Detecting Engineered Nanomaterials in Processed Foods From Australia

Final Report

Prepared by:

Robert Reed, Jared Schoepf, Marisa Masles, and Paul Westerhoff

Arizona State University (Tempe, AZ)

Prepared For:

Friends of the Earth

August 18, 2015

Executive Summary

Fourteen processed food samples commercially available in Australia were obtained by Friends of the Earth Australia. Samples were shipped to Arizona State University, where they were prepared and analyzed for the presence of engineered nanomaterials using transmission electron microscopy (TEM) with energy dispersive x-ray (EDX) detection and by Inductively coupled plasma mass spectrometry (ICP-MS). With one exception, labels on the food samples listed E551, E171, SiO_2, titanium dioxide or TiO_2 as an ingredient. The samples were chemically digested for bulk elemental analysis by inductively coupled plasma mass spectrometry to quantify the amount of metals present, with a focus on titanium and silicon. All samples were found to contain Si or Ti above instrument detection limits. Transmission electron microscopy was performed to determine the presence or absence of nanomaterials. Nanomaterials were detected in all cases, and energy dispersive X-ray analysis was used to determine the elemental composition of these detected particles. Based upon the morphology of the Si- or Ti-containing particles, they are consistent with our experience and characterization of engineered nanomaterials commonly used within the global food industry. The percentage of Si- or Ti- containing particles less than 100 nm were determined by counting primary particle numbers from TEM images. For products (8 of 14 samples) containing Ti- nanoparticles, 10% to 50% of the primary particles were below 100 nm in width. For products containing Si- nanoparticles, their average sizes were smaller (averaged around 18 nm) than Ti-nanoparticles in other samples (averaged around 130 nm), and when Si-nanoparticles were detected in foods (6 of 14 samples) nearly 100% of the primary particles were below 100 nm in width. The conclusion of this study is that each of the foods tested contain nanomaterials. The accompanying Table E-1 summarizes the findings from these measurements.

Table E-1: Summary of Observations and Upper Bound on Si or Ti Content within the Food Matrix

Food ID	Ingredient listed on label	Nanoparticles observed by TEM?	Particle diameter by TEM (nm)	Percent of particles below 100nm	Number of particles counted	Elements detected by EDS	Element & concentration by ICP-MS (µg/g food dry mass)
Allen's kool mints	E171 (TiO_2)	Yes	150	14%	250	Ti, O	(Ti) 211
Caesar dressing	E171 (TiO_2)	Yes	112	41%	241	Ti, O	(Ti) 970
Chicken salt	E551 (SiO_2)	Yes	21	100%	166	Si, O	(Si) 1090
Coffee Mate creamer	E551 (SiO_2)	Yes	24	100%	92	Si, O	(Si) 944
Duncan Hines frosting	E171 (TiO_2)	Yes	148	29%	122	Ti, O	(Ti) 2440
Eclipse mints	E171 (TiO_2)	Yes	131	21%	24	Ti, O	(Ti) 262
M&Ms	E171 (TiO_2)	Yes	123	34%	175	Ti, O	(Ti) 1040
Mentos gum	E171 (TiO_2)	Yes	105	42%	45	Ti, O	(Ti) 3070
Moccona cappuccino	E551 (SiO_2)	Yes	15	100%	50	Si, O	(Si) 648
Roast meat gravy	E551 (SiO_2)	Yes	15	100%	145	Si, O	(Si) 305
Skittles	E171 (TiO_2)	Yes	177	10%	21	Ti, O	(Ti) 37.6
Sour straps	E171 (TiO_2)	Yes	103	50%	250	Ti, O	(Ti) 548
Taco mix	E551 (SiO_2)	Yes	21	100%	250	Si, O	(Si) 2700
White Sauce	E551 (SiO_2)	Yes	10	100%	50	Si, O	(Si) 234

Contents

Methods and Materials

Transmission electron microscopy and energy dispersive X-ray spectroscopy

The foods (~0.125 g each) were suspended in 40 mL ultrapure water and sonicated for 30 minutes to suspend particles. These samples were centrifuged at 15,000 G for 15 minutes to settle any particles present. The organics-rich supernatant was poured off, leaving a pellet of particulate matter in the centrifuge tube. This was re-suspended in 20 mL ultrapure water and sonicated for 5 minutes, then 100 uL volumes were pipetted onto a copper/lacey carbon transmission electron microscopy grid and allowed to dry. Microscopy was performed on a Philips CM200 transmission electron microscope with energy dispersive spectroscopy. Mean particle diameter was measured manually with ImageJ software. Particle number size distributions were developed and the percentage of particles less than 100 nm in width determined. The percentage of Si- or Ti- containing particles less than 100 nm were determined by counting primary particle numbers from TEM images.

Our laboratory has analyzed, separately, multiple food grade stocks of TiO_2 and SiO_2 carrying E171 and E551 designation, respectively [1-3]. These separate analyses are used in assessing the similarities of TEM images of particles to these globally used and common food additives.

Digestion of dried foods and ICP-MS analysis

Dry food samples of ~0.25 g each were microwave digested in a mixture of 8 mL concentrated nitric acid and 2 mL hydrofluoric acid (both acids ultra trace metal grade, JT Baker). The microwave program was set to ramp the temperature to 150°C in 15 minutes, 180 °C in another 15 minutes, and hold this temperature for 20 minutes. To remove hydrofluoric acid from solution after digestion, it was reacted with boric acid (10 mL). The digested samples were analyzed by inductively coupled plasma mass spectrometry (ICP-MS, Thermo Fisher X-Series II) for elemental composition.

Concentrations of Si or Ti analyzed on digested food samples have units of μg/g food. These were converted to equivalent weight percentages of TiO_2 or SiO_2 according to the following equations:

$$\frac{X\ ugTi}{g\ FOOD} \times \frac{79.9\ ugTiO_2}{47.9\ ugTi} \times \frac{1\ gTiO_2}{10^6 ugTiO_2} \times 100\% = Y\ wt\%\ TiO_2$$

$$\frac{A\ ugSi}{g\ FOOD} \times \frac{60.1\ ugSiO_2}{28.1\ ugSi} \times \frac{1\ gSiO_2}{10^6 ugSiO_2} \times 100\% = B\ wt\%\ SiO_2$$

Individual food data

Allen's kool mints

This product was labeled as containing "Colour (171)". The E171 food designation represents TiO_2. Particles in the nanoscale range were observed by TEM and point analysis using EDX of the particles indicated they contained titanium and oxygen, at ratios consistent with TiO_2. Figure 4 shows the distribution of particle sized by TEM and at least 14% of the primary particles were below 100 nm in size, with an average particle size of 150 nm. After acid digestion, the product was found to contain 211 μgTi/gram of dry food (0.035 wt% as TiO_2).

Figure 1: Allen's Kool Mints and ingredient list

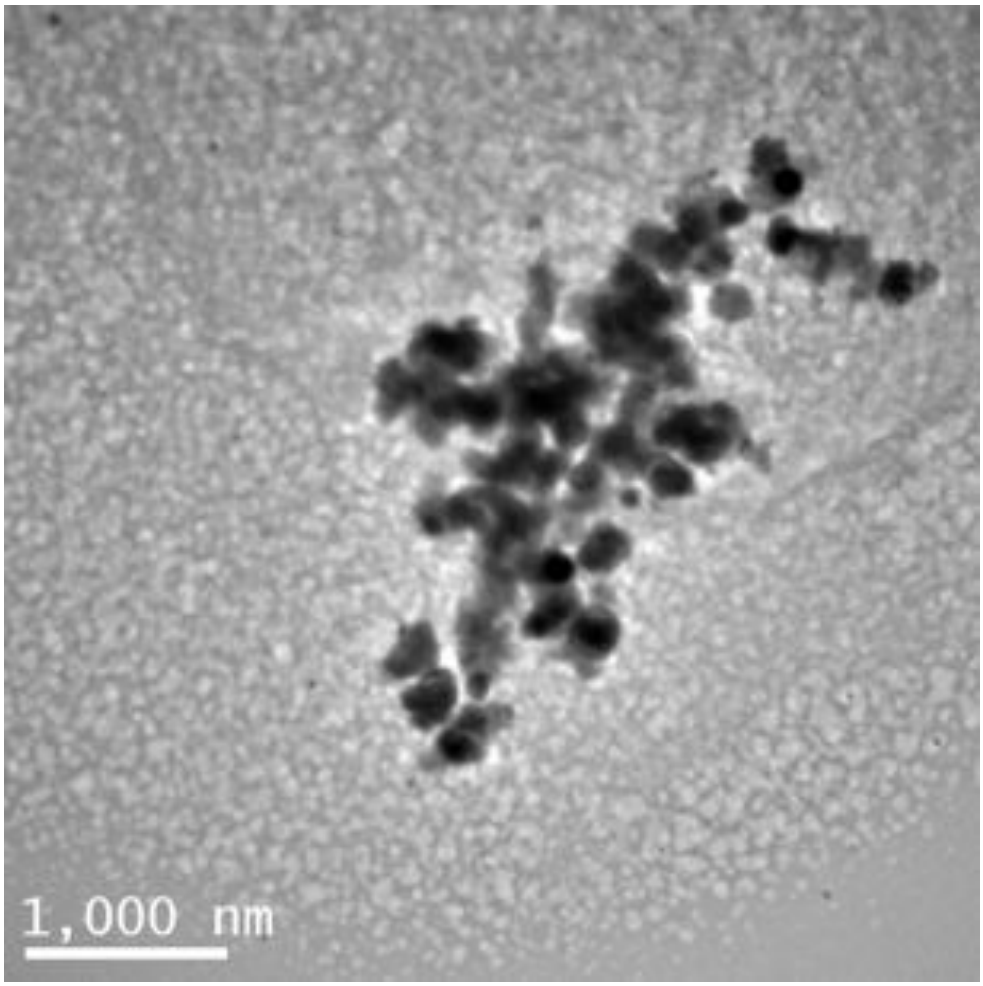

Figure 2: TEM of Kool Mints

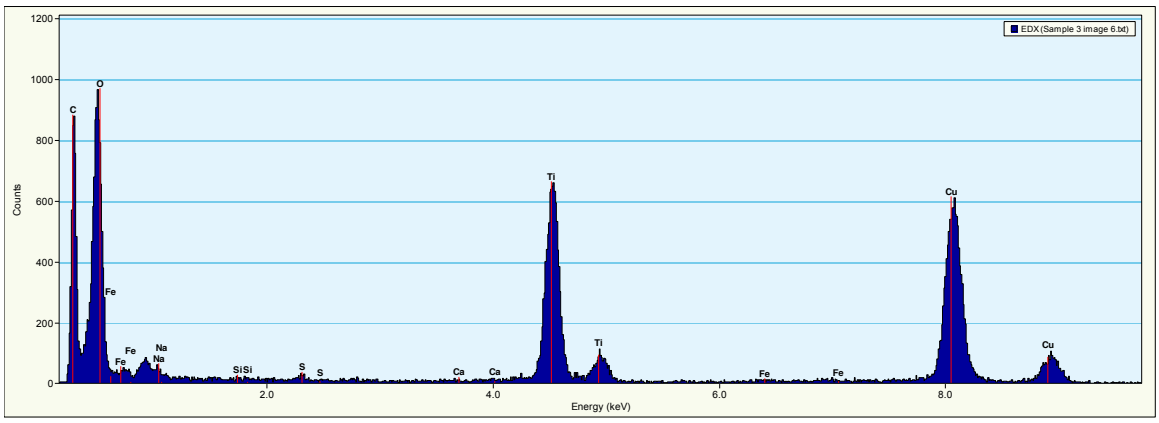

Figure 3: Presence of titanium dioxide in Kool Mints by EDS

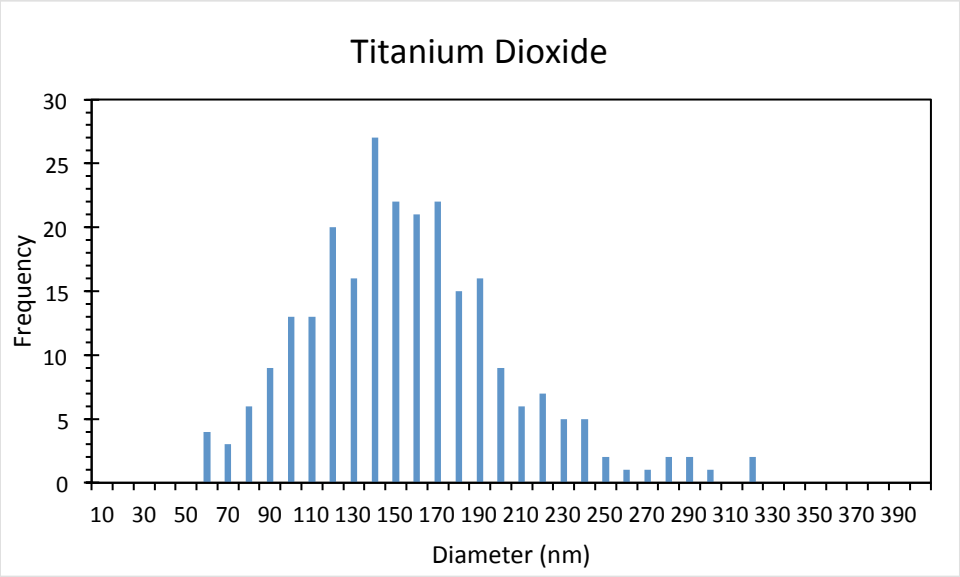

Figure 4: Titanium dioxide particle size distribution in Kool Mints

Caesar dressing

This product was labeled as containing "Colour (171)". The E171 food designation represents TiO_2. Particles in the nanoscale range were observed by TEM and point analysis using EDX of the particles indicated they contained titanium and oxygen, at ratios consistent with TiO_2. Figure 8 shows the distribution of particle sized by TEM and at least 41% of the primary particles were below 100 nm in size, with an average particle size of 112 nm. After acid digestion, the product was found to contain 970 μgTi/gram of dry food (0.16 wt% as TiO_2).

Figure 5: Praise Caesar Dressing and ingredient list

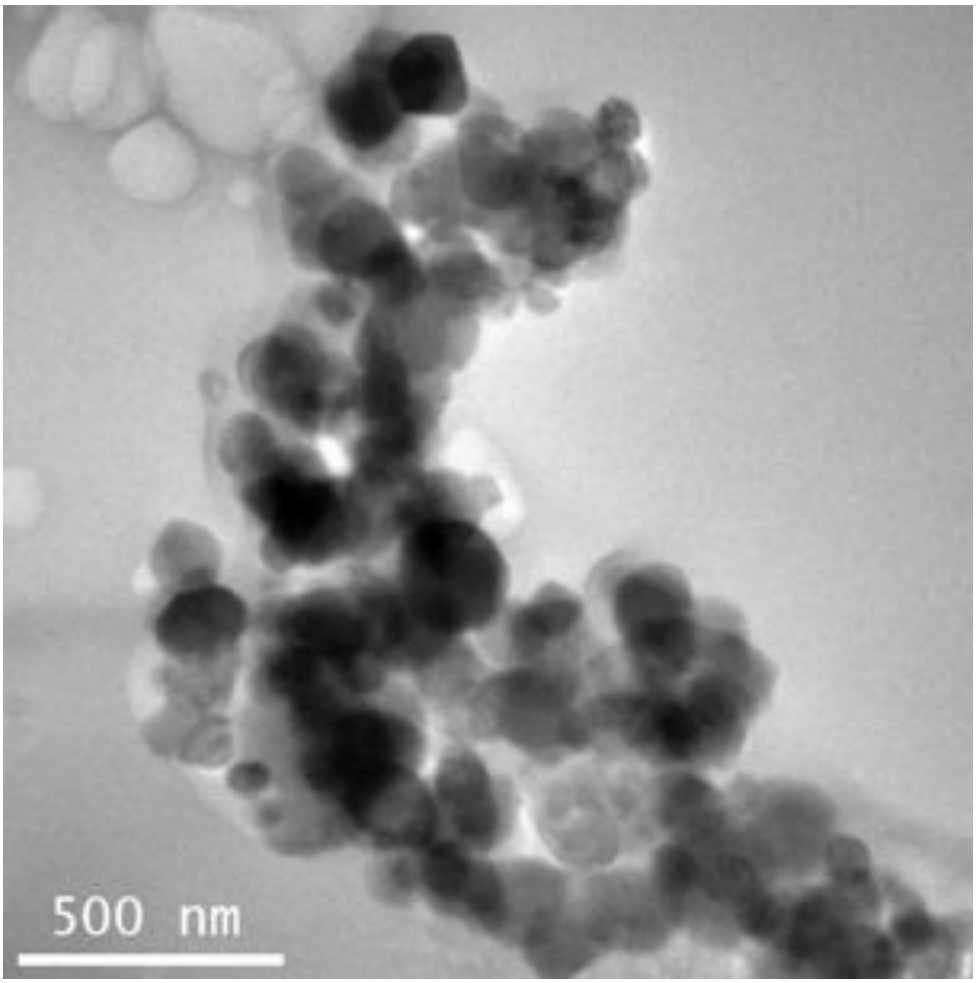

Figure 6: TEM of Praise Caesar Dressing

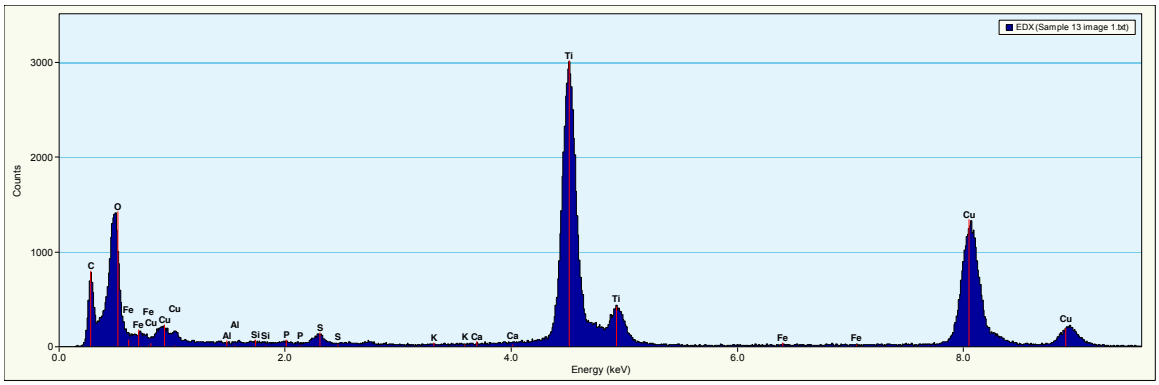

Figure 7: Presence of titanium dioxide in Praise Caesar Dressing by EDS

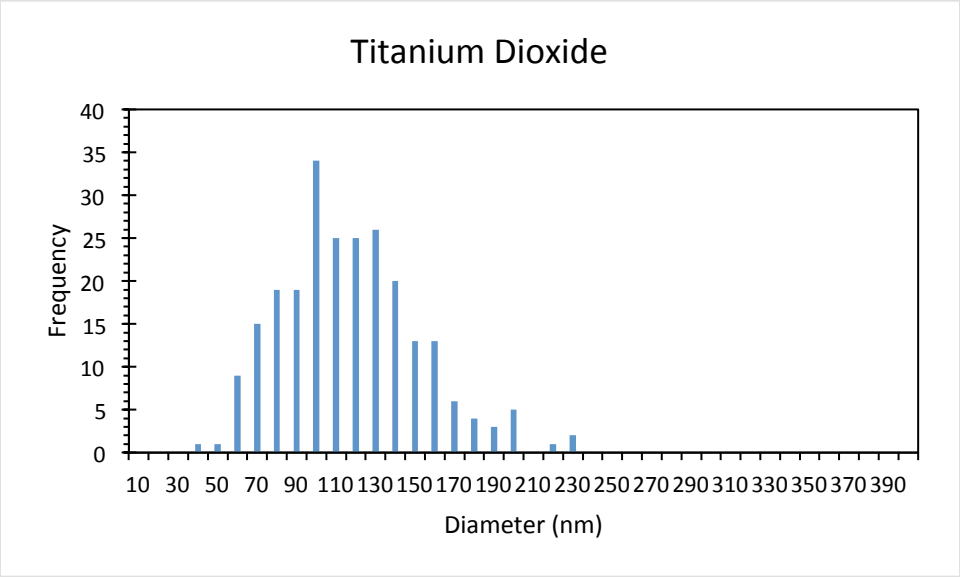

Figure 8: Titanium dioxide particle size distribution in Praise Caesar Dressing

Chicken salt

This product was labeled as containing "Anti Caking Agent (151)". The E151 food designation represents SiO_2. Particles in the nanoscale range were observed by TEM and point analysis using EDX of the particles indicated they contained silica and oxygen, and is probably SiO_2. Figure 12 shows the distribution of particle sized by TEM and 100% of the primary particles were below 100 nm in size, with an average particle size of 21 nm. After acid digestion, the product was found to contain 1090 μgSi/gram of dry food (0.23 wt % as SiO_2).

Figure 9: Nice N' Tasty Chicken Salt and ingredients list

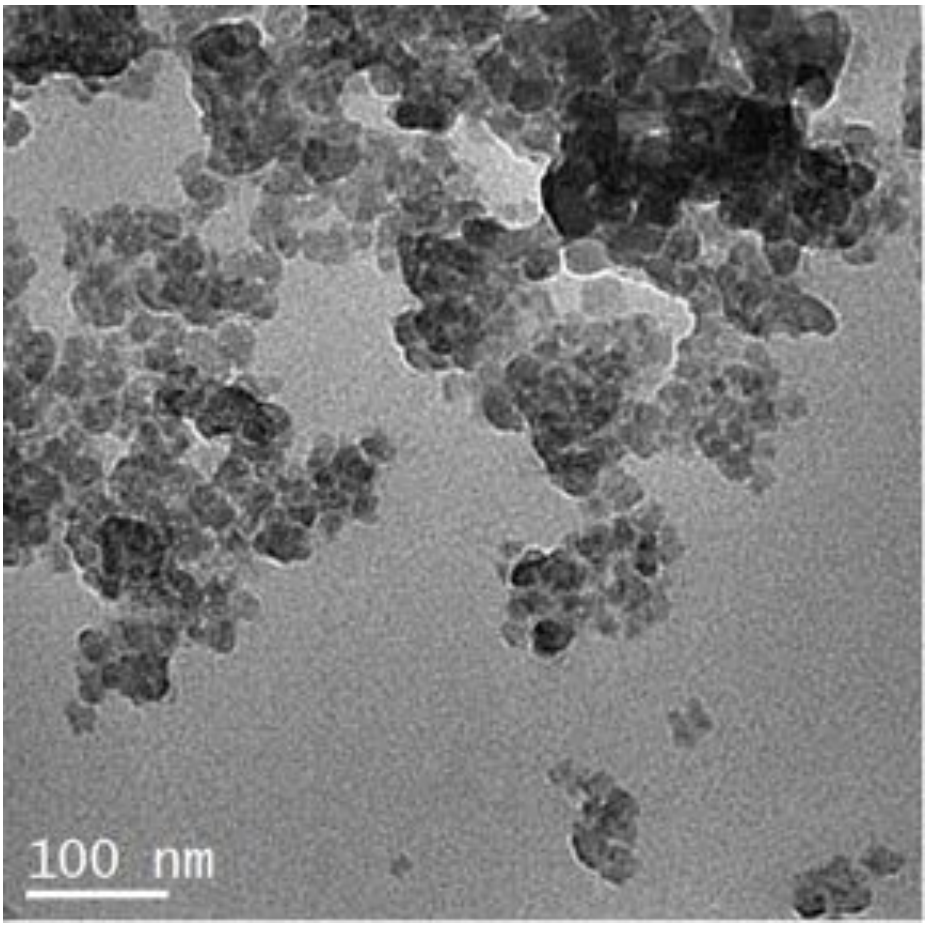

Figure 10: TEM of Chicken Salt

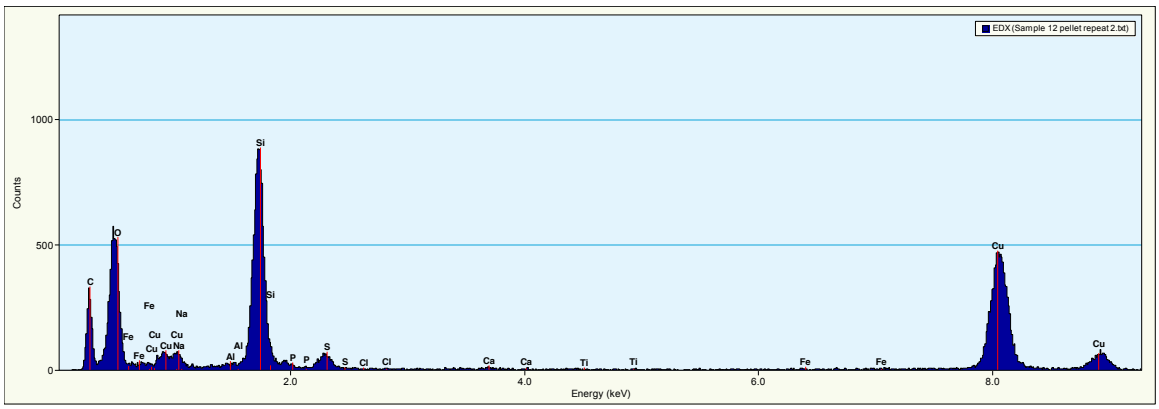

Figure 11: Presence of silicon dioxide in Chicken Salt by EDS

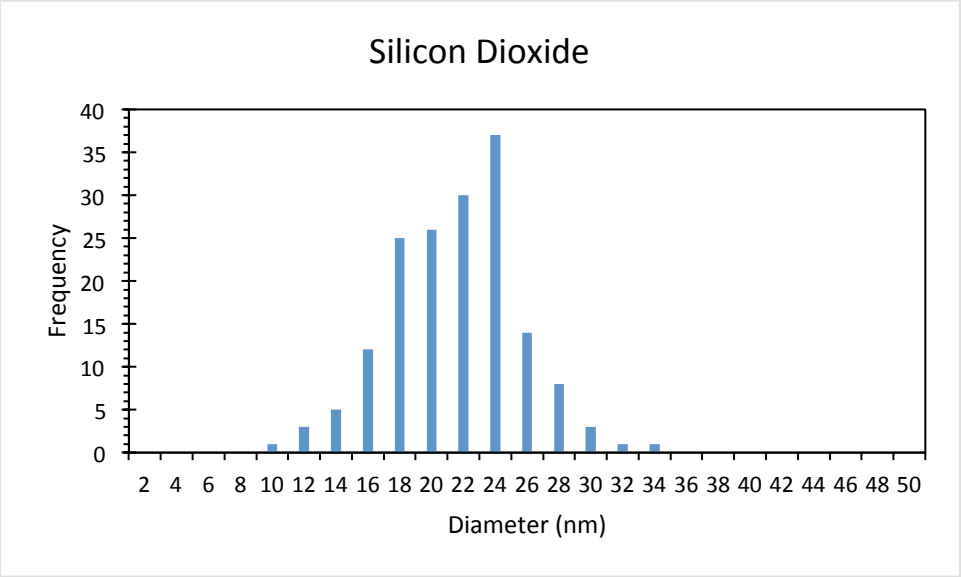

Figure 12: Silicon dioxide particle size distribution in Chicken Salt

Coffee Mate creamer

This product was labeled as containing "Anti-caking agent (551)". The E551 food designation represents SiO_2. Particles in the nanoscale range were observed by TEM and point analysis using EDX of the particles indicated they contained silica and oxygen, and is probably SiO_2. Figure 16 shows the distribution of particle sized by TEM and 100% of the primary particles were below 100 nm in size, with an average particle size of 24 nm. After acid digestion, the product was found to contain 944 μgSi/gram of dry food (0.20 wt% as SiO_2).

Figure 13: Coffee-mate and ingredients list

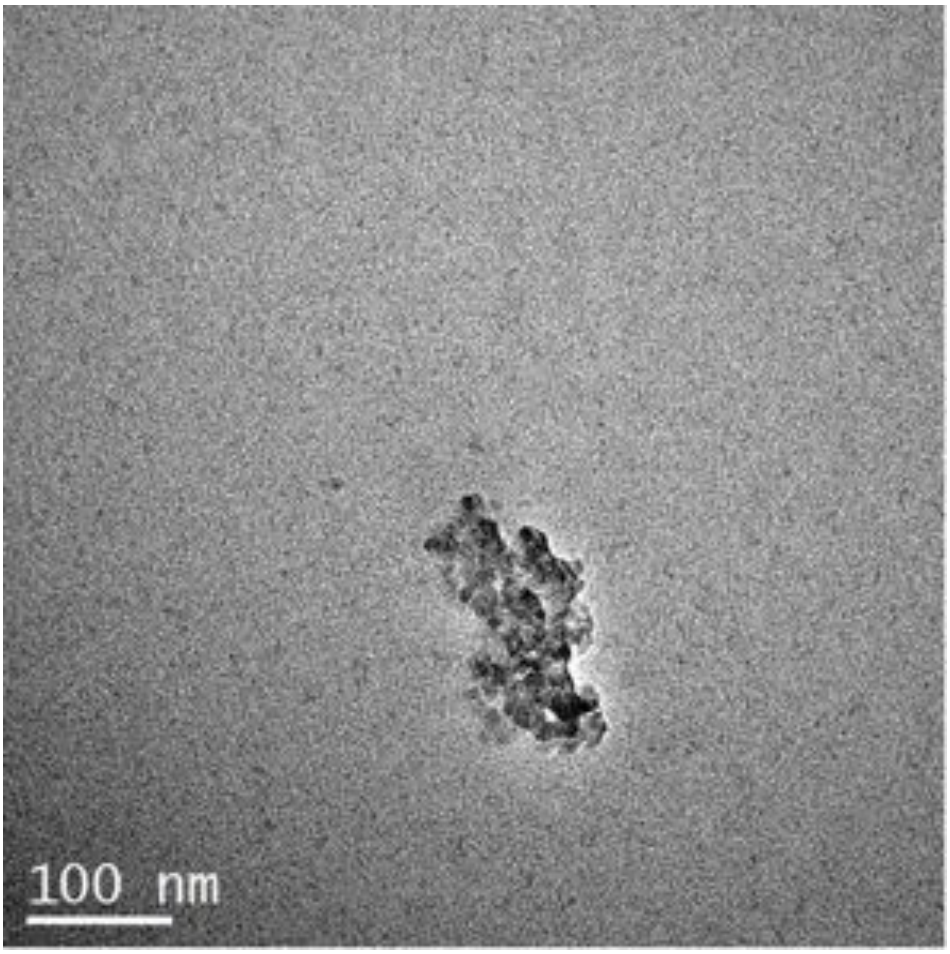

Figure 14: TEM of Coffee-mate

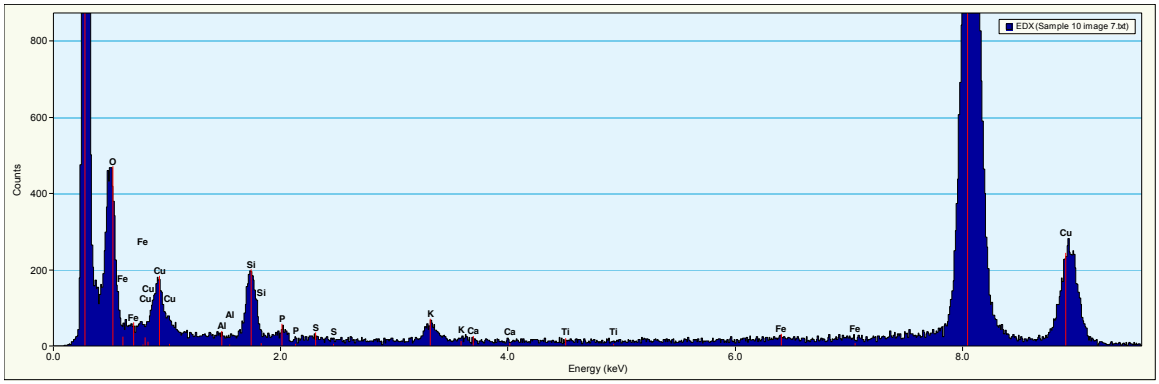

Figure 15: Presence of silicon dioxide in Coffee-mate by EDS

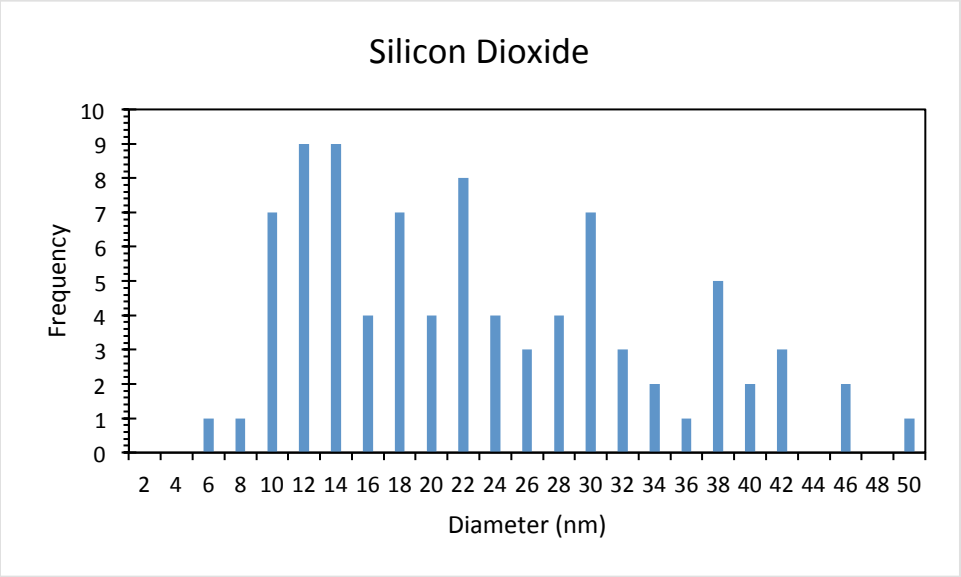

Figure 16: Silicon dioxide particle size distribution in Coffee-mate

Duncan Hines frosting

This product was labeled as containing "Colored with (Titanium Dioxide..)". Particles in the nanoscale range were observed by TEM and point analysis using EDX of the particles indicated they contained titanium and oxygen, at ratios consistent with TiO_2. Figure 20 shows the distribution of particle sized by TEM and at least 29% of the primary particles were below 100 nm in size, with an average particle size of 148 nm. After acid digestion, the product was found to contain 2440 μgTi/gram of dry food (0.41 wt% as TiO_2).

Figure 17: Duncan Hines Creamy Frosting and ingredients list

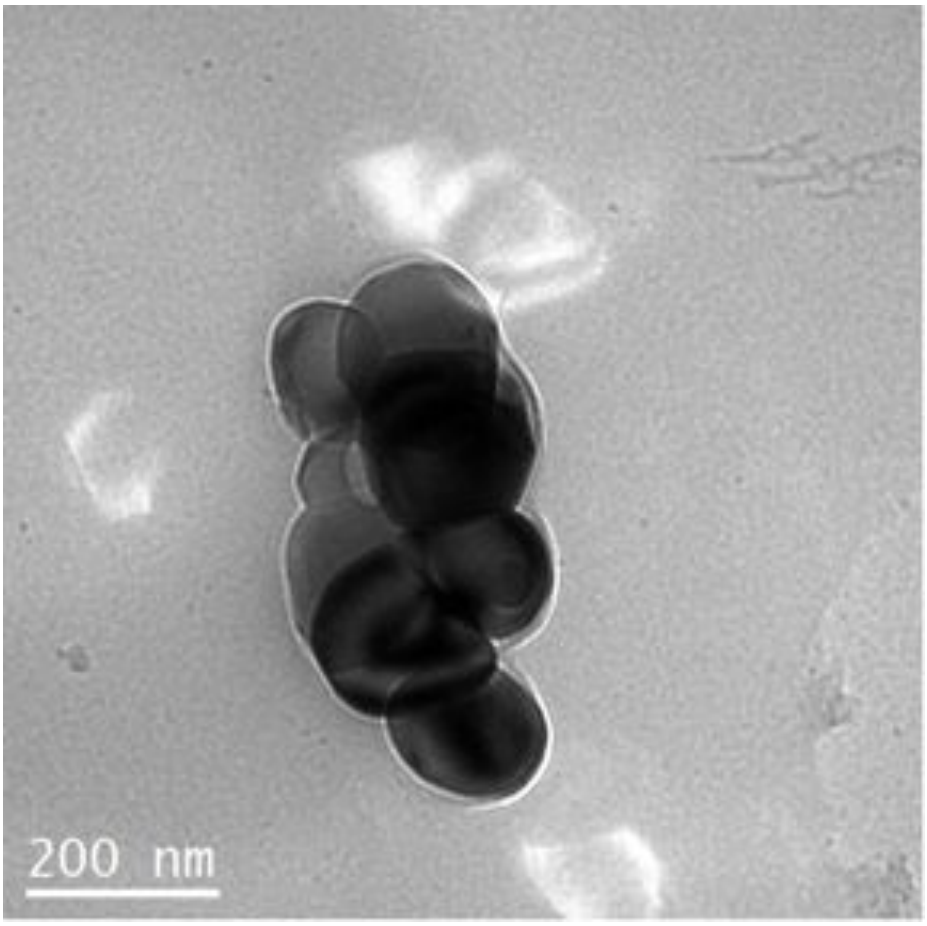

Figure 18: TEM of Duncan Hines Creamy Frosting

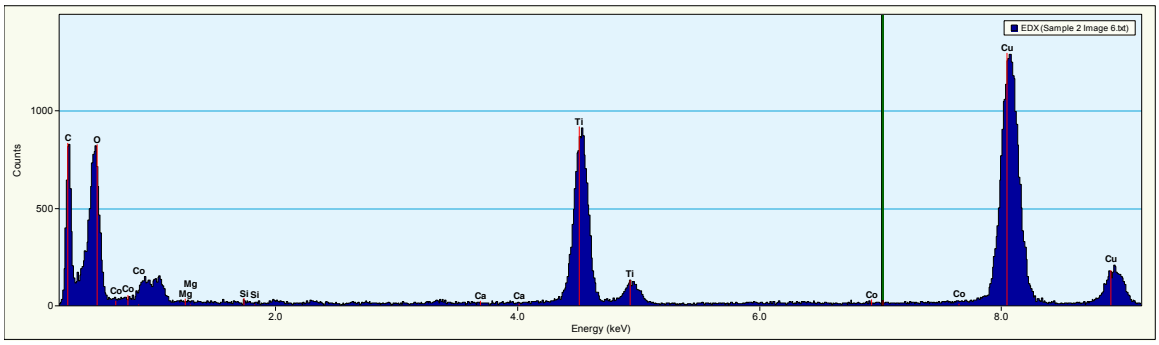

Figure 19: Presence of titanium dioxide in Duncan Hines Creamy Frosting by EDS

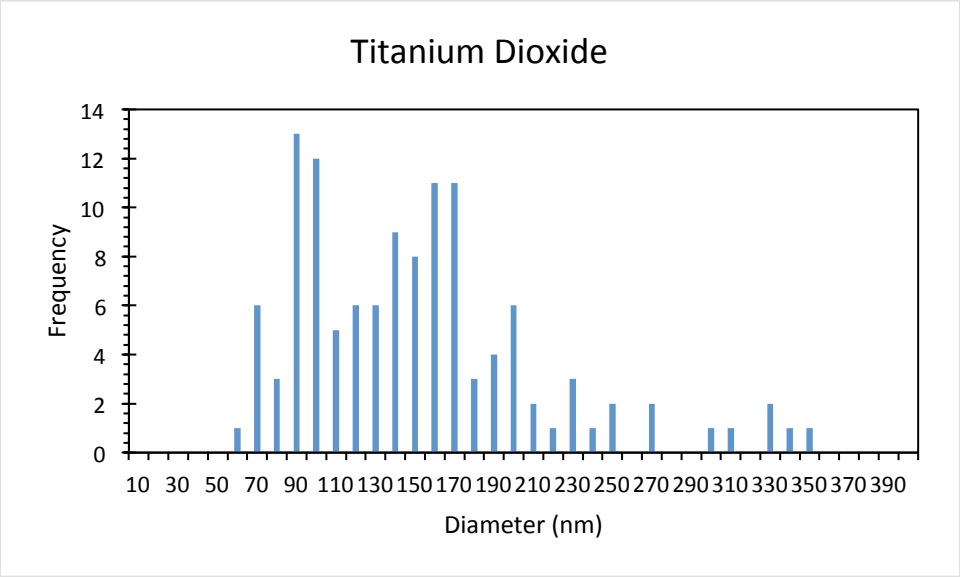

Figure 20: Titanium dioxide particle size distribution in Duncan Hines Creamy Frosting

Eclipse mints

This product was labeled as containing "Colours (171, …)". The E171 food designation represents TiO_2. Particles in the nanoscale range were observed by TEM and point analysis using EDX of the particles indicated they contained titanium and oxygen, at ratios consistent with TiO_2. Figure 24 shows the distribution of particle sized by TEM and at least 21% of the primary particles were below 100 nm in size, with an average particle size of 131 nm. After acid digestion, the product was found to contain 262 μgTi/gram of dry food (0.044 wt% as TiO_2).

Figure 21: Eclipse Chewy Mints and ingredients list

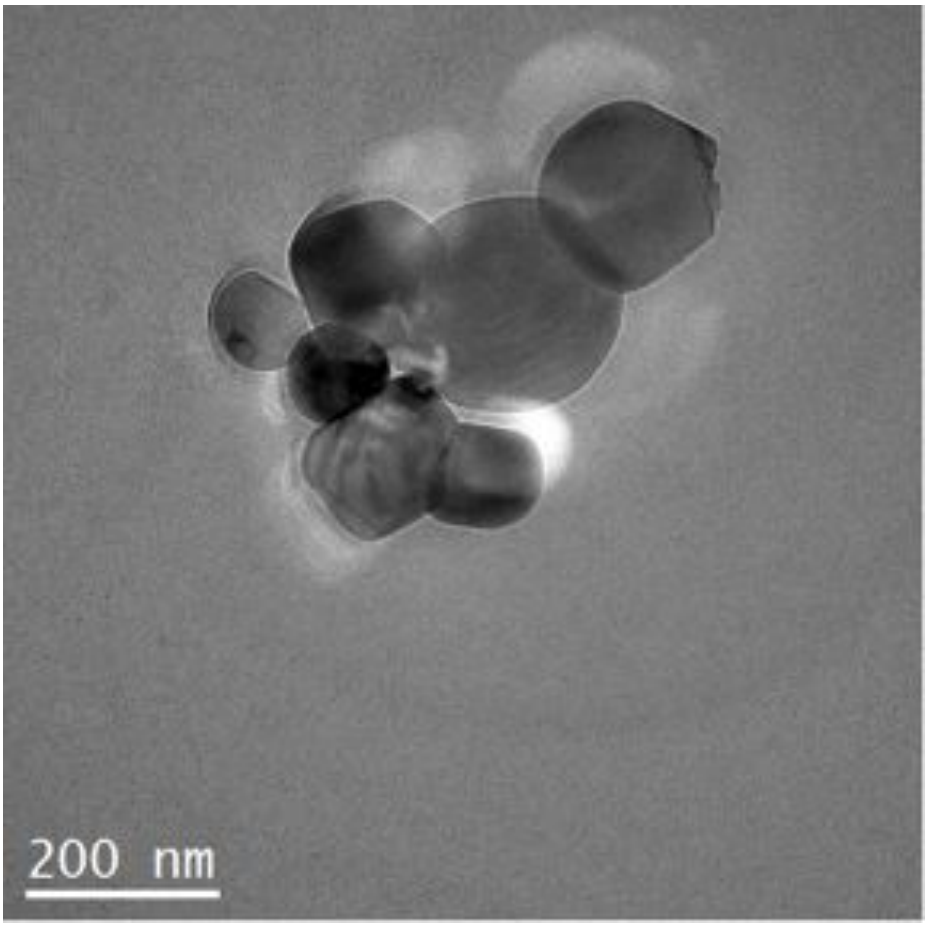

Figure 22: TEM of Eclipse Chewy Mints

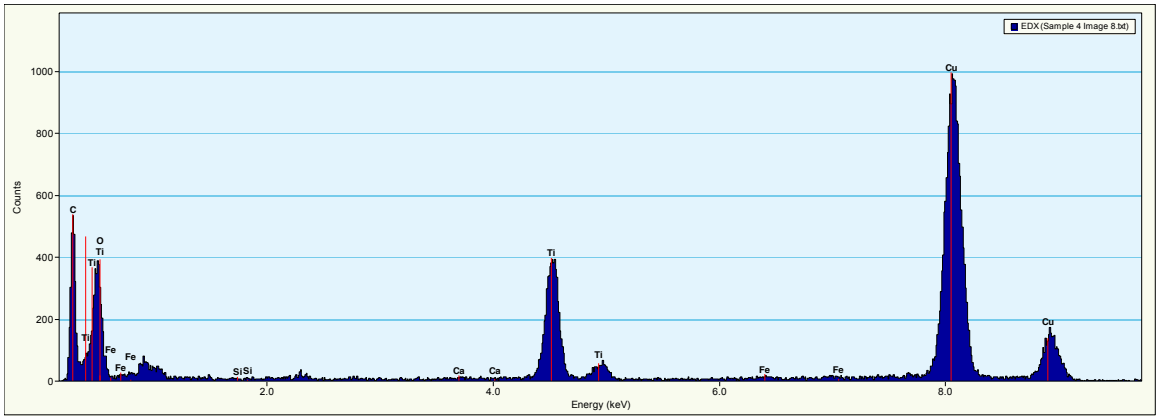

Figure 23: Presence of titanium dioxide in Eclipse Chewy Mints by EDS

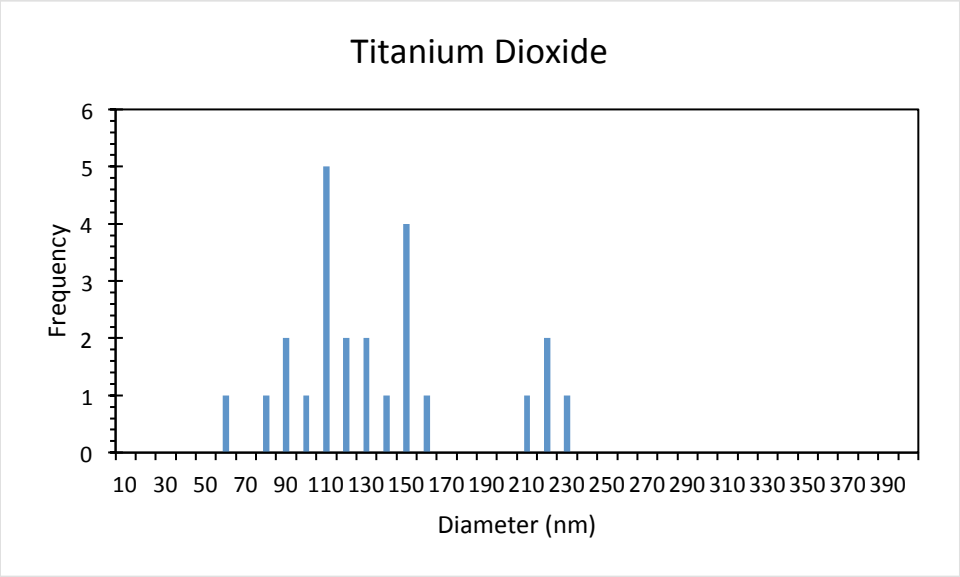

Figure 24: Titanium dioxide particle size distribution in Eclipse Chewy Mints

M&M's

This product was labeled as containing "Colours (171, …)". The E171 food designation represents TiO_2. Particles in the nanoscale range were observed by TEM and point analysis using EDX of the particles indicated they contained titanium and oxygen, at ratios consistent with TiO_2. Figure 28 shows the distribution of particle sized by TEM and at least 34% of the primary particles were below 100 nm in size, with an average particle size of 123 nm. After acid digestion, the product was found to contain 1040 μgTi/gram of dry food (0.17 wt% as TiO_2).

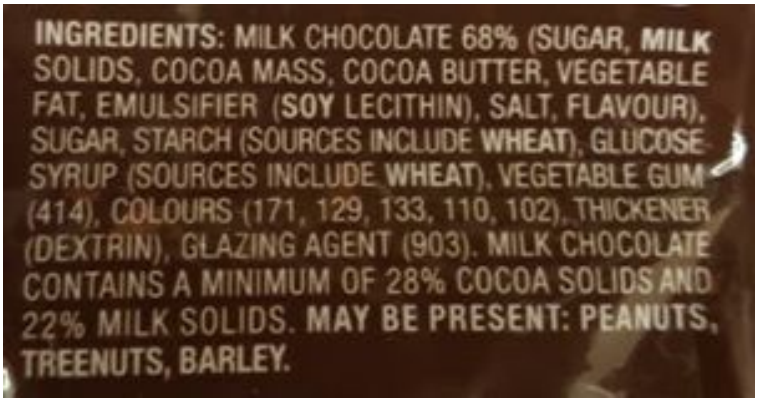

Figure 25: M&M's and ingredient list

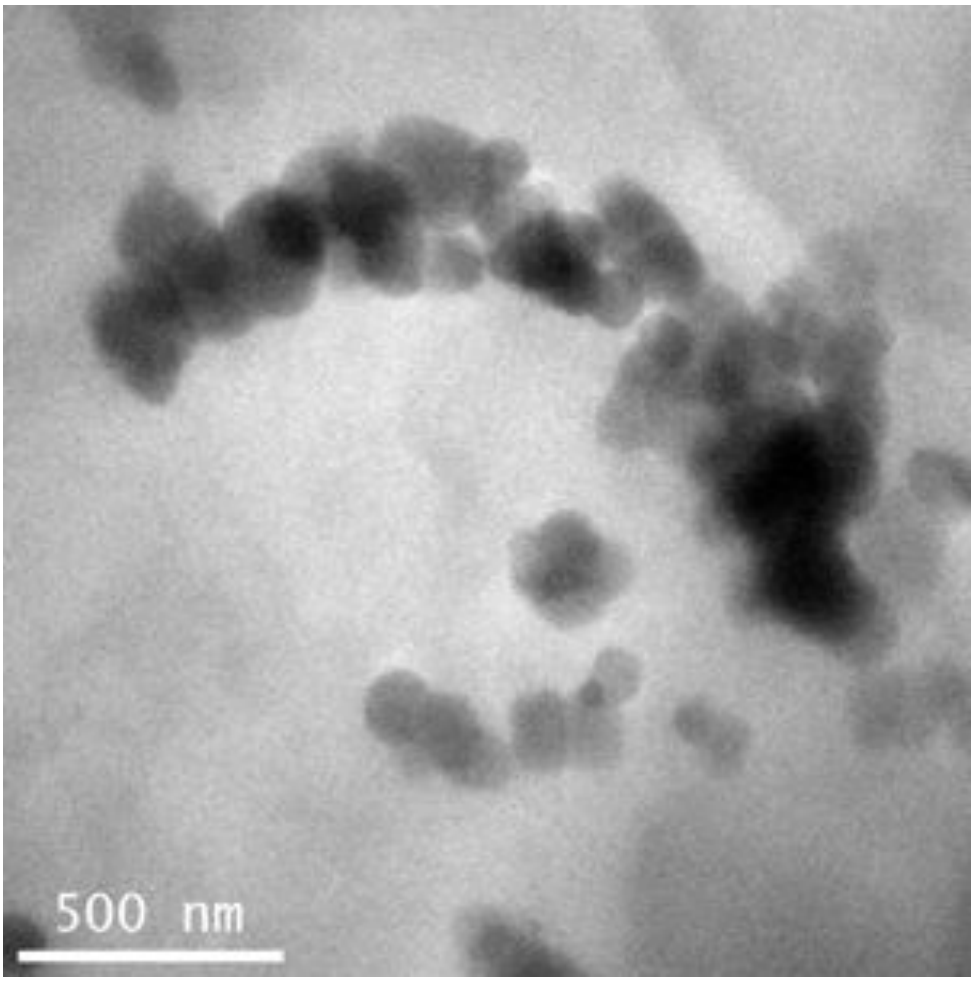

Figure 26: TEM of M&M's

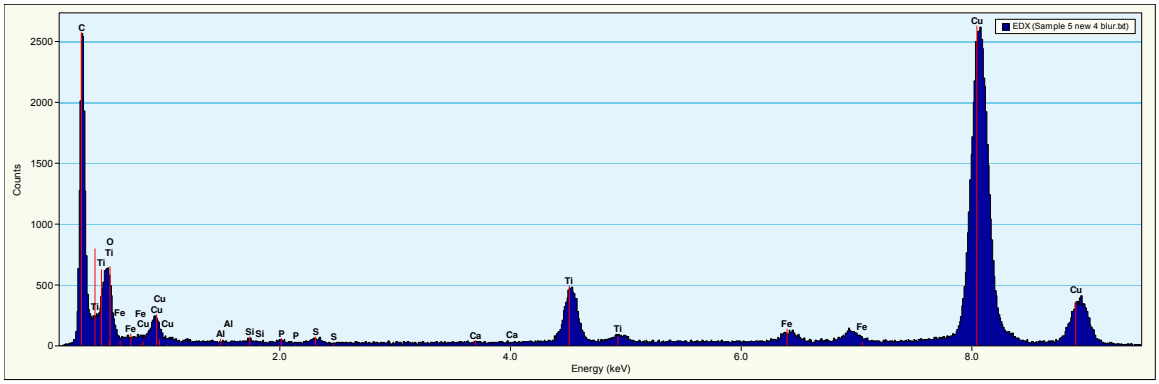

Figure 27: Presence of titanium dioxide in M&M's by EDS

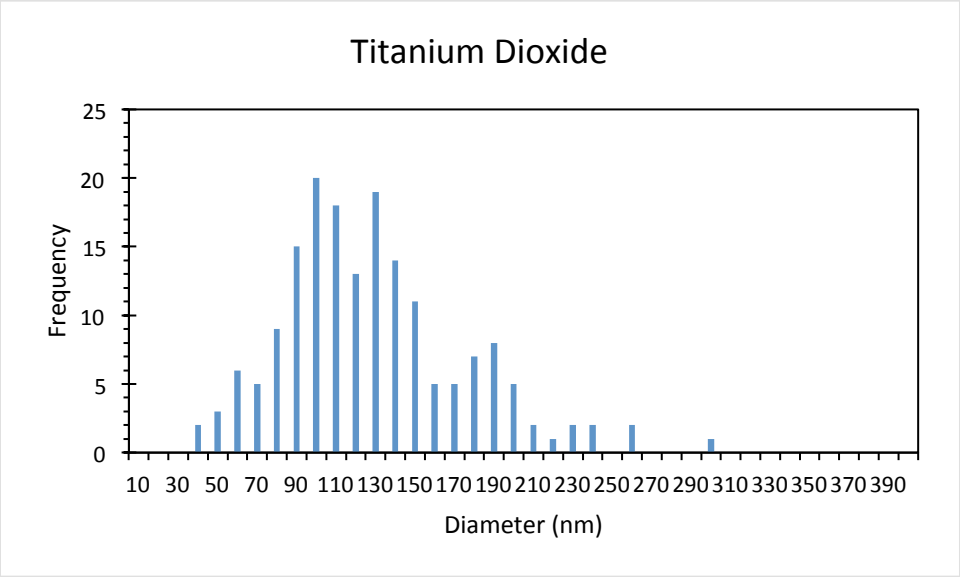

Figure 28: Titanium dioxide particle size distribution in M&M's

Mentos gum

This product was labeled as containing "Colours (171, …)". The E171 food designation represents TiO_2. Particles in the nanoscale range were observed by TEM and point analysis using EDX of the particles indicated they contained titanium and oxygen, at ratios consistent with TiO_2. Figure 32 shows the distribution of particle sized by TEM and at least 42% of the primary particles were below 100 nm in size, with an average particle size of 105 nm. After acid digestion, the product was found to contain 3070 μgTi/gram of dry food (0.51 wt% as TiO_2).

Ingredients

Sorbitol, gum base, humectant (422), xylitol, flavourings, mannitol, sweetener (951), colours (171, 100, 133), vegetable oil, instant green tea powder (0.2%), antioxidant (320). Phenylketonurics: contains phenylalanine. Excess consumption may have a laxative effect. Source: http://www.mentos.com.au/products/mentos-pure-fresh-wallets/

Figure 29: Mentos Pure Fresh Gum

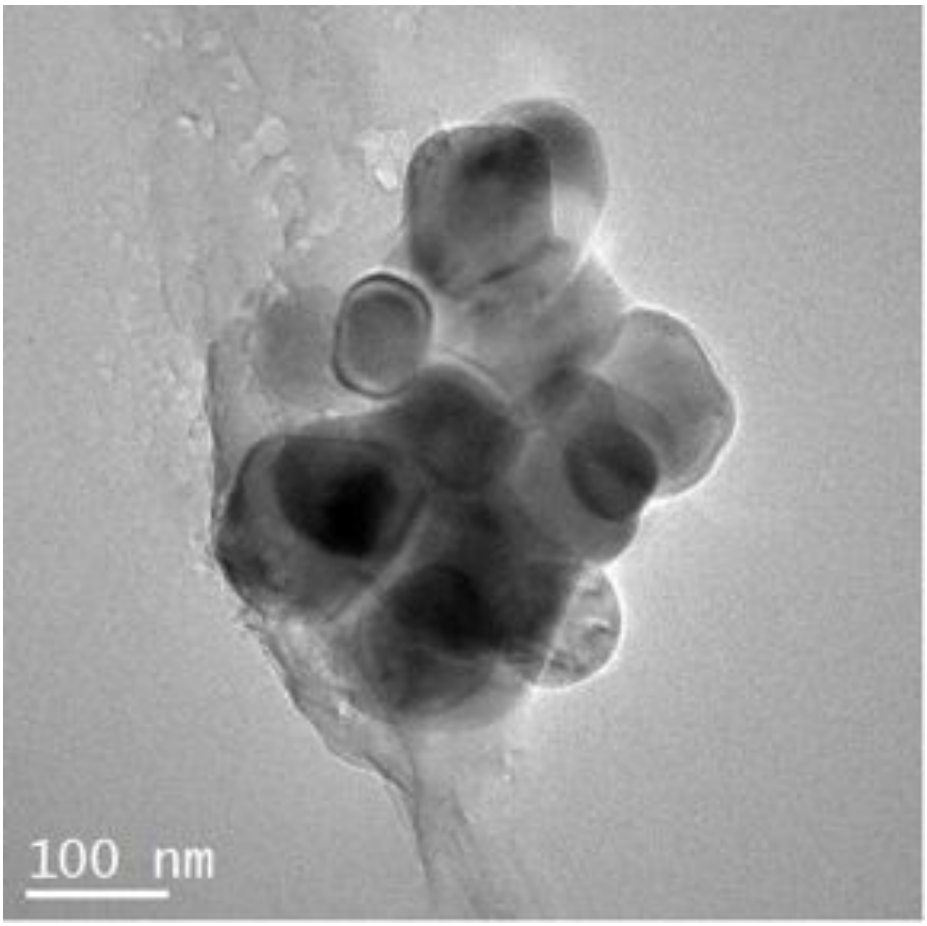

Figure 30: TEM of Mentos gum

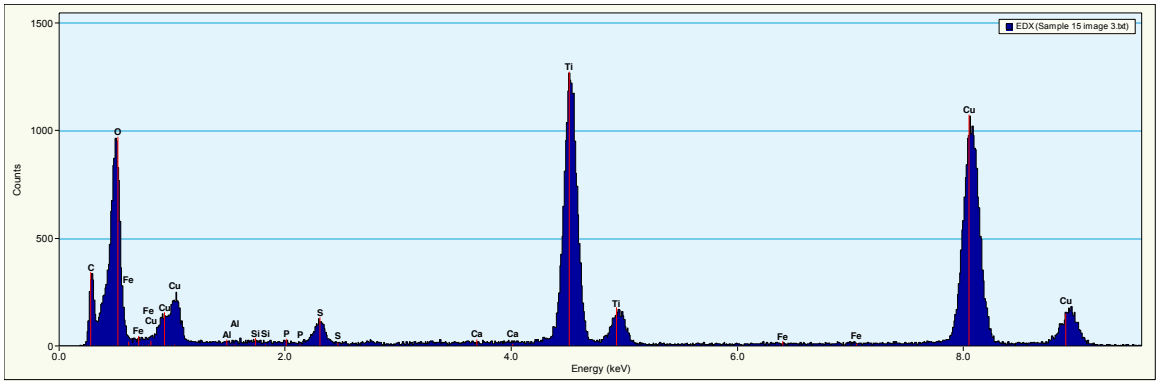

Figure 31: Presence of titanium dioxide in Mentos gum by EDS

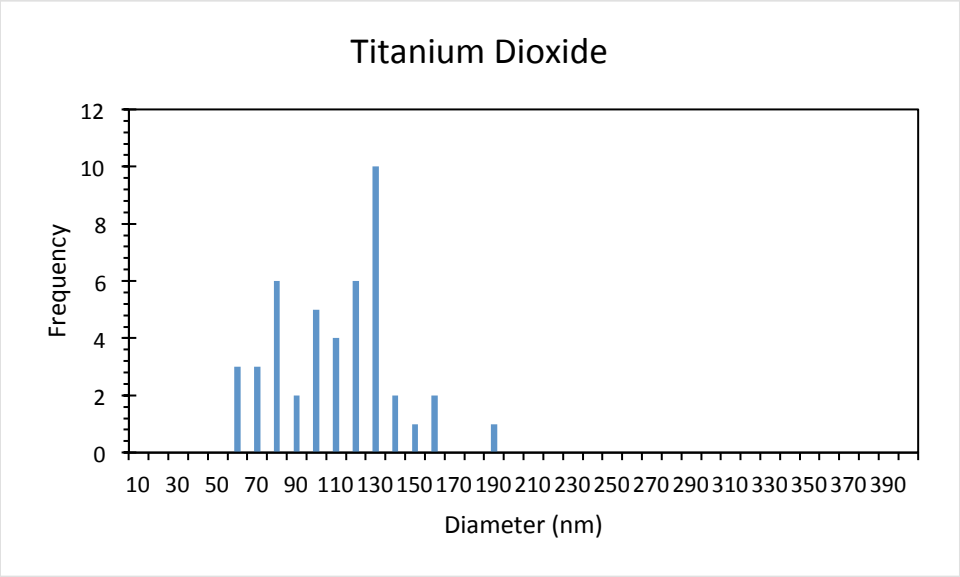

Figure 32: Titanium dioxide particle size distribution in Mentos gum

Moccona Cappuccino

This product had limited labeling (Figure 33) because it was purchased in a container with multiple single serving packages. Particles in the nanoscale range were observed by TEM and point analysis using EDX of the particles indicated they contained silica and oxygen, and is probably SiO_2. Figure 36 shows the distribution of particle sized by TEM and 100% of the primary particles were below 100 nm in size, with an average particle size of 15 nm. After acid digestion, the product was found to contain 648 μgSi/gram of dry food (0.14 wt% as SiO_2).

Figure 33: Moccona Cappuccino and ingredients list

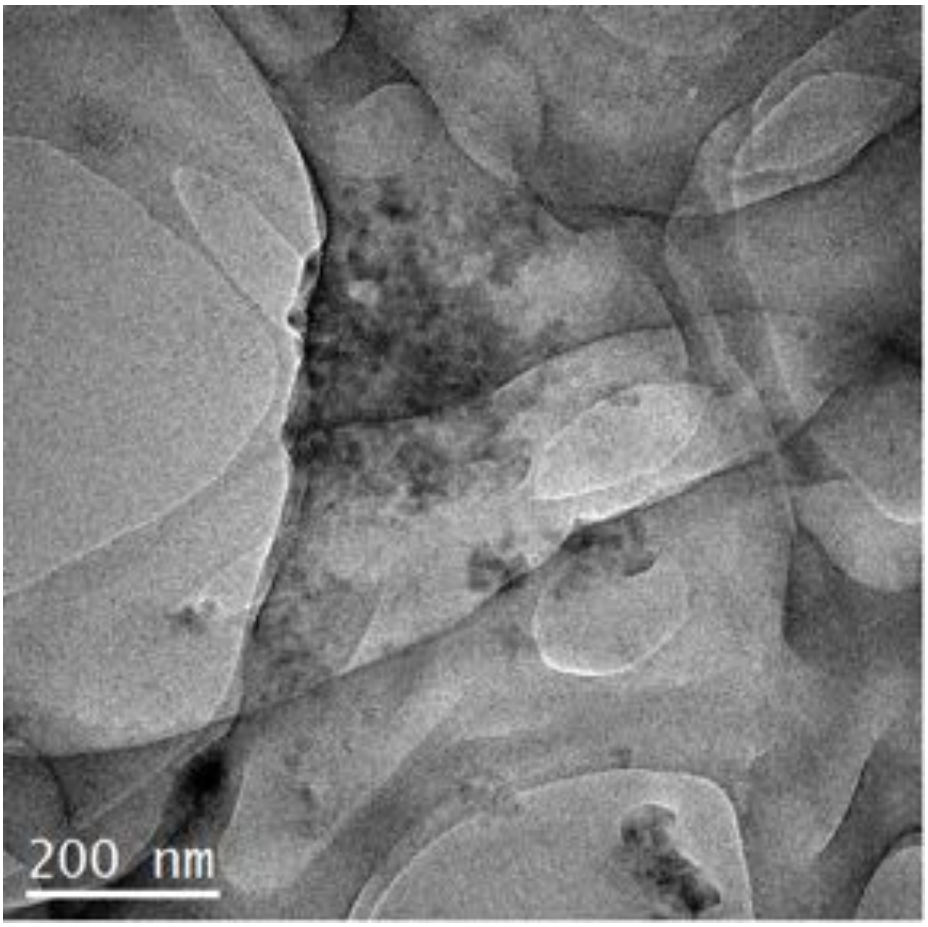

Figure 34: TEM of Moccona Cappuccino

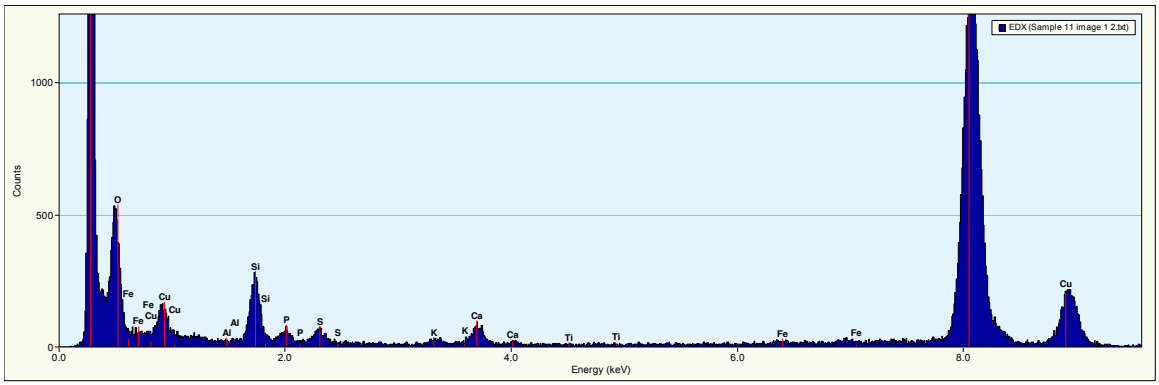

Figure 35: Presence of silicon dioxide in Moccona Cappuccino by EDS

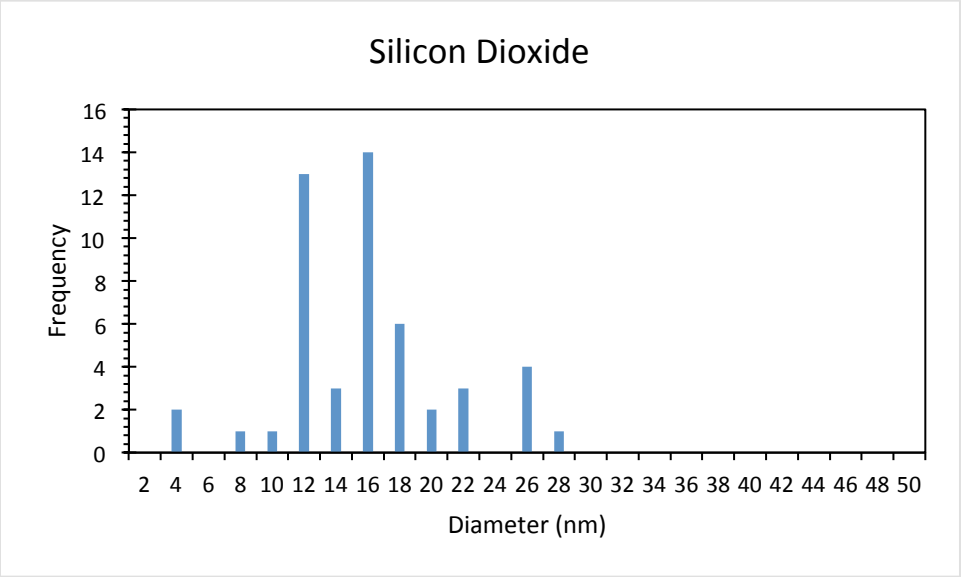

Figure 36: Silicon dioxide particle size distribution in Moccona Cappuccino

Roast meat gravy

This product was labeled as containing "Anti-caking agent (551)". The E551 food designation represents SiO_2. Particles in the nanoscale range were observed by TEM and point analysis using EDX of the particles indicated they contained silica and oxygen, and is probably SiO_2. Figure 40 shows the distribution of particle sized by TEM and 100% of the primary particles were below 100 nm in size, with an average particle size of 15 nm. After acid digestion, the product was found to contain 305 μgSi/gram of dry food (0.065 wt% as SiO_2).

Figure 37: Roast Meat Gravy and ingredients list

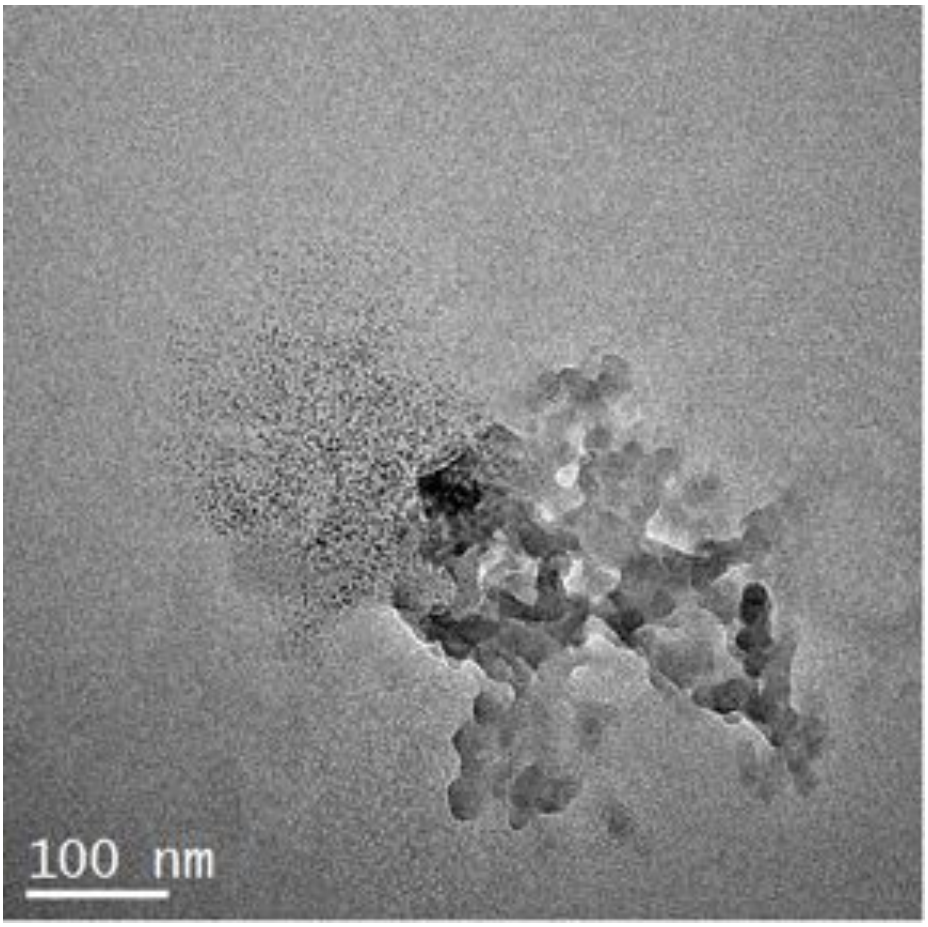

Figure 38: TEM of Roast Meat Gravy

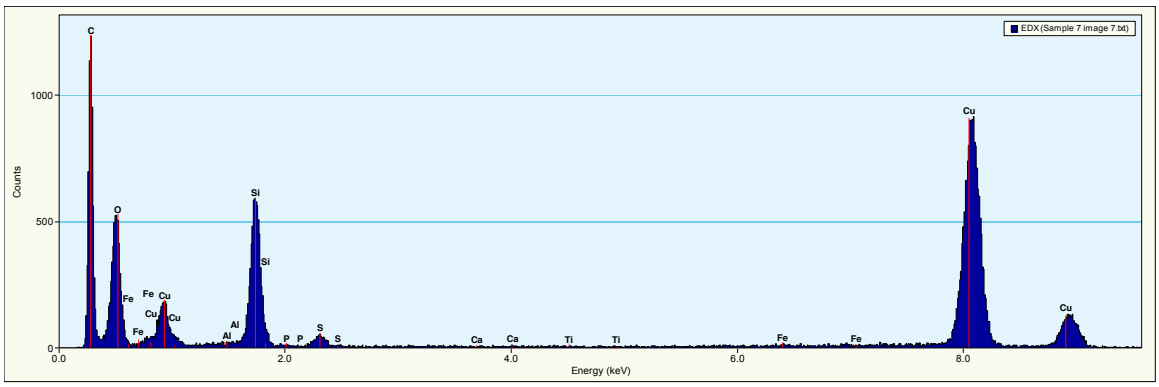

Figure 39: Presence of silicon dioxide in Meat Gravy by EDS

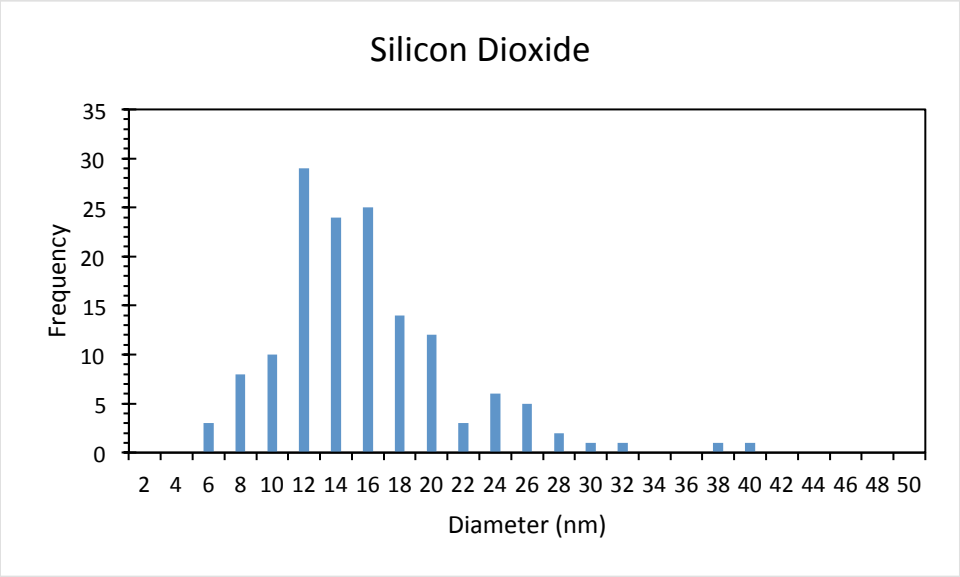

Figure 40: Silicon dioxide particle size distribution in Meat Gravy

Skittles

This product was labeled as containing "Colours (171, …)". The E171 food designation represents TiO_2. Particles in the nanoscale range were observed by TEM and point analysis using EDX of the particles indicated they contained titanium and oxygen, at ratios consistent with TiO_2. Figure 44 shows the distribution of particle sized by TEM and at least 10% of the primary particles were below 100 nm in size, with an average particle size of 177 nm. After acid digestion, the product was found to contain 37.6 μgTi/gram of dry food (0.0063 wt% as TiO_2).

Figure 41: Skittles and ingredients list

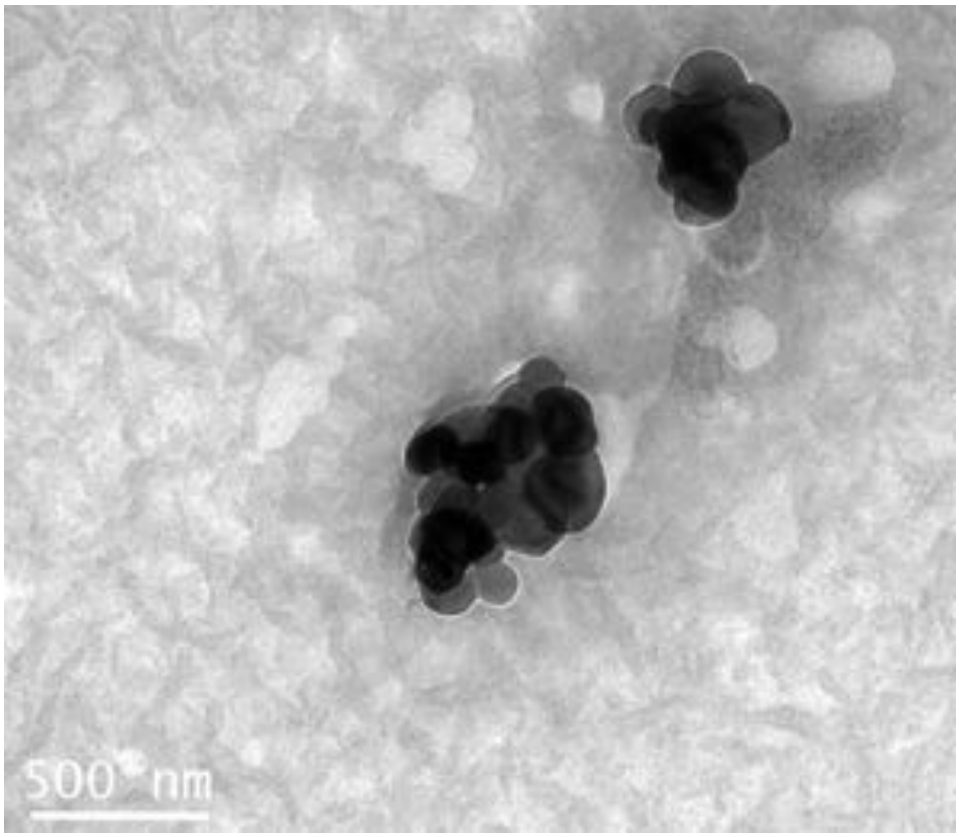

Figure 42: TEM of Skittles

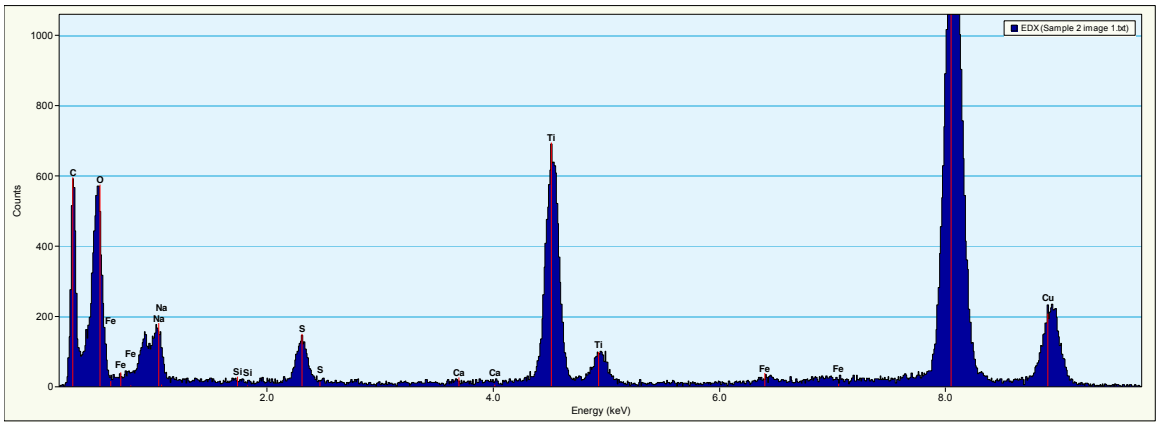

Figure 43: Presence of titanium dioxide in Skittles by EDS

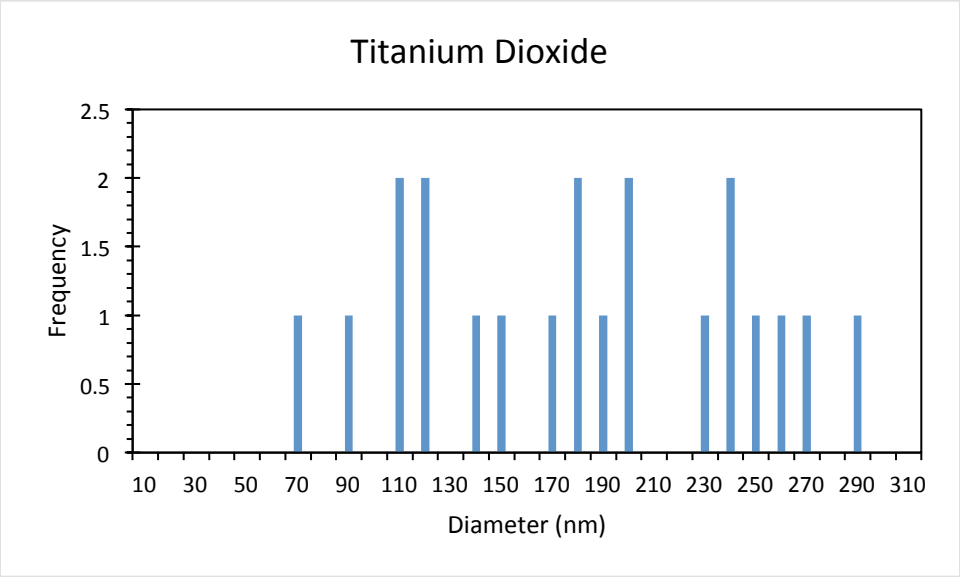

Figure 44: Titanium dioxide particle size distribution in Skittles

Sour straps

This product was labeled as containing "Colours (…, 171)". The E171 food designation represents TiO_2. Particles in the nanoscale range were observed by TEM and point analysis using EDX of the particles indicated they contained titanium and oxygen, at ratios consistent with TiO_2. Figure 48 shows the distribution of particle sized by TEM and at least 50% of the primary particles were below 100 nm in size, with an average particle size of 103 nm. After acid digestion, the product was found to contain 548 μgTi/gram of dry food (0.091 wt% as TiO_2).

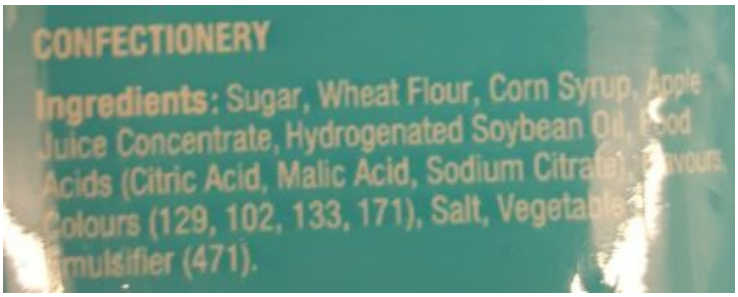

Figure 45: Sour Straps and ingredients list

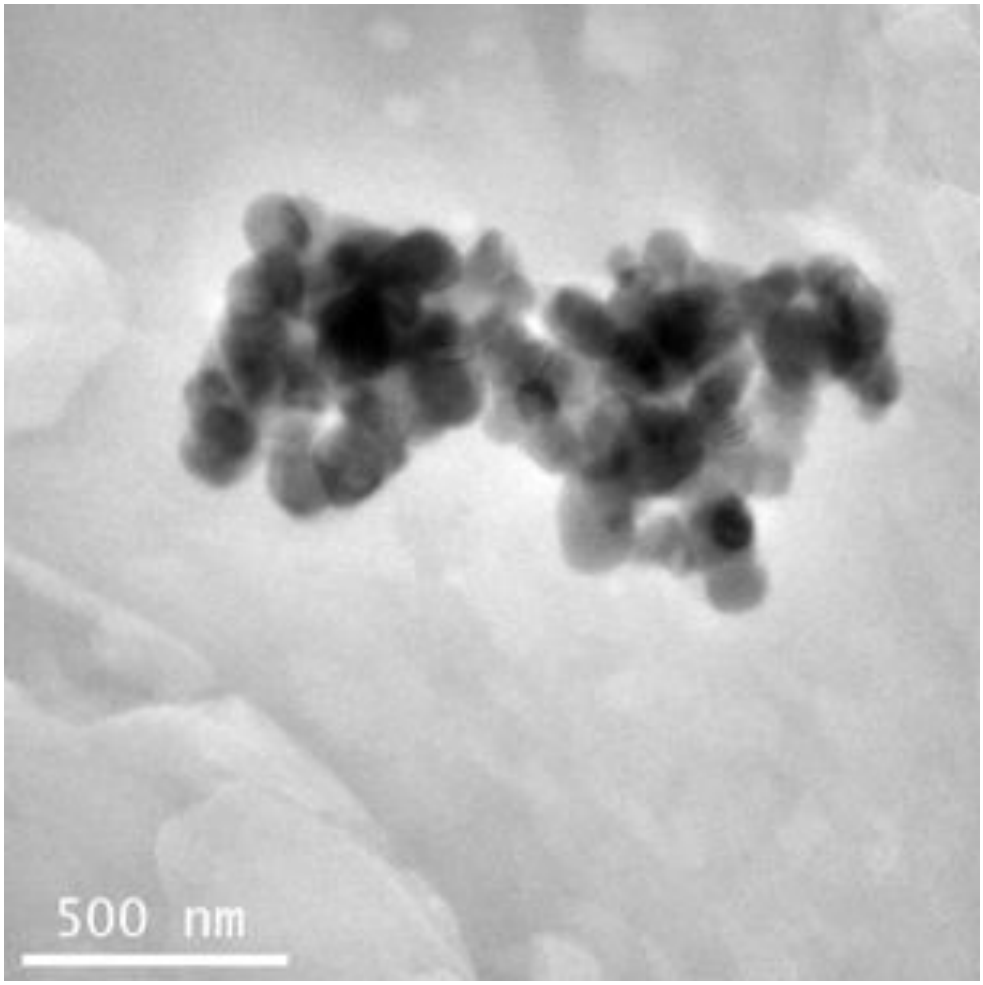

Figure 46: TEM of Sour Straps

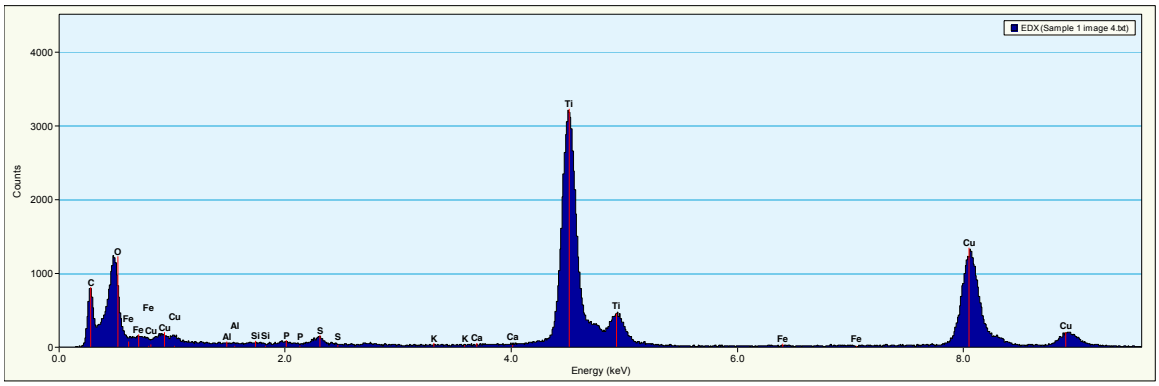

Figure 47: Presence of titanium dioxide in Sour Straps by EDS

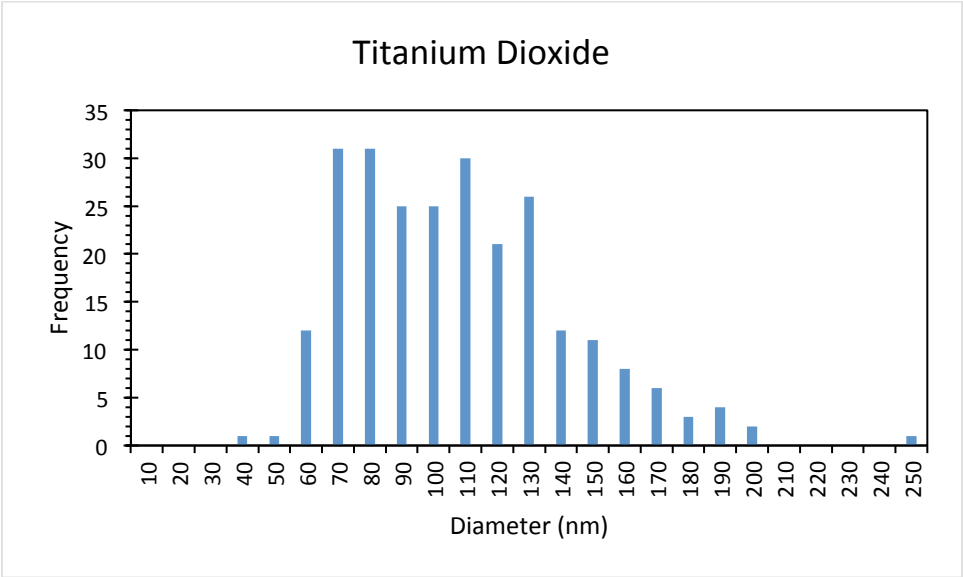

Figure 48: Titanium dioxide particle size distribution in Sour Straps

Taco mix

This product was labeled as containing "Anti-caking agent (551)". The E551 food designation represents SiO_2. Particles in the nanoscale range were observed by TEM and point analysis using EDX of the particles indicated they contained silica and oxygen, and is probably SiO_2. Figure 52 shows the distribution of particle sized by TEM and 100% of the primary particles were below 100 nm in size, with an average particle size of 21 nm. After acid digestion, the product was found to contain 2700 μgSi/gram of dry food (0.58 wt% as SiO_2).

Figure 49: Taco Spice Mix and ingredients list

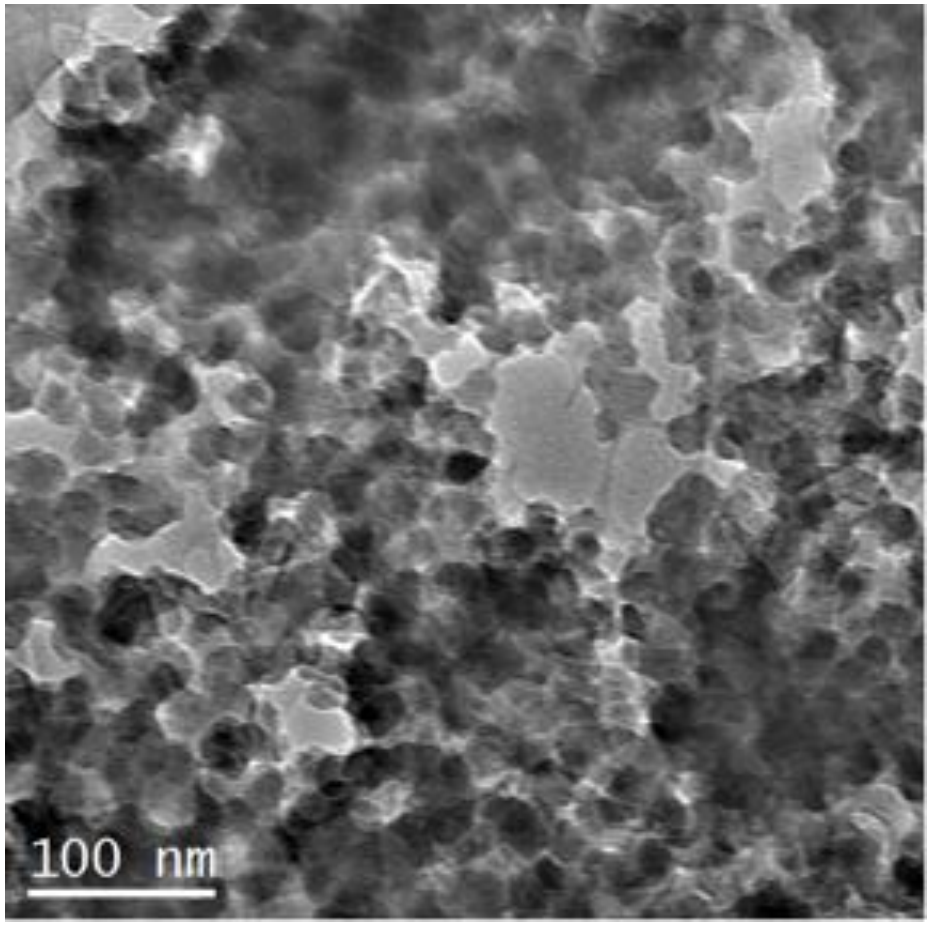

Figure 50: TEM of Taco Spice Mix

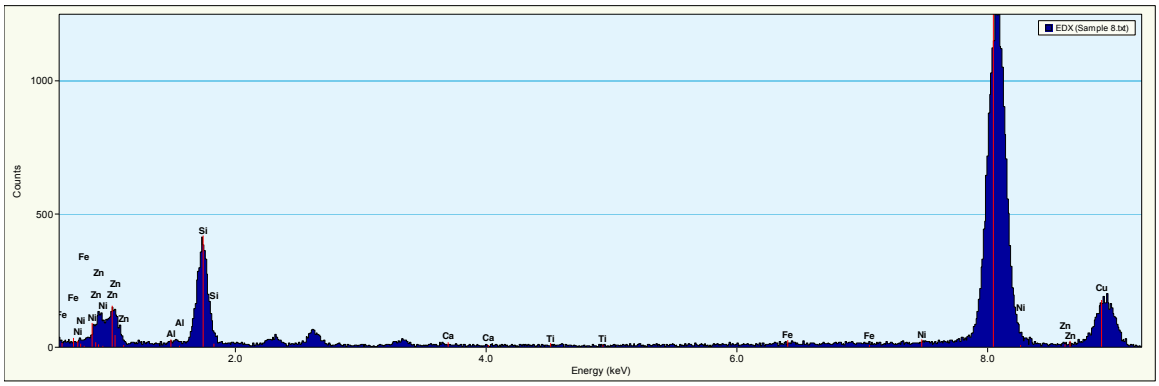

Figure 51: Presence of silicon dioxide in Taco Spice Mix by EDS

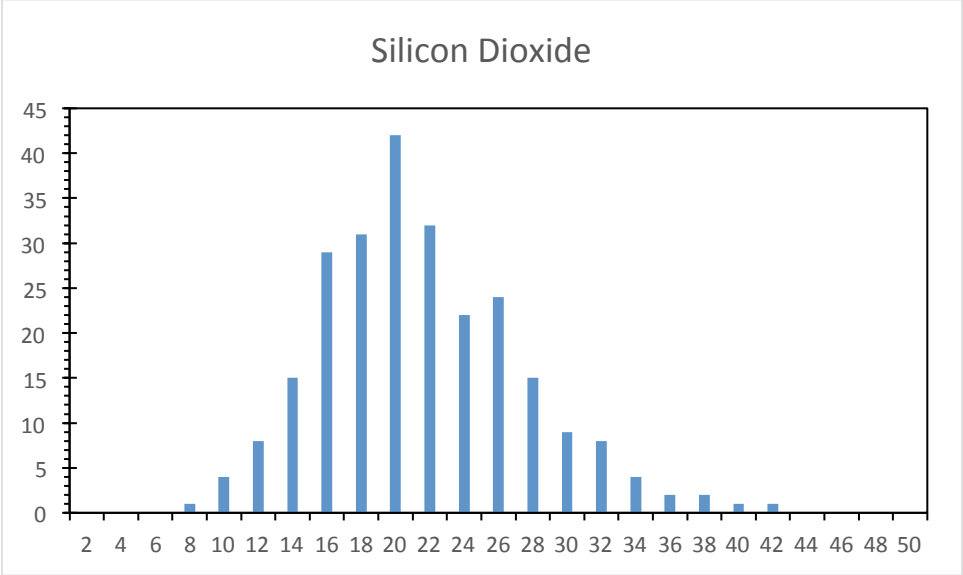

Figure 52: Silicon dioxide particle size distribution in Taco Spice Mix

White Sauce

This product was labeled as containing "Anticaking agent (551)". The E551 food designation represents SiO_2. Particles in the nanoscale range were observed by TEM and point analysis using EDX of the particles indicated they contained silica and oxygen, and is probably SiO_2. Figure 40 shows the distribution of particle sized by TEM and 100% of the primary particles were below 100 nm in size, with an average particle size of 10 nm. After acid digestion, the product was found to contain 234 μgSi/gram of dry food (0.050 wt% as SiO_2).

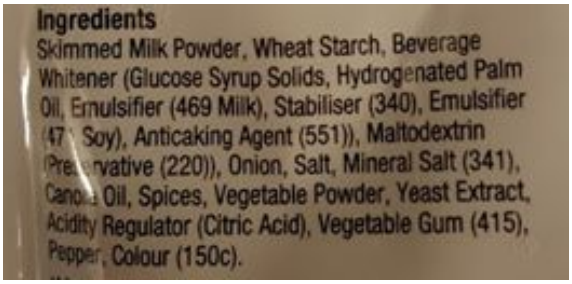

Figure 53: White Sauce and ingredients list

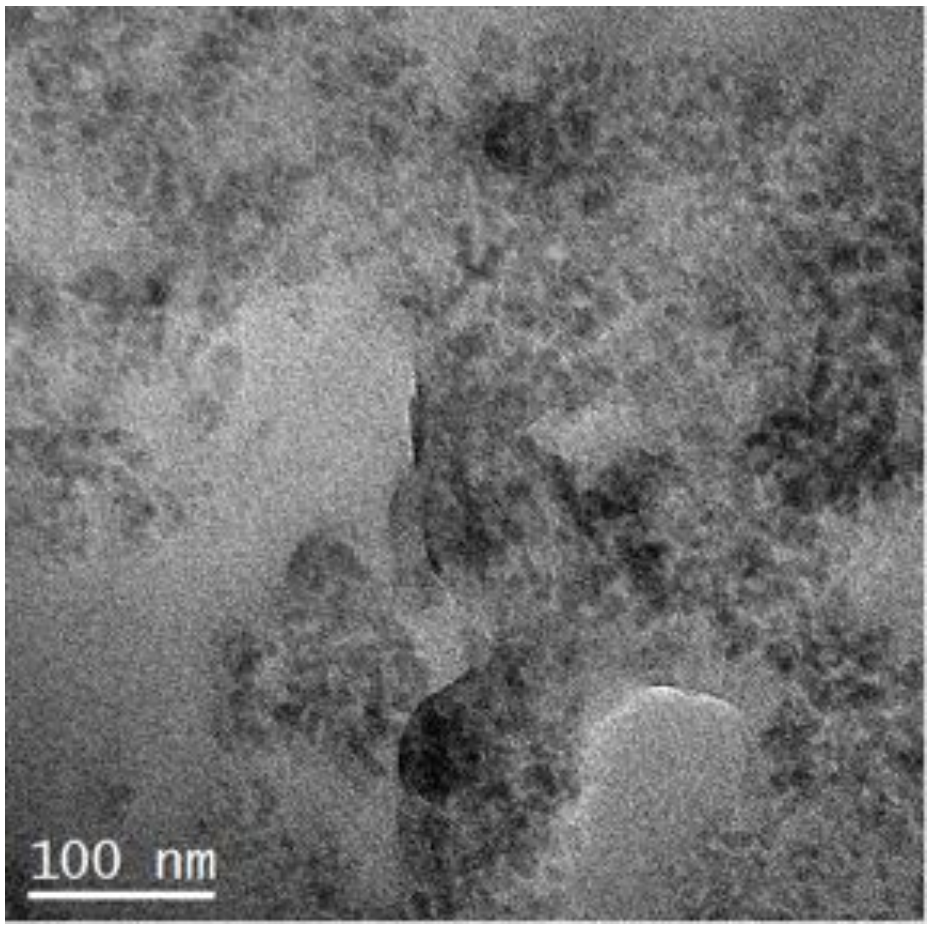

Figure 54: TEM of White Sauce

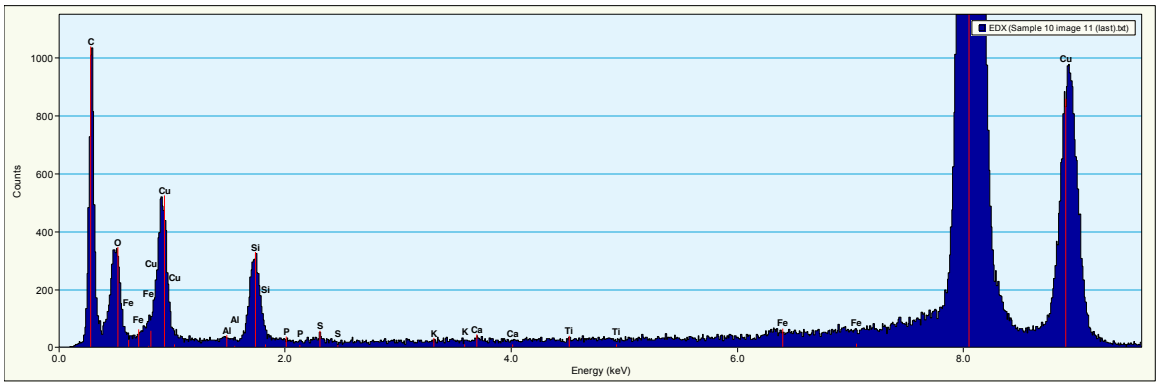

Figure 55: Presence of silicon dioxide in White Sauce by EDS

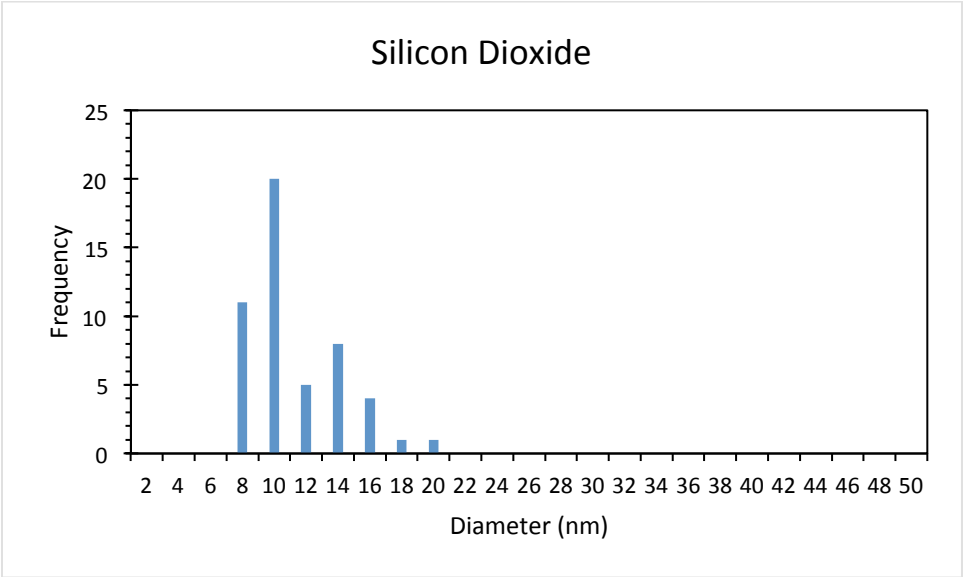

Figure 56: Silicon dioxide particle size distribution in White Sauce

References

1. Yang, Y.; Doudrick, K.; Bi, X. Y.; Hristovski, K.; Herckes, P.; Westerhoff, P.; Kaegi, R., Characterization of Food-Grade Titanium Dioxide: The Presence of Nanosized Particles. *Environmental Science & Technology* **2014,** *48*, (11), 6391-6400.
2. Yang, Y.; Faust, J. J.; Schoepf, J. J.; Hristovski, K.; Capco, D.; Herckes, P.; Westerhoff, P., Characterization, Human Impacts, and Environmental Fate of Food-grade Silicon Dioxide Nanomaterials throughout the Food Supply Value Chain. *Envir. Sci. & Tech.* **under review**.
3. Weir, A.; Westerhoff, P.; Fabricius, L.; Hristovski, K.; von Goetz, N., Titanium Dioxide Nanoparticles in Food and Personal Care Products. *Environ. Sci. Tech.* **2012,** *46*, (4), 2242-2250.