

## Supplementary Information

### **Impact of polyphenol oxidase on flavan-3-ol bioavailability from fruit smoothies: a controlled, randomized, single blinded, cross-over study.**

Javier I. Ottaviani<sup>a,b</sup>, Jodi L. Ensunsa<sup>b</sup>, Reedmond Y. Fong<sup>b</sup>, Jennifer Kimball<sup>b</sup>, Valentina Medici<sup>c</sup>, Alan Crozier<sup>b,d</sup>, Hagen Schroeter<sup>a</sup>, Catherine Kwik-Urbe<sup>a</sup>

<sup>a</sup>*Mars Inc., McLean, VA 22101, USA*

<sup>b</sup>*Department of Nutrition, University of California, Davis, CA 95616, USA*

<sup>c</sup>*Department of Internal Medicine, Division of Gastroenterology and Hepatology, University of California Davis, Sacramento, CA 05817, USA*

<sup>d</sup>*Department of Chemistry, King Saud University, Riyadh 11451, Saudi Arabia*

**Supplemental Table 1** Conditions for the detection and quantification of structurally related (-)-epicatechin metabolites and 5-(3',4'-dihydroxyphenyl)- $\gamma$ -valerolactone metabolites by multiple reaction monitoring using a triple quadrupole mass spectrometer.

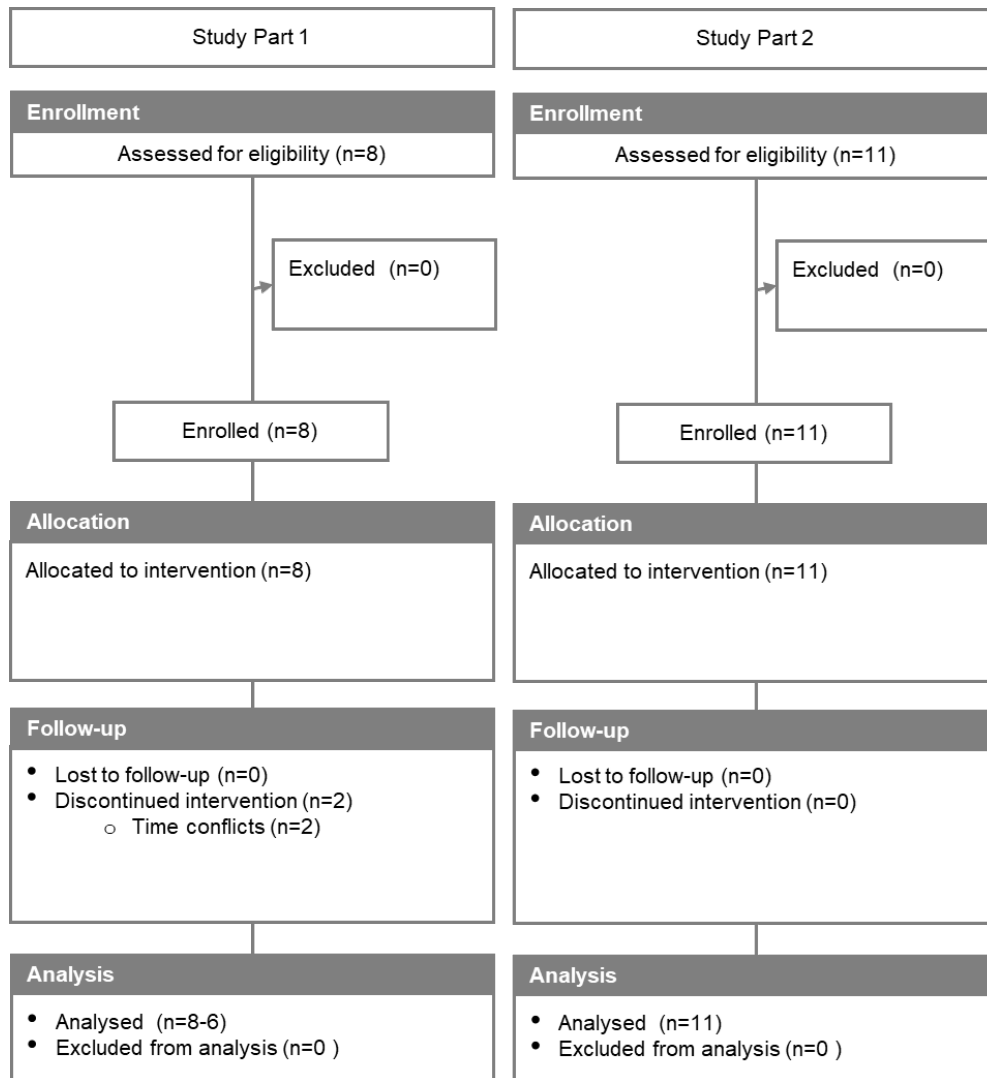
Metabolites	Retention time (min)	Parent ( <i>m/z</i> )	Daughter ( <i>m/z</i> )	Cone (V)	Collision (V)
(-)-Epicatechin-5-sulfate	3.92	369.1	289.2	35	26
(-)-Epicatechin-3'-sulfate	4.17	369.1	289.2	35	26
(-)-Epicatechin-3'-glucuronide	3.92	465.2	289.2	35	24
3'-Methoxy(-)-epicatechin-4'-sulfate	4.18	383.1	303.3	40	22
3'-Methoxy(-)-epicatechin-5-sulfate	4.35	383.1	303.3	40	22
3'-Methoxy(-)-epicatechin-7-sulfate	4.49	383.1	303.3	40	22
4'-Methoxy(-)-epicatechin-5-sulfate	4.63	383.1	303.3	40	22
4'-Methoxy(-)-epicatechin-7-sulfate	4.80	383.1	303.3	40	22
5-(3'-Hydroxyphenyl)- $\gamma$ -valerolactone-4'-sulfate	3.85	287.0	207.0	32	22
5-(4'-Hydroxyphenyl)- $\gamma$ -valerolactone-3'-sulfate	3.93	287.0	207.0	32	22
5-(3'-Hydroxyphenyl)- $\gamma$ -valerolactone-4'-glucuronide	3.69	383.2	207.2	32	20
5-(4'-Hydroxyphenyl)- $\gamma$ -valerolactone-3'-glucuronide	3.85	383.2	207.2	32	20
4'-Methoxy-5-(Phenyl)- $\gamma$ -valerolactone-3'-sulfate	4.10	301.0	245.2	18	20
3'-Methoxy-5-(Phenyl)- $\gamma$ -valerolactone-4'-sulfate	4.32	301.0	245.2	18	20
4'-Methoxy-5-(phenyl)- $\gamma$ -valerolactone-3'-	3.97	397.0	221.0	15	20
3'-Methoxy-5-(phenyl)- $\gamma$ -valerolactone-4'-	4.25	397.0	221.0	15	20

**Supplemental Table 2** Accuracy and precision of the method used for the quantification of structurally related (-)-epicatechin metabolites and 5-(3',4'-dihydroxyphenyl)- $\gamma$ -valerolactone metabolites in plasma and urine by UPLC-MS/MS.

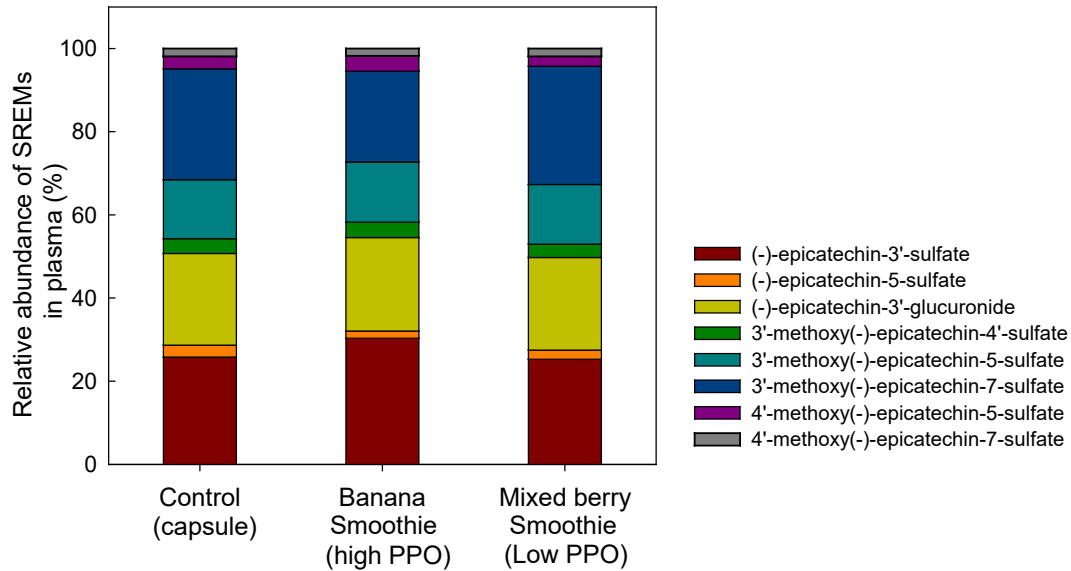
Metabolites	Plasma		Urine	
	Accuracy	Precision	Accuracy	Precision
(-)-Epicatechin-3'-sulfate	93%	6%	89%	5%
(-)-Epicatechin-3'-glucuronide	93%	5%	84%	3%
3'-Methoxy(-)-epicatechin-5-sulfate	96%	4%	99%	5%
3'-Methoxy(-)-epicatechin-7-sulfate	99%	5%	90%	3%
4'-Methoxy(-)-epicatechin-5-sulfate	93%	7%	94%	4%
4'-Methoxy(-)-epicatechin-7-sulfate	98%	6%	91%	4%
5-(4'-Hydroxyphenyl)- $\gamma$ -valerolactone-3'-sulfate	96%	5%	91%	2%
5-(4'-Hydroxyphenyl)- $\gamma$ -valerolactone-3'-glucuronide	86%	5%	86%	3%
5-(3'-Hydroxyphenyl)- $\gamma$ -valerolactone-4'-glucuronide	83%	7%	81%	3%

<b><u>Do not eat:</u></b>	<b><u>Types of foods you may eat:</u></b>
Fruit (other than those specified)	Iceberg lettuce, watermelon, cucumber, white endives, bananas
Vegetables (other than those specified)	Corn, potatoes, rice, plain white bread and bagels
Cocoa	Milk, cream, cheese, butter
Chocolate products	kefir and yogurt without fruit
Tea, both black and herbal such as chamomile	Pasta [non-enriched], macaroni and cheese
Nuts	Rice, couscous, potatoes
Herbs (parsley, oregano, marjoram, thyme etc.)	White sauces
Jelly or Jam	Oatmeal [non-fortified]
Preserves	“Cream of wheat” or Rice,
Fruit or nut-based candies	Grits or polenta [non-enriched]
Cereals	Fish, chicken, beef, lamb, pork, bacon, ham, eggs, sausages
<u>Fruit juices</u>	Cheeseburgers (go light on the spices and omit tomatoes and onions)
<u>Wine or Beer</u>	Potato chips, fries, crackers, plain tortilla chips, pretzels
<u>Alcohol</u>	Cakes and sweets, which do not contain fruit, fruits juice, or fruit extracts
Spaghetti sauce	
Fruit or nut-containing breads	
Whole grain breads or crackers	

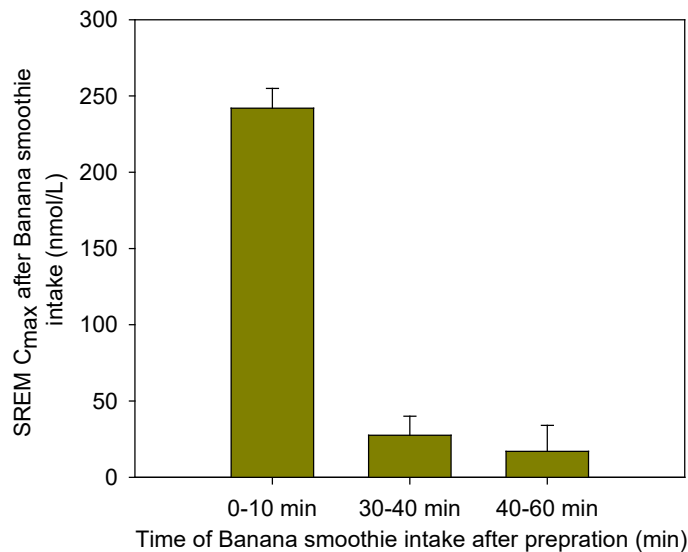
**Supplemental Figure 1:** Directions provided to study volunteers to follow a low-flavanol diet in preparation of study visits.



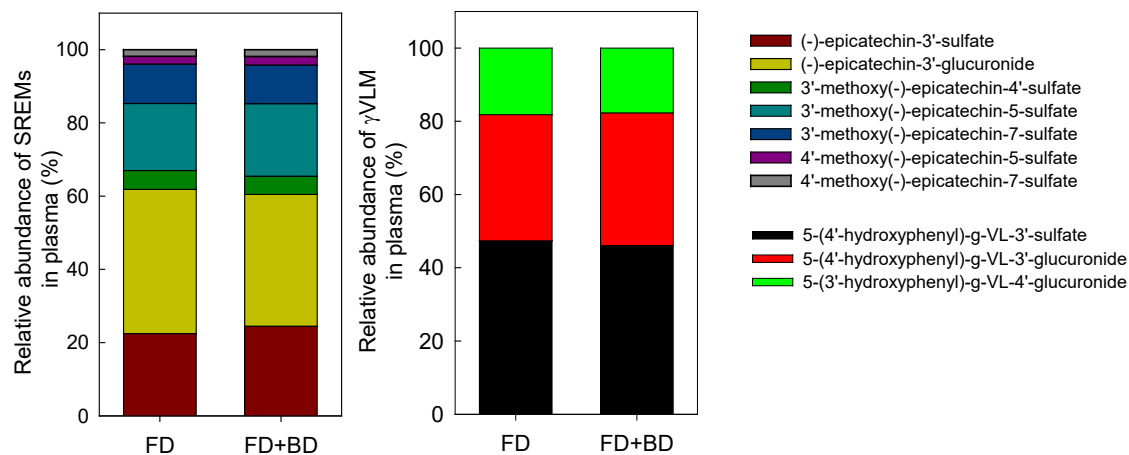
**Suppl. Figure 2:** CONSORT flow chart for study part 1 and 2 of the study.



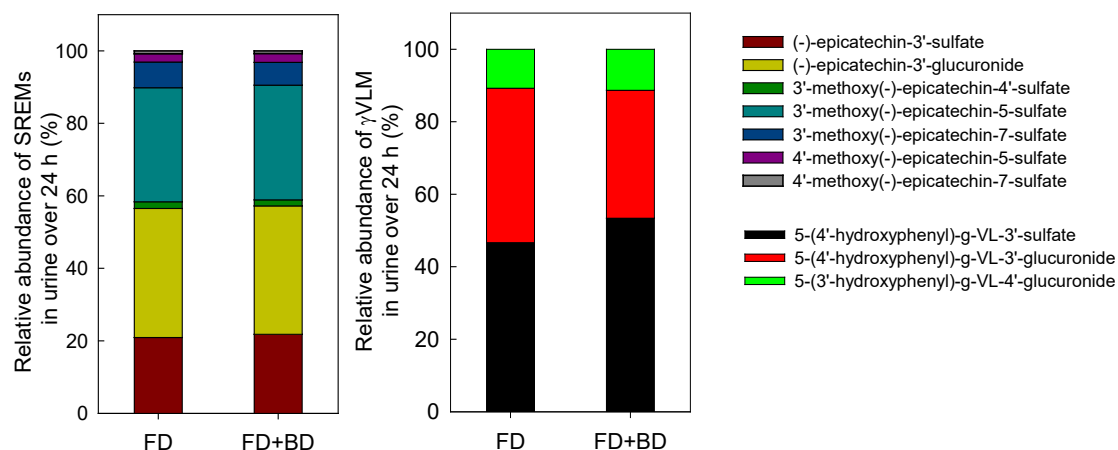
**Supplemental Figure 3:** Relative amounts of individual structurally related (-)-epicatechin metabolites (SREMs) in plasma after the intake of flavan-3-ols in capsule form and flavan-3-ols mixed in different fruit smoothies. Data are expressed as percentage of the area under the curve described by the concentration of individual SREMs up to 6 h after intake relative to that obtained for the sum of SREMs (n = 8).



**Supplemental Figure 4:** Maximum plasma concentration ( $C_{max}$ ) of structurally related (-)-epicatechin metabolites (SREMs) after intake of banana smoothie consumed within different time periods after preparation of the smoothie. Data are presented as mean  $\pm$  SE (n = 2 per time period).



**Supplemental Figure 5:** Relative amounts of individual structurally related (-)-epicatechin metabolites (SREMs) and 5-(3',4'-dihydroxyphenyl)- $\gamma$ -valerolactone metabolites ( $\gamma$ VLMs) in plasma after the intake of a flavan-3-ol drink (FD) and the intake of a flavan-3-ol drink simultaneously consumed with a banana drink (FD+BD). Data are expressed as percentage of the area under the curve described by the concentration of individual SREMs up to 6 h after intake relative to that obtained for the sum of SREMs, and as percentage of the plasma concentration of individual  $\gamma$ VLMs 6 h after intake relative to that obtained for the sum of  $\gamma$ VLMs (n = 11).



**Supplemental Figure 6:** Relative amounts of individual structurally related (-)-epicatechin metabolites (SREMs) and 5-(3',4'-dihydroxyphenyl)- $\gamma$ -valerolactone metabolites ( $\gamma$ VLMs) excreted in urine over 24 h after the intake of a flavan-3-ol drink (FD) and the intake of a flavan-3-ol drink simultaneously consumed with a banana drink (FD+BD). Data are expressed as percentage of the amount of individual SREMs and  $\gamma$ VLMs excreted over 24 h after intake relative to that obtained for the sum of SREMs and  $\gamma$ VLMs, respectively (n=11).

### Supplementary Test Materials:

In addition to banana smoothie and mixed berry smoothie, 5 additional foods added with flavan-3-ols were tested during study part 1. This aimed at assessing the effect of a broad range of food matrixes on flavan-3-ol bioavailability. These additional test foods included i) peanut butter toast (serving: 132 g), ii) oatmeal (serving: 277 g), iii) yogurt (serving: 227 g), iv) a high protein drink (serving: 236 mL) and v) sports drink (serving: 355 mL). Peanut butter toast was prepared with 32 g of peanut butter spread added with flavan-3-ols on a 50 g white bread toast with 50 g of sliced strawberries. Oatmeal was prepared with 40 g of instant oats and 237 g of boiling water, following flavan-3-ols were added. Yogurt, High protein drink and sports drink (Gatorade) were directly added with flavan-3-ols. All foods and beverages were commercially available, obtained from a local grocery store and stored according to manufacturer directions. The content of macronutrients as well as the amount of flavan-3-ols added in the foods tested are detailed in Table I.

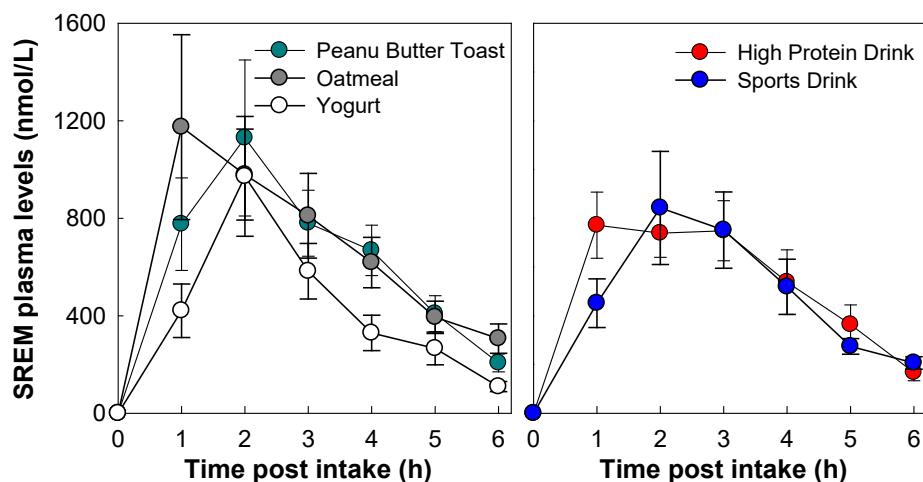
**Table I** Content of macronutrients and amount of flavan-3-ols added to the additional test foods evaluated during study part 1. Nutritional content was obtained from labels and from USDA Food Data Central.

	Peanut Butter-Toast	Oatmeal	Yogurt	High Protein Drink	Sports Drink
Energy (Kcal)	386	176	159	240	112
Protein (g)	14	8	24	15	0
Fat (g)	19	3	0	6	0
Carbohydrate (g)	41	31	15	33	29
Fiber (g)	5	5	0	0	0
Sugar (g)	7	0.4	9	27	27
Flavan-3-ols added (mg)	642	642	484	565	565
(-)-Epicatechin (mg)	85	85	68	78	78
(+)-Epicatechin (mg)	b.l.d.	b.l.d.	b.l.d.	b.l.d.	b.l.d.
(-)-Catechin (mg)	7	7	9	8	8
(+)-Catechin (mg)	3	3	3	3	3
DP2 (mg)	101	101	75	90	90
DP3 (mg)	146	146	91	103	103
DP4 (mg)	97	97	78	91	91
DP5 (mg)	85	85	67	80	80
DP6 (mg)	68	68	53	64	64
DP7 (mg)	50	50	40	48	48

DP: degree of polymerization; b.l.d.:below level of detection

## Supplementary Results

The intake of additional test foods added with flavan-3-ols significantly increased SREM levels in plasma (Figure I). Pharmacokinetic parameters after the intake of additional test foods are shown in Table II. When accounting for differences in time to reach peak plasma concentrations and differences in the amount of (-)-epicatechin in each test foods (as reflected by  $AUC_{0-inf}/75 \text{ mg Epi}$ ; Table II), no significant differences were observed in SREM levels between additional test foods and control. Concurrently, no differences in the profile of SREMs were detected (Figure II).



**Figure I:** Concentration of structurally related (-)-epicatechin metabolites (SREMs) in plasma after the intake of additional test foods added with flavan-3-ols. Data are expressed as means  $\pm$  SEM (n=6).

**Conclusion:** The intake of flavan-3-ols added to different foods did not significantly affect the absorption and metabolism of these compounds.

**Table II:** Pharmacokinetic parameters determined for the sum of structurally related (-)-epicatechin metabolites (SREM) after the different foods added with flavan-3-ols. Data presented as mean values  $\pm$  SEM (n = 6)<sup>a</sup>.

Test products	$C_{max}$ (nmol/L)	$T_{max}$ (h)	$t_{1/2}$ (h)	$AUC_{0-6h}$ (nmol/L*6 h)	$AUC_{0-inf}$ (nmol/L* h)	$AUC_{0-inf}/75 \text{ mg Epi}$ (nmol/L* h)
Capsule (control)	680 $\pm$ 78	3.6 $\pm$ 0.2	2.4 $\pm$ 0.4	2,259 $\pm$ 279	3,860 $\pm$ 622	3,860 $\pm$ 622
Peanut Butter-Toast	1,288 $\pm$ 292*	2.2 $\pm$ 0.4	1.5 $\pm$ 0.1	3,930 $\pm$ 646*	4,433 $\pm$ 709	3,932 $\pm$ 629
Oatmeal	1,339 $\pm$ 353*	1.5 $\pm$ 0.2*	2.5 $\pm$ 0.4	4,192 $\pm$ 863*	5,311 $\pm$ 931*	4,711 $\pm$ 826
Yogurt	1,006 $\pm$ 242	2.2 $\pm$ 0.2	1.5 $\pm$ 0.1	2,683 $\pm$ 556	3,030 $\pm$ 625	3,342 $\pm$ 689
High Protein Drink	980 $\pm$ 133	2.0 $\pm$ 0.5*	1.7 $\pm$ 0.2	3,245 $\pm$ 381	3,640 $\pm$ 416	3,496 $\pm$ 400
Sports Drink	971 $\pm$ 192	2.2 $\pm$ 0.3	1.7 $\pm$ 0.4	2,998 $\pm$ 523	3,515 $\pm$ 541	3,376 $\pm$ 520

<sup>a</sup>not determined, n.d.; peak plasma concentration,  $C_{max}$ ; time to reach  $C_{max}$ ,  $T_{max}$ ; apparent elimination half-life,  $AT_{1/2}$ ; 0-6 h plasma area under the curve,  $AUC_{0-6h}$ ; plasma area under the curve extrapolated to infinity,  $AUC_{0-inf}$ ; plasma area under the curve extrapolated to infinity and normalized to an intake of 75 mg (-)-epicatechin,  $AUC_{0-inf}/75 \text{ mg Epi}$ ; \* $p < 0.05$  vs. control (Capsule); ANOVA-RM, Bonferroni post-hoc.



