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### Physicochemical behaviour of TiO<sub>2</sub> particles in simulated digestive fluids.

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Titanium dioxide is a white metal oxide employed as a pigment, which is commonly used in coatings of candies and chewing-gum. Food-grade TiO<sub>2</sub>, referred to as E171 in Europe and INS171 in North America, includes a nano-sized fraction, representing less than 44% of the particles. Due to concerns about TiO<sub>2</sub> nanoparticles (NPs) as potentially hazardous, and in particular after ingestion, the fate of particles in the gastro-intestinal tract is under investigation. In this study, the behavior of food grade TiO<sub>2</sub> and TiO<sub>2</sub> NPs (P25, 25nm) were determined through a standardized static in vitro digestion protocol and compared to the control digestion (with and without the digestion enzymes). After each step of digestion (i.e. saliva, gastric and intestinal step), the size of the particles and the surface charge changes were characterized using laser particle size distribution analysis and zeta potential measurements. Finally, the nature of the adsorbed proteins was determined by denaturing gradient gel electrophoresis (DGGE) after separation from the TiO<sub>2</sub> particles.

Our results showed that the agglomeration states and surface charge were found to be dependent on the medium composition. Both food grade and P25 particles agglomerate more strongly in the digestive fluids due to the presence of salts and proteins. Moreover, all  $TiO_2$  samples formed the largest agglomerates once in the intestinal fluid, up to 593 µm for food grade, and 77 µm for P25 particles. DGGE analysis showed that food grade and P25 could interact with different enzymes during the simulated digestion. All  $TiO_2$  samples interacted with the alpha amylase, and even with pepsin for the second batch of one of them.

Food grade and nano- $TiO_2$  do not appear to exist as nanometric entity all along the steps of the in vitro digestion protocol, which is good news considering the concerns about the effects of ingested nano-products. However, these large agglomerates could still be transformed in contact with the epithelium and the adsorption of enzymes could have a major impact on the digestion process, if this interaction was responsible for the inactivation of the enzyme.

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