The influence of food matrix on the bioavailability of curcuminoids from a dried colloidal turmeric suspension: A randomized, crossover, clinical trial

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Supplementary Table 1: Turmeric-free meal compositions

	Energy, kJ	Carbohydrate, g	Fat, g	Protein, g
Dinner (evening before all experimental sessions)				
Bottle of water, 500 mL	0	0	0	0
Beef ravioli, 400 g, or	1536	52	9	15
spaghetti bolognaise, 400 g	1232	40	7	14
Applesauce without added sugar, 100 g	243	12	1	0
Shortbread biscuits, 25 g	463	18	3	2
Lunch (day of all experimental sessions)				
Bottle of water, 500 mL	0	0	0	0
Wholegrain club sandwich chicken and mayonnaise, 1 or 2 servings of 160 g	1818	40	22	16
Applesauce without added sugar, 100 g	243	12	1	0
Shortbread biscuits, 25 g	463	18	3	2
Afternoon snack (day of all experimental sessions)				
Orange juice, 20 cL	360	20	0	0
Chocolate chip cake, 30 g	554	15	7	2
Dinner (day of all experimental sessions)				
Bottle of water, 500 mL	0	0	0	0
Veal Milanese and tomato spaghetti, 300 g	1888	44	19	22
Applesauce without added sugar, 100 g	243	12	1	0
Chocolate chip cake, 30 g	554	15	7	2

Investigational products

List of SBar ingredients. One portion (32 g) consists of 300 mg turmeric formulation, 28% glucose fructose syrup, 16% oat flakes, 10% sunflower oil, 10% pea protein, 10% sorbitol syrup, 8% hydrolysed wheat protein, 8% puffed rice, 5% cocoa powder, 4% soluble fibre, soy lecithin, salt, coffee cappuccino flavour.

List of Gum ingredients. One portion (10 g) consists of 300 mg turmeric formulation, 49% sucrose, 30% glucose syrup, 20% water, 3.6% pectin, citric acid, trisodium citrate, lemon juice flavour.

Supplementary Table 2: Nutritional values for one serving of investigational products

Nutrient	**RTD (60 mL)**	**SBar (32 g)**	**DA (240 mL)**	**Gum (10 g)**	**Prob (100 g)**
Energy, kJ	49	434	444	123	307
Fat, g	0	2.0	3.6	0	1.6
of which saturated, g	0	0.6	0.2	0	1.0
Carbohydrate, g	2.9	16.6	16.3	7.4	10.8
of which sugar, g	2.9	9.2	7.9	5.7	10.8
Protein, g	0	6.0	3.4	0	3.0
Dietary fibre, g	N/A	2.4	0.7	0.2	N/A

DA, dairy analogue; Gum, gummies; N/A, not available; Prob, probiotic drink; RTD, ready to drink; SBar, sports nutrition bar.

Analytical materials & methods

Chemicals and study materials

Curcumin (USP #1151855), DMC (USP #1173100), and BDMC (USP #1075305) analytical standards were purchased from Merck (St Quentin Fallavier, France), HHC (#82559) and THC (#82665) were purchased from Phytolab (Vestenbergsgreuth, Germany), curcumin-d6 (#C-838502), DMC-d7 (#D-230817), BDMC-d8 (#B-425802), HHC-d6 (#H-281277),THC-d6 (#T-293387), curcumin glucuronide (#C-693) and curcumin sulphate trimethylamine salt (#C-6925) were purchased from Toronto Research Chemicals (TRC, North York, Canada). ULC-MS Acetonitrile 0.1% formic acid and ULC-MS water 0.1% formic acid solvents used as eluent for HPLC-HRMS as well as ULC-MS Methanol and ULS-MS Acetonitrile were purchased from Biosolve (Dieuze, France). Merck (St Quentin Fallavier, France) supplied citric acid (#27109).

Extraction of curcuminoids from plasma

After deconjugation, plasma underwent protein precipitation, solid-phase extraction and filtration. Briefly, 100 µL of deconjugated plasma or untreated plasma was loaded and mixed with 300 µL of cold methanol/acetonitrile (50:50; v/v) containing internal standards [d_6]-curcumin, [d_7]-DMC, [d_8]-BDMC-, [d_6]-THC, and [d_6]-HHC at 33.50 ng/mL on each solid phase extraction cartridge. Curcuminoids were eluted with 200 µL of methanol/acetonitrile (50:50; v/v) prior to analysis using HRMS.

HPLC-HRMS of curcuminoids in plasma

Unconjugated curcumin, DMC, BDMC, THC, and HHC concentrations were measured in plasma using HRMS on a Thermofisher Orbitrap Q Exactive Focus equipped with a Vanquish UHPLC system. Chromatographic separation was obtained using a Waters BEH-C_{18} column (2.1 x 100 mm, 1.7 µm) fitted with a precolumn cartridge containing the same stationary phase. The column temperature was 35°C, and the injection volume was 2 µL, and the

autosampler was maintained at 10°C. The mobile phase consisted of a linear gradient from solvent A (water 0.1% formic acid) to solvent B (acetonitrile 0.1% formic acid) at a flow rate of 400 µL/min as follows: 40% to 60% B over 3 min, 60% to 80% B from 3 to 4 min, and 80% to 100% B from 4 to 6 min. The column re-equilibration time was 4 min between analyses.

Quantification is performed with accurate mass extraction at *m/z* 367.11871 for curcumin, *m/z 337.10875* for DMC, *m/z* 307.09758 for BDMC, *m/z* 371.15001 for THC, *m/z* 373.16566 for HHC with a maximum tolerance of 5 ppm.

A stock solution was prepared in methanol containing each curcuminoid standard at 250 µg/mL, and aliquots were diluted using plasma matrix to prepare 6 calibration solutions from 10 to 200 ng/mL. The calibration curves were linear (r = 0.99) for all components. The method was qualified based on linearity, specificity, precision, accuracy, and robustness according to ICH guidelines (Supplementary Table 3). Quality control samples (blank human plasma spiked with known curcumin concentration) were analyzed every 10 samples, and a standard control at the limit of quantification (LOQ) was measured at the beginning and at the end of the sequence. The limit of detection (LOD defined as signal to noise of 3) was 1.677 ng/mL for curcumin, 1.525 ng/mL for DMC, 1.453 ng/mL for BDMC, 6.351 ng/mL for THC, and 5.534 ng/mL for HHC, and the LOQ values were defined as signal to noise of 10.

Supplementary Table 3: HRMS parameters for curcuminoid quantification

Compound	EIC *m/z*	Polarity
[d_6]-Curcumin	373.15637	Negative
Curcumin	367.11871	Negative
[d_7]-DMC	344.15208	Negative
DMC	337.10875	Negative
[d_8]-BDMC	315.14870	Negative
BDMC	307.09758	Negative
[d_6]-HHC	379.20332	Negative
HHC	373.16566	Negative
[d_6]-THC	377.18767	Negative
THC	371.15001	Negative

BDMC, bisdemethoxycurcumin; DMC, demethoxycurcumin; HHC, hexahydrocurcumin; THC, tetrahydrocurcumin.

Supplementary Table 4: HPLC-HRMS assay validation

Compound	LOD/LOQ [ng/mL]	Precision (intra-day) Conc. vs RSD	Precision (inter-day) Conc. vs RSD [ng/mL / %]	Accuracy level vs recovery
Curcumin	1.677 / 5.591	100 / 2.48 150 / 4.43	130 / 1.88	10 / 108.12 200 / 103.52
DMC	1.525 / 5.085	100 / 3.27 150 / 3.11	130 / 1.53	10 / 102.68 200 / 101.01
BDMC	1.453/ 5.085	100 / 4.67 150 / 3.99	130 / 0.79	10 / 99.55 200 / 98.68
HHC	5.534 / 18.447	100 / 6.38 150 / 3.88	150 / 2.67	20/ 83.78 220 / 98.53
THC	6.351 / 21.170	100 / 6.71 150 / 5.90	150 / 4.49	25 / 103.26 220 / 101.18

Precision (n = 6), RSD%: Relative standard deviation (SD/Average x 100).
BDMC, bisdemethoxycurcumin; Conc., concentration; DMC, demethoxycurcumin; HHC, hexahydrocurcumin; THC, tetrahydrocurcumin.

Calculation of conjugated curcuminoids

The specificity and cross-activity of β-glucuronidase and sulfatase enzymes were evaluated using a mixture of curcumin glucuronide and curcumin sulphate analytical standards. The activity of the β-glucuronidase on curcumin glucuronide as substrate was found to be 96.2% and the activity of the sulphatase on curcumin sulphate trimethylamine salt was found to be 95.1%. The unspecific/cross-activity for β-glucuronidase on sulphated compounds was negligible. Sulphatase (≥10 U/mg) is also acting as a β-glucuronidase (≥300 U/mg). Therefore, the concentration of glucuronide and sulphate conjugates were calculated by subtracting the

concentration of native curcuminoids (without deconjugation) from the concentration of curcuminoids obtained after the hydrolysis with β-glucuronidase or sulphatase, respectively. In addition, the differential concentrations were corrected for the molecular weight. Specifically, the calculation was carried out as follows:

$$glucuronide\ compounds = \left(unconjugated\ compounds_{(obtained\ after\ glucuronidase\ hydrolysis)} - native\ compounds\right) \cdot 1.48$$

$$sulphate\ compounds = \left(unconjugated\ compounds_{(obtained\ after\ sulphate\ hydrolysis)} - unconjugated\ compounds_{(obtained\ after\ glucuronidase\ hydrolysis)}\right) \cdot 1.22$$

Extraction of curcuminoids from investigational products

Extraction of curcuminoids from Caps. For the quantification of curcuminoids in Caps, the contents of 10 capsules were mixed, and 50 mg were weighed into a 50 mL volumetric flask. The sample was extracted with 2 mL of water in an ultrasonic bath for 5 minutes. Subsequently, 40 mL of methanol was added, and the mixture was sonicated for an additional 5 minutes. After cooling to room temperature, the solution was brought to volume with methanol. The solution was then filtered and analyzed using UHPLC for the quantification of curcuminoids (see *UPLC of curcuminoids extracted from dietary supplements* below).

Extraction of curcuminoids from RTD, DA, and Prob. For the quantification of curcuminoids in liquid food matrices, 2500 mg of the sample were weighed into a 50 mL Falcon tube. The sample was extracted with 20 mL of methanol in an ultrasonic bath for 5 minutes. The mixture was then centrifuged for 4 minutes at 4000 rpm. The supernatant was transferred into a 50 mL volumetric flask, and the extraction was repeated once before bringing the solution to volume with methanol. The solution was then filtered and analyzed using UHPLC for the quantification of curcuminoids (see *UPLC of curcuminoids extracted from dietary supplements* below).

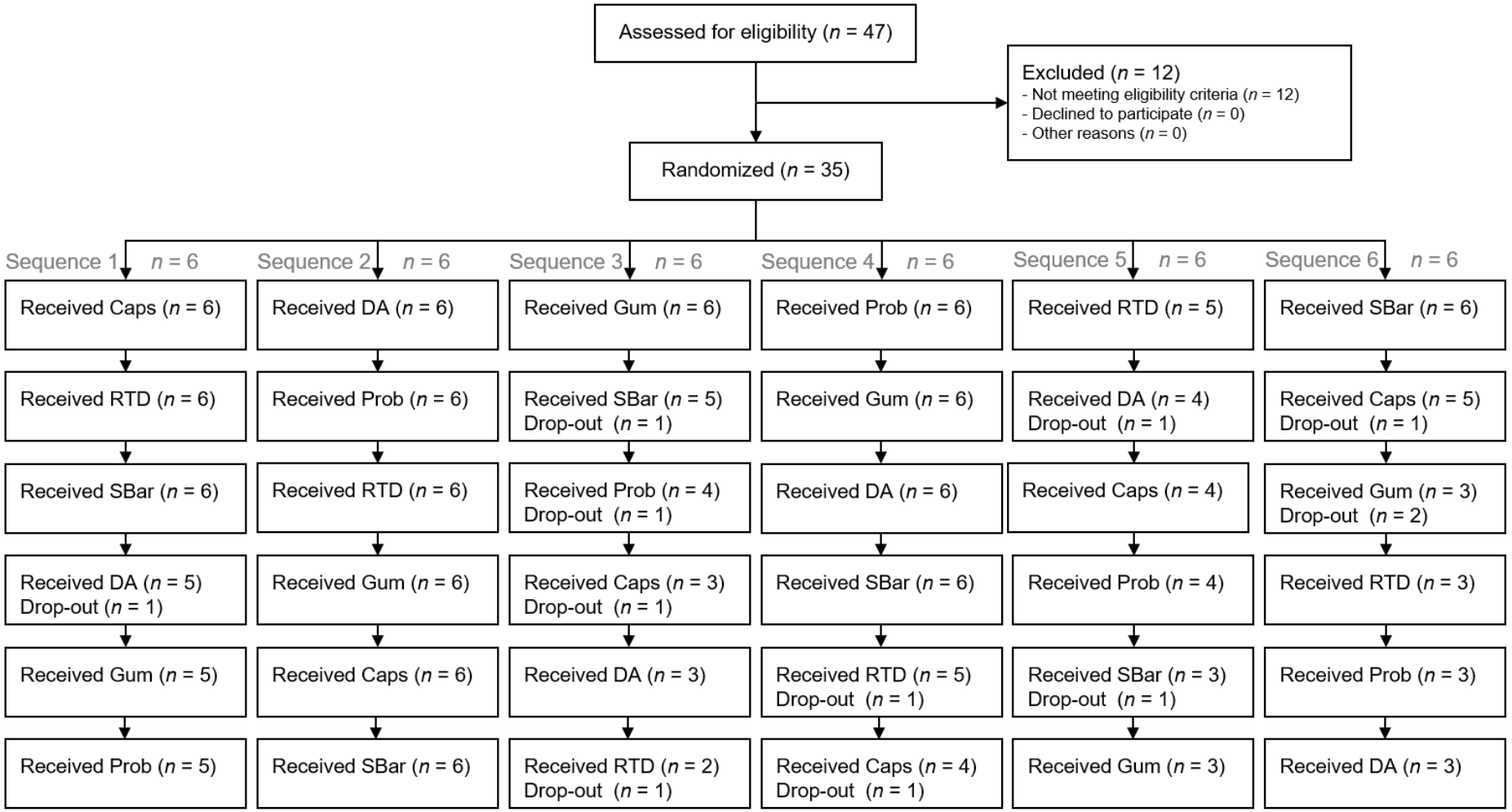
Extraction of curcuminoids from SBar. For the quantification of curcuminoids in SBar, 750 mg of crushed SBar were weighed into a 50 mL Falcon tube. The sample was extracted with 20 mL of methanol in an ultrasonic bath for 5 minutes. The mixture was then centrifuged for 4 minutes at 4000 rpm. The supernatant was transferred into a 50 mL volumetric flask, and the extraction was repeated once before bringing the solution to volume with methanol. The solution was then filtered and analyzed using UHPLC for the quantification of curcuminoids (see *UPLC of curcuminoids extracted from dietary supplements* below).

Extraction of curcuminoids from Gum. For the quantification of curcuminoids in gummies, 300 mg of the sample were weighed into a 20 mL volumetric flask. The sample was extracted with 2 mL of water in an ultrasonic bath for 5 minutes. Subsequently, 15 mL of acetone was added, and the mixture was sonicated for an additional 5 minutes. After cooling to room temperature, the solution was brought to volume with acetone. The solution was then filtered and analyzed using UHPLC for the quantification of curcuminoids (see *UPLC of curcuminoids extracted from dietary supplements* below).

UPLC of curcuminoids extracted from investigational products

Curcumin, DMC, and BDMC were quantified on an Agilent 1260 system (Santa Clara, CA, United States) equipped with a binary pump, an autosampler (maintained at 15°C), and a DAD detector. Separation was performed on a Zorbax Eclipse-C18 column (150 x 4.6 mm, 5 µm) at a flow rate of 1 mL/min in isocratic elution mode (1 mg/mL citric acid in water / acetonitrile (60:40; v/v) that allowed for the resolution of the three curcuminoids detected at 420 nm. The column temperature was 25°C, and the injection volume was 2 µL.

Supplementary results



Supplementary Figure 1: Study flowchart
Reasons for dropouts were: Intercurrent pathology not present at inclusion (n = 6), lost to follow-up (n = 1); participant's decision (n = 3); discomfort / poor tolerance on catheter placement (n = 2)
Caps, capsules; DA, dairy analogue; Gum, gummies; Prob, probiotic drink; RTD, ready to drink; SBar, sports nutrition bar.

Supplementary Table 5: Observed mean and SD of plasma concentration of total curcuminoids over 24 hours after product intake

Time, hours	Caps	RTD	SBar	DA	Gum	Prob
Non-normalized, ng/mL						
0	59 (128)	77 (151)	59 (167)	91 (178)	63 (125)	52 (161)
0.25	24 (47)	128 (124)	151 (140)	262 (199)	121 (87)	204 (135)
0.5	115 (143)	379 (245)	276 (163)	621 (303)	254 (186)	529 (340)
0.75	226 (200)	443 (203)	382 (230)	817 (428)	359 (219)	587 (313)
1	288 (236)	435 (171)	493 (259)	860 (440)	384 (222)	584 (312)
1.5	400 (304)	394 (201)	516 (258)	749 (410)	388 (231)	569 (259)
2	392 (277)	374 (181)	521 (230)	660 (327)	395 (198)	499 (252)
2.5	403 (256)	382 (213)	487 (202)	571 (299)	358 (210)	474 (225)
3	385 (243)	333 (201)	442 (138)	493 (203)	292 (191)	403 (186)
3.5	352 (210)	301 (169)	384 (164)	476 (144)	286 (174)	362 (167)
4	302 (198)	242 (146)	330 (146)	406 (219)	276 (169)	309 (148)
6	269 (124)	259 (148)	314 (146)	433 (220)	273 (188)	330 (121)
8	239 (161)	301 (175)	259 (126)	361 (194)	264 (178)	332 (137)
24	127 (156)	112 (172)	74 (165)	188 (259)	130 (149)	121 (133)
Dose-normalized (per mg of ingested curcuminoids), ng/mL/mg						
0	0.58 (1.26)	0.77 (1.51)	0.74 (2.09)	0.91 (1.78)	0.67 (1.33)	0.52 (1.63)
0.25	0.24 (0.47)	1.28 (1.24)	1.89 (1.76)	2.62 (1.99)	1.29 (0.93)	2.06 (1.36)
0.5	1.13 (1.41)	3.79 (2.45)	3.45 (2.04)	6.19 (3.02)	2.71 (1.98)	5.34 (3.43)
0.75	2.23 (1.97)	4.43 (2.03)	4.78 (2.88)	8.15 (4.27)	3.83 (2.34)	5.92 (3.15)
1	2.85 (2.33)	4.35 (1.71)	6.17 (3.24)	8.58 (4.39)	4.09 (2.37)	5.89 (3.15)
1.5	3.96 (3.01)	3.94 (2.01)	6.46 (3.23)	7.47 (4.09)	4.14 (2.47)	5.74 (2.62)
2	3.88 (2.73)	3.74 (1.81)	6.52 (2.88)	6.59 (3.26)	4.22 (2.12)	5.03 (2.54)
2.5	3.98 (2.53)	3.82 (2.13)	6.09 (2.53)	5.70 (2.99)	3.82 (2.24)	4.78 (2.27)
3	3.81 (2.41)	3.33 (2.01)	5.53 (1.73)	4.91 (2.03)	3.11 (2.04)	4.06 (1.88)
3.5	3.48 (2.07)	3.01 (1.69)	4.80 (2.05)	4.75 (1.44)	3.05 (1.86)	3.65 (1.69)
4	2.99 (1.95)	2.42 (1.46)	4.13 (1.83)	4.05 (2.18)	2.95 (1.80)	3.12 (1.49)
6	2.66 (1.23)	2.59 (1.48)	3.93 (1.83)	4.32 (2.19)	2.91 (2.00)	3.33 (1.22)
8	2.36 (1.59)	3.01 (1.75)	3.25 (1.58)	3.60 (1.94)	2.81 (1.90)	3.35 (1.38)
24	1.25 (1.54)	1.12 (1.72)	0.93 (2.06)	1.87 (2.59)	1.39 (1.59)	1.22 (1.34)

Caps, capsules; DA, dairy analogue; Gum, gummies; Prob, probiotic drink; RTD, ready to drink; SBar, sports nutrition bar.

Supplementary Table 6: Range of exposures to total curcuminoids

	Caps	RTD	SBar	DA	Gum	Prob
Norm $AUC_{24\ h}$, ng·h/mL/mg	13.09 ; 139.57	12.08 ; 162.91 (1.2)	13.70 ; 135.08 (1.0)	42.02 ; 125.48 (0.9)	8.45 ; 99.33 (0.7)	22.89 ; 121.16 (0.9)
Norm $AUC_{8\ h}$, ng·h/mL/mg	7.08 ; 44.48	4.56 ; 48.65 (1.1)	13.70 ; 61.34 (1.4)	17.93 ; 87.18 (2.0)	8.45 ; 50.24 (1.1)	11.53 ; 60.95 (1.4)
Norm AUC_{∞}, ng·h/mL/mg	18.14 ; 230.97	26.20 ; 575.70 (2.5)	14.87 ; 110.68 (0.5)	44.36 ; 701.66 (3.0)	8.99 ; 972.85 (4.2)	32.82 ; 652.67 (2.8)
$AUC_{24\ h}$, ng·h/mL	1325.01 ; 14122.83	1208.21 ; 16287.82 (1.2)	1094.20 ; 10790.41 (0.8)	4210.91 ; 12575.60 (0.9)	792.03 ; 9307.07 (0.7)	2269.58 ; 12013.11 (0.9)
$AUC_{8\ h}$, ng·h/mL	716.58 ; 4500.66	455.44 ; 4864.39 (1.1)	1094.20 ; 4899.56 (1.1)	1796.67 ; 8736.92 (1.9)	792.03 ; 4707.94 (1.0)	1143.04 ; 6043.30 (1.3)
AUC_{∞}, ng·h/mL	1835.13 ; 23372.35	2619.70 ; 57558.45 (2.5)	1188.10 ; 8840.89 (0.4)	4446.01 ; 70320.10 (3.0)	842.19 ; 91155.68 (3.9)	3254.11 ; 64712.04 (2.8)
Norm C_{max}, ng/mL/mg	2.06 ; 11.00	1.86 ; 10.15 (0.9)	4.29 ; 15.43 (1.4)	5.10 ; 24.19 (2.2)	2.25 ; 10.69 (1.0)	3.88 ; 19.38 (1.8)
C_{max}, ng/mL	207.96 ; 1113.08	185.68 ; 1014.82 (0.9)	342.50 ; 1232.27 (1.1)	510.89 ; 2424.38 (2.2)	210.58 ; 1002.09 (0.9)	385.17 ; 1921.56 (1.7)

Ranges are displayed as min ; max with the ratio of the maximal value for each matrix to the maximal value obtained for capsules in brackets. AUC, area under the concentration-time curve from 0 to 24 hours, 0 to 8 hours, and total area from 0 to extrapolated infinite time; Caps, capsules; C_{max}, peak concentration; DA, dairy analogue; Gum, gummies; Norm, dose-normalized; Prob, probiotic drink; RTD, ready to drink; SBar, sports nutrition bar.

Supplementary references

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