

**GPM AND NON-GPM VITAMINS & MINERALS
COMPARATIVE DIGESTION STABILITY AND BIOAVAILABILITY**

To compare GPM fermented vitamins and minerals to non-fermented vitamins and minerals, in vitro digestion stability and bioavailability testing was conducted using two published methods. The vitamins and minerals evaluated were vitamin B2, vitamin B3, vitamin B6, vitamin C, calcium, magnesium, copper, and zinc.

Digestion stability

Digestion stability testing was performed according to a modified published two-stage in vitro digestion model¹ with enzymes, bile, and pH adjustments simulating gastric and small intestinal digestion. Samples were blanketed in nitrogen and incubated in a dark shaking water bath at 37°C. Incubation time was either 1 or 2 hours depending on the nutrient. Each vitamin and mineral were analyzed across multiple incubation concentrations that were identical between the (+) GPM and (-) GPM groups. Samples were analyzed using HPLC with diode array detection (DAD) to quantify vitamin and mineral levels pre and post digestion. Results are shown below in Table 1.

Table 1-Digestion Stability

Sample	Incubation concentration (µmol/l)	Concentration post digestion (µmol/l)	GPM % increase
(-) GPM Riboflavin	4	4.22	
(+) GPM Riboflavin	4	4.36	3%
(-) GPM Riboflavin	10	11.25	
(+) GPM Riboflavin	10	11.78	5%
(-) GPM Riboflavin	100	99.55	
(+) GPM Riboflavin	100	98.82	-1%
(-) GPM Niacinamide	0.8	0.76	
(+) GPM Niacinamide	0.8	0.77	1%
(-) GPM Niacinamide	1.6	1.57	
(+) GPM Niacinamide	1.6	1.56	-1%
(-) GPM Niacinamide	3.2	3.19	
(+) GPM Niacinamide	3.2	3.24	2%
(-) GPM Pyridoxine	10	10.52	
(+) GPM Pyridoxine	10	11.42	9%
(-) GPM Pyridoxine	42	42.23	
(+) GPM Pyridoxine	42	42.82	1%
(-) GPM Pyridoxine	195	196.85	
(+) GPM Pyridoxine	195	199.98	2%
(-) GPM Ascorbic acid	113.6	4.39	
(+) GPM Ascorbic acid	113.6	51.02	1062%
(-) GPM Ascorbic acid	227.2	7.39	
(+) GPM Ascorbic acid	227.2	145.35	1867%
(-) GPM Calcium	4.4	4.26	
(+) GPM Calcium	4.4	4.50	6%
(-) GPM Calcium	44	45.47	
(+) GPM Calcium	44	44.21	-3%

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(-) GPM Calcium	440	442.03	
(+) GPM Calcium	440	446.67	1%
(-) GPM Magnesium	4	5.75	
(+) GPM Magnesium	4	5.50	-4%
(-) GPM Magnesium	20	21.00	
(+) GPM Magnesium	20	21.75	4%
(-) GPM Magnesium	100	100.50	
(+) GPM Magnesium	100	106.50	6%
(-) GPM Copper	18	18.70	
(+) GPM Copper	18	18.30	-2%
(-) GPM Copper	36	36.10	
(+) GPM Copper	36	38.30	6%
(-) GPM Copper	71	71.15	
(+) GPM Copper	71	73.00	3%
(-) GPM Zinc	2	1.95	
(+) GPM Zinc	2	2.02	4%
(-) GPM Zinc	20	20.23	
(+) GPM Zinc	20	19.94	-1%
(-) GPM Zinc	200	202.67	
(+) GPM Zinc	200	203.33	0%

Nutrient levels after simulated digestion were stable and consistent for both GPM and non-GPM versions for seven of the eight nutrients analyzed, indicating that both types are equivalently available for absorption. However, ascorbic acid post-digestion levels (highlighted) were over 10X and 30X higher for the GPM samples compared to the non-GPM samples, allowing a much higher level of this nutrient to be available for absorption.

Bioavailability

Bioavailability testing was conducted using a modified published method² measuring vitamin and mineral uptake into porcine red blood cells (RBC). Samples were incubated at 37°C. Incubation time was either 0.5 or 1 hour depending on the nutrient. Each vitamin and mineral were analyzed across multiple incubation concentrations. The vitamin and mineral incubation concentrations were identical between the (+) GPM and (-) GPM groups. After incubation samples were washed and precipitated and the supernatant was analyzed. Vitamin and mineral absorption was measured by atomic absorption spectrophotometry or HPLC. Results are shown below in Table 2.

Table 2-Bioavailability

Sample	Incubation concentration (µmol/l)	Concentration absorbed into RBC (µmol/l)	GPM % increase
(-) GPM Riboflavin	2	0.69	
(+) GPM Riboflavin	2	0.82	19%
(-) GPM Riboflavin	5	1.61	
(+) GPM Riboflavin	5	1.76	9%
(-) GPM Riboflavin	25	5.24	
(+) GPM Riboflavin	25	5.03	-4%
(-) GPM Niacinamide	2500	19.39	
(+) GPM Niacinamide	2500	25.23	30%
(-) GPM Niacinamide	5000	87.50	
(+) GPM Niacinamide	5000	96.74	11%
(-) GPM Niacinamide	25000	155.58	

(+) GPM Niacinamide	25000	181.50	17%
(-) GPM Pyridoxine	500	7.02	
(+) GPM Pyridoxine	500	11.09	58%
(-) GPM Pyridoxine	2500	21.93	
(+) GPM Pyridoxine	2500	61.78	182%
(-) GPM Pyridoxine	12500	299.79	
(+) GPM Pyridoxine	12500	498.45	166%
(-) GPM Ascorbic acid	10	1.68	
(+) GPM Ascorbic acid	10	2.42	44%
(-) GPM Ascorbic acid	50	12.9	
(+) GPM Ascorbic acid	50	18.76	45%
(-) GPM Ascorbic acid	100	34.74	
(+) GPM Ascorbic acid	100	39.05	12%
(-) GPM Calcium	44	0.026	
(+) GPM Calcium	44	0.051	96%
(-) GPM Calcium	110	0.077	
(+) GPM Calcium	110	0.117	52%
(-) GPM Calcium	220	0.175	
(+) GPM Calcium	220	0.222	27%
(-) GPM Magnesium	40	6.67	
(+) GPM Magnesium	40	24.49	267%
(-) GPM Magnesium	200	56.16	
(+) GPM Magnesium	200	79.23	41%
(-) GPM Magnesium	1000	118.49	
(+) GPM Magnesium	1000	148.99	26%
(-) GPM Copper	1	0.034	
(+) GPM Copper	1	0.1	194%
(-) GPM Copper	2	0.184	
(+) GPM Copper	2	0.592	222%
(-) GPM Copper	4	0.67	
(+) GPM Copper	4	2.22	231%
(-) GPM Zinc	1	0.056	
(+) GPM Zinc	1	0.154	175%
(-) GPM Zinc	5	0.231	
(+) GPM Zinc	5	0.418	81%
(-) GPM Zinc	10	0.422	
(+) GPM Zinc	10	0.756	79%

Nutrient uptake was consistently higher for the GPM nutrients compared to the non-GPM nutrients for all the vitamins and minerals tested and all the concentrations tested, except for the high concentration of riboflavin. GPM nutrient uptake was notably much higher for vitamin B6, magnesium, copper and zinc.

References

1. Neilson AP, Hopf AS, Cooper BR, et al. Catechin degradation with concurrent formation of homo- and heterocatechin dimers during *in vitro* digestion. *Journal of Agricultural and Food Chemistry* 2007, 55; 8941-8949.
2. Fiorani, M, Accorsi A and Cantoni O. Human red blood cells as a natural flavonoid reservoir. *Free Radical Research* 2003, 37; 12:1331-1338.

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