

# Exploring Dental Calculus and Diet Through an Oral Biofilm Model

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Including dietary research by extraction of plant microremains

However...



## What we know

- microremains preserve well in calculus
- they can be extracted from calculus to infer diet

## What we don't know

- mechanism(s) of incorporation (and biases)
- methodological biases of extraction methods
- how diet affects calculus growth
- and a whole lot more...



# So what are we going to do about it?

Presenting an oral biofilm model to grow and 'feed' dental calculus in a highly controlled setting.



# An oral what-now?

Biofilm: Communities of microbial cells within extracellular polymers.

Biofilm model: growing a biofilm in (or moving to) a lab for analysis.



# An oral what-now?

Biofilm: Communities of microbial cells within extracellular polymers.

Biofilm model: growing a biofilm in (or moving to) a lab for analysis.

Popular in clinical studies

- study the behaviour of microbiota in biofilms
- the affect of diet on dental disease,
- and the effect of various treatments on (opportunistic) pathogens.



# Calculus models

Also known as *in vitro* dental calculus

Common until the 90s,

then focus shifted to short-term biofilm models for testing treatments (caries, periodontitis, etc.)

Lack of interest from dental research since then



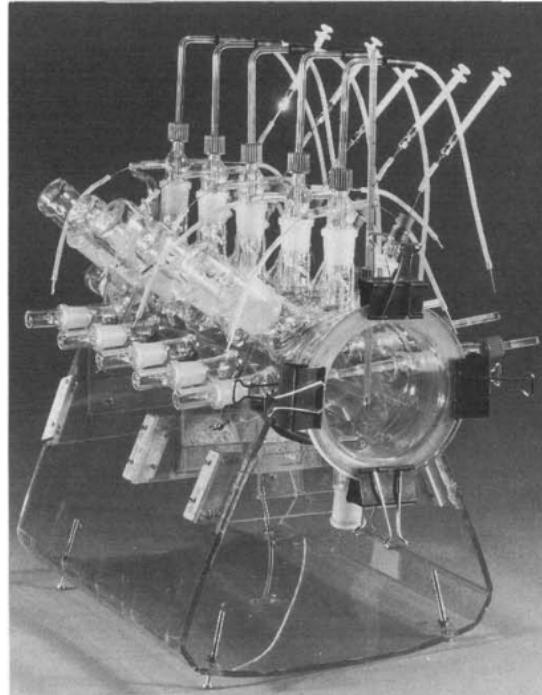
# Types of biofilm models

## Simple

- agar plate
- multiplate

## Complex

- constant depth film fermenter
- modified Robbins device
- artificial mouth



Multiple artificial mouth setup. Sissons et al.  
1991.



# Our setup



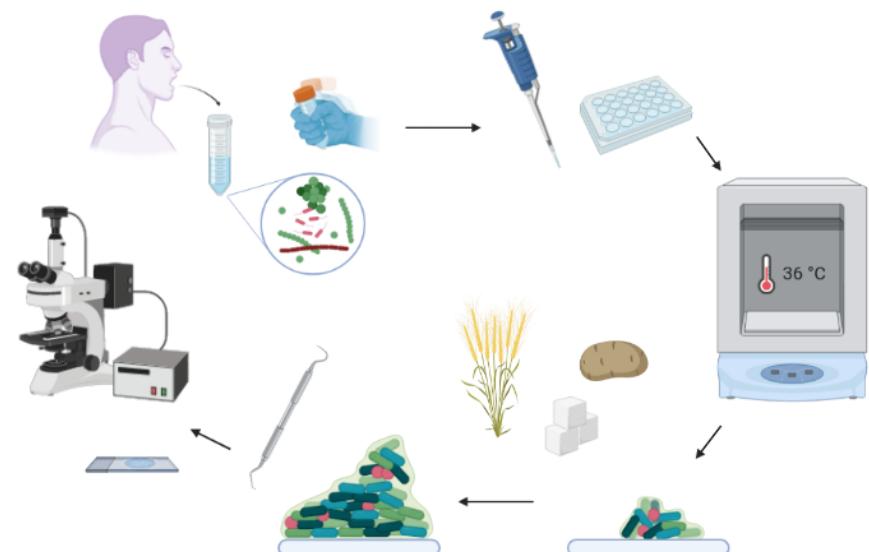
Polypropylene 24 deepwell plate with "teeth" attached to lid.

- multiwell plate with suspended substrata
  - polypropylene 24 deepwell plate with lid
- substrata suspended vertically for active attachment
- high throughput model
- consistent treatment(s) across all samples



# Protocol

- inoculated with whole saliva
- incubated for 25 days ( $36^{\circ}\text{C}$ , 30 rpm)
- sucrose for bacterial growth
- wheat and potato treatments
- increase mineralisation with CPMU
- extraction (EDTA) and quantification of starches



Created in BioRender.com 



# Goal

To test fundamental aspects of dental calculus analysis by using the oral biofilm model as a proxy.



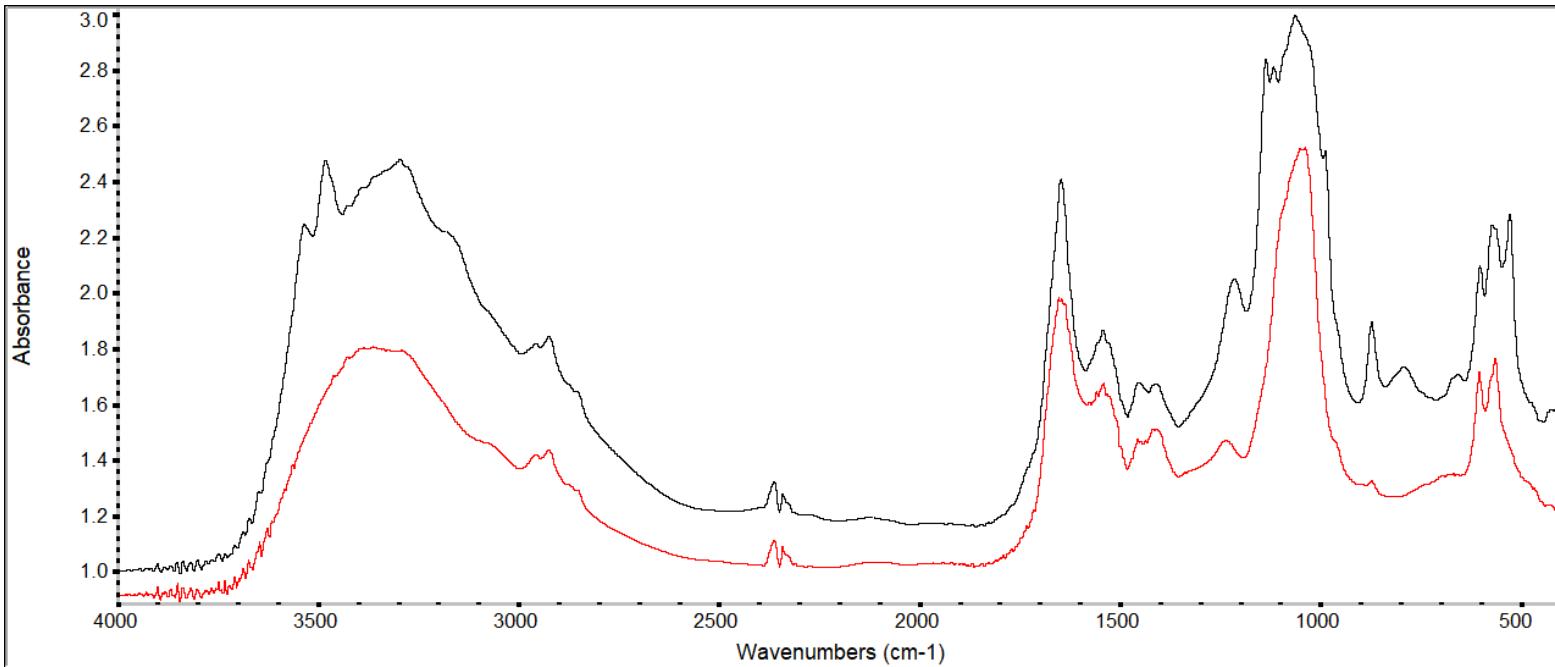
# Results



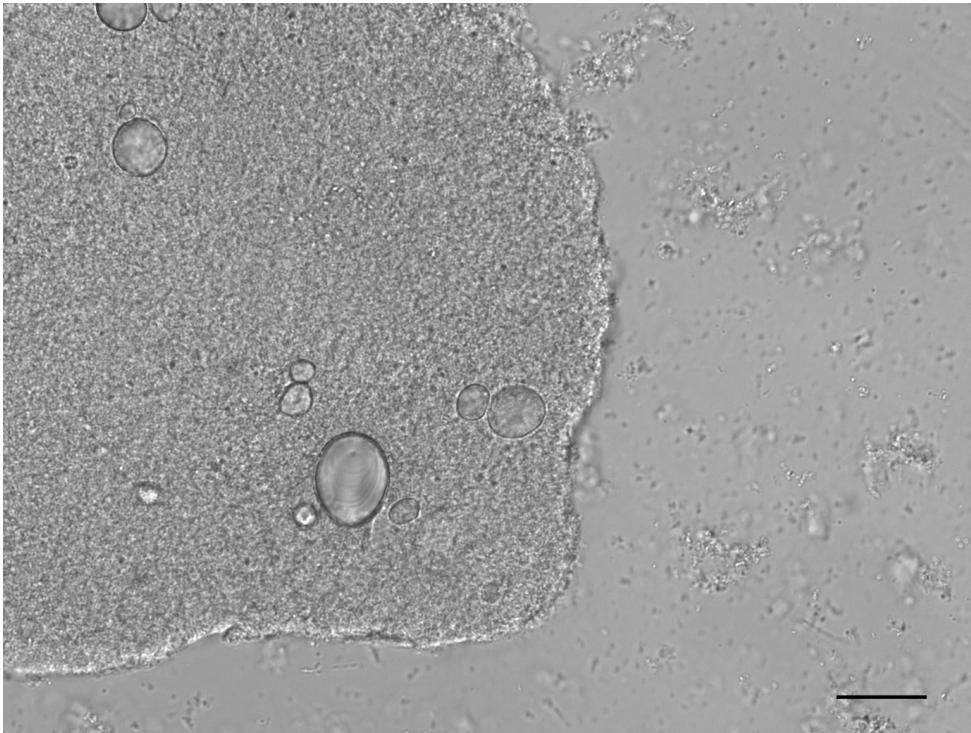
# End product

It certainly looks like dental calculus.

**Red** = model calculus, **black** = real calculus comparison.



# Extraction and quantification of starches

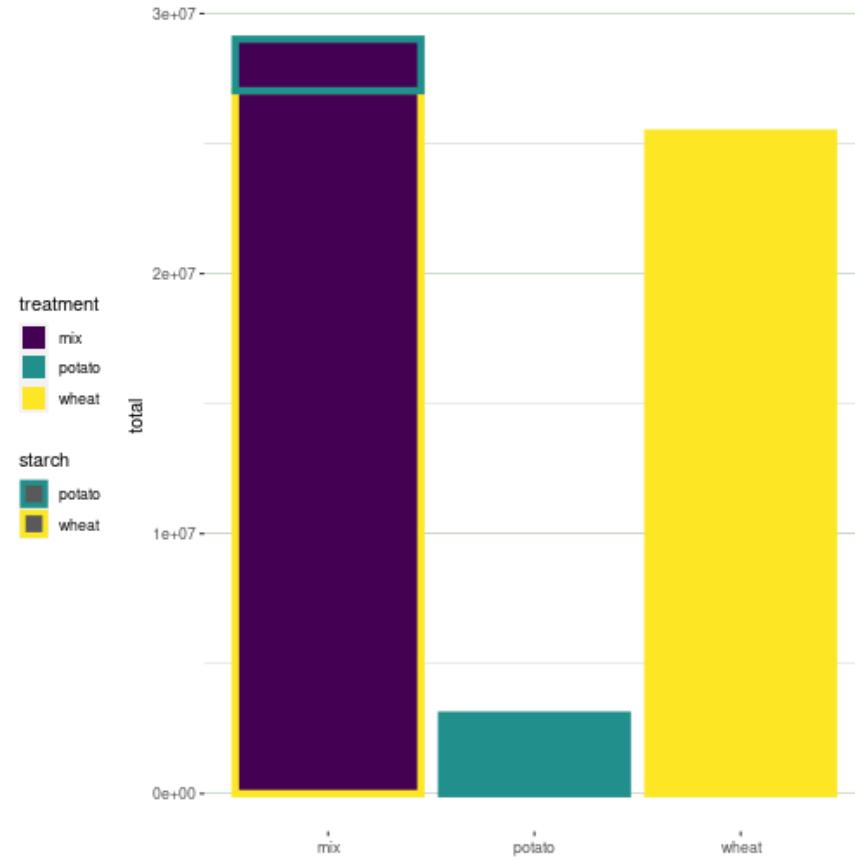


Microscope image of biofilm sample with potato starches and bacteria.

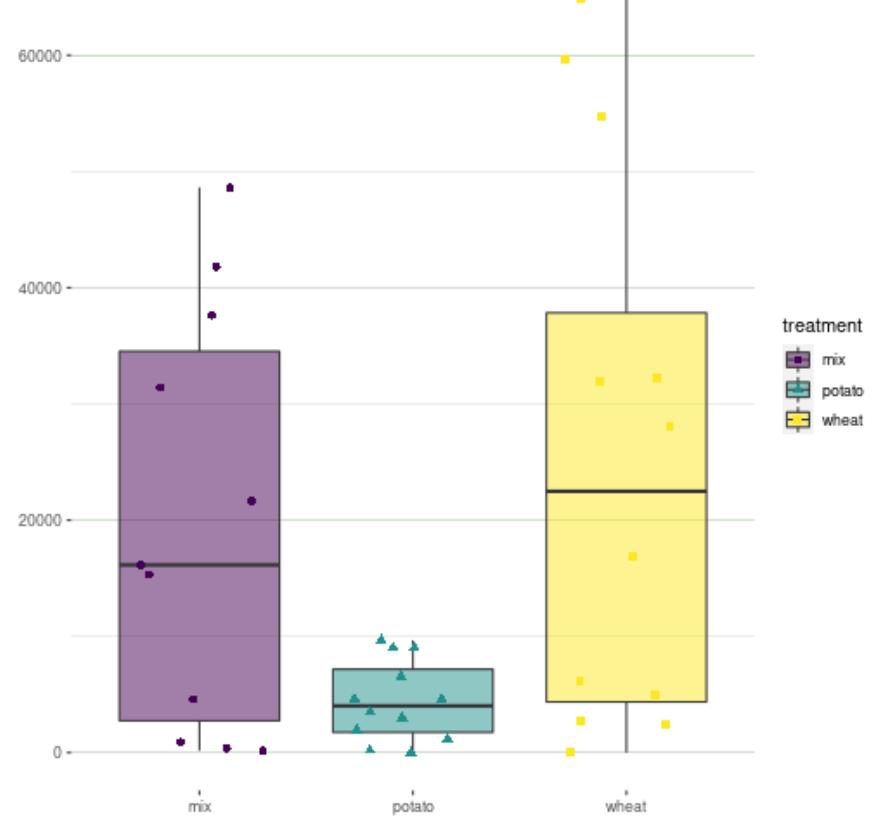
Scale bar = 20  $\mu\text{m}$



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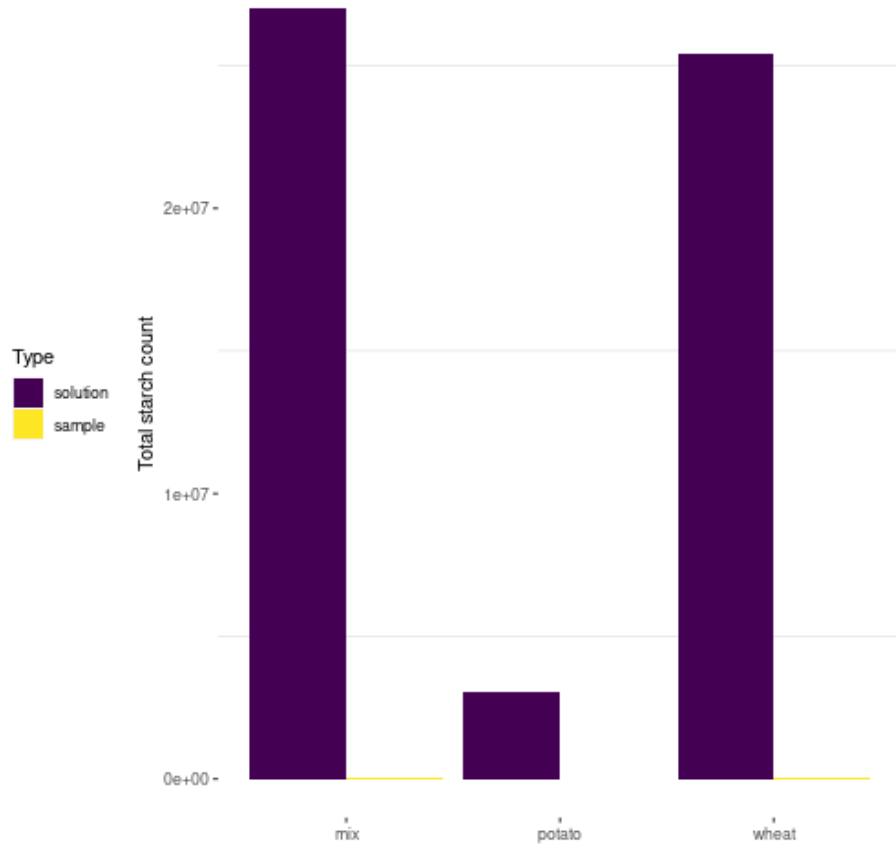


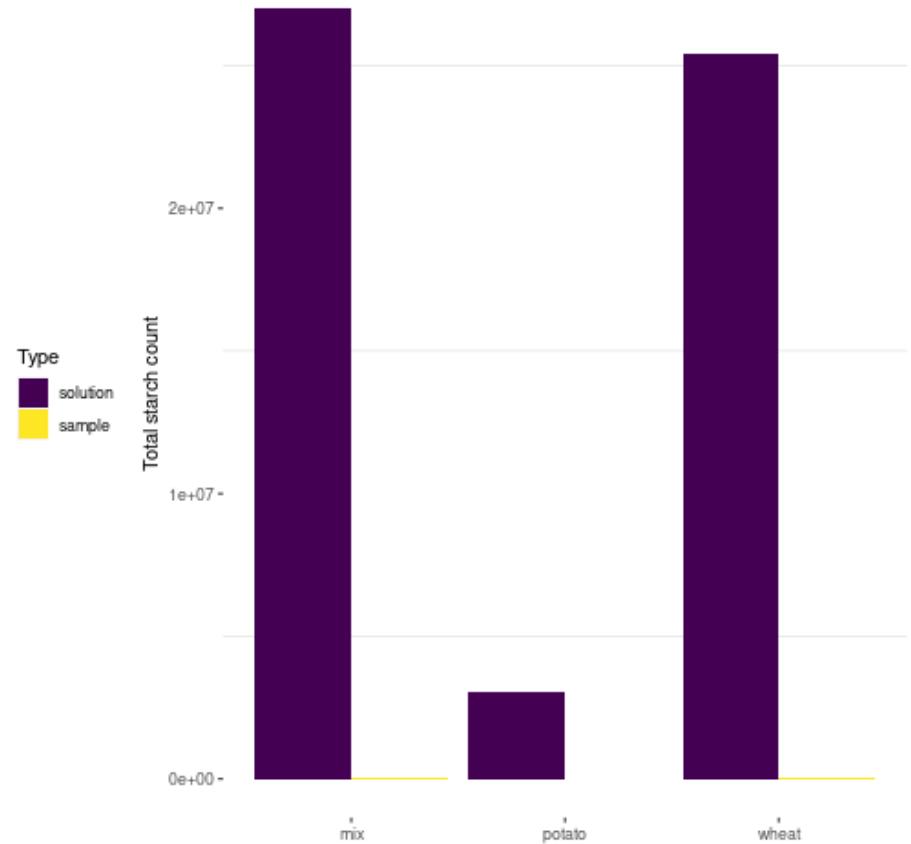
Mean extrapolated counts for solutions



Mean extrapolated counts for granules extracted from the samples.







Incorporation is so low it's not visible in the plot

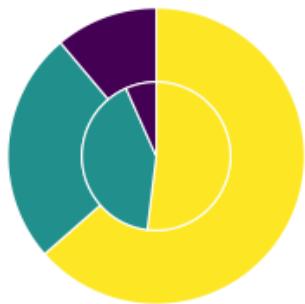
so here's a table

treatment	starch	s	m	l	total
mix	potato	NA	0.19%	0.05%	0.12%
mix	wheat	0.05%	0.10%	0.08%	0.06%
potato	potato	0.28%	0.27%	0.06%	0.16%
wheat	wheat	0.09%	0.19%	0.07%	0.11%

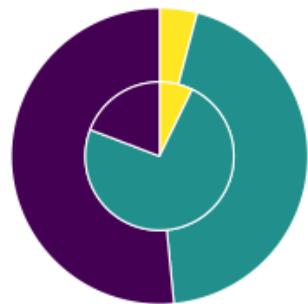
mean percentage of starches from solutions extracted from the calculus.



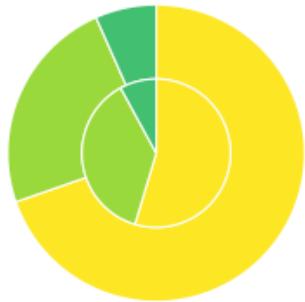
Separate - wheat



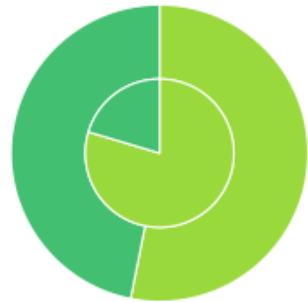
Separate - potato



Mix - wheat



Mix - potato



Size  
large  
medium  
small

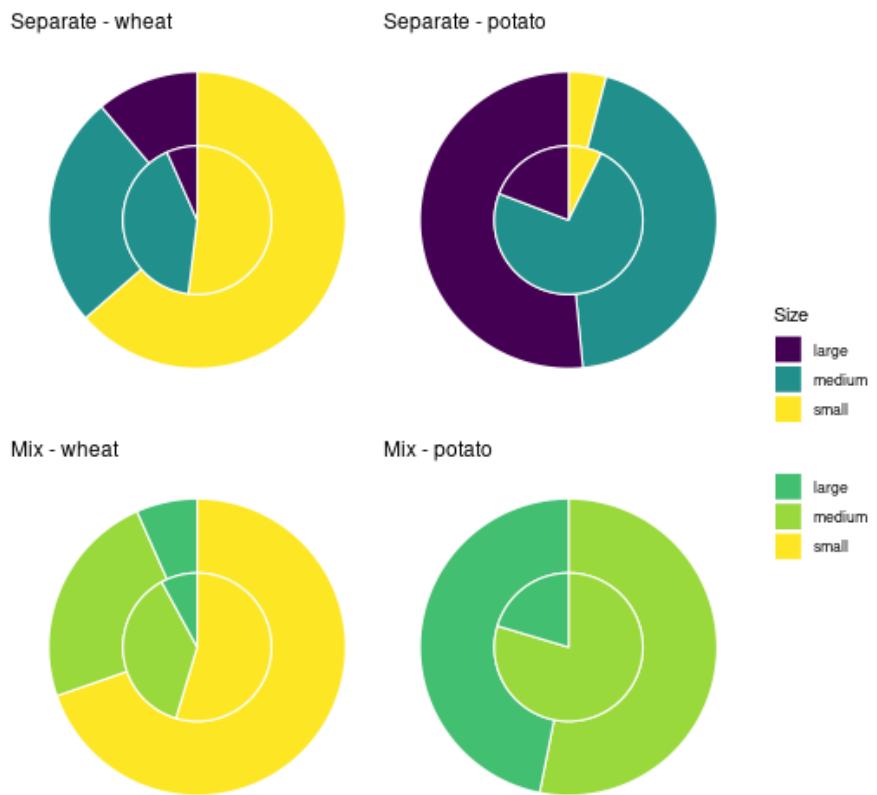
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Shift in size ratios from solution (outer ring) to sample (inner ring)

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## Size bias

Small ( $<10 \mu\text{m}$ ) and medium ( $10\text{-}20 \mu\text{m}$ ) starches over-represented

Large ( $>20 \mu\text{m}$ ) starches under-represented

- especially potato



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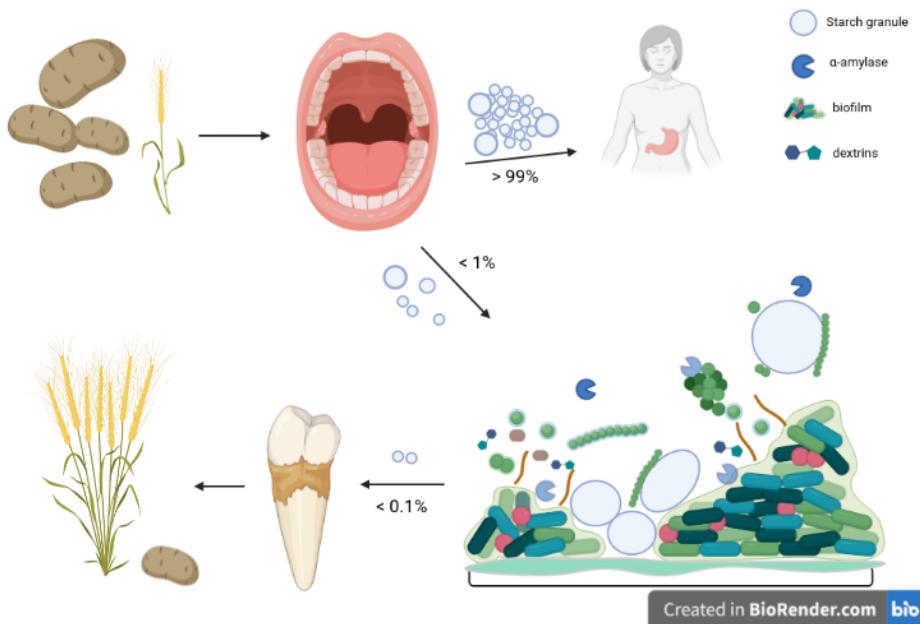
- further compounded by diagenesis

And the size of granules matters

- large-granule producers are under-represented



# Starch incorporation



◀ Based on our results it may look something like this

But no one really seems to know



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Starch delivery system is not perfect

- substrata are transferred to plate with starch solutions
- rpm is increased to double speed



# Potential of the model

## Methods testing

- extraction using various dissolution agents (e.g. EDTA and HCl)
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## High degree of control over multiple factors

- dietary input
- bacterial composition (sort of...)



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- size affects likelihood of incorporation

We still know very little, but...

our model can supplement archaeological studies

...and help us learn a little more



# Open Science Statement

Open Data

Open Methods

Open Access



Slides available (with data and code):

<https://bbartholdy.github.io/capa2021-pres/CAPA-pres.html#1>

<https://osf.io/5fbxw/>

Protocols available:

<https://www.protocols.io/workspaces/byoc>

Pre-print available:

<https://www.biorxiv.org/content/10.1101/2021.10.27.466104v1>



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