

1 *Supporting Information*

2 **High-Throughput Quantitation of Proline Betaine in Foods and**
3 **Suitability as a Valid Biomarker for Citrus Consumption**

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20 **Supplemental table 1: Precision and Accuracy of standard solutions over 3 months.**
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Nominal (pmol/mL)	First measurement (n=5)		Second measurement (after 2 months, n=3)		Third measurement (after 3 months, n=3)	
	Calculated (pmol/mL, RSD)	Accuracy (%)	Calculated (pmol/mL, RSD)	Accuracy (%)	Calculated (pmol/mL, RSD)	Accuracy (%)
10	10.1 (4.7%)	101	10.7 (4.4%)	107	10.5 (0.8%)	105
19	19.8 (2.0%)	105	19.1 (6.6%)	101	20.3 (1.9%)	107
39	39.5 (4.0%)	101	39.7 (4.8%)	102	38.9 (0.7%)	99.6
78	78.5 (2.8%)	101	80.3 (5.1%)	103	77.9 (0.9%)	99.8
156	150.1 (1.9%)	96.2	148.8 (4.1%)	95.4	149.3 (0.9%)	95.7
313	314.7 (1.3%)	101	305.4 (0.9%)	97.6	307.8 (0.8%)	98.3
625	611.3 (1.2%)	97.8	608.6 (1.5%)	97.4	601.5 (3.5%)	97.4
1250	1218.5 (2.6%)	97.5	1179.6 (0.5%)	94.4	1200.2 (0.8%)	96.0
2500	2469.9 (2.9%)	98.8	2384.7 (1.1%)	95.4	2469.1 (2.8%)	98.8
5000	5096.9 (2.5%)	102	5221.7 (0.6%)	104	5107.5 (2.6%)	102

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26 **Supplemental table 2:** Validation data for quantification of proline betaine in fruit juice
 27 (pineapple juice serves as matrix)

	Nominal (mg/L)	Calculated (mg/L) ^b	Precision (%) ^c	Accuracy (%) ^d
Aqueous QC	620	647±4.02	0.6	104
Pineapple juice ^a	72.0	76.2±0.6	0.9	106
		73.8±0.8	1.1	102
		75.4±1.4	1.9	105
Pineapple juice ^a	720	744±8.1	1.1	103
		721±13.9	1.9	100
		689±8.2	1.2	95.7

^a pineapple juice with no detectable amount of PB was used as matrix for spiking; each spiking level was prepared in triplicate and each analyzed in replicates (n=5), ^b ±standard deviation, ^c precision calculated as relative standard deviation (RSD,%), ^d accuracy calculated as agreement of nominal and determined concentration.

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Supplemental Table 3: details on proline betaine in citrus and other fruit juices.

Citrus		Proline Betaine (mg/L)
White Grapefruit	Freshly squeezed	409 (± 10 , n=3) ^a
		307
	Commercial juice	476
		325
		342
		507
398		
Pink Grapefruit	Freshly squeezed	597
	Commercial juice	413
		358
		495
		470
		301
		340
583		
Lime	Freshly squeezed	431 (± 21 , n=3) ^a
		330
	Commercial juice	459
		357
		347
322		
Lemon	Freshly squeezed	143
	Commercial juice	186
		281
		253
		257
Orange	Freshly squeezed	881 (± 38 , n=5) ^a
		1091 (± 4 , n=3) ^a
	Commercial juice	345
		751
		825
		702
		613
		717
		546
		462
798		
Tangerine		800 (± 11 , n=3) ^a
Assorted juices/drinks, and seasonings		Proline Betaine (mg/L)
Pineapple	Commercial juice	< 0.1
Apple	Commercial juice	0.2
Grapes	Commercial juice	0.1
Carbonated soft drink containing orange juice		14.1
Lemonade containing grapefruit juice		26.5
Radler (50/50 mixture of beer and lemonade)		32.2
Mai tai (commercial cocktail containing lime juice)		302
Roast coffee brew (Arabica)		0.25
White vinegar		< 0.1
Vinegar made from raspberry		0.4
Soy sauce		8.8

Quantitative data are from n=1 unless noted, criteria for unequivocal identification of proline betaine were retention time, quantifier, qualifier and their ratio, ^a Standard-Deviation

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34 **Supplemental Table 4:** details on proline betaine in beer and wine.

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Beer samples	Beer class/type	EtOH (%) ^a	Proline Betaine (µg/L)
	Red ale	4.2	241
		4.5	43
	Pils	4.9	77
		4.8	25
	Stout	4.1	226 (±9, n=4)
	Lager	5.7	64
		4.9	90 (±3, n=4)
		5.2	41
		5.1	30
		4.9	43
		5.0	53
	Alcohol free beer	0.0	27
	Wheat Beer	4.9	44
	Dark Lager	5.0	46
		5.1	42
		4.9	133
	Strong Beer	5.8	27
		7.9	58

Wine samples	Origin, year	EtOH (%)	Proline Betaine (µg/L)
Red Wine	South Africa, 2011	14.0	181 (±4, n=3) ^b
	Italia, 2010	16.0	3572
	Italia, 2009	16.0	6966
	Italia, 2011	14.0	111
	Italia, 2010	14.0	1412
	Italia, 2010	13.0	232
	Italia, 2012	14.0	1019
	Italia, 2011	14.0	1910
	Italia, 2011	14.0	1164
	Italia, 2011	13.5	144
	Italia, 2010	16.0	2868
	USA, 2008	14.5	114
	USA, 2009	14.5	63
	USA, 2011	13.5	383
	USA, 2013	14.4	377
	USA, 2012	14.5	217
	USA, 2013	13.8	881
	Chile, 2013	13.5	640
	Chile, 2011	13.5	351
	Argentina, 2010	14.0	117
Australia, 2010	14.5	164	
Austria, 2011	13.5	180	
Germany, 2011	13.5	123	
White Wine	USA, 2013	14.5	155
	Italia, 2014	13.0	152
	Italia, 2013	13.0	67

Quantitative data are from n=1 unless noted, criteria for unequivocal identification of Proline Betaine were retention time, quantifier, qualifier and their ratio, ^a EtOH according to the label; ^b Standard-Deviation

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37 **Supplemental Table 5:** details on proline betaine in solid food products.
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Citrus containing Sweets		Proline Betaine (mg/kg)
Mousse au Citron		25.0
Marmelade (from orange)		73.8
Yoghurt containing orange		15.0
Chocolade containing orange		75.2
Gummy bear orange color		12.4
Gummy bear yellow color		4.1
Orange biscuit (Jaffa cake)		92.6
Orange flavored infant food		193
Orange flavor for baking		174
Animal source food		Proline Betaine (mg/kg)
Milk (1.5% fat)		<1
Curd (40% fat in drymass)		<1
Cheese (Gouda)		<1
Cheese (Gorgonzola)		138
Goat Ceese		<1
Meat (beef)		<1
Meat (pork)		1.3
Meat (chicken)		<1
Other Plant source food/plant products		Proline Betaine (mg/kg)
Carrot	Raw	<1
Cucumber	Raw, with peel	<1
Broccoli	Raw	<1
Potatoe	Raw, with peel	<1
Black Tea	Powder	<1
Raw coffee	Robusta, washed	<1
	Robusta, unwashed	3.9
	Arabica, dry processed	1.2
	Arabica, wet processed	<1
Fungi		Proline Betaine (mg/kg)
Chanterelle		<1
Champignon		1.3
Fish		Proline Betaine (mg/kg)
Rainbow trout (<i>Oncorhynchus mykiss</i>)	Frozen	0.3
Alaska Pollock (<i>Gadus chalcogrammus</i>)	Frozen	1.5
Codfish (<i>Gadus morhua</i>)	Frozen	0.2
Chum salmon (<i>Oncorhynchus keta</i>)	Frozen	0.8
European perch (<i>Perca fluviatilis</i>)	Frozen	<0.1
Tuna (<i>Thunnus albacares</i>)	Frozen	0.2
Haddock (<i>Malanogrammus aeglefinus</i>)	Frozen	2.8
Zander (<i>Stizostedion lucioperca</i>)	Frozen	0.1
Caviar from trout		0.6
Seafood		Proline Betaine (mg/kg)
Whiteleg shrimp (<i>Litopenaeus vannamei</i>)	blanched, husked	1.8
	blanched, husked, from india	8.3 (±0.7, n=2) ^a
	raw	3.4
	blanched, from india	3.9
Tiger shrimp (<i>Penaeus vannamei</i>)	Blanched, husked, from vietnam	3.6
	Blanched, from india	1.7 (±0.1, n=2)
Giant tiger Shrimp (<i>Penaeus monodon</i>)	Raw, husked, from india	6.7 (±0.1, n=2) ^a
Shrimp (<i>Pleoticus muelleri</i>)	Raw, husked, from argentina	24.9 (±0.7, n=2) ^a
	Raw, husked, from argentina	34.7
Composition of shrimps (<i>Penaeidae</i> + <i>L. vannamei</i>)	from indian ocean	6.6 (±0.6, n=2) ^a
Clam (<i>Placopecten magallanicus</i>)	Raw, without shell	61.3 (±0.8, n=2) ^a
Blue mussel (<i>Mytilus edulis</i>)	Raw, without shell, from north eastern atlantic	15.0 (±0.4, n=2) ^a
	Raw, without shell, from north eastern atlantic	26.9 (±1.9, n=2) ^a
	Raw, without shell	75.7 (±7.4, n=3) ^a
	Cooked, from New Zealand	49.5 (±3.9, n=2) ^a
Greenshell mussel (<i>Perna canalicus</i>)		
Pharaoh cuttlefish (<i>Sepia pharaonic</i>)	Raw, from indian ocean	2.0 (±0.2, n=2)
Indian ocean squid (<i>Loligo duvauceli</i>)	Raw, from indian ocean	2.3 (±0.1, n=2) ^a
American Lobster (<i>Homarus americanus</i>)		95.2 (±4.9, n=2) ^a

Quantitative data are from n=1 unless noted, criteria for unequivocal identification of Proline Betaine were retention time, quantifier, qualifier and their ratio, ^a Standard-Deviation

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