

# Physico-chemical Approach for Characterizing Probiotics: Example of High Concentrated Multistrain-Based Formulation

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## ABSTRACT

A new approach based on the physico-chemical characterization of microorganisms was applied to a high concentrated multistrain probiotic formulation containing 8 strains. Such a methodology included thermal and particle analyses of sample in the powder state (solid-in-gaseous phase), and the bacteria surface characterization in the dispersed state (solid-in aqueous phase). Decomposition and transition phases from thermogravimetry and scanning calorimetry analyses of the powder provided qualitative and quantitative information, which could serve as identity for the probiotic sample, and may be used in the product quality control. Cell surface properties of the dispersed sample, investigated by nanosizer and contact angle measurements, allow the evaluation of the film forming probiotic hydrophobicity, cell electrophoretic mobility, and particle size. These physico-chemical parameters govern the most crucial cell functionalities, like bacteria adhesion and aggregation, and constitute, therefore, the key factors to control important phenomena such as the biofilm formation and destruction.

**Keywords:** Multistrain probiotics; Physico-chemical characterization; Thermogravimetry; Differential scanning Calorimetry; Contact angle; Electrokinetic potential; Particle size; Scanning Electronic Microscopy

## INTRODUCTION

Probiotics are receiving today unprecedented growing interests in Europe, Asia, and in the rest of the world. Their applications are not only limited to food and health sectors, but cover also agriculture and aquaculture areas. The quality of probiotic products depends on many factors such as, the properties of each individual strain and its proportion in mixed products, the viable probiotic dose, and other selective ingredients like prebiotics and protectant agents incorporated into the formulation [1]. Several strategies are employed for ensuring high product qualities, which can be controlled by different methods and techniques [2]. To date, the physico-chemical approach for characterizing and controlling probiotic qualities and performances appears very attractive, but less exploited. It particularly consists in characterizing probiotic products in terms of thermal, surface and colloidal properties, which could be correlated to probiotic viability and functionalities. In this communication, we report the efficiency of such an approach when applied to a high concentrated multistrain-based formulation, for which has been shown a metabolic variability impacting on the inflammatory response, depending on the production site [3].

## EXPERIMENTATION

**MATERIAL:** Multistrain probiotics

Exp. date: 11/2017  
Lot: VSM003NM (Vivomixx)  
State: powder 4,4g/sachet  
Dose: 8 strains (450 billions)  
Source: ITALY

**METHOD:** Physico-chemical approach

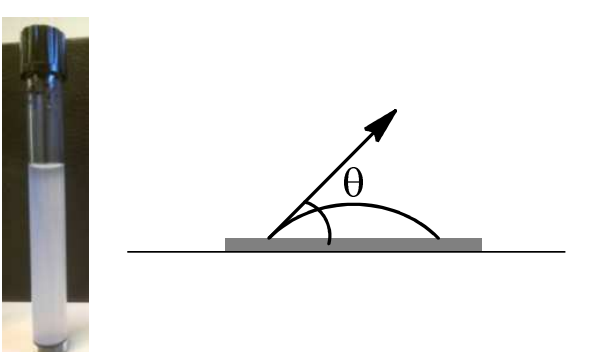
### 1. Powder state

- SEM
- Size
- TGA/DSC



### 2. Dispersed state

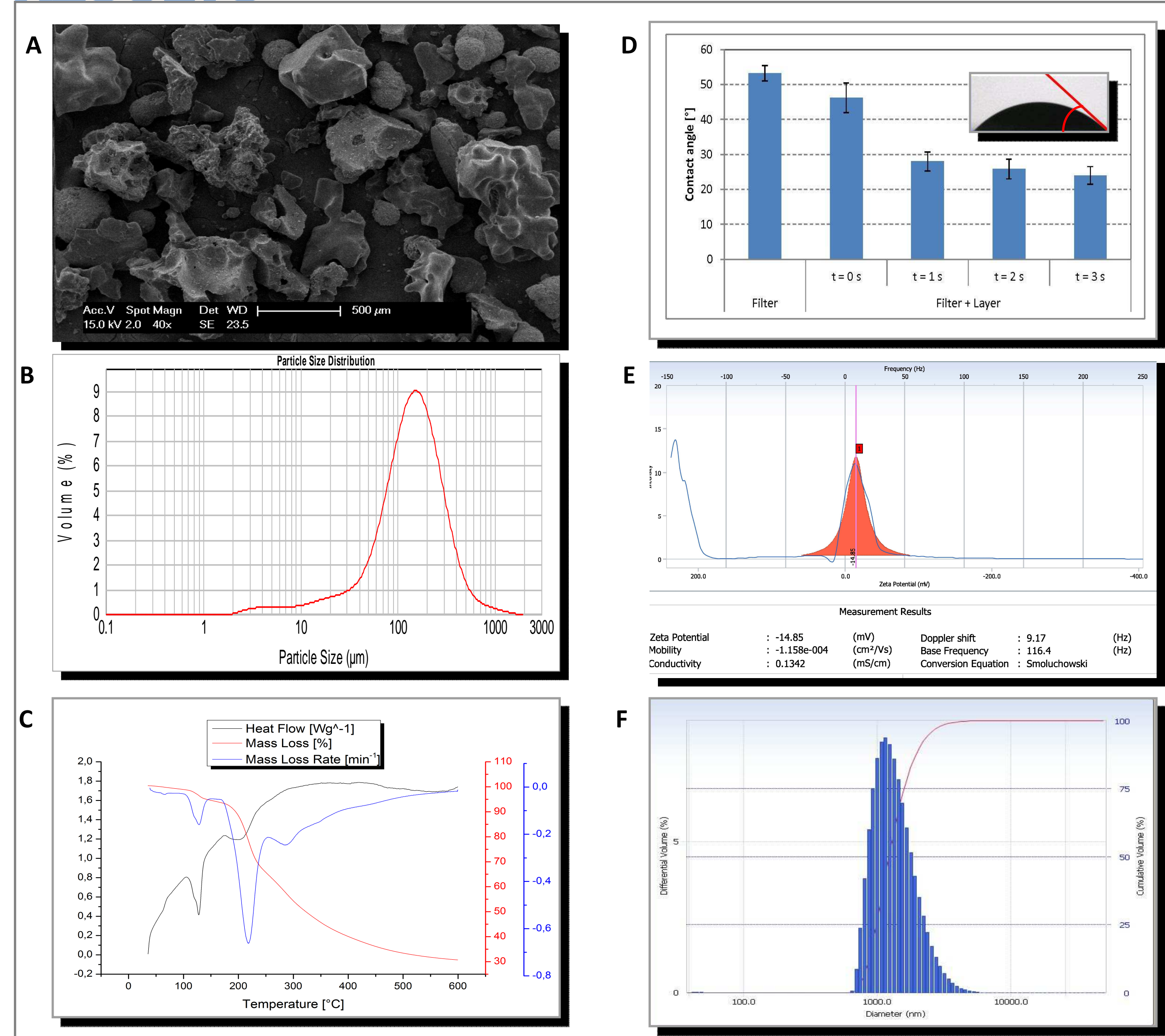
- Hydrophobicity
- Electrokinetic potential
- Size



SEM: Scanning Electronic Microscopy

TGA/DSC: Thermogravimetry analysis/Differential Scanning Calorimetry

## RESULTS



**Figure 1.** Illustrations of the physico-chemical characterization of multistrain probiotic in the **solid state**: **A.** SEM image; **B.** Solid particle size distribution; **C.** TGA/DTG/DSC plots (5°C/min), and in the **aqueous dispersed form**: **D.** Contact angle of probiotic layers; **E.** Electrophoretic mobility distribution; **F.** Hydrodynamic size distribution in volume percentage.

## CONCLUSION

Our new approach based on the physico-chemical screening of probiotic properties shows that it is possible to characterize in qualitative (thermogram profile) and quantitative (data) ways a high concentrated multistrain sample (vivomixx). Such a methodology includes the analysis of probiotics in their powder and dispersed state by thermogravimetry and scanning calorimetry, particle size and electrical potential analyzer, and cell surface hydrophobicity characterization through the contact angle measurement. Some data from TGA and DSC analyses (decomposition and transition characteristics) can be used as fingerprints of the characterized sample, and will be useful for the probiotic-based product quality control in manufacturing sites. Others, such as surface hydrophobicity, zeta potential and cell size, may be exploited for better understanding the action mechanism of probiotics, because these physico-chemical quantities control most crucial cell functionalities like bacteria adhesion and aggregation, the key factors to the biofilm formation and destruction.

## REFERENCES

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Table 1: Physico-chemical data of multistrain probiotic			
Physico-chemical characteristics	mean	±	sd
<b>SOLID in GAS (powder)</b>			
<i>Particle size</i>	d0,5 (µm)	±	3,7
<b>TGA</b>			
-H <sub>2</sub> O (%)	97,5	±	0,7
L1 (%)	-3,0	±	0,2
V1max (10 <sup>-3</sup> /min)	-7,8	±	0,2
T1max (°C)	126,8	±	0,2
L2 (%)	-21,2	±	0,8
V2max (10 <sup>-3</sup> /min)	-31,9	±	1,8
T2max	216,6	±	0,7
L3 (%)	-7,8	±	0,7
V3max(10 <sup>-3</sup> /min)	-12,4	±	0,1
T3max (°C)	285,7	±	0,2
BC (%)	30,5	±	0,2
<b>DSC</b>			
ΔH1m (J/g)	-104,1	±	7,2
T1m (°C)	127,7	±	0,3
ΔH2m (J/g)	-78	±	8,3
T2m (°C)	208,2	±	1,4
<b>SOLID in LIQUID (dispersion)</b>			
<i>Hydrophobicity</i> θ <sub>c</sub> (t=0)	46,2	±	4,2
<i>Zeta potential</i> (mV)	-16,0	±	0,7
<i>Diameter</i> (µm)	1,87	±	0,03

**Legends.** -H<sub>2</sub>O: dehydration; L<sub>n</sub>: mass loss; V<sub>n</sub>max: L<sub>n</sub> maximum rate; T<sub>n</sub>max: temperature at V<sub>n</sub>max; BC: black carbon at 600°C; ΔH<sub>n</sub>m: transition enthalpy; T1m: transition temperature; θ<sub>c</sub>: instantaneous contact angle. n = 1, 2 and 3 correspond to 100-150°C, 150-250°C and 250-350°C temperature ranges by heating at 5°C/min rate, respectively.