



## Correct labelling? A full analytical pathway for silica and titania particles in food products

Christina Glaubitz<sup>a</sup>, Amélie Bazzoni<sup>a</sup>, Christoph Neururer<sup>b</sup>, Raphael Locher<sup>c</sup>, Kata Dorbić<sup>c</sup>, Jessica Caldwell<sup>a</sup>, Miguel Spuch-Calvar<sup>d</sup>, Laura Rodriguez Lorenzo<sup>e</sup>, Sandor Balog<sup>a</sup>, Vincent Serneels<sup>b</sup>, Barbara Rothen-Rutishauser<sup>a</sup>, Alke Petri Fink<sup>a,c,\*</sup>

<sup>a</sup> Adolphe Merkle Institute, University of Fribourg, Chemin des Verdiers 4, 1700, Fribourg, Switzerland

<sup>b</sup> Geology Department, University of Fribourg, Chemin Du Musée 4, 1700, Fribourg, Switzerland

<sup>c</sup> Chemistry Department, University of Fribourg, Chemin Du Musée 9, 1700, Fribourg, Switzerland

<sup>d</sup> TeamNanoTech / Magnetic Materials Group, CINBIO-Universidade de Vigo, Campus Universitario Lagoas Marcosende, 36310, Vigo, Spain

<sup>e</sup> INL - International Iberian Nanotechnology Laboratory, Avda. Mestre José Veiga S/n, Braga, Portugal

### ARTICLE INFO

Handling Editor: Professor Aiqian Ye

#### Keywords:

Nanoparticles  
Food products  
Particle analysis  
X-ray fluorescence  
Food additives

### ABSTRACT

Introducing particles as additives, specifically engineered nanoparticles, in the food industry has improved food properties. Since 2014, alongside the presence of these added particles, there has been a mandatory requirement to disclose if those additives are nanomaterials in the ingredient list of food products. However, detecting and characterizing nanomaterials is time-consuming due to their small sizes, low concentrations, and diverse food matrices.

We present a streamlined analytical process to detect the presence of silica and titania particles in food, applicable for food regulation and control. Using X-ray Fluorescence Spectrometry for screening enables quick categorization of inorganic particles labeling accuracy, distinguishing products with and without them. For the former, we develop matrix-independent digestion and introduce time-effective statistics to evaluate the median particle size using a reduced number of particles counted, ensuring accurate “nano” labeling.

Through the implementation of this work, our objective is to simplify and facilitate verifying the proper labeling of food products.

### 1. Introduction

The food sector has been revolutionized by introducing inorganic particles as additives to enhance properties such as texture, color, flavor, or shelf life (Weir et al., 2012; Lomer et al., 2000; Go et al., 2017a). Those particles—e.g., SiO<sub>2</sub> E551 (Commission Regulation (EU), 2012) as an anti-caking agent (Younes et al., 2018) or the now forbidden TiO<sub>2</sub> E171 (Commission Regulation (EU), 2012) for food whitening (Younes et al., 2021)—come as powders, with particles potentially in the nano-range. Manufacturers are legally obliged to clearly indicate the presence of those additives in food products on the ingredient list (Regulation (EU), 2011).

Despite the benefits of these additives, there are growing concerns over their potential health hazards, especially because proper risk assessment of oral exposure to nanoparticles is complex due to the lack of conclusive data (Peters et al., 2014; Bouwmeester et al., 2009). The

Scientific Committee on Emerging and Newly Identified Health Risks (SCENHIR) was asked to provide a scientific opinion on the most appropriate metrics for identifying which size range could be problematic. As a minimal fraction of the mass could contain large numbers of elements in the small size range, and hazard is associated with the number of particles, they recommended a definition based on the number-based particle size distribution rather than a mass fraction (Scientific Committee on Emerging and Newly Identified Health Risks SCENHIR, 2010).

Based on those recommendations, the European Commission defines nanomaterials as a natural, incidental, or manufactured material consisting of solid particles and where 50 % or more of these particles in the number-based size distribution in one or more external dimensions of the particle are in the size range 1 nm–100 nm (Commission Recommendation of 10 June 2022). Therefore, the food labeling regulation was expanded to require mentioning “nano” on the ingredient list when the

\* Corresponding author. Adolphe Merkle Institute, University of Fribourg, Chemin des Verdiers 4, 1700, Fribourg, Switzerland.

E-mail address: [alke.fink@unifr.ch](mailto:alke.fink@unifr.ch) (A.P. Fink).

<https://doi.org/10.1016/j.crf.2024.100808>

Received 7 November 2023; Received in revised form 4 July 2024; Accepted 8 July 2024

Available online 10 July 2024

2665-9271/© 2024 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC license (<http://creativecommons.org/licenses/by-nc/4.0/>).

used additives fulfill the nanomaterials definition (Regulation (EU), 2011).

It is necessary (Weir et al., 2012; Lomer et al., 2000; Le contrôle des nanomatériaux par la DGCCRF, 2018) that food products are controlled for compliance with regulations, which would require a multitude of robust protocols that are adaptable to different product types, capable of quickly detecting nanoparticles, and offering their size information for nanomaterial labeling.

Much research has been channeled to detecting nanoparticles in food products and characterizing their size, size distribution, and concentration (Weir et al., 2012; Peters et al., 2014; Dudkiewicz et al., 2011; Dekkers et al., 2011; Grombe et al., 2014; Yu et al., 2021; López-Heras et al., 2014; Loeschner et al., 2018; Heroult et al., 2014). However, the analytical process is complicated because the nanoparticles must first be extracted from the food matrix (Peters et al., 2014; Dudkiewicz et al., 2011; Dekkers et al., 2011; Grombe et al., 2014). This poses multiple challenges, including the requirement to preserve particle sizes and suspension behavior, as well as the utilization of harsh chemicals and reaction conditions (Weir et al., 2012; Peters et al., 2014; López-Heras et al., 2014; Loeschner et al., 2018; Heroult et al., 2014). Furthermore, some previously developed extraction protocols are only suitable for certain types of food products, (Grombe et al., 2014; Loeschner et al., 2018; Heroult et al., 2014; Kim et al., 2018) limiting their applicability. However, the need to apply different protocols and, in some cases, develop new ones for novel food products can be labor-intensive and time-consuming.

Our study presents a simple yet robust protocol where Wavelength Dispersive X-Ray Fluorescence Spectrometry (WD-XRF) is used as an initial analytical step to provide semi-quantitative and qualitative information about the presence of additives. XRF determines the chemical composition of a sample by measuring the emission of fluorescent X-rays produced when the sample is excited by a primary X-ray source. The emitted X-rays are element-specific, and their intensity is related to the concentration (Potts and Webb, 1992). Although XRF is a well-established technique in the food industry, used to detect traces of heavy metals in plants, (Mijovilovich et al., 2020; Gutiérrez-Ginés et al., 2013) soils (Gutiérrez-Ginés et al., 2013; Li et al., 2021; Ene et al., 2010) and vegetables, (Byers et al., 2019) its application for additives is relatively new. Synchrotron XRF analysis has been utilized to demonstrate the transfer of engineered nanoparticles from the environment into food products, (Hernandez-Viezas et al., 2013; Servin et al., 2013, 2017; Zhao et al., 2013, 2015) while Linder et al. quantified silver nanoparticles using a simple, portable XRF instrument (Sánchez-Pomales et al., 2013).

Benefitting from the fast and reduced sample preparation of XRF (Potts and Webb, 1992; Hall, 2017) the here proposed solution is to detect the presence of inorganic nanoparticles used as food additives. We confirmed our method by comparing the WD-XRF results with data obtained by Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES), which is a common technique for elemental quantification in food products (Weir et al., 2012; Lomer et al., 2000; Go et al., 2017b). Although XRF does not provide any size information, it allows us to target only the products that need further size determination to verify whether a food product is correctly labeled according to regulatory standards. Once a food product is flagged as containing inorganic nanoparticles, Electron Microscopy (EM) techniques are necessary to establish a number-based size distribution (Labuda et al., 2023). (More et al., 2021; Calzolari et al., 2012)

To meet the criteria for labeling as "nano", the proportion of particles smaller than 100 nm must be determined (Commission Recommendation of 10 June 2022). Therefore, we have developed a rigorously tested protocol that employs acidic microwave-assisted digestion to remove all organic matrices effectively while maintaining the original additive size of silica and titania particles, ensuring accurate analysis.

Counting and sizing nanoparticles in food samples can be challenging as they often form particle clusters due to various factors, such

as sample preparation, (Michen et al., 2015) extraction process, particle treatment during food manufacturing, or particle synthesis (Glaubitz et al., 2022). As a result, particle counting and sizing using programs for automated analysis can be difficult, and the analysis is often done manually (de Temmermann et al., 2012). To ensure reliable data, previous protocols have recommended particle counts ranging from 100 to 1000,<sup>43-45</sup> making manual analysis time-consuming and impractical for routine analysis. Our here presented study demonstrates that a particle count of 30 is adequate for evaluating the probability of finding particles below 100 nm, the crucial requirement to fulfill the definition of a nanomaterial. By counting 30 particles, the relative standard deviation we found was between 1 and 7 %, which is within the published uncertainty range of measuring the particle size using EM (Verleyesen et al., 2019; De Temmerman et al., 2014; Rice et al., 2013).

## 2. Method

1. *Sample Preparation for the WD-XRF Measurements:* Flour, instant coffee, seasoning salt and fondant were selected, two with E171, one with E551 additives labeled on the ingredient list, and one with food samples without additives listed. To prepare the samples, 12 g of the food product was mixed with 3 g of binder (Licowax/Hoechst wax C micro powder) and pressed into pellets using a hydraulic press. The pellets had a 4 cm diameter and a height of 0.8–1.2 cm and were dried before analysis at 60 °C for 24 h to ensure a loss of pore water. WD-XRF measurements were done using a Zetium X-ray spectrometer equipped with a rhodium X-ray tube and the Omnia standardless analysis package (Malvern-PANalytical). The intensities are measured at specific conditions for each element (see Table SI 1). The concentrations calculated with the two-point calibration are not normalized to consider the mainly organic matrix. This remains a semi-quantitative approach but allows the detection of the elements of interest.
2. *Acid-Resistant Particle Extraction:* To digest the food samples, 3 mL of nitric acid (65 %, Sigma Aldrich) was added to 0.5 g of the sample in PTFE tubes. The matrix was eliminated using a microwave (Anton Paar), following the temperature and pressure profile in Figure SI 1. After digestion, solids were purified with MilliQ water by centrifuging thrice at 8000 g for 10 min to remove the acidic solution. The detailed protocol is in the Supporting Information, Protocol for Detection of Silicon Dioxide (SiO<sub>2</sub>) and Titanium Dioxide (TiO<sub>2</sub>) Particles in Food Products. To demonstrate that the developed protocol for particle extraction does not result in a size degradation, we studied the size of five different representative types of particles (40 nm, 95 nm, and 400 nm spherical SiO<sub>2</sub> particles, as well as the two commercially available Aerosil 200 SiO<sub>2</sub> (Evonik) and P25 TiO<sub>2</sub> (Degussa)) before and after digestion (see Supporting Information, Particle Size Alteration Due to Digestion).
3. *Characterization of Extracted Nanoparticles:* After purification, the particles were characterized by Transmission Electron Microscopy (TEM) using a FEI Tecnai Spirit microscope operating at 120 kV. Images were acquired using a Veleta wide-angle camera 2048 x 2048.
4. *Particles Size Analysis:* To evaluate the particle counting method, we conducted tests on a sample size of 1000 manually counted particles, including 400 nm spherical SiO<sub>2</sub> particles, as well as commercially available Aerosil 200 SiO<sub>2</sub> and P25 Degussa TiO<sub>2</sub> and the particles found in fondant and a seasoning salt counting process involved randomly selecting between 3 and 100 particles from the total pool of 1000 counted particles by employing 1000 rounds of bootstrapped sampling (Efron, 1988).
5. *Sample Preparation for ICP-OES:* To digest the particles, 0.3 g of food product was digested with 1.8 mL nitric acid using a microwave (Anton Paar). Then, 200 µL hydrofluoric acid (48 %, Sigma Aldrich) and 2 mL MilliQ water was added to the sample before additional digestion assisted by the microwave, as described in Figure SI 1. 80

mg boric acid (Sigma Aldrich) was added to quench non-reacted hydrofluoric acid, and the samples were measured with ICP-OES after 24 h when the boric acid was completely dissolved. The ICP-OES measurements of Si and Ti were performed with a Perkin Elmer Avio-200 (radio frequency power: 1500 W, gas flow rates: Ar for plasma 8 L min<sup>-1</sup>, N<sub>2</sub> as auxiliary gas 0.2 L min<sup>-1</sup>, nebulizer pump 0.8 L min<sup>-1</sup>, sample flow 1 L min<sup>-1</sup>), using the spectral lines  $\lambda_{\text{Si}} = 251.611 \text{ nm}$ ,  $\lambda_{\text{Ti}} = 334.94 \text{ nm}$  (see Fig. 1).

### 3. Results and discussion

We tested SiO<sub>2</sub> and TiO<sub>2</sub> content in four food products using WD-XRF and ICP-OES (Fig. 2) and found that WD-XRF could accurately detect the presence of additives in seven out of eight cases. However, we encountered a false positive for the TiO<sub>2</sub> content in the seasoning salt. The subsequent size analysis would confirm the absence of particles, ensuring the correct labeling of the product.

As previously reported in the literature, (Chojnacka et al., 2018; Cataldo, 2012; Arenas et al., 2011; Kilbride et al., 2006) we observed that the concentrations measured by WD-XRF were higher than those obtained with ICP-OES, likely due to matrix effects that occur during WD-XRF measurements. Despite these differences, there is an agreement between ICP-OES and WD-XRF results, with high correlation coefficients—except the false positive—between 0.75 and 1.0 (see Table SI 2).

Given that some food crops have been found to naturally contain silica particles, (Liu et al., 2013; Savant et al., 1999) we suggest a threshold of 0.05 w% for flagging food products that contain SiO<sub>2</sub>. This value is based on the levels detected for flour and fondant in our study.

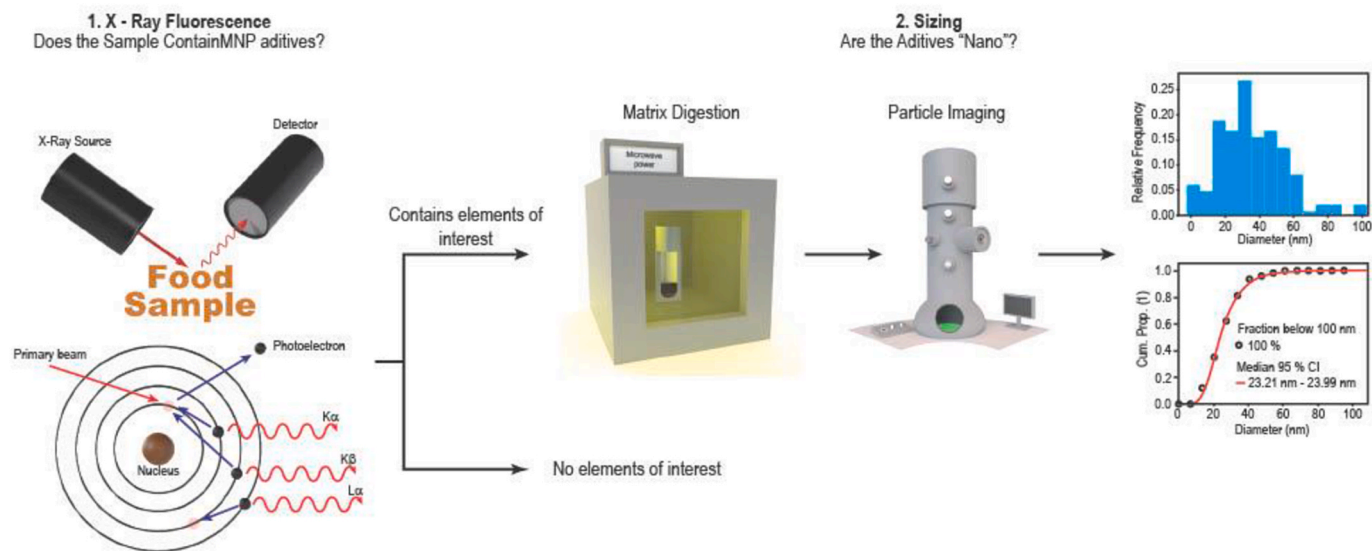
Like ICP-OES, WD-XRF showed an absence of TiO<sub>2</sub> particles in flour and coffee samples. In contrast, the other samples were identified as containing particles, necessitating size analysis to evaluate if they meet the nanomaterial definition. An example comparison of the size distribution for the 40 nm SiO<sub>2</sub> particles can be seen in Fig. 3a, demonstrating that no significant size alterations (Ho et al., 2019) occurred during the digestion process. Additional examples of extracted particles are given in the Supporting Information. A micrograph of extracted particles from a fondant product, together with their size distribution, is depicted in Fig. 3b (additional micrographs can be found in the Supporting Information, Representative TEM Micrographs of Extracted Particles).

As the amount of analyzed particles increases, we gain more

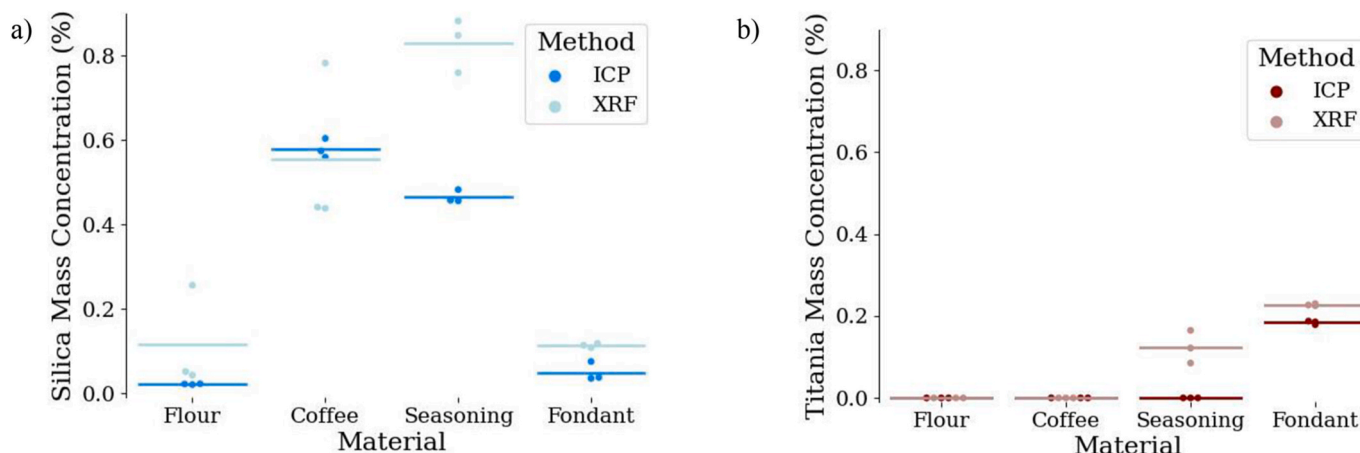
confidence in our estimate and experience reduced uncertainty, resulting in improved precision (Israel, 1992). Therefore, the precision of a measurement, such as the median size of a nanoparticle sample—the criteria for defining a material as nanomaterial, is influenced by various factors. These factors include the vastness of the population being studied, the risk associated with selecting an unfavorable sample, and the acceptable level of sampling error. Fig. 4 highlights that as the number of analyzed particles increases, the certainty in determining the median improves, following this fundamental statistical concept. However, our results show that counting as few as 30 particles can yield statistically significant results, providing a more efficient means of analysis than counting 100–1000 particles while still being able to classify a material as “nano” with 95 % confidence. Additional examples of the influence of particle counting on the median for different particle samples are shown in Figure SI 10. In Table 1, we present the particle median and the relative standard deviation obtained from comparing 30 and 100 counted particles. Notably, the obtained mean of the median remains highly consistent, showing little to no change. Additionally, the relative standard deviation for 30 counted particles is sufficiently low, indicating that it can be comparable to the overall precision achievable through EM (Verleysen et al., 2019; De Temmerman et al., 2014; Rice et al., 2013).

### 4. Conclusion

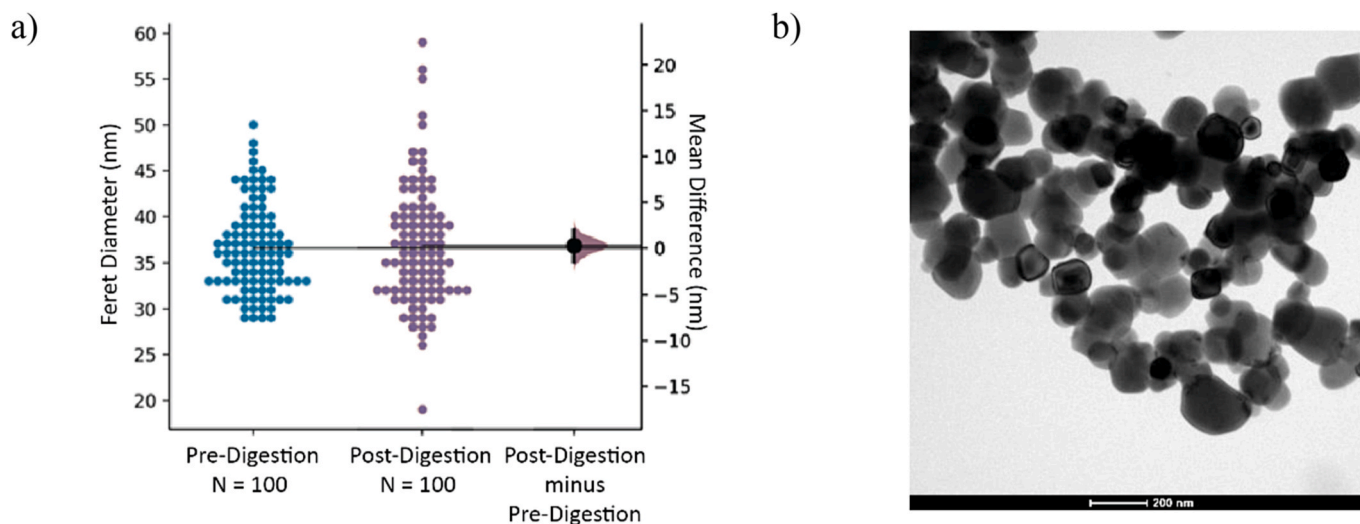
This study presents a comprehensive analysis of the presence of nanoparticles as additives in food products and offers protocols that are fit for routine measurements for food regulators in Europe. Using WD-XRF as a preliminary screening tool effectively identifies food products containing SiO<sub>2</sub> and TiO<sub>2</sub> particles, thus reducing the time and cost of subsequent size analysis. However, due to false positives, especially for SiO<sub>2</sub> particles, a threshold should be applied and a subsequent particle extraction with TEM analysis is recommended. For particle sizing, we offer robust protocols for inorganic particle extraction, applicable to many food products available. The study also proposes that counting as many as 30 particles is sufficient for the size analysis to evaluate if they are nanomaterials and thus requires the “nano” labeling, which will further reduce the time and labor needed to assess the particle size of a food sample accurately. These findings contribute to a safer and more transparent food industry, which empowers consumers to make informed food choices.



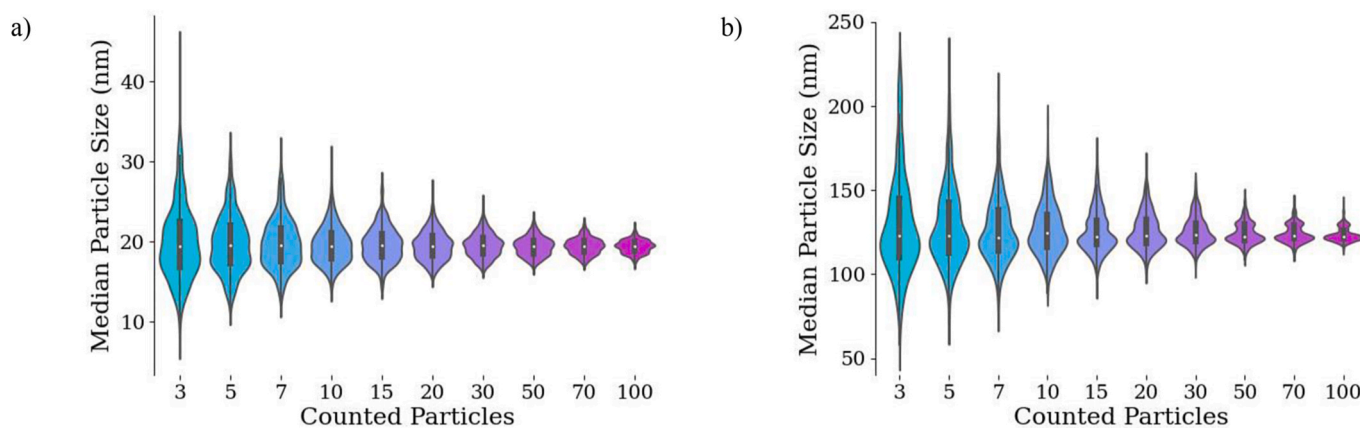
**Fig. 1.** To ensure regulatory requirements, analyzing the addition of inorganic particle additives to food products is necessary. We propose a two steps analytical pathway, starting with WD-XRF as a screening method. WD-XRF uses X-rays to detect the elements and their concentrations in the sample. Once a product is identified as containing particles, we offer protocols for extracting and sizing the particles.



**Fig. 2.** The SiO<sub>2</sub> (panel a) and TiO<sub>2</sub> (panel b) contents were compared for the four food products measured. The comparison displays the mass concentration obtained through WD-XRF (lighter color) and ICP-OES (darker color), with the lines representing the average of the three measurement replicas symbolized with dots. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)



**Fig. 3.** To validate the efficacy of our digestion protocol, we performed a student t-test (95 % confidence interval) (Gosset, 1908) presented as a Gardner-Altman (Ho et al., 2019) estimation plot in panel a), which demonstrates that the mean particle size before (blue) and after (lilac) digestion remains unaltered. The mean difference between the two groups is plotted on the right-hand side as a bootstrap sampling distribution, with the average difference indicated as a dot, with 95 % confidence interval error bars. In panel b), a TEM micrograph using a 200 nm scalebar of a TiO<sub>2</sub> particle cluster inside a fondant sample is displayed. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)



**Fig. 4.** The results of counting different numbers of P25 Degussa TiO<sub>2</sub> reference particles (panel a) and TiO<sub>2</sub> particles found inside fondant (panel b) were obtained by randomly resampling the counted particles 1000 times using the Bootstrap method from a total pool of 1000 counted particles per sample. The density of each violin indicates the frequency of the data points, with a grey box indicating the 95% confidence interval and a white dot representing the median of all data points.



**Table 1**

Comparison of the obtained particle median and the relative standard deviation (STD) of 30 and 100 analyzed particles, which were obtained by randomly resampling the counts 1000 times using the Bootstrap method.

Particle Type	Particle Median and Relative STD of 30 counted Particles	Particle Median and Relative STD of 100 counted Particles
Aerosil SiO <sub>2</sub>	44 ± 3.9 %	44 ± 1.7 %
Seasoning Salt	24 ± 7.4 %	24 ± 3.8 %
TiO <sub>2</sub> P25	20 ± 7.5 %	19 ± 4.1 %
SiO <sub>2</sub> 400 nm	409 ± 1.2 %	409 ± 0.5 %

## Funding sources

The authors are grateful for the financial support of the Adolphe Merkle Foundation, the University of Fribourg, and the Swiss National Science Foundation through the National Centre of Competence in Research Bio-Inspired Materials and the grant SNF no. 200020\_184,635. The authors would also like to thank the Federal Food Safety and Veterinary Office for generous funding of parts of this research.

## CRedit authorship contribution statement

**Christina Glaubitz:** Conceptualization, Methodology, Data curation, Writing – original draft. **Amélie Bazzoni:** Conceptualization, Data curation, Writing – review & editing. **Christoph Neururer:** Data curation. **Raphael Locher:** Data curation. **Kata Dorbić:** Data curation. **Jessica Caldwell:** Writing – review & editing. **Miguel Spuch-Calvar:** Visualization. **Laura Rodriguez Lorenzo:** Data curation. **Sandor Balog:** Investigation, Writing – review & editing. **Vincent Serneels:** Supervision, Conceptualization. **Barbara Rothen-Rutishauser:** Supervision, Writing – review & editing. **Alke Petri Fink:** Supervision, Writing – review & editing.

## Declaration of competing interest

The authors have no interests to declare. This includes external finances, additional relationships, patents, or other activities.

## Data availability

Data will be made available on request.

## Acknowledgments

The authors are grateful for the support of Aurélien Crochet, Laetitia Haeni, and Liliane Ackermann.

## Abbreviations

EDS	Energy Dispersive X-Ray Spectroscopy
EM	Electron Microscopy
ICP-OES	Inductively Coupled Plasma-Optical Emission Spectroscopy
STD	standard deviation
XRF	X-Ray Fluorescence
WD-XRF	Wavelength Dispersive X-Ray Fluorescence

## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.crfs.2024.100808>.

## References

- Arenas, L., Ortega, M., García-Martínez, M.J., Querol, E., Llamas, J.F., 2011. Geochemical characterization of the mining district of Linares (Jaen, Spain) by means of XRF and ICP-AES. *J. Geochem. Explor.* 108 (1), 21–26.
- Bouwmeester, H., Dekkers, S., Noordam, M.Y., Hagens, W.I., Bulder, A.S., de Heer, C., ten Voorde, S.E.C.G., Wijnhoven, S.W.P., Marvin, H.J.P., Sips, A.J.A.M., 2009. Review of health safety aspects of nanotechnologies in food production. *Regul. Toxicol. Pharmacol.* 53 (1), 52–62.
- Byers, H.L., McHenry, L.J., Grundl, T.J., 2019. XRF techniques to quantify heavy metals in vegetables at low detection limits. *Food Chem. X* 1, 100001.
- Calzolari, L., Gilliland, D., Rossi, F., 2012. Measuring nanoparticles size distribution in food and consumer products: a review. *Food Addit. Contam.* 29 (8), 1183–1193.
- Cataldo, F., 2012. Multielement analysis of a municipal landfill leachate with total reflection X-ray fluorescence (TXRF). A comparison with ICP-OES analytical results. *J. Radioanal. Nucl. Chem.* 293 (1), 119–126.
- Chojnacka, K., Samoraj, M., Tuhy, L., Michalak, I., Mironiuk, M., Mikulewicz, M., 2018. Using XRF and ICP-oes in biosorption studies. *Molecules* 23 (8), 2076.
- Commission Recommendation of 10 June 2022 on the Definition of Nanomaterial (Text with EEA Relevance) 2022/C 229/01, 2022. European Commission, pp. 1–5.
- Commission Regulation (EU), 2012. No 231/2012 of 9 March 2012 Laying Down Specifications for Food Additives Listed in Annexes II and III to Regulation (EC) No 1333/2008 of the European Parliament and of the Council. *OJ L*, 83. European Commission, pp. 1–295.
- De Temmerman, P.-J., Lammertyn, J., De Ketelaere, B., Kestens, V., Roebben, G., Verleysen, E., Mast, J., 2014. Measurement uncertainties of size, shape, and surface measurements using transmission electron microscopy of near-monodisperse, near-spherical nanoparticles. *Journal of Nanoparticle* 16 (1), 2177.
- de Temmermann, P.J., Mast, J., Guiot, C., Spalle, O., Rousset, D., Shivachev, B., Tarasov, M., Jensen, K.A., 2012. Deliverable 4.2: Transmission electron microscopic characterization of NANOGENOTOX nanomaterials. *Nanogen Tox* 1–76. Grant Agreement no 2009 21 01.
- Dekkers, S., Krystek, P., Peters, R.J.B., Lankveld, D.P.K., Bokkers, B.G.H., van Hoven-Arentzen, P.H., Bouwmeester, H., Oomen, A.G., 2011. Presence and risks of nanosilica in food products. *Nanotoxicology* 5 (3), 393–405.
- Dudkiewicz, A., Tiede, K., Loeschner, K., Jensen, L.H.S., Jensen, E., Wierzbicki, R., Boxall, A.B.A., Molhave, K., 2011. Characterization of nanomaterials in food by electron microscopy. *TrAC, Trends Anal. Chem.* 30 (1), 28–43.
- Efron, B., 1988. Bootstrap confidence intervals: good or bad? *Psychol. Bull.* 104 (2), 293–296.
- Ene, A., Bosneaga, A., Georgescu, L., 2010. Determination of heavy metals in soils using XRF technique. *Rom. J. Phys.* 55 (7–8), 815–820.
- Glaubitz, C., Rothen-Rutishauser, B., Lattuada, M., Balog, S., Petri-Fink, A., 2022. Designing the ultrasonic treatment of nanoparticle-dispersions via machine learning. *Nanoscale* 14 (35), 12940–12950.
- Go, M.-R., Bae, S.-H., Kim, H.-J., Yu, J., Choi, S.-J., 2017a. Interactions between food additive silica nanoparticles and food matrices. *Front. Microbiol.* 8.
- Go, M.-R., Bae, S.-H., Kim, H.-J., Yu, J., Choi, S.-J., 2017b. Interactions between food additive silica nanoparticles and food matrices. *Front. Microbiol.* 8, 1013.
- Gosset, W.S., 1908. The probable error of a mean. *Biometrika* 6 (1), 1–25.
- Grombe, R., Charoud-Got, J., Emteborg, H., Linsinger, T.P.J., Seghers, J., Wagner, S., von der Kammer, F., Hofmann, T., Dudkiewicz, A., Llinas, M., Solans, C., Lehner, A., Allmaier, G., 2014. Production of reference materials for the detection and size determination of silica nanoparticles in tomato soup. *Anal. Bioanal. Chem.* 406, 3895–3907.
- Gutiérrez-Ginés, M.J., Pastor, J., Hernández, A.J., 2013. Assessment of field portable X-ray fluorescence spectrometry for the in situ determination of heavy metals in soils and plants. *Environ. Sci. J. Integr. Environ. Res.: Process. Impacts* 15 (8), 1545.
- Hall, M., 2017. X-ray fluorescence-energy dispersive (ED-XRF) and wavelength dispersive (WD-XRF) spectrometry. In: *The Oxford Handbook of Archaeological Ceramic Analysis*. Oxford University Press, pp. 343–381.
- Hernandez-Viezas, J.A., Castillo-Michel, H., Andrews, J.C., Cotte, M., Rico, C., Peralta-Videa, J.R., Ge, Y., Priester, J.H., Holden, P.A., Gardea-Torresdey, J.L., 2013. In situ synchrotron X-ray fluorescence mapping and speciation of CeO<sub>2</sub> and ZnO nanoparticles in soil cultivated soybean (*Glycine max*). *ACS Nano* 7 (2), 1415–1423.
- Heroult, J., Nischwitz, V., Bartczak, D., Goenaga-Infante, H., 2014. The potential of asymmetric flow field-flow fractionation hyphenated to multiple detectors for the quantification and size estimation of silica nanoparticles in a food matrix. *Anal. Bioanal. Chem.* 406 (16), 3919–3927.
- Ho, J., Tunkaya, T., Aryal, S., Choi, H., Claridge-Chang, A., 2019. Moving beyond P values: data analysis with estimation graphics. *Nat. Methods* 16 (7), 565–566.
- Israel, G.D., 1992. Determining Sample Size, Program Evaluation and Organizational Development, IFAS. PEOD-6. University of Florida, Florida (FL).
- Kilbride, C., Poole, J., Hutchings, T.R., 2006. A comparison of Cu, Pb, As, Cd, Zn, Fe, Ni and Mn determined by acid extraction/ICP-OES and ex situ field portable X-ray fluorescence analyses. *Environ. Pollut.* 143 (1), 16–23.
- Kim, N., Kim, C., Jung, S., Park, Y., Lee, Y., Jo, J., Hong, M., Lee, S., Oh, Y., Jung, K., 2018. Determination and identification of titanium dioxide nanoparticles in confectionery foods, marketed in South Korea, using inductively coupled plasma optical emission spectrometry and transmission electron microscopy. *Food Addit. Contam.* 35 (7), 1238–1246.
- Labuda, J., Barek, J., Gajdosechova, Z., Goenaga-Infante, H., Johnston, L.J., Mester, Z., Shtykov, S., 2023. Analytical chemistry of engineered nanomaterials: Part 1. Scope, regulation, legislation, and metrology (IUPAC Technical Report). *Pure Appl. Chem.* 95 (2), 133–163.

- Le contrôle des nanomatériaux par la DGCCRF, 2018. Conseil National de la Consommation. In: Direction générale de la consommation.
- Li, W., Niu, N., Guo, N., Zhou, H., Bu, J., Ding, A., 2021. Comparative study on the determination of heavy metals in soil by XRF and ICP-MS. *J. Phys. Conf. Ser.* 2009 (1), 012075.
- Liu, N., Huo, K., McDowell, M.T., Zhao, J., Cui, Y., 2013. Rice husks as a sustainable source of nanostructured silicon for high performance Li-ion battery anodes. *Sci. Rep.* 3 (1), 1919.
- Loeschner, K., Correia, M., López Chaves, C., Rokkjær, I., Sloth, J.J., 2018. Detection and characterisation of aluminium-containing nanoparticles in Chinese noodles by single particle ICP-MS. *Food Addit. Contam.* 35 (1), 86–93.
- Lomer, M.C.E., Thompson, R.P.H., Comisso, J., Keen, C.L., Powell, J.J., 2000. Determination of titanium dioxide in foods using inductively coupled plasma optical emission spectrometry. *The Analyst*. 125 (12), 2339–2343.
- López-Heras, I., Madrid, Y., Cámara, C., 2014. Prospects and difficulties in TiO<sub>2</sub> nanoparticles analysis in cosmetic and food products using asymmetrical flow field-flow fractionation hyphenated to inductively coupled plasma mass spectrometry. *Talanta* 124, 71–78.
- Michen, B., Geers, C., Vanhecke, D., Endes, C., Rothen-Rutishauser, B., Balog, S., Petri-Fink, A., 2015. Avoiding drying-artifacts in transmission electron microscopy: characterizing the size and colloidal state of nanoparticles. *Sci. Rep.* 5 (1), 9793.
- Mijovilovich, A., Morina, F., Bokhari, S.N., Wolff, T., Küpper, H., 2020. Analysis of trace metal distribution in plants with lab-based microscopic X-ray fluorescence imaging. *Plant Methods* 16 (1), 82.
- More, S., Bampidis, V., Benford, D., Bragard, C., Halldorsson, T., Hernández-Jerez, A., Bennekou, S.H., Koutsoumanis, K., Lambre, C., Macher, K., Naegeli, H., Nielsen, S., Schlatter, J., Schrenk, D., Silano (deceased), V., Turck, D., Younes, M., Castenmiller, J., Chaudhry, Q., Cubadda, F., Franz, R., Gott, D., Mast, J., Mortensen, A., Oomen, A.G., Weigel, S., Barthelemy, E., Rincon, A., Tarazona, J., Schoonjans, R., 2021. Guidance on technical requirements for regulated food and feed product applications to establish the presence of small particles including nanoparticles. *EFSA J.* 19 (8).
- Peters, R.J.B., van Bommel, G., Herrera-Rivera, Z., Helsper, H.P.F.G., Marvin, H.J.P., Weigel, S., Tromp, P.C., Oomen, A.G., Rietveld, A.G., Bouwmeester, H., 2014. Characterization of titanium dioxide nanoparticles in food products: analytical methods to define nanoparticles. *J. Agric. Food Chem.* 62 (27), 6285–6293.
- Potts, P.J., Webb, P.C., 1992. X-ray fluorescence spectrometry. *J. Geochem. Explor.* 44 (1–3), 251–296.
- Regulation (EU), 2011. No 1169/2011 of the European parliament and of the council of 25 october 2011 on the provision of food information to consumers, amending regulations (EC) No 1924/2006 and (EC) No 1925/2006 of the European parliament and of the council, and repealing commission directive 87/250/EEC, council directive 90/496/EEC, commission directive 1999/10/EC, directive 2000/13/EC of the European parliament and of the council, commission directives 2002/67/EC and 2008/5/EC and commission regulation (EC) No 608/2004 text with EEA relevance. *Orkesterjournalen L 304* (2011), 18–63.
- Rice, S.B., Chan, C., Brown, S.C., Eschbach, P., Han, L., Ensor, D.S., Stefaniak, A.B., Bonevich, J., Vladar, A.E., Walker, A.R.H., Zheng, J., Starnes, C., Stromberg, A., Ye, J., Grulke, E.A., 2013. Particle size distributions by transmission electron microscopy: an interlaboratory comparison case study. *Metrologia* 50 (6), 663–678.
- Sánchez-Pomales, G., Mudalige, T.K., Lim, J.-H., Linder, S.W., 2013. Rapid determination of silver in nanobased liquid dietary supplements using a portable X-ray fluorescence analyzer. *J. Agric. Food Chem.* 61 (30), 7250–7257.
- Savant, N.K., Korndörfer, G.H., Datnoff, L.E., Snyder, G.H., 1999. Silicon nutrition and sugarcane production: a review 1. *J. Plant Nutr.* 22 (12), 1853–1903.
- Scientific Committee on Emerging and Newly Identified Health Risks SCENIHR, 2010. Scientific Basis for the Definition of the Term “Nanomaterial. European Commission DG Health & Consumers.
- Servin, A.D., Morales, M.I., Castillo-Michel, H., Hernandez-Viezas, J.A., Munoz, B., Zhao, L., Nunez, J.E., Peralta-Videa, J.R., Gardea-Torresdey, J.L., 2013. Synchrotron verification of TiO<sub>2</sub> accumulation in cucumber fruit: a possible pathway of TiO<sub>2</sub> nanoparticle transfer from soil into the food chain. *Environ. Sci. Technol.* 47 (20), 11592–11598.
- Servin, A.D., Pagano, L., Castillo-Michel, H., De la Torre-Roche, R., Hawthorne, J., Hernandez-Viezas, J.A., Loreda-Portales, R., Majumdar, S., Gardea-Torresdey, J., Dhankher, O.P., White, J.C., 2017. Weathering in soil increases nanoparticle CuO bioaccumulation within a terrestrial food chain. *Nanotoxicology* 11 (1), 98–111.
- Verleysen, E., Wagner, T., Lipinski, H.-G., Kägi, R., Koerber, R., Boix-Sanfeliu, A., De Temmerman, P.-J., Mast, J., 2019. Evaluation of a TEM based approach for size measurement of particulate (Nano)materials. *Materials* 12 (14), 2274.
- Weir, A., Westerhoff, P., Fabricius, L., Hristovski, K., von Goetz, N., 2012. Titanium dioxide nanoparticles in food and personal care products. *Environ. Sci. Technol.* 46 (4), 2242–2250.
- Younes, M., Aggett, P., Aguilar, F., Crebelli, R., Dusemund, B., Filipic, M., Frutos, M.J., Galtier, P., Gott, D., Gundert-Remy, U., Kuhnle, G.G., Leblanc, J.C., Lillegaard, I.T., Moldeus, P., Mortensen, A., Oskarsson, A., Stankovic, I., Waalkens-Berendsen, I., Woutersen, R.A., Wright, M., Boon, P., Chrysafidis, D., Gürtler, R., Mosesso, P., Parent-Massin, D., Tobback, P., Kovalkovicova, N., Rincon, A.M., Tard, A., Lambre, C., 2018. Re-evaluation of silicon dioxide (E 551) as a food additive. *EFSA J.* 16 (1).
- Younes, M., Aquilina, G., Castle, L., Engel, K.H., Fowler, P., Frutos Fernandez, M.J., Fürst, P., Gundert-Remy, U., Gürtler, R., Husøy, T., Manco, M., Mennes, W., Moldeus, P., Passamonti, S., Shah, R., Waalkens-Berendsen, I., Wölflé, D., Corsini, E., Cubadda, F., De Groot, D., FitzGerald, R., Gunnare, S., Gutleb, A.C., Mast, J., Mortensen, A., Oomen, A., Piersma, A., Plichta, V., Ulbrich, B., Van Loveren, H., Benford, D., Bignami, M., Bolognesi, C., Crebelli, R., Dusinska, M., Marcon, F., Nielsen, E., Schlatter, J., Vleminckx, C., Barmaz, S., Carfi, M., Civitella, C., Giarola, A., Rincon, A.M., Serafimova, R., Smeraldi, C., Tarazona, J., Tard, A., Wright, M., 2021. Safety assessment of titanium dioxide (E171) as a food additive. *EFSA J.* 19 (5).
- Yu, J., Jeon, Y.-R., Kim, Y.-H., Jung, E.-B., Choi, S.-J., 2021. Characterization and determination of nanoparticles in commercial processed foods. *Foods* 10 (9), 2020.
- Zhao, L., Sun, Y., Hernandez-Viezas, J.A., Servin, A.D., Hong, J., Niu, G., Peralta-Videa, J.R., Duarte-Gardea, M., Gardea-Torresdey, J.L., 2013. Influence of CeO<sub>2</sub> and ZnO nanoparticles on cucumber physiological markers and bioaccumulation of Ce and Zn: a life cycle study. *J. Agric. Food Chem.* 61 (49), 11945–11951.
- Zhao, L., Sun, Y., Hernandez-Viezas, J.A., Hong, J., Majumdar, S., Niu, G., Duarte-Gardea, M., Peralta-Videa, J.R., Gardea-Torresdey, J.L., 2015. Monitoring the environmental effects of CeO<sub>2</sub> and ZnO nanoparticles through the life cycle of corn (*Zea mays*) plants and in situ  $\mu$ -XRF mapping of nutrients in kernels. *Environ. Sci. Technol.* 49 (5), 2921–2928.