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PHYSICO-CHEMISTRY, PROTEOMICS AND IN VIVO COMPARATIVE TESTS TO REVEAL VARIABILITY IN MULTISTRAIN PROBIOTIC FORMULATIONS

Microbiome in Gastrointestinal and Liver Diseases
Prebiotics, Probiotics and Synbiotics in Health and Disease
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VSL#3 powder formulations originated from two production sites (USA- and ITALY-made products) were subjected to a physicochemical characterization based on thermal and colloidal properties [1], proteomics analyses, and *in vivo*intestinal permeability tests on animal models. Thermogravimetry-Differential Scanning Calorimetry (TGA-DSC) analyses provided powder sample profiles in mass and internal energy changes under temperature scan, and thermophysical data in terms of material decomposition and transition phases. Sample particle sizes, size distributions, and charges at the dispersed state informed on their capacity to remain stable in aqueous suspension. Table 1 compares physicochemical data generated from USA- and ITALY-made formulations obtained with different lots (lots DM538 and 10151198 analyzed after 0 and 3 months for USA *vs.*lots 512058 and 3302E10 analyzed after 0 and 3 months for ITALY). Even though both formulations were qualitatively similar in thermal and colloidal profiles, significant differences were quantitatively observed in terms of maximum decomposition temperature *T*max ($p < 0.05$), and transition phase temperature *T*m ($p < 0.01$), as illustrated in Figure 1. Further comparative analyses by proteomics showed 99 proteins decreased abundances and 38 increased ones in ITALY-made batch (lot 512058) compared to the US-made one (lot DM538), and revealed differences in protein identities and origins. *Invivo*intestinal permeability tests with old mice confirmed such significant differences between US-made lot DM538 and IT-made lot 512058. In conclusion, the thermal scan data and shotgun proteomic comparisons demonstrate significant physicochemical and chemical variability between multistrain probiotic VSL#3 formulations from two manufacturer countries. Such differences have an impact on the intestinal permeability *in vivo*.

[1] Razafindralambo, H. et al. (2018). Physico-chemical Approach for Characterizing Probiotics at the Solid and Dispersed States. Food Research International (*In press*).

At the time of publication, litigation between the manufacturer of VSL#3 and Dr. De Simone/ExeGi Pharma over intellectual property claims was ongoing.

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Techniques	Physicochemical data	US-MEAN (n ≥ 6)	±	SD	IT-MEAN (n ≥ 6)	±	SD
TGA							
→ 100°C	- H_2O (%)	97.64	±	0.98	98.20	±	1.03
100 →150°C	*L*1 (%)	-4.18	±	0.09	-4.15	±	0.22^b
150 →250°C	*L*2 (%)	-18.39	±	1.31^a	-18.76	±	1.98
250 →350°C	*L*3 (%)	-8.18	±	0.87	-6.86	±	1.51
600°C	*BC* (%)	32.78	±	0.76	32.52	±	1.95^a
DTG							
	*A*t1 (µg)	-16.75	±	0.62	-19.48	±	6.34^b
100 →150°C	*V*1max (10^{-3}.min^{-1})	4.27	±	0.28	5.02	±	2.04^b
	*T*1max (°C)	128.69	±	$0.38^{b,c}$	129.21	±	0.34^a
	*A*t2 (mg)	-0.15	±	0.01	-0.16	±	0.02
150 →250°C	*V*2max (10^{-3}.min^{-1})	24.62	±	1.56	24.75	±	1.04
	*T*2max (°C)	207.72	±	2.54	209.45	±	7.41^a
	*A*t3 (mg)	-0.40	±	0.13	-0.34	±	0.09^a
250 →350°C	*V*3max (10^{-3}.min^{-1})	3.04	±	0.78	2.63	±	0.63
	*T*3max (°C)	277.97	±	1.54	278.94	±	6.91^a
DSC(TGA)							
	*T*1m (°C)	127.39	±	0.28	127.93	±	0.24
100 →150°C	ΔH1m (J/g)	-83.66	±	3.87^a	-99.01	±	22.05
	*T*2m (°C)	207.00	±	6.51	200.12	±	10.06
150 →250°C	ΔH2m (J/g)	-34.34	±	13.53^b	-51.33	±	37.82
	$d_H$cum (µm)	2.71	±	0.68^b	2.46	±	0.77^b
Nanosizer	$d_H$ave (µm)	1.41	±	0.57^a	1.18	±	0.50^a
	ζ (mV)	-17.64	±	1.09	-16.68	±	0.75

Table 1:TGA, DTG, DSC and nanosizer analysis data of VSL#3 samples made in USA and in Italy

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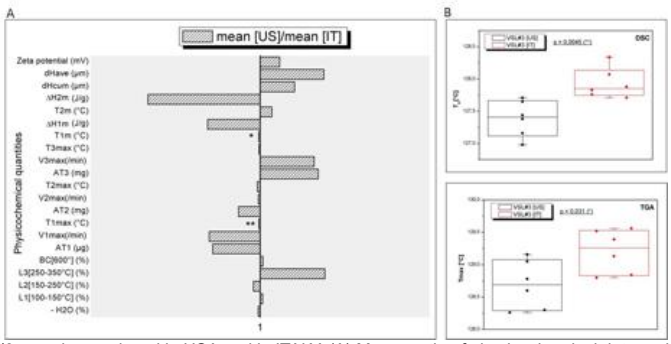

Figure 1: Comparison of VSL#3 samples produced in USA and in ITALY. (A) Means ratio of physicochemical data and (B) Thermal variables with significant differences (*T*1m, *T*1max) analysed by ANOVA 1-way Tukey test with (*) significant and (**) highly significant differences (p-value = 0.05, n>=6)