°C, 90°C with the incubation performed in a GC oven (GC) or with agitation in a Thermomixer (TM).



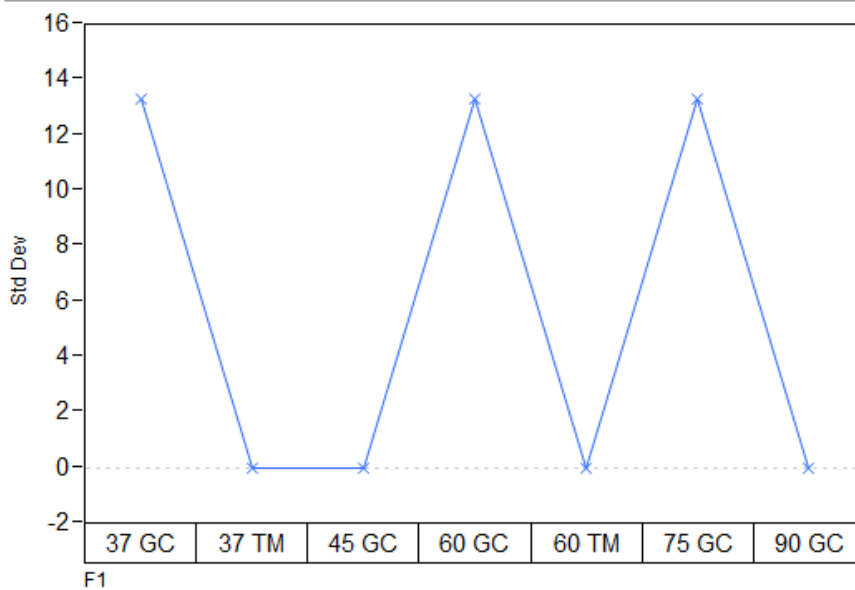
Appendix 3.5d Standard deviation of the measured peak (n=3) of Aspartic acid (2 TMS) using MSTFA incubation temperatures of 37°C, 45°C, 60°C, 75°C, 90°C with the incubation performed in a GC oven (GC) or with agitation in a Thermomixer (TM).



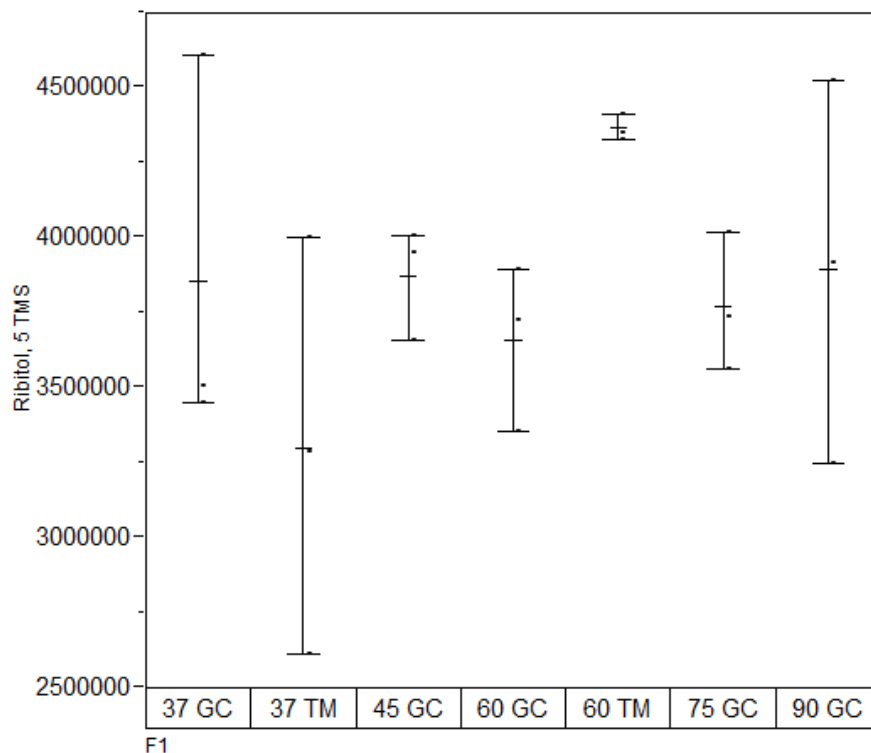
Appendix 3.5e Measured peak area of Mix N Unknown 1 using MSTFA incubation temperatures of 37°C, 45°C, 60°C, 75°C, 90°C with the incubation performed in a GC oven (GC) or with agitation in a Thermomixer (TM).



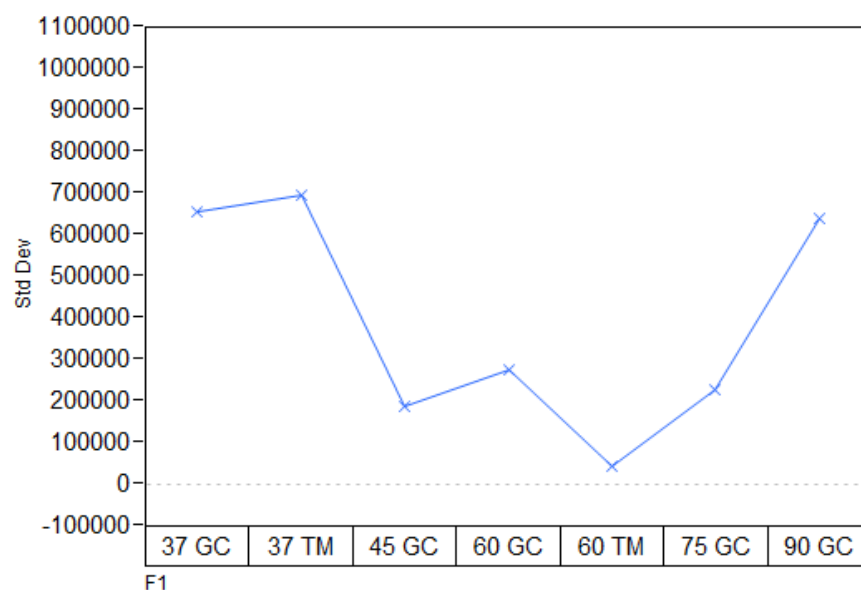
Appendix 3.5f Standard deviation of the measured peak area (n=3) of Mix N Unknown 1 using MSTFA incubation temperatures of 37°C, 45°C, 60°C, 75°C, 90°C with the incubation performed in a GC oven (GC) or with agitation in a Thermomixer (TM).



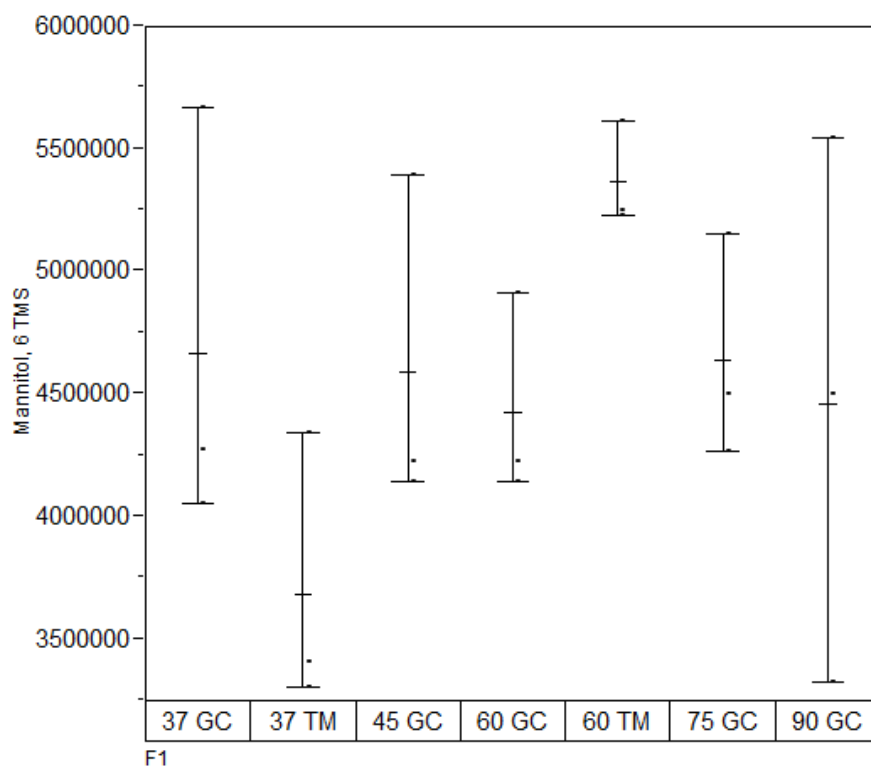
Appendix 3.5g Measured peak area of Ribitol (5 TMS) using MSTFA incubation temperatures of 37°C, 45°C, 60°C, 75°C, 90°C with the incubation performed in a GC oven (GC) or with agitation in a Thermomixer (TM).



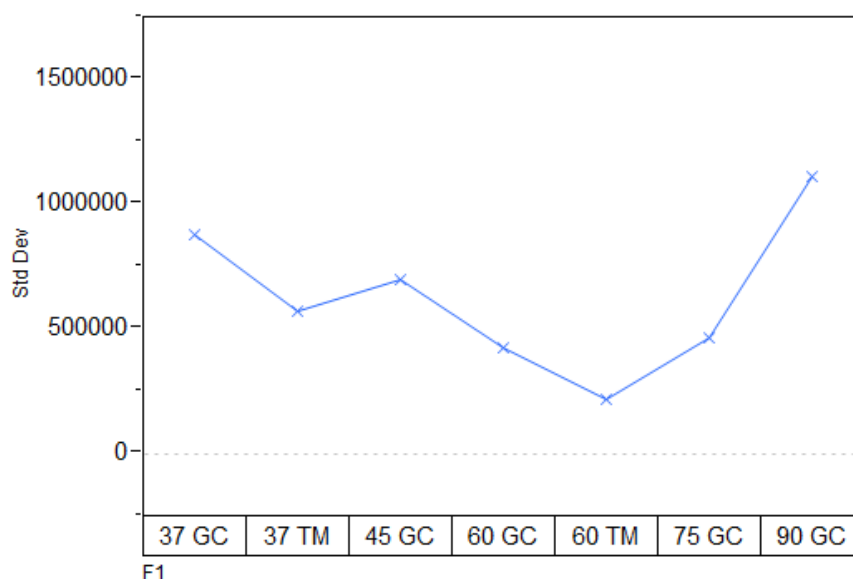
Appendix 3.5h Standard deviation of the measured peak area (n=3) of Ribitol (5 TMS) using MSTFA incubation temperatures of 37°C, 45°C, 60°C, 75°C, 90°C with the incubation performed in a GC oven (GC) or with agitation in a Thermomixer (TM).



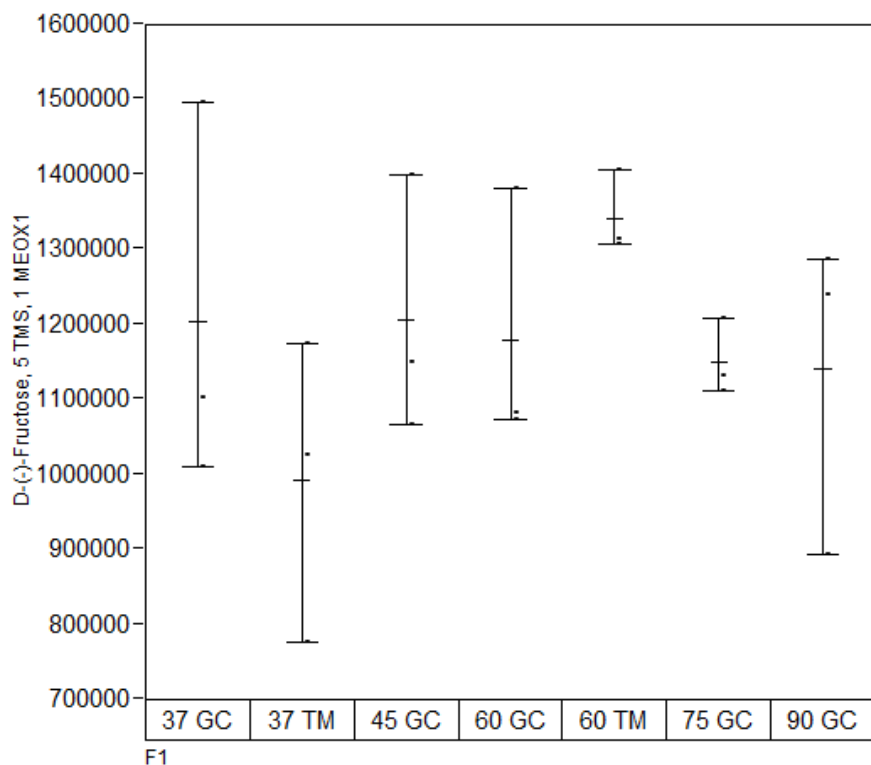
Appendix 3.5i Measured peak area of Mannitol (6 TMS) using MSTFA incubation temperatures of 37°C, 45°C, 60°C, 75°C, 90°C with the incubation performed in a GC oven (GC) or with agitation in a Thermomixer (TM).



Appendix 3.5j Standard deviation of the measured peak area (n=3) of Mannitol (6 TMS) using MSTFA incubation temperatures of 37°C, 45°C, 60°C, 75°C, 90°C with the incubation performed in a GC oven (GC) or with agitation in a Thermomixer (TM).

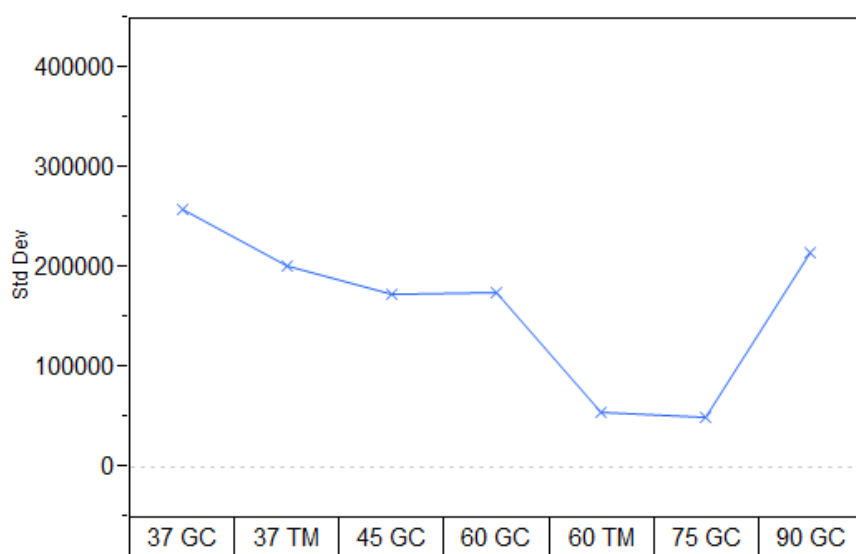


Appendix 3.5k Measured peak area of D(-)-Fructose (5 TMS, 1 MEOX) using MSTFA incubation temperatures of 37°C, 45°C, 60°C, 75°C, 90°C with the incubation performed in a GC oven (GC) or with agitation in a Thermomixer (TM).



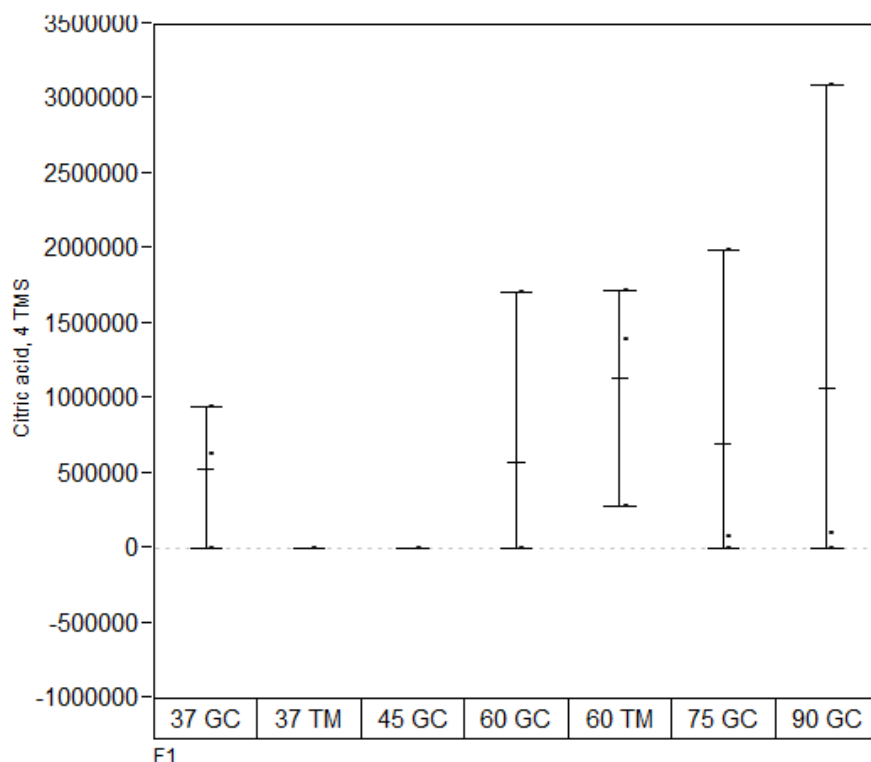
F1

Appendix 3.5l Standard deviation of the measured peak area (n=3) of D(-)-Fructose (5 TMS, 1 MEOX) using MSTFA incubation temperatures of 37°C, 45°C, 60°C, 75°C, 90°C with the incubation performed in a GC oven (GC) or with agitation in a Thermomixer (TM).

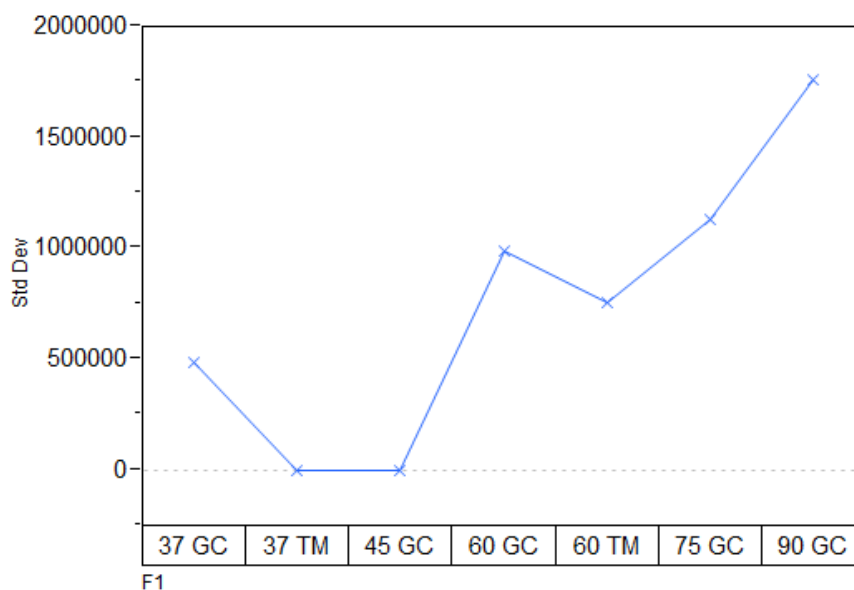


F1

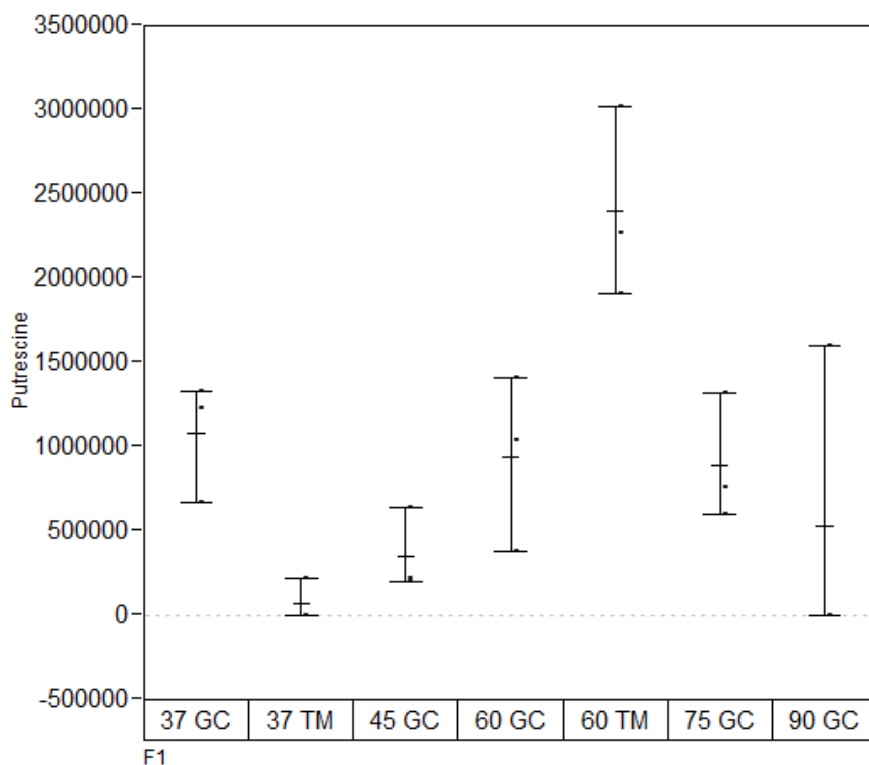
Appendix 3.5m Measured peak area of Citric acid (4 TMS) using MSTFA incubation temperatures of 37°C, 45°C, 60°C, 75°C, 90°C with the incubation performed in a GC oven (GC) or with agitation in a Thermomixer (TM).



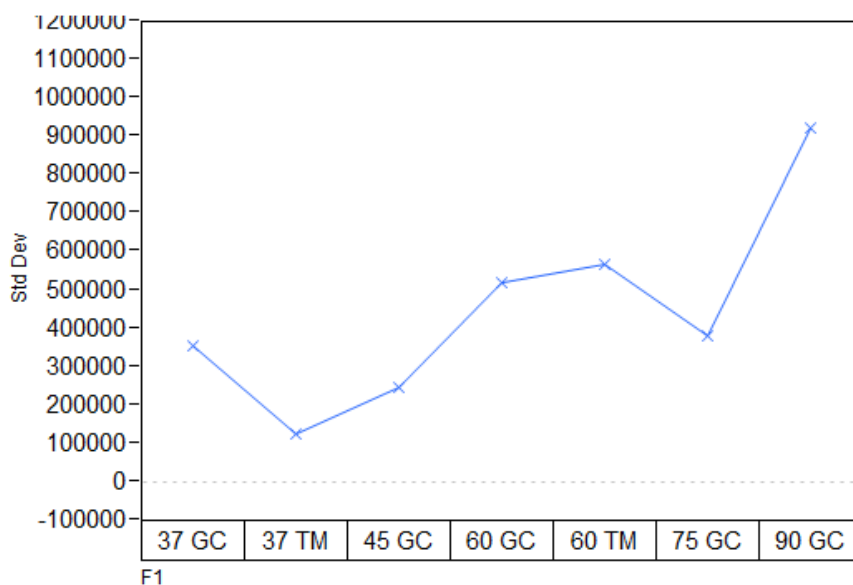
Appendix 3.5n Standard deviation of the measured peak area (n=3) of Citric acid (4 TMS) using MSTFA incubation temperatures of 37°C, 45°C, 60°C, 75°C, 90°C with the incubation performed in a GC oven (GC) or with agitation in a Thermomixer (TM).



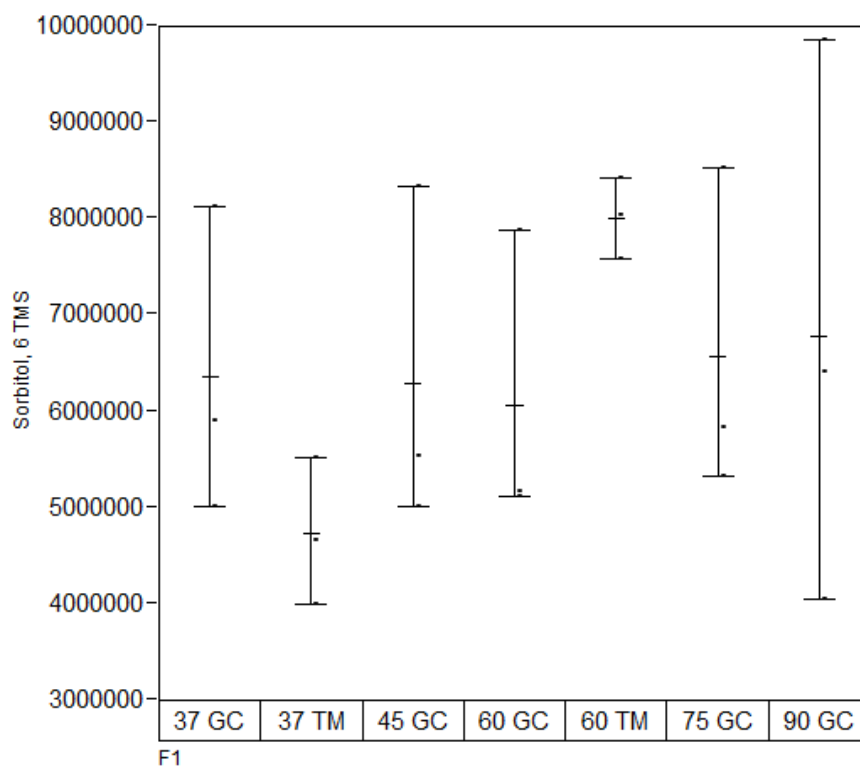
Appendix 3.5o Measured peak area of Putrescine using MSTFA incubation temperatures of 37°C, 45°C, 60°C, 75°C, 90°C with the incubation performed in a GC oven (GC) or with agitation in a Thermomixer (TM).



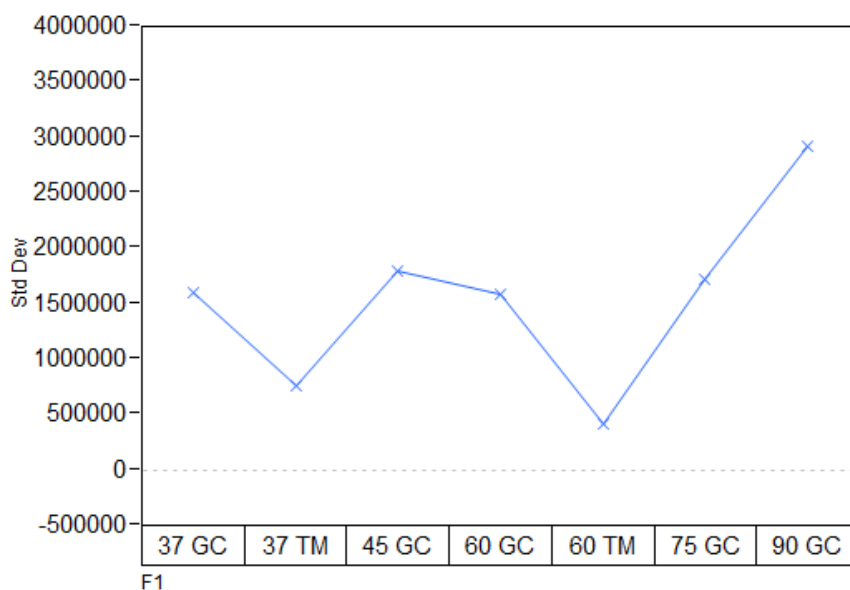
Appendix 3.5p Standard deviation of the measured peak area (n=3) of Putrescine using MSTFA incubation temperatures of 37°C, 45°C, 60°C, 75°C, 90°C with the incubation performed in a GC oven (GC) or with agitation in a Thermomixer (TM).



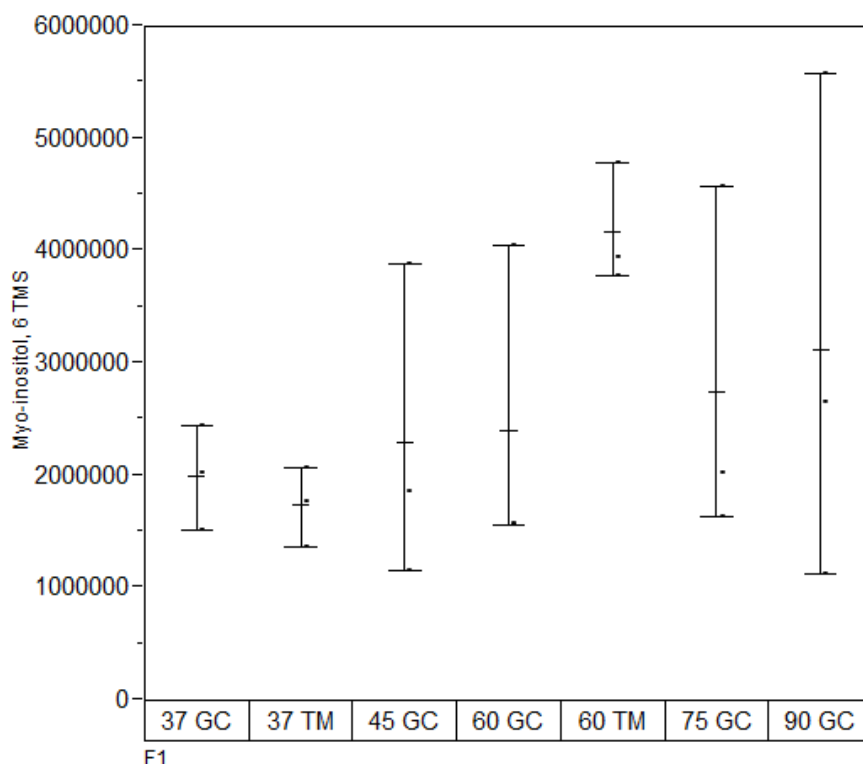
Appendix 3.5q Measured peak area of Sorbitol (5 TMS) using MSTFA incubation temperatures of 37°C, 45°C, 60°C, 75°C, 90°C with the incubation performed in a GC oven (GC) or with agitation in a Thermomixer (TM).



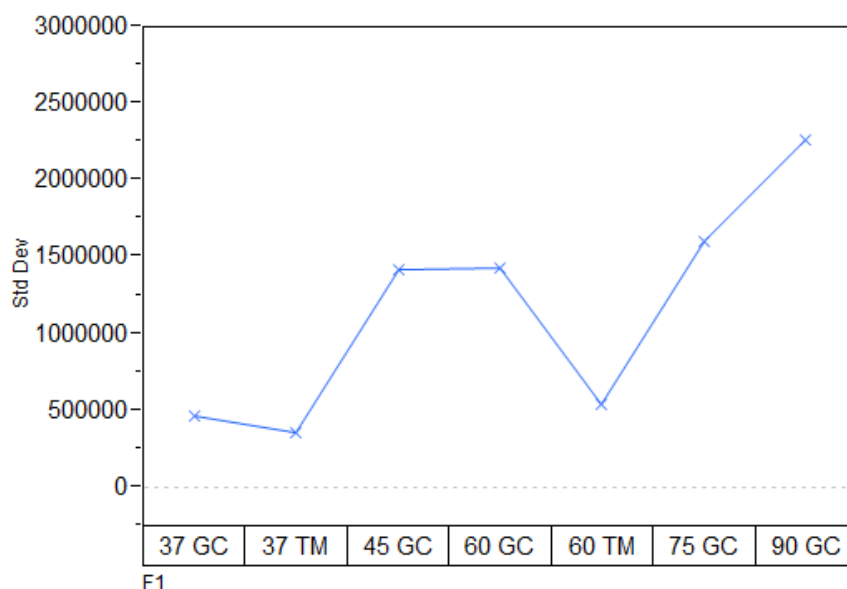
Appendix 3.5r Standard deviation of the measured peak area (n=3) of Sorbitol (5 TMS) using MSTFA incubation temperatures of 37°C, 45°C, 60°C, 75°C, 90°C with the incubation performed in a GC oven (GC) or with agitation in a Thermomixer (TM).



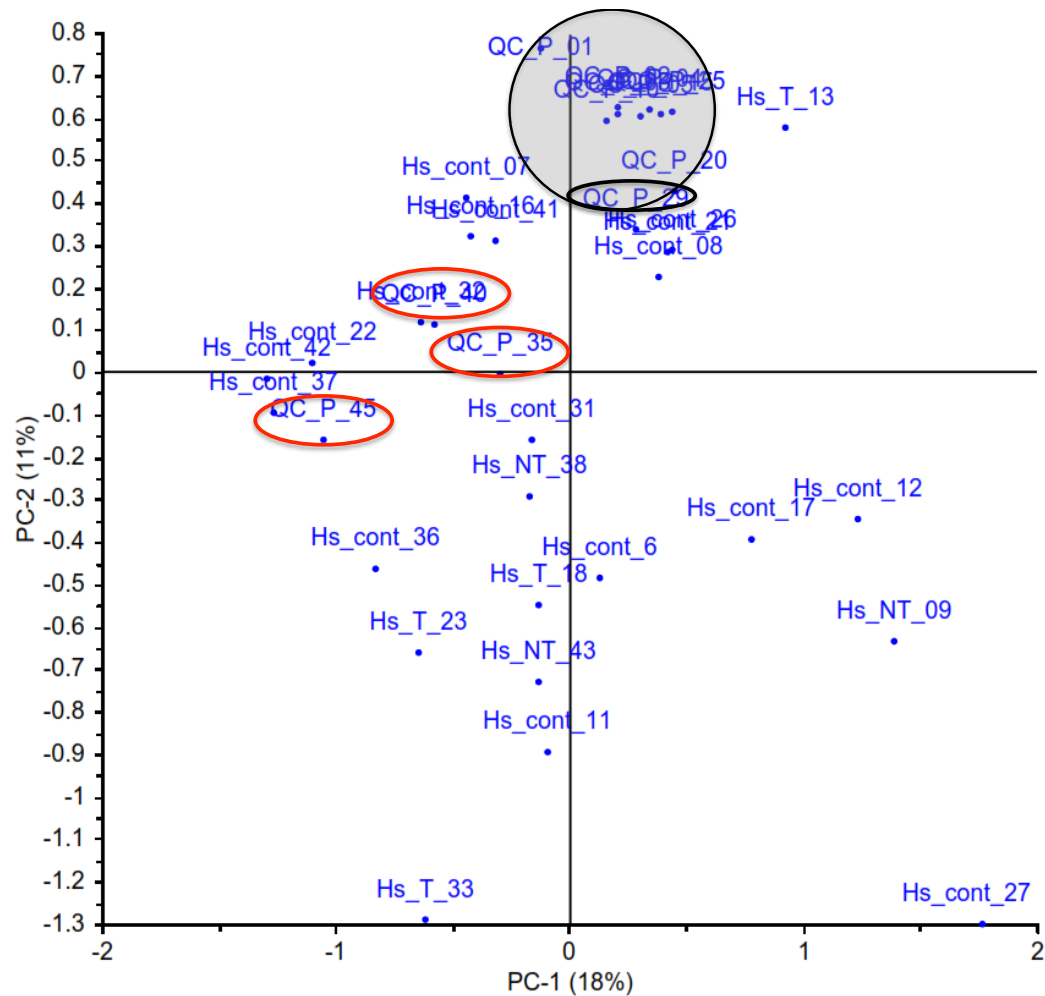
Appendix 3.5s Measured peak area of Myo-inositol (6 TMS) using MSTFA incubation temperatures of 37°C, 45°C, 60°C, 75°C, 90°C with the incubation performed in a GC oven (GC) or with agitation in a Thermomixer (TM).



Appendix 3.5t Standard deviation of the measured peak area (n=3) of Myo-inositol (6 TMS) using MSTFA incubation temperatures of 37°C, 45°C, 60°C, 75°C, 90°C with the incubation performed in a GC oven (GC) or with agitation in a Thermomixer (TM).



Appendix 3.6 Scores plot of PC-1 (18%) vs. PC-2 (11%) illustrating the reproducibility of the pooled quality controls



The black circle indicates the cluster of QCs with comparatively lower variance; the red circles indicate where variation in QCs is introduced by instrumental maintenance cycles.