

Article title: Development, validation, and use of ¹H-NMR spectroscopy for evaluating the quality of acerola-based food supplements and quantifying ascorbic acid

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Table S1. Calculated (AA area/Choline area) ratio for ¹H-NMR spectra of 117 samples of acerola concentrate juice

Sample code	AA area B4_8905	Choline area B3_2595	Ratio (AA area/ Choline area)
Acerola EC 2021139LC01-E1_2	117.96	52.96	2.228
Acerola EC 2021139LC01-E2_2	96.96	43.66	2.221
Acerola EC 2021139LC02-E1_2	111.55	51.70	2.158
Acerola EC 2021139LC02-E2_2	128.62	59.60	2.158
Acerola EC 2021174LC01-E1_2	114.65	56.95	2.013
Acerola EC 2021174LC01-E2_2	137.49	68.60	2.004
Acerola EC 2021189LC01-E1_2	126.72	64.59	1.962
Acerola EC 2021189LC01-E2_2	126.71	64.21	1.973
Acerola EC 2021202LC01-E1_2	136.79	66.63	2.053
Acerola EC 2021202LC01-E2_2	142.14	69.60	2.042
Acerola EC 20190-1 E1_2	119.69	60.52	1.978
Acerola EC 20190-1 E2_2	117.15	58.75	1.994
Acerola EC 20197-1 E1_2	138.29	72.33	1.912
Acerola EC 20197-1 E2_2	92.91	48.44	1.918
Acerola EC 20197-2 E1_2	96.37	46.60	2.068
Acerola EC 20197-2 E2_2	111.99	53.60	2.089
Acerola EC 2021006LC01-E1_2	94.14	48.41	1.945
Acerola EC 2021006LC01-E2_2	102.61	52.69	1.947
Acerola EC 2021006LC02-E1_2	87.86	44.60	1.970
Acerola EC 2021006LC02-E2_2	106.21	53.64	1.980
Acerola EC 2021013LC01-E1_2	96.86	54.86	1.765
Acerola EC 2021013LC01-E2_2	108.22	61.02	1.773
Acerola EC 2021013LC02-E1_2	103.23	57.76	1.787
Acerola EC 2021013LC02-E2_2	111.15	62.11	1.789
Acerola EC 2021042LC03-E1_2	86.61	55.47	1.561
Acerola EC 2021042LC03-E2_2	117.39	75.28	1.559
Acerola EC 2021056LC01-E1_2	107.97	44.93	2.403
Acerola EC 2021056LC01-E2_2	106.04	44.20	2.399

Acerola EC 2021069LC01-E1_2	92.50	42.37	2.183
Acerola EC 2021069LC01-E2_2	115.93	53.12	2.182
Acerola EC 2021070LC01-E1_2	102.51	56.02	1.830
Acerola EC 2021070LC01-E2_2	128.48	70.29	1.828
Acerola EC 2021076LC02-E1_2	95.93	43.46	2.207
Acerola EC 2021076LC02-E2_2	119.06	53.60	2.221
Acerola EC 2021077LC02-E1_2	86.81	39.62	2.191
Acerola EC 2021077LC02-E2_2	110.72	50.74	2.182
Acerola EC 2021096LC03-E1_2	91.26	46.59	1.959
Acerola EC 2021096LC03-E2_2	87.59	44.49	1.969
Acerola EC 2021132LC01-E1_2	159.56	81.76	1.952
Acerola EC 2021132LC01-E2_2	127.81	65.28	1.958
Acerola EC 2021223LC01-E1_2	145.11	86.41	1.679
Acerola EC 2021223LC01-E2_2	116.56	68.57	1.700
Acerola EC 2021274LC02-E1_2	117.31	69.10	1.698
Acerola EC 2021274LC02-E2_2	121.46	71.69	1.694
Acerola EC 2021288LC01-E1_2	106.02	62.38	1.700
Acerola EC 2021288LC01-E2_2	106.71	63.49	1.681
Acerola EC 2021302LC01-E1_2	128.33	71.24	1.801
Acerola EC 2021302LC01-E2_2	116.17	64.15	1.811
Acerola EC 2021309LC01-E1_2	125.04	65.70	1.903
Acerola EC 2021309LC01-E2_2	138.24	72.08	1.918
Acerola EC 2021323LC01-E1_2	146.81	80.24	1.830
Acerola EC 2021323LC01-E2_2	123.68	68.01	1.818
Acerola EC 2021323LC02-bis-E1_2	143.16	77.47	1.848
Acerola EC 2021323LC02-bis-E2_2	152.26	82.45	1.847
Acerola EC 2021363LC01-E1_2	106.71	47.51	2.246
Acerola EC 2021363LC01-E2_2	116.17	52.91	2.196
Acerola EC 2021363LC02-E1_2	97.76	44.17	2.213
Acerola EC 2021363LC02-E2_2	130.41	59.18	2.203
Acerola EC 2022040LC03-E1_2	110.94	47.67	2.327
Acerola EC 2022040LC03-E2_2	129.82	55.76	2.328
Acerola EC 2022040LC04-E1_2	102.21	43.75	2.336
Acerola EC 2022040LC04-E2_2	107.64	45.99	2.341
Acerola EC 2022041LC01-E1_2	112.39	43.83	2.564

Acerola EC 2022041LC01-E2_2	96.47	37.51	2.572
Acerola EC 2022041LC02-E1_2	101.10	37.24	2.715
Acerola EC 2022041LC02-E2_2	156.56	58.14	2.693
Acerola EC 2022091LC02-E1_2	125.32	55.45	2.260
Acerola EC 2022091LC02-E2_2	116.21	51.31	2.265
Acerola EC 2022101LC01-E1_2	112.76	53.09	2.124
Acerola EC 2022101LC01-E2_2	141.67	66.73	2.123
Acerola EC 2022103LC01-E1_2	132.07	51.95	2.542
Acerola EC 2022103LC01-E2_2	143.58	56.74	2.531
Acerola EC 2022103LC02-E1_2	136.13	54.26	2.509
Acerola EC 20303-1 E1_2	117.65	72.66	1.619
Acerola EC 20303-1 E2_2	121.81	75.28	1.618
Acerola EC 20303-2 E1_2	101.32	62.72	1.615
Acerola EC 20303-2 E2_2	104.45	65.09	1.605
Acerola EC 20315-1-E1_2	146.95	86.61	1.697
Acerola EC 20315-1-E2_2	131.09	78.35	1.673
Acerola EC 20316-1-E1_2	113.92	64.70	1.761
Acerola EC 20364-1-E1_2	106.63	55.90	1.908
Acerola EC 20364-1-E2_2	113.11	58.84	1.922
Acerola EC 20364-2-E1_2	94.18	48.77	1.931
Acerola EC 20364-2-E2_2	114.37	59.24	1.931
Acerola EC B0296600-E1_2	103.82	53.25	1.950
Acerola EC B0296600-E2_2	126.36	64.84	1.949
Acerola EC B1186687-E1_2	103.64	46.35	2.236
Acerola EC B1186687-E2_2	120.47	53.79	2.240
Acerola EC F0296602-E1_2	128.59	57.19	2.248
Acerola EC F0296602-E2_2	135.06	60.29	2.240
Acerola EC H0296602-E1_2	103.97	45.45	2.287
Acerola EC H0296602-E2_2	113.13	49.25	2.297
Acerola EC J0296604-E1_2	123.37	50.94	2.422
Acerola EC J0296604-E2_2	100.18	41.55	2.411
Acerola EC K0296605-E1_2	100.29	49.43	2.029
Acerola EC K0296605-E2_2	109.36	53.67	2.038
Acerola EC L0296606-E1_2	112.60	58.23	1.934
Acerola EC L0296606-E2_2	105.38	54.36	1.939

Acerola EC L20310-1-E1_2	108.52	67.10	1.617
Acerola EC L20310-1-E2_2	104.85	63.79	1.644
Acerola EC L20315-2-E1_2	92.18	63.10	1.461
Acerola EC L20315-2-E2_2	111.62	76.19	1.465
Acerola EC L20316-1-E2_2	109.41	62.50	1.751
Acerola EC L20331-1-E1_2	126.44	67.36	1.877
Acerola EC L20331-1-E2_2	119.95	63.20	1.898
Acerola EC N0296607-E1_2	112.20	56.65	1.981
Acerola EC N0296607-E2_2	120.74	60.81	1.986
Acerola EC X0296699-E1_2	110.62	58.62	1.887
Acerola EC X0296699-E2_2	120.10	63.30	1.897
Acerola EC Z1156679-E1_2	103.66	49.18	2.108
Acerola EC Z1156679-E2_2	104.27	49.37	2.112
Acerola EC2021019LC01-E1_2	83.35	51.78	1.610
Acerola EC2021019LC01-E2_2	113.57	70.58	1.609
Acerola EC2021019LC02-E1_2	91.02	52.76	1.725
Acerola EC2021019LC02-E2_2	95.33	55.18	1.728
Acerola EC2021042LC02-E1_2	98.48	60.78	1.620
Acerola EC2021042LC02-E2_2	102.59	62.89	1.631
Mean			1.995
SD			0.275
RSD (%)			13.80%

B4_8905: bucket centered at 4.89 ppm containing the doublet of ascorbic acid H1; B3_2595: bucket centered at 3.26 ppm containing the singlet of choline (CH₃)₃; NMR area in arbitrary unit.

Table S 2. Ingredients listed on product manufacturer labels

Food supplements (D.S.)	Composition	Galenic form
Acerola reference powder	Organic acerola extract powder titrated to 17% of Vitamin C	Powder
D.S.1	Organic acerola extract powder titrated to 17% Vitamin C	Powder
D.S.2	Acerola extract powder titrated to 15.44% of Vitamin C Royal jelly <i>Citrus paradisi</i> fruit and seed extract	Tablet
D.S.3	Acerola extract powder titrated to 16.99% Vitamin C Maltodextrin	Tablet

	Cane Sugar Dried rice syrup Dextrose Gum acacia Anti-caking agents: magnesium carbonate. Talc Natural flavor: berries Sunflower oil	
D.S.4	Sucrose Vitamin C Dextrose Anti-caking agents: calcium stearate. silicon dioxide Acidifying agent: citric acid Flavor Acerola extract Cynorrhodon extract Sweet orange extract	Tablet
D.S.5	Sorbitol Acerola extract powder titrated to 25% Vitamin C Xylitol Natural flavor: berries Anti-caking agents: magnesium Stearate Stevia	Tablet
D.S.6	Acerola extract powder titrated to 25% Vitamin C Sorbitol Xylitol Cane Sugar Anti-caking agents: magnesium stearate Cherry flavor <i>Citrus sinensis</i> bioflavonoids	Tablet
D.S.7	Acerola extract powder titrated to 17% Vitamin C Vegetal cover	Capsule
D.S.8	Acerola extract powder with added Vitamin C titrated to 17% Vitamin C Blackcurrant powder Cranberry powder Anti-caking agents: calcium salts of fatty acids Sodium saccharin	Tablet
D.S.9	Acerola extract powder Sorbitol Sucralose Acesulfame potassium	Tablet

D.S.10	Anti-caking agents: calcium salts of fatty acids	Tablet
	Naturel red fruit flavor	
	Anti-caking agents: calcium salts of fatty acids	
	Sorbitol	
	Acerola extract powder	
	Anti-caking agents: calcium stearate	
	Vitamin C	
	Cherry flavor	

Table S3. Calculated (AA area/Choline area) ratio for ¹H-NMR spectra of 50 samples prepared from acerola powder

Sample code	Choline area B3_2490	AA area B4_8730	Ratio (AA area/ Choline area)
acerola bio b1388901 c11_2	20.14	52.31	2.597
acerola bio c2118201 c11_2	23.19	46.32	1.997
acerola bio f1465301 c21_2	21.69	45.10	2.080
acerola bio f1465301 c31_2	23.61	48.89	2.071
acerola bio f1465301 c41_2	22.00	45.65	2.075
acerola bio f2118101 c41_2	20.54	48.76	2.374
acerola bio f2118202 c11_2	23.26	42.82	1.841
acerola bio h2096801 c21_2	21.98	47.93	2.180
acerola bio k1377601 c241_2	20.70	46.50	2.247
acerola bio k2011701 c101_2	23.72	42.52	1.793
acerola bio k2011701 c21_2	23.82	42.75	1.794
acerola bio k2011701 c61_2	23.20	41.59	1.793
acerola bio k2263301 c11_2	20.26	44.79	2.211
acerola bio l1388601 c41_2	20.77	45.15	2.174
acerola bio l1388601 c71_2	20.40	43.49	2.132
acerola bio n1185401 c51_2	19.19	43.04	2.242
acerola bio n1185401 c81_2	18.32	41.46	2.263
acerola bio n2253001 c21_2	20.43	51.92	2.542
acerola bio n2253001 c61_2	19.59	50.13	2.559
acerola Bio R0294501-C21_2	19.09	39.78	2.083
acerola Bio R0294501-C41_2	19.36	28.88	1.491
Acerola Bio R0294501-C61_2	19.63	28.01	1.427
acerola bio r2252901 c111_2	20.14	46.56	2.312
acerola bio r2252901 c151_2	20.31	46.37	2.283
acerola bio r2252901 c71_2	19.68	45.27	2.300
acerola bio t1185201 c111_2	18.59	44.78	2.408
acerola bio t1185201 c131_2	18.34	44.18	2.409

acerola bio t2142301 c41_2	19.27	47.29	2.454
acerola bio t2142301 c61_2	19.10	47.06	2.464
acerola bio t2142301 c81_2	18.70	45.86	2.452
acerola bio u1399301 c1_2	20.57	45.87	2.230
acerola bio u1399301 c11_2	20.43	46.18	2.260
acerola bio u1399301 c31_2	20.26	45.95	2.268
acerola bio u2020701 c101_2	23.17	41.99	1.812
acerola bio u2020701 c141_2	23.21	42.69	1.840
acerola bio u2020701 c21_2	23.25	42.22	1.816
acerola bio u2020701 c61_2	22.87	41.42	1.811
acerola bio w0262001 c101_2	18.07	41.54	2.299
acerola bio w0262001 c11_2	17.83	40.87	2.292
acerola bio w0262001 c41_2	17.88	40.13	2.244
acerola bio w0262001 c81_2	18.19	41.30	2.270
acerola bio w0262001-c121_2	18.31	41.64	2.274
acerola bio w0262001-c141_2	18.43	41.92	2.275
acerola bio w0262001-c161_2	17.73	40.03	2.258
acerola bio w0262001-c181_2	18.29	40.44	2.211
acerola bio w0262001-c211_2	18.35	40.75	2.221
acerola bio w0262001-c51_2	17.72	40.19	2.268
acerola bio x1422201 c111_2	21.19	44.67	2.108
acerola bio x1422201 c131_2	22.00	46.19	2.099
acerola bio x1422201 c231_2	21.97	45.89	2.089
Mean			2.159
SD			0.254
RSD (%)			11.77%

B3_2490: bucket centered at 3.25 ppm containing the singlet of choline (CH₃)₃; B4_8730: bucket centered at 4.87 ppm containing the doublet of ascorbic acid H1; NMR area in arbitrary unit

Table S4. Repeatability of the qNMR method based on ¹H-NMR

Experiments	AA area B4_8695	TSP area B0_0170
Ascorbic Acid (Batch 1966)-1	133.26	25.34
Ascorbic Acid (Batch 1966)-2	132.63	25.13
Ascorbic Acid (Batch 1966)-3	132.08	25.19
Ascorbic Acid (Batch 1966)-4	131.44	24.99
Ascorbic Acid (Batch 1966)-5	132.48	25.01
Ascorbic Acid (Batch 1966)-6	131.38	24.94
Ascorbic Acid (Batch 1966)-7	132.43	25.03
Ascorbic Acid (Batch 1966)-8	131.79	24.76

Ascorbic Acid (Batch 1966)-9	132.32	25.05
Ascorbic Acid (Batch 1966)-10	131.15	24.85
Mean (n=10)	132.09	25.03
SD	0.656	0.166
RSD (%)	0.50%	0.66%

B4_8695: bucket centered at 4.87 ppm containing the doublet of ascorbic acid H1; B-0_0170: bucket centered at 0 ppm containing the singlet of TSP; NMR area in arbitrary unit

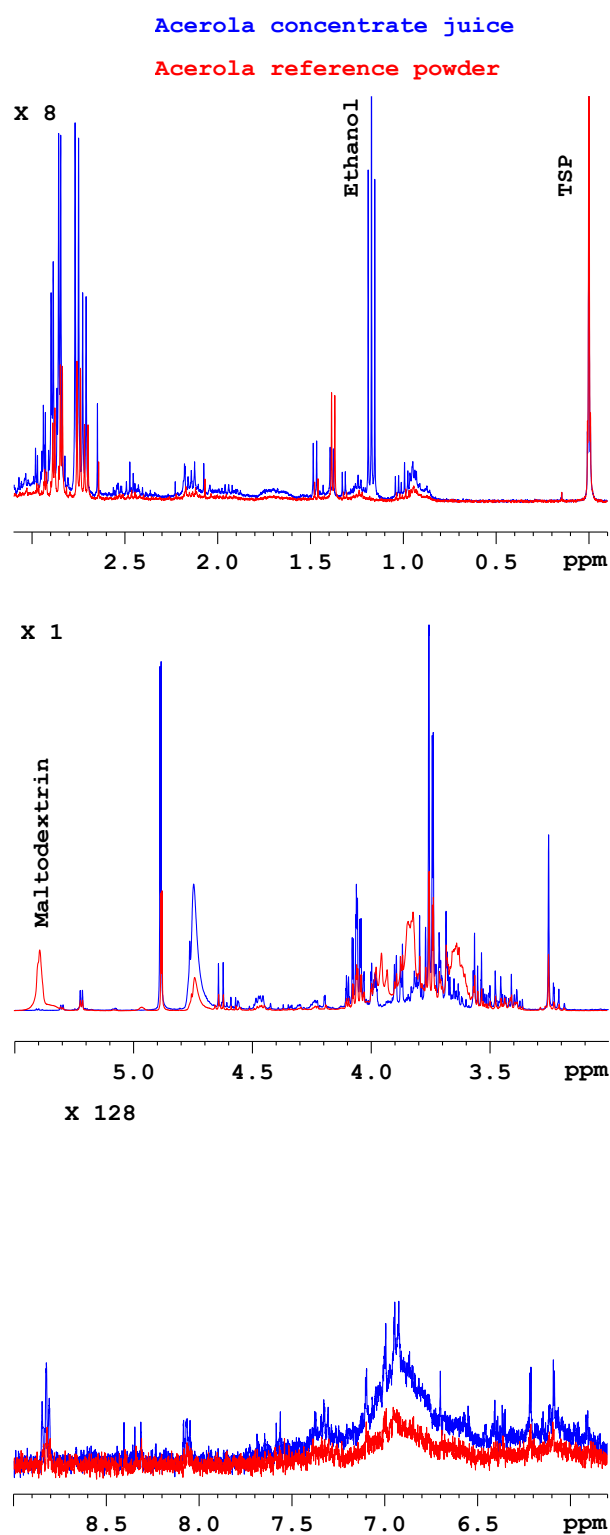


Figure S1. Overlay of 1D ^1H -NMR spectra of acerola concentrate juice (blue) and acerola reference powder (red) (400 MHz, 300 K, D_2O , pH 3.35–20 mM TSP)

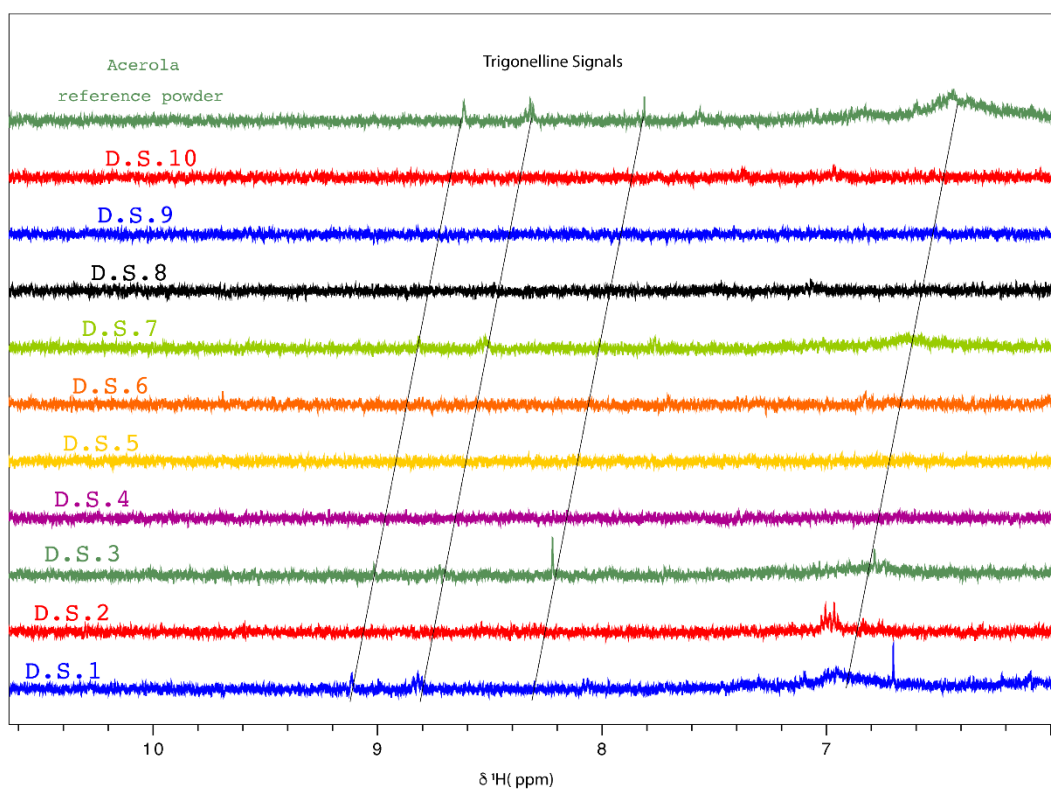


Figure S2. Zoom in on signals observed between 6 and 10 ppm (aromatic region) on food supplement ^1H -NMR spectra (400 MHz, 300 K. D_2O , pH 3.35, 20 mM TSP)

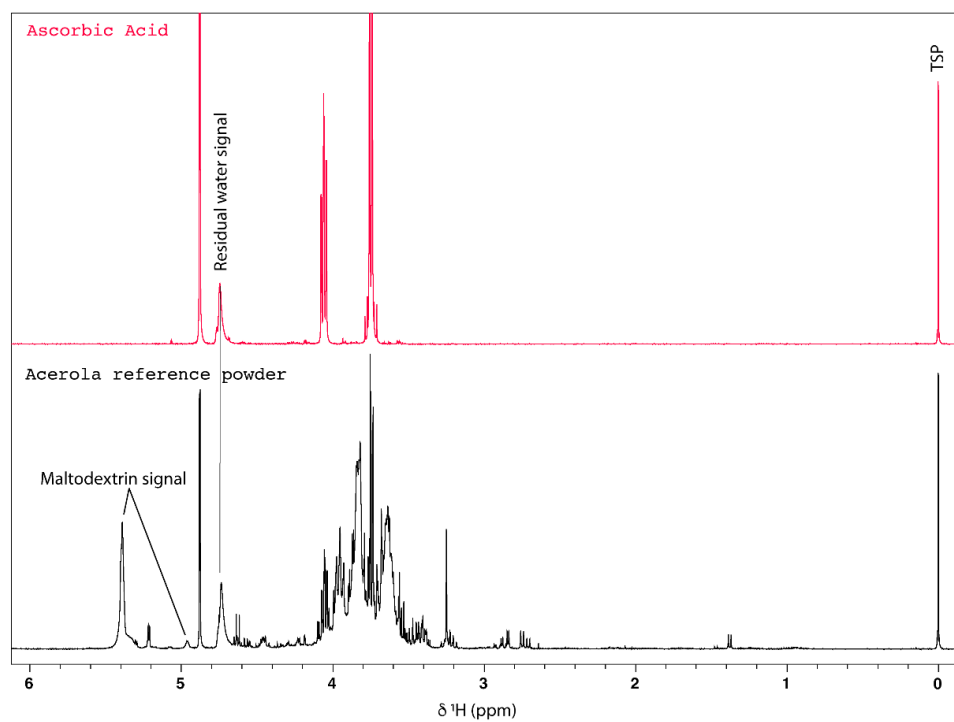


Figure S3. Comparison between spectra of ascorbic acid (red) and of the sample of solution prepared from acerola reference powder (black) (400MHz, 300 K, D₂O pH 3.35, 20 mM TSP)