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In vitro duodenal digestion of raw and roasted almond lipids

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In order to be digested, lipids require to be in the form of emulsions. However, a great proportion of lipid in some food matrices such as almonds, remain undisturbed after mastication. Indeed, the data from our mastication study showed that half of the almond bolus was composed of particles with a size superior to 500 µm. We also revealed that negligible changes in microstructure, in particular particle size, occur during gastric digestion. According to these results, we anticipate that lipids present on the surface of the chewed almond particle are hydrolysed, whereas the intracellular lipids of the intact cells remained undigested [1].

The purpose of the present study was therefore to compare the lipid digestion on almond materials with different degrees of bioaccessibility, namely separated cells (i.e. intact cell walls) and particles of different sizes (i.e. different proportion of intact and fractures cells).

The *in vitro* digestion studies were performed using a pH-stat device to follow the FFA release.

The final composition for the digestion (total volume 37.5 mL, pH 7 at 37°C) was as follows (adapted from [2]):

- 300 mg of lipids (emulsions, almond separated cells or almond particles)
- 20 mg/mL bile extract solution in 10 mM phosphate buffer
- 150 mM NaCl
- 10 mM CaCl₂
- 2.4 mg/mL pancreatin solution in 10 mM phosphate buffer

The titrations were performed using 0.15 M NaOH for 1h

METHODS



pH-stat: Methrom 848 Tritrino plus

-Almond cells were separated by leaving almond particles for 4 weeks in a solution containing 50 mM CDTA and 5 mM metabisulfite (Na₂S₂O₅), pH 7, grinding the pieces using mortar and pestle and then sieving them before performing a dialysis to remove CDTA.

-Almond particles of different sizes were obtained by grinding almonds in a blender, sieving and collecting the particles from sieves of 1, 0.5 and 0.25 mm aperture and a sieve base.

The microstructure of almond cells before and after digestion was studied using either light or confocal laser scanning (CLM) microscope. Cells observed with CLM were stained with Nile red (1 mg/mL in dimethyl sulfoxide, λ = 366 nm).

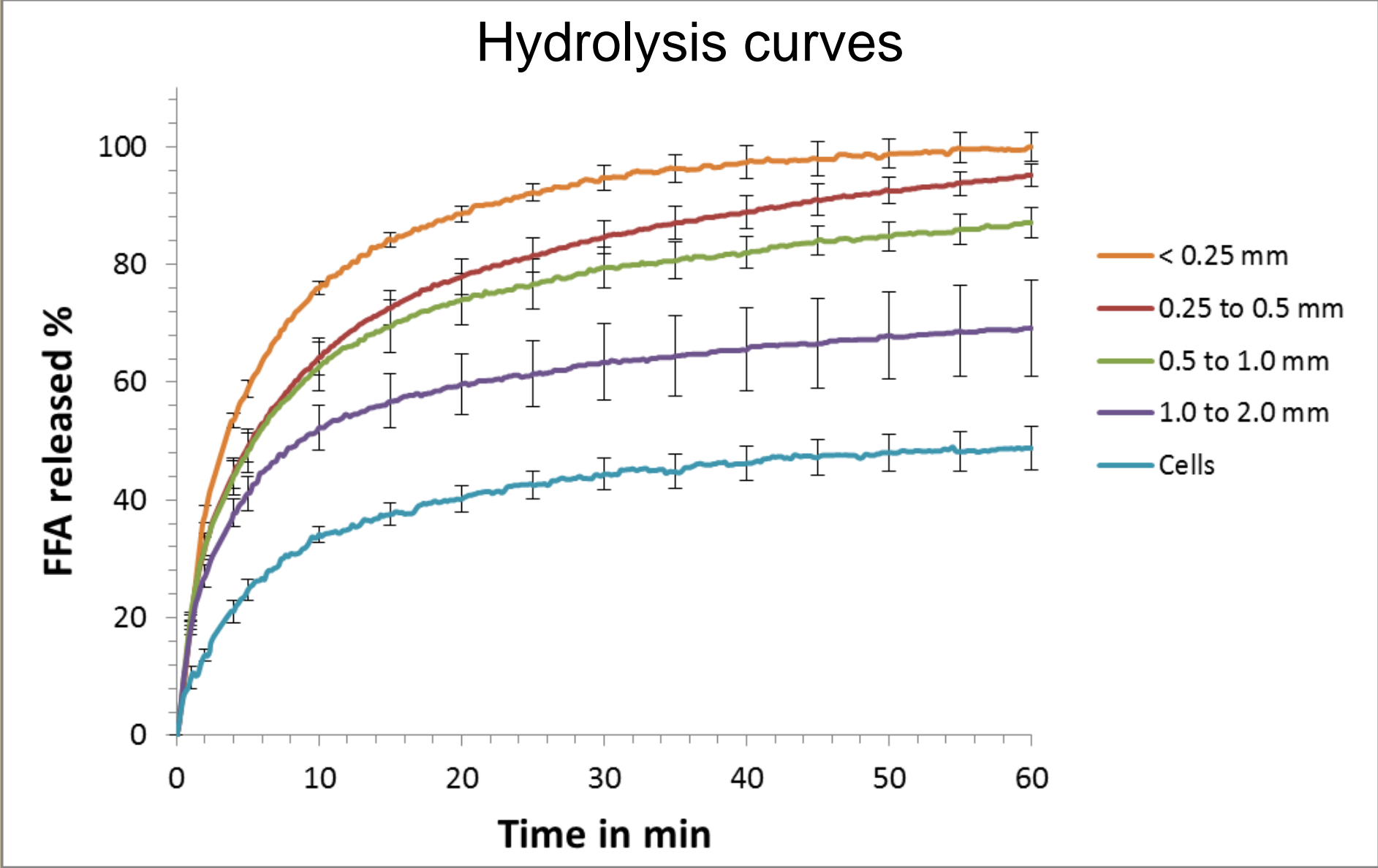
RESULTS AND DISCUSSION

Percentage of free fatty acid (%FFA) release using raw or roasted almond particles of different sizes and separated cells as a source of lipid substrate

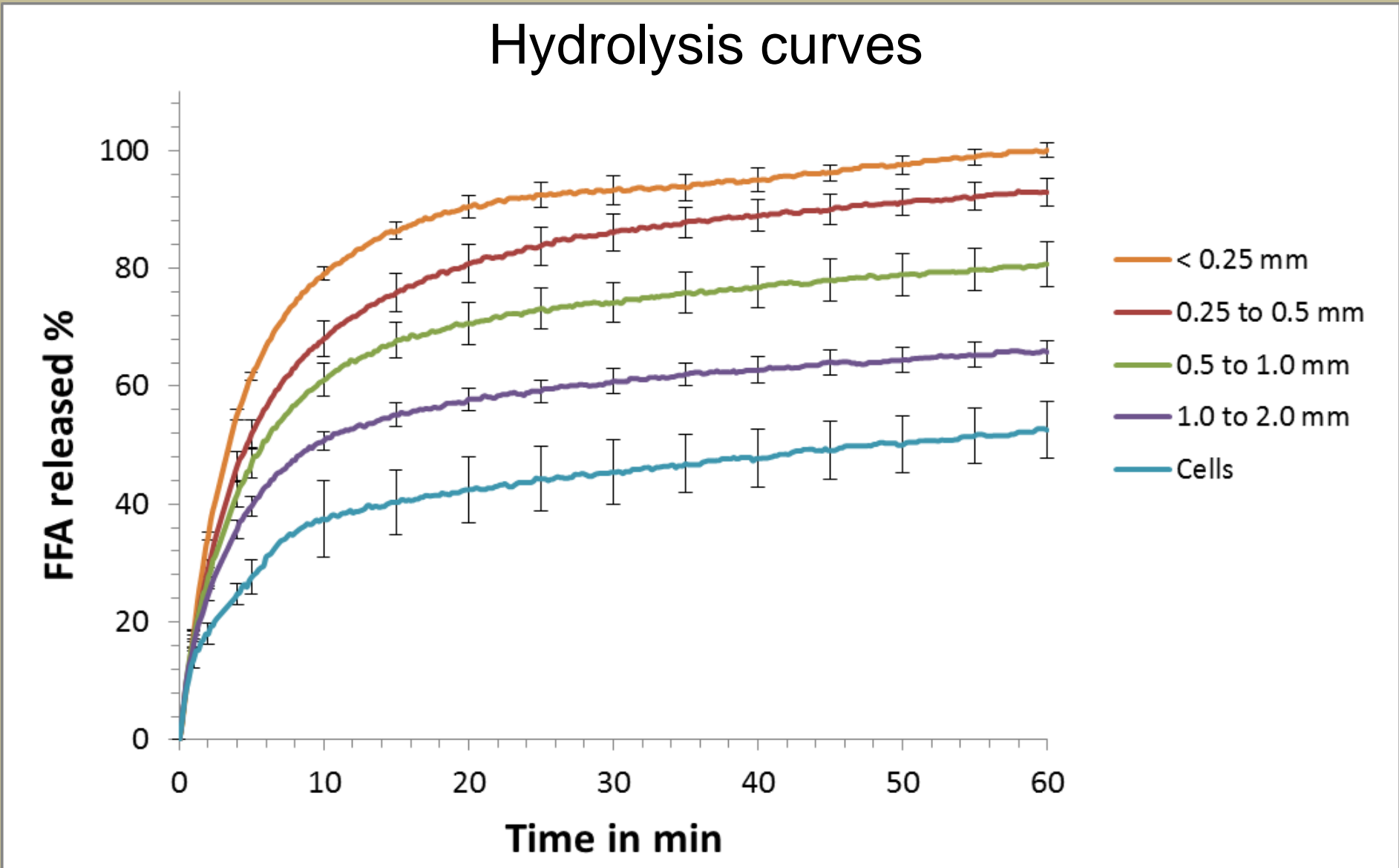
The maximum amount of FFA produced during the duodenal digestion of **almond flour (< 0.25 mm) was used as a reference (100%)** for the subsequent materials.



RAW almond

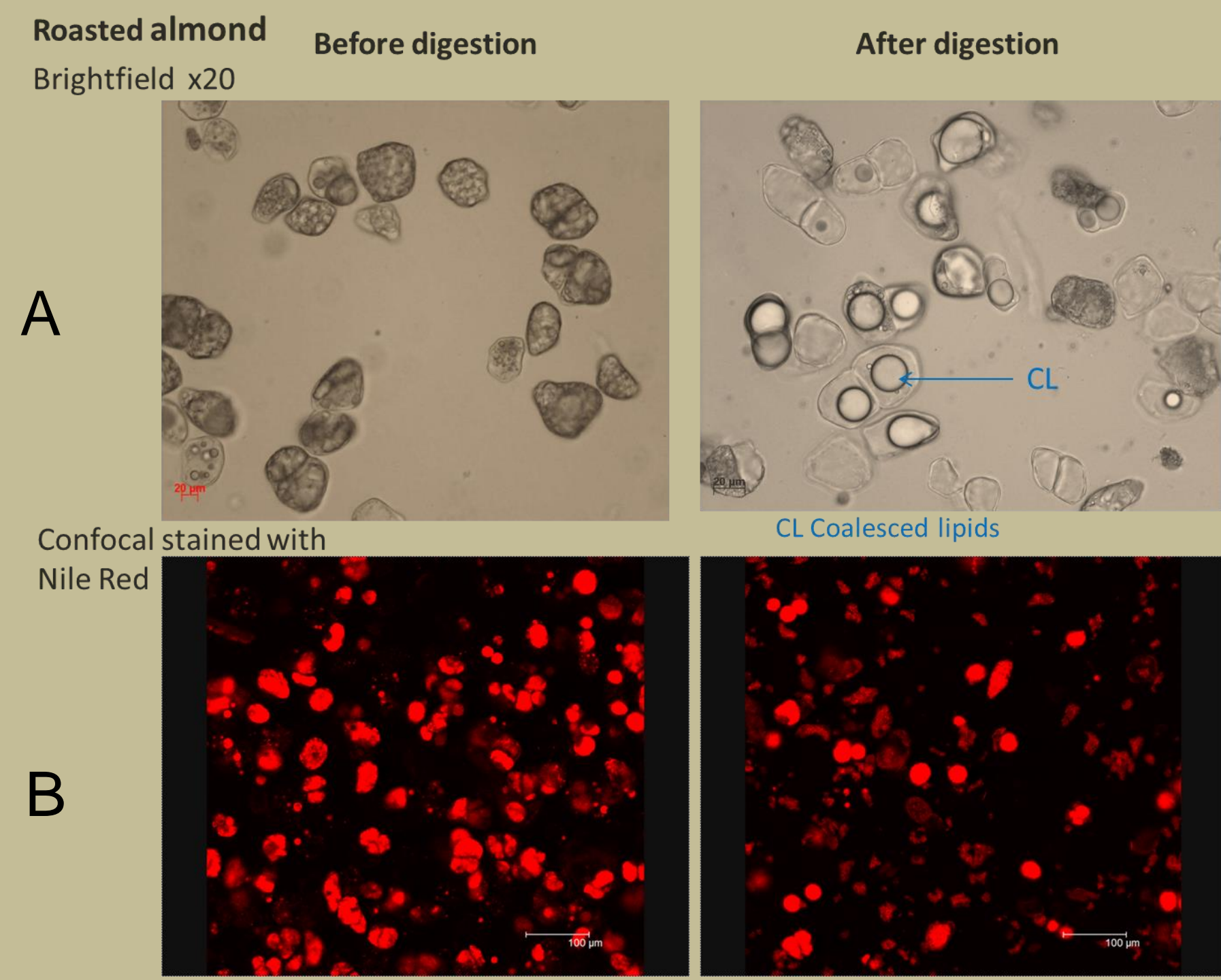
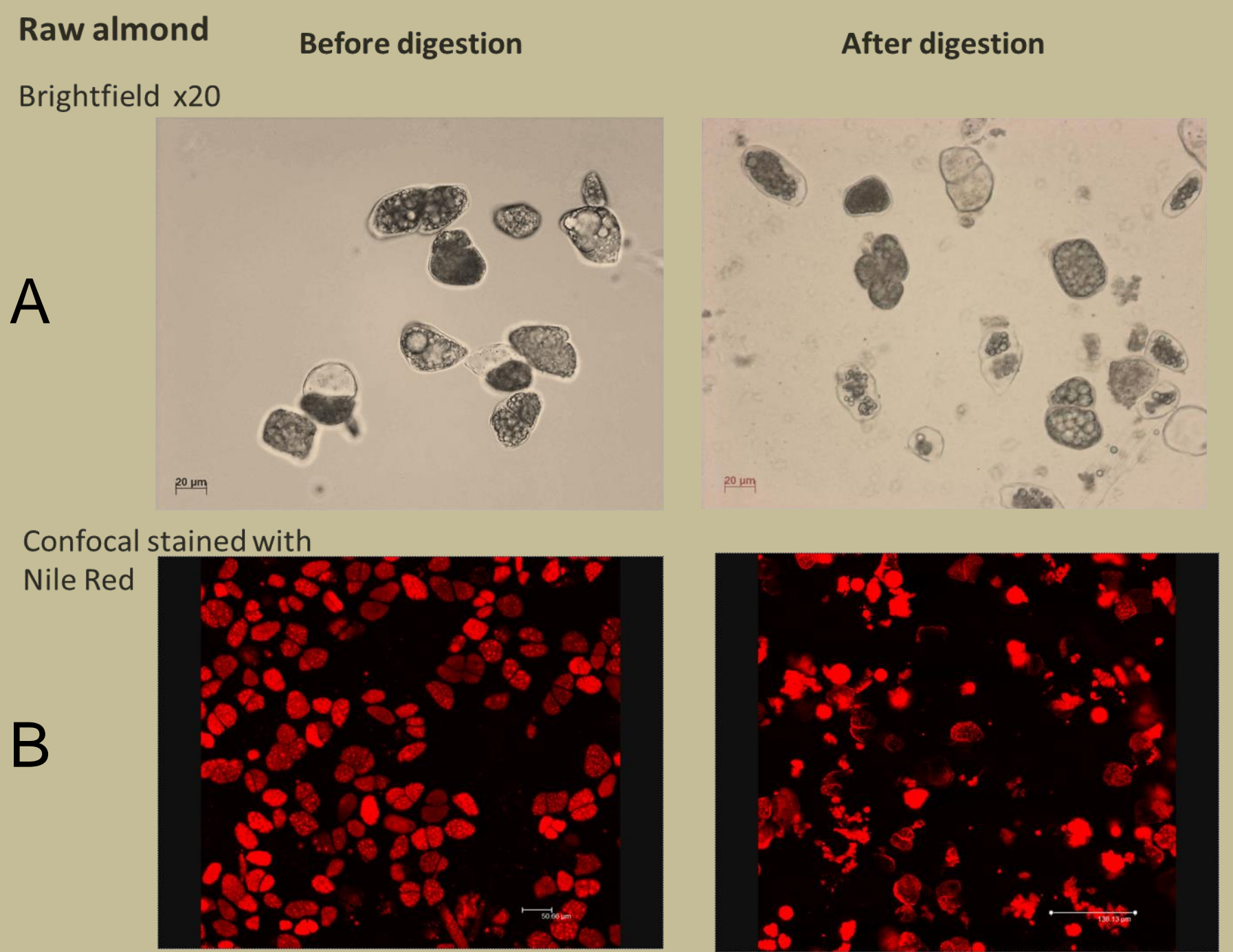


ROASTED almond



Representative image of separated, raw and roasted almond cells before and after digestion in bright field (A) or confocal (B) microscopy

Most of the lipids remain undigested as encapsulated by the cell walls.



Amount of fatty acid release (%) and reaction rate (µmol/mL/min) after 1 hour hydrolysis with pancreatic (mean ± SEM, n = 3)

	%FFA produced	Reaction rate in µmol/mL/min
RAW		
Separated cells	21.2 ± 1.59	0.97 ± 0.140
Particles < 0.25 mm	43.3 ± 1.08	1.54 ± 0.021
Particles 0.25 to 0.5 mm	41.2 ± 0.85	1.61 ± 0.046
Particles 0.5 to 1.0 mm	37.8 ± 1.10	1.60 ± 0.064
Particles 1.0 to 2.0 mm	26.1 ± 1.04	1.45 ± 0.064
ROASTED		
Separated cells	22.0 ± 2.04	1.13 ± 0.089
Particles < 0.25 mm	42.0 ± 0.55	1.34 ± 0.043
Particles 0.25 to 0.5 mm	39.0 ± 1.00	1.35 ± 0.071
Particles 0.5 to 1.0 mm	33.9 ± 1.56	1.32 ± 0.099
Particles 1.0 to 2.0 mm	27.6 ± 0.79	1.26 ± 0.059

- Particle size had a major impact on the rate and extent of digestion
- The rate of reaction and the amount of FFA released was markedly reduced in separated cells
- No significant difference between raw and roasted almonds were found. Roasting did not seem to have an effect on the rate and extent of lipid digestion.

CONCLUSION

These results showed that depending on the almond structure and degree of processing, the amount of fatty acids produced from the hydrolysis of TAG substantially varied. The concentration of FFA release was greater for smaller particles compared to larger particles due to the **greater proportion of ruptured cells**, and thus lipids available for digestion. This study showed that cell walls act as a physical barrier that limits nutrient digestion.

RELEVANCE TO INDUSTRY

Controlling the particle size of plant-based ingredients such as almonds could be used to develop novel food ingredients and products, that can be used to increase or decrease the uptake of lipids and other nutrients. This could potentially have a range of health benefits including appetite control, weight management, prevention of CVD and diabetes or even increasing nutrient uptake as needed by undernourished populations (i.e. elderly).

Future work

- *In vivo* study in ileostomy individuals measuring blood responses and nutrient losses in digesta following the ingestion of almond meals with different degree of bioaccessibility
- Kinetic studies using antibodies to measure cellular uptake of lipase on almond cells

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References:

- [1] Ellis PR, Kendall CW, Ren Y, Parker C, Pacy JF, Waldron KW *et al* (2004). *Am J Clin Nutr* **80**: 604-613, [2] McClements DJ, Li Y (2010). Review of in vitro digestion models for rapid screening of emulsion-based systems. *Food & Function* **1**: 32-59.

