

# Supplementary Material

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## 1 R Session info

```
print(sessionInfo(), locale = FALSE)

## R version 4.1.2 (2021-11-01)
## Platform: x86_64-pc-linux-gnu (64-bit)
## Running under: Pop!_OS 21.10
##
## Matrix products: default
## BLAS:    /usr/lib/x86_64-linux-gnu/openblas-pthread/libblas.so.3
## LAPACK:  /usr/lib/x86_64-linux-gnu/openblas-pthread/libopenblas-p0.3.13.so
##
## attached base packages:
## [1] stats      graphics   grDevices  utils      datasets   methods    base
##
## other attached packages:
## [1] broom_0.7.11    patchwork_1.1.1 forcats_0.5.1  stringr_1.4.0
## [5] dplyr_1.0.8     purrrr_0.3.4   readr_2.1.1    tidyverse_1.3.1 here_1.0.1
## [9] tibble_3.1.6    ggplot2_3.3.5  tidyverse_1.3.1 haven_2.4.3
## [13] glue_1.6.1      DBI_1.1.2      pillar_1.7.0   colorspace_2.0-2
## [17] vctrs_0.3.8     generics_0.1.2 htmltools_0.5.2 yaml_2.2.2
## [21] utf8_1.2.2      rlang_1.0.1     pillar_1.7.0   withr_2.4.3
## [25] dbplyr_2.1.1    bit64_4.0.5    dbplyr_2.1.1
```

```
## [17] modelr_0.1.8      readxl_1.3.1      lifecycle_1.0.1   munsell_0.5.0
## [21] gtable_0.3.0       cellranger_1.1.0  rvest_1.0.2      evaluate_0.14
## [25] knitr_1.37         tzdb_0.2.0        fastmap_1.1.0    parallel_4.1.2
## [29] fansi_1.0.2        Rcpp_1.0.8        backports_1.4.1  scales_1.1.1
## [33] vroom_1.5.7        jsonlite_1.7.3    bit_4.0.4        fs_1.5.2
## [37] hms_1.1.1          digest_0.6.29    stringi_1.7.6   bookdown_0.24
## [41] rprojroot_2.0.2    grid_4.1.2        cli_3.1.1        tools_4.1.2
## [45] magrittr_2.0.2     crayon_1.4.2    pkgconfig_2.0.3  ellipsis_0.3.2
## [49] xml2_1.3.3         reprex_2.0.1    lubridate_1.8.0  assertthat_0.2.1
## [53] rmarkdown_2.11      httr_1.4.2       rstudioapi_0.13  R6_2.5.1
## [57] compiler_4.1.2
```

## 2 Experimental setup

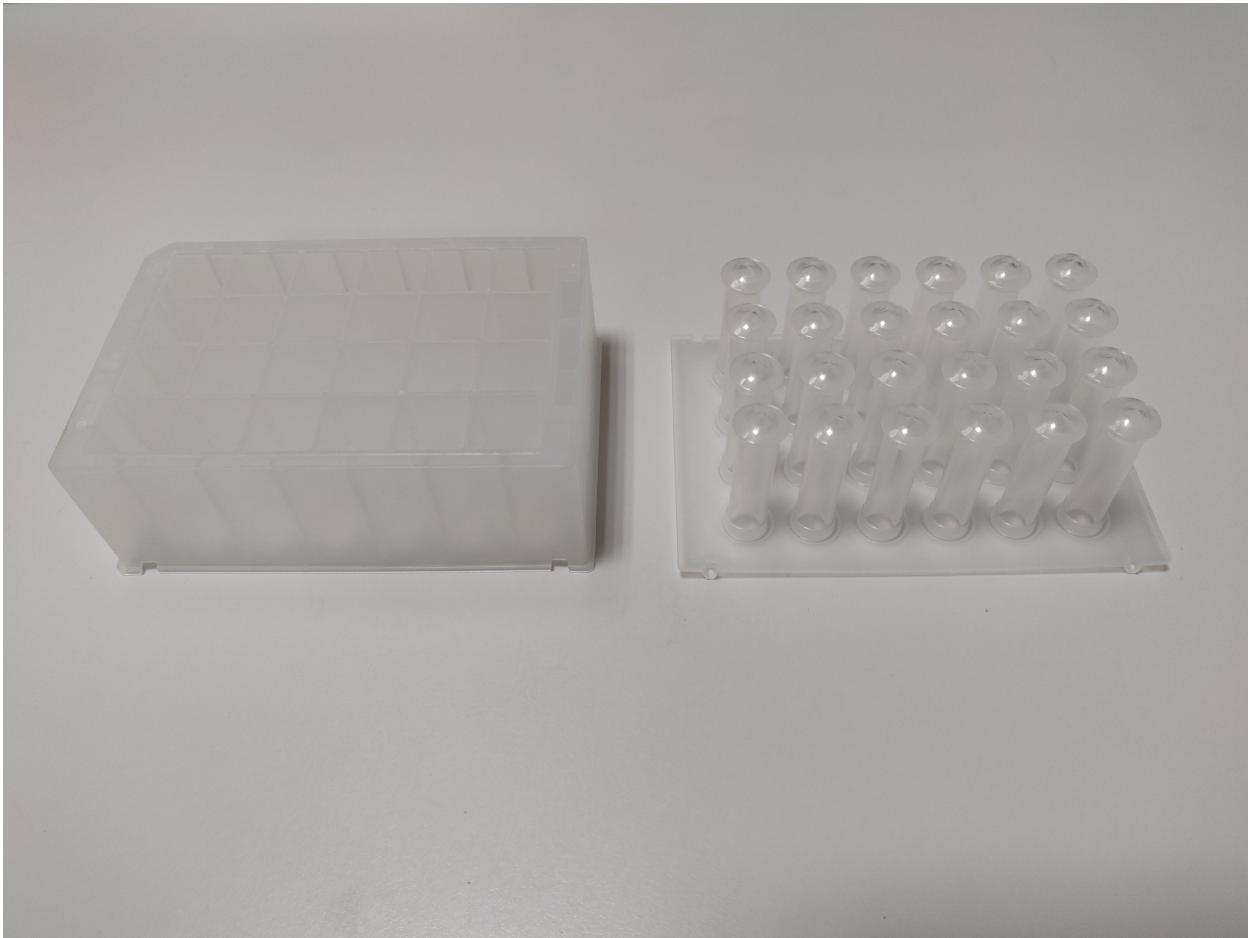


Figure 1: The 24 deepwell plate and the lid with pegs (substrata)

## 3 Protocols

All protocols are available on protocols.io.

Creating the artificial saliva: <https://www.protocols.io/view/artificial-saliva-bva9n2h6>



Figure 2: The 24 deepwell plate with the lid (almost) on.

Creating the CPMU solution: <https://www.protocols.io/view/cpmu-bv8pn9vn>

Biofilm growth protocol: <https://www.protocols.io/view/biofilm-growth-with-starch-treatment-bu7jnzkn>

Amylase activity assay: <https://www.protocols.io/view/amylase-activity-bw8jphun>

## 4 Raw data

The raw data can be downloaded from OSF:

Solution counts: <https://osf.io/kz3b2/>

Sample counts: <https://osf.io/kz3b2/>

```
# solution counts
wget https://osf.io/kz3b2/download -O solution_counts.csv

# sample counts
wget https://osf.io/kz3b2/download -O starch_counts.csv
```

### 4.1 Metadata for raw data files

Counts represent the absolute number of starches counted on a slide

**starch\_counts.csv**

variable	description
sample	Sample number.
plate	Plate number that the sample came from.
row	Which row on the plate the sample came from.
s	Small starch count.
m	Medium starch count.
l	Large starch count.
total	Sum of s, m, and l.
treatment	Treatment solution to which the samples were exposed.
starch	Type of starch that was counted.
weight	Weight of the biofilm sample.
vol	Total volume of EDTA in which the sample was dissolved.
portion_slide	Proportion of the microscope slide that was counted. Total transects on slide divided by counted transects.

**solution\_counts.csv**

variable	description
solution	Type of starch in solution.
concentration	Concentration (%w/v) of starch in solution.
vol_slide	Volume of solution added to slide.
vol_total	Total volume of solution in aliquot.
portion_slide	Proportion of slide that was counted. Total transects on slide divided by counted transects.
slide	Slide number.
starch	Starch type counted.
s	Small starch count.

variable	description
m	Medium starch count.
l	Large starch count.
total	Sum of s, m, and l.

## 5 Microscope images

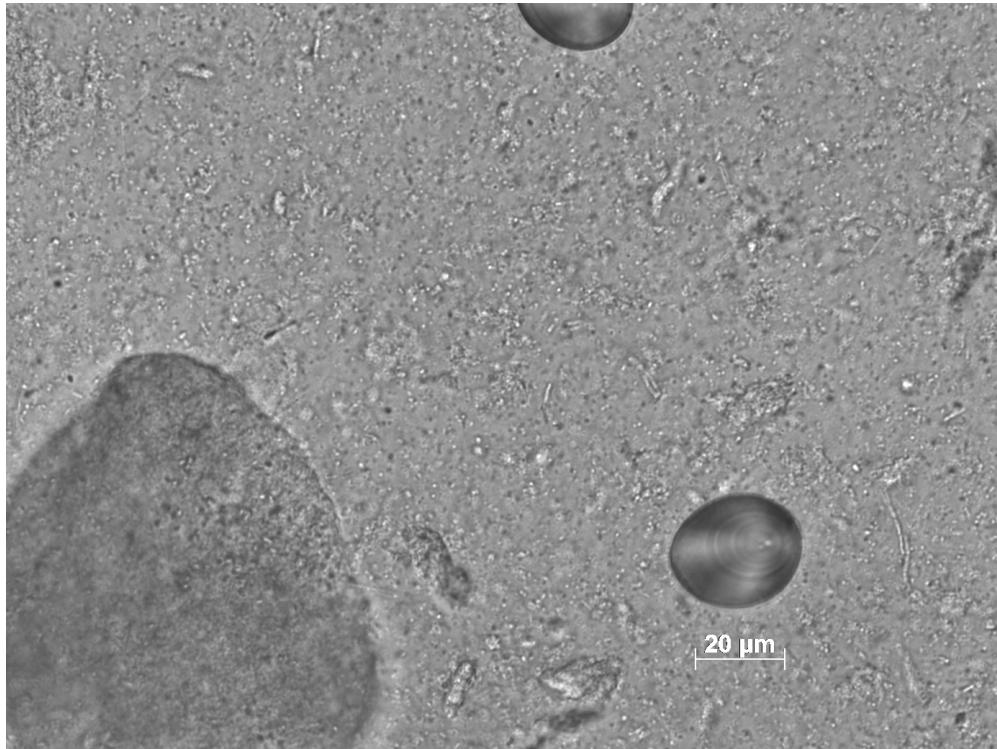


Figure 3: Microscope image of wheat starch from a Potato treatment sample.

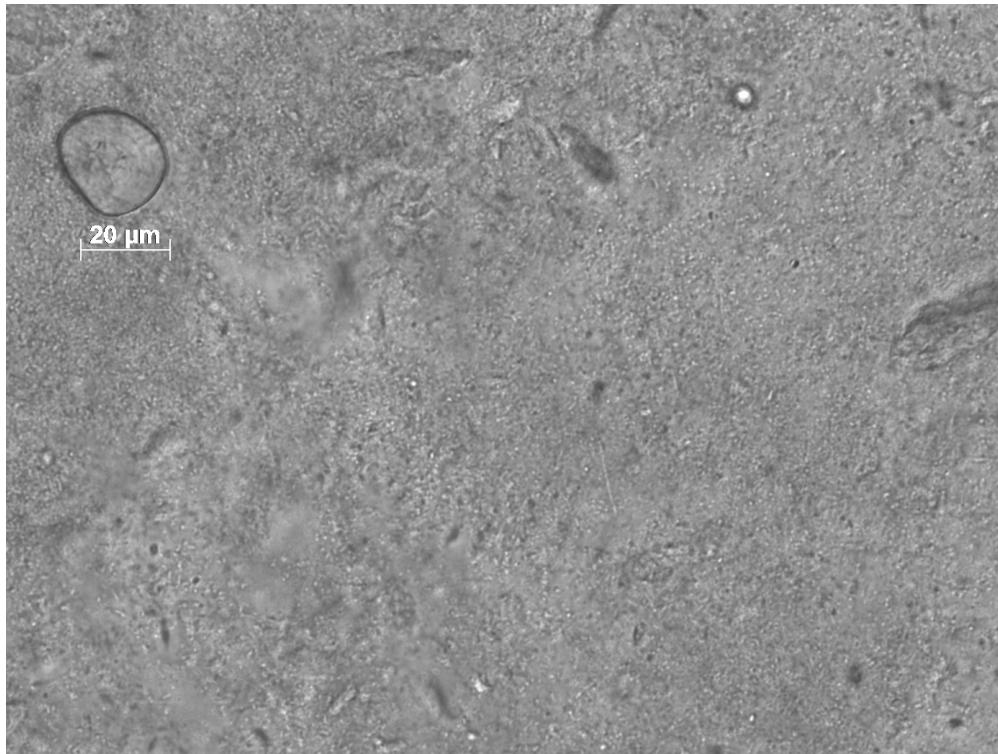


Figure 4: Microscope image of wheat starch from a wheat treatment sample.

## 6 Amylase activity

Amylase activity in U/mL enzyme, where U is the amount of enzyme needed to release 1  $\mu$ mole maltose from starch in six minutes at 36 °C.

Tables containing the amylase activity results for both plates and both photometric readings conducted on each plate. Samples (rows) were analysed in triplicates (columns).

```
# table of results reported in units amylase per mL enzyme (but let's be honest,
# ...it doesn't really matter what the unit is. No activity is no activity)
cols <- c("1", "2", "3") # sample triplicates
rows <- c("S1", "S2", "S3", "B1", "B2", "B3", "B4", "B5", "BT1", "BT2", "BT3")
plt1_ph1_result <- rbind(sal1_ph1, bmm1_ph1)
rownames(plt1_ph1_result) <- rows
plt1_ph2_result <- rbind(sal1_ph2, bmm1_ph2)
rownames(plt1_ph2_result) <- rows
plt2_ph1_result <- rbind(sal2_ph1, bmm2_ph1)
rownames(plt2_ph1_result) <- rows
plt2_ph2_result <- rbind(sal2_ph2, bmm2_ph2)
rownames(plt2_ph2_result) <- rows
```

## 7 Control samples

```
raw_counts %>%
  filter(treatment == "control") %>%
  select(!c(vol, portion_slide, s, m, l))
```

Table 3: Amylase activity in U/mL for plate 1, photometric read 1.

	V1	V2	V3
S1	28.2306584	10.0605214	28.4572187
S2	30.0884529	13.8667346	28.0720662
S3	26.8486405	15.0448483	28.2533144
B1	-0.8602506	-0.7072591	-0.7837549
B2	-0.5670169	-1.0642394	-0.7200084
B3	-0.6180140	-1.2299802	-0.4395239
B4	-0.7965042	-1.2809774	-0.9112478
B5	-0.9877436	-1.0514901	-0.6435126
BT1	-1.4467182	-1.2809774	-1.4212196
BT2	-1.4212196	-0.8857492	-1.4467182
BT3	-1.4849661	-1.2682281	-1.3702225

Table 4: Amylase activity in U/mL for plate 1, photometric read 2.

	V1	V2	V3
S1	27.9846495	9.9310259	28.0075022
S2	29.8128646	13.8159829	28.1217656
S3	26.7506043	15.0500280	28.3959979
B1	-0.8003496	-0.6852669	-0.7364148
B2	-0.4934625	-1.0305148	-0.6852669
B3	-0.5446104	-1.1967453	-0.4167408
B4	-0.7236278	-1.2478932	-0.8642844
B5	-0.9410061	-1.0305148	-0.6341191
BT1	-1.4013367	-1.2478932	-1.3885497
BT2	-1.3757627	-0.8514974	-1.4013367
BT3	-1.4396975	-1.2351062	-1.3246149

Table 5: Amylase activity in U/mL for plate 2, photometric read 1.

	V1	V2	V3
S1	28.0673333	10.3602545	26.9474345
S2	30.1803498	13.6354301	27.7503808
S3	26.2501390	15.3892339	27.8560316
B1	-0.7162472	-0.8020671	-0.8020671
B2	-0.5813875	-1.1453464	-0.7652871
B3	-0.5200876	-1.3660261	-0.5446075
B4	-0.8756269	-1.3782860	-0.9859667
B5	-0.7530272	-1.1698664	-0.6794673
BT1	-1.4641059	-1.1943864	-1.3905460
BT2	-1.4641059	-0.8511070	-1.4273259
BT3	-1.5499257	-0.9614468	-1.5131458

Table 6: Amylase activity in U/mL for plate 2, photometric read 2.

	V1	V2	V3
S1	28.0794000	10.3989036	26.7014237
S2	30.0085669	13.5364497	27.3798120
S3	26.0442349	15.3172192	27.5494091
B1	-0.6620533	-0.7725806	-0.7602997
B2	-0.5515260	-1.1041625	-0.7111765
B3	-0.4778411	-1.3374979	-0.4901219
B4	-0.8462654	-1.3374979	-0.9322311
B5	-0.7234573	-1.1410049	-0.6374916
BT1	-1.4480252	-1.1532857	-1.3620595
BT2	-1.4357443	-0.7971422	-1.3866211
BT3	-1.5094292	-0.9076695	-1.4725868

sample	plate	row	total	treatment	starch	weight
st1D1	1	D	1	control	none	6.51
st1D2	1	D	0	control	none	4.42
st1D3	1	D	0	control	none	5.01
st1D4	1	D	0	control	none	5.14
st1D5	1	D	0	control	none	4.51
st1D6	1	D	0	control	none	1.67
st2D1	2	D	0	control	none	8.32
st2D2	2	D	0	control	none	11.18
st2D3	2	D	NA	control	none	3.43
st2D4	2	D	NA	control	none	5.76
st2D5	2	D	NA	control	none	3.66
st2D6	2	D	NA	control	none	5.67

Only the total starch count was considered for control samples, as size was deemed irrelevant.

## 8 Count corrections

Slide transects were calculated by counting the number of transects on the cover slip under the microscope. This was done by starting in the bottom-left corner, and counting the total number of full fields-of-view across the cover slip to the bottom-right corner. The total number of transects was 29 (verified multiple times).

A 1 mL aliquot of each of the original treatment solutions was taken, from which 10  $\mu\text{L}$  was taken and mounted on a microscope slide and mixed with 10  $\mu\text{L}$  20% (v/v) glycerol. Solution counts were extrapolated from a slide (10  $\mu\text{L}$ ) to the quantity in a 1 ml solution, and then multiplied by 16 days to achieve the total number of granules that were exposed to the samples:

$$\text{corrected count} = \text{raw count} \times \frac{\text{total slides}}{\text{counted slides}} \times 100\mu\text{L} \times 16 \text{ days}$$

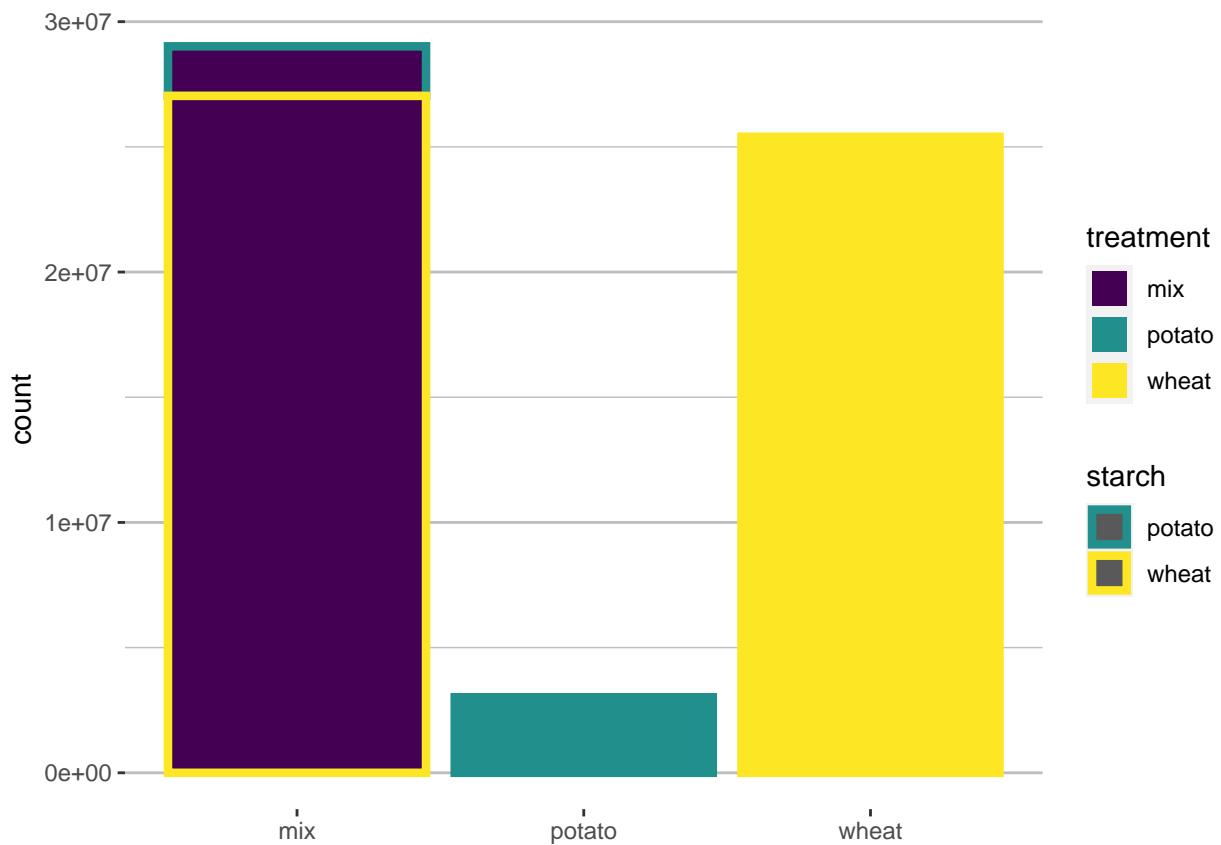
Samples were submerged in 50–100  $\mu\text{L}$  EDTA, from which 20  $\mu\text{L}$  was mounted on a microscope slide ( $V_{slide}$ ) and counted. Sample counts were extrapolated to the full volume of EDTA ( $V_{sample}$ ) in which the sample was submerged (i.e. 50–100  $\mu\text{L}$ ).

$$\text{Corrected count} = \text{raw count} \times (\text{portion of slide})^{-1} \times \frac{V_{sample}}{V_{slide}}$$

## 9 Some additional plots

Bar plot for the total count of granules exposed to the samples over the duration of the experiment,

```
sol_long %>%
  filter(size == "total") %>%
  group_by(treatment, starch) %>%
  ggplot(aes(x = treatment, y = count, fill = treatment, col = starch)) +
  geom_col(size = 1.5) +
  theme(panel.background = element_rect(fill = "white"),
        panel.grid = element_line(colour = "grey"),
        panel.grid.major.x = element_blank(),
        axis.title.x = element_blank()) +
  scale_fill_viridis_d() +
  scale_color_viridis_d(begin = 0.5)
```



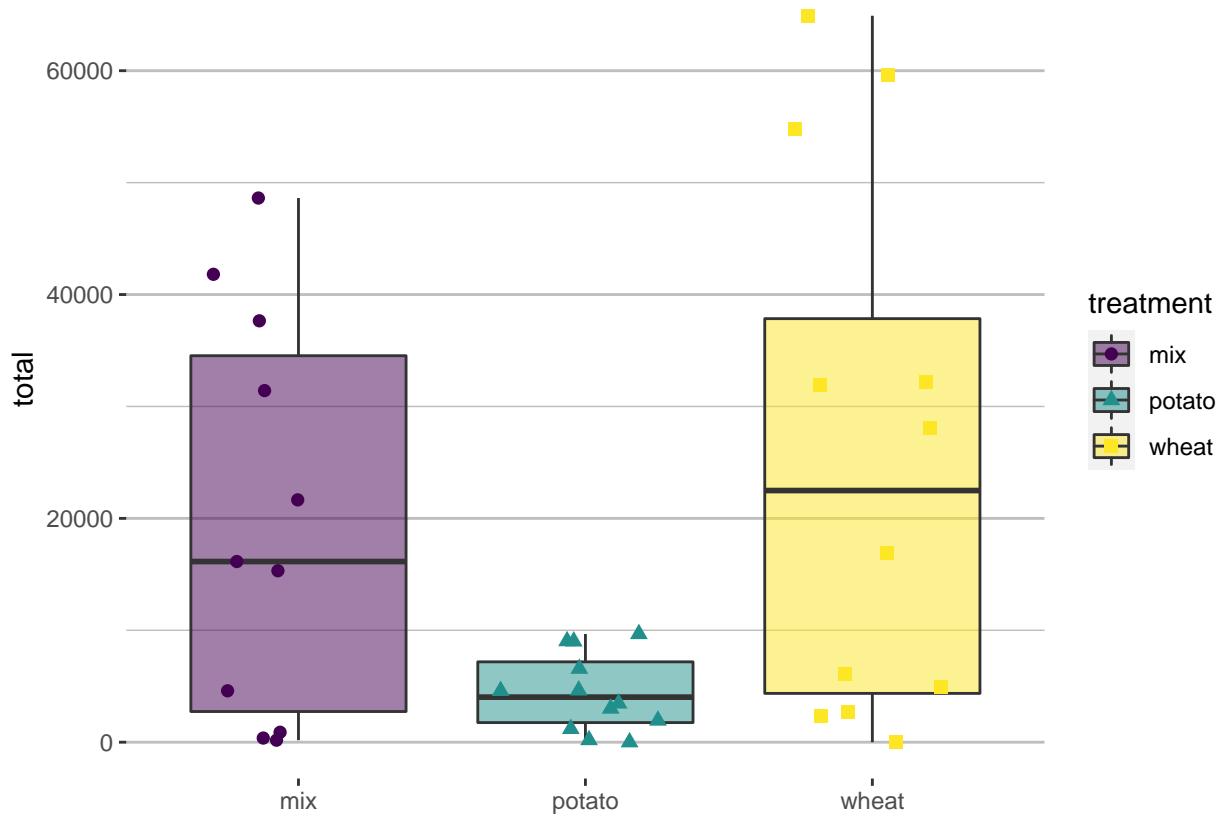
and box plot with superimposed points (with added jitter) for the extrapolated mean counts of granules extracted from the samples.

```
corr_comb %>%
  filter(treatment != "control") %>%
  ggplot(aes(x = treatment, y = total,
             shape = treatment)) +
  geom_boxplot(aes(fill = treatment), alpha = 0.5) +
  geom_jitter(aes(col = treatment), width = 0.3, size = 2) +
  scale_color_viridis_d() +
  theme(panel.background = element_rect(fill = "white"),
        panel.grid = element_line(colour = "grey"),
```

```

  panel.grid.major.x = element_blank(),
  axis.title.x = element_blank() + # remove y-axis title
scale_fill_viridis_d()

```



Extracted-granule counts separated by treatment and size, including error bars:

```

corr_counts_long %>%
  filter(size != "total",
        treatment != "control") %>%
  group_by(treatment, starch, size) %>%
  summarise(sd = sd(count, na.rm = T),
            count = mean(count, na.rm = T)) %>%
  #mutate(percent = count / sum(count, na.rm = T) * 100) %>%
  ggplot(aes(x = starch, y = count, fill = size)) +
  geom_col(position = "dodge") +
  geom_errorbar(aes(ymin = count, ymax = count + sd), width = 0.2, position = position_dodge(0.9)) +
  facet_wrap(~ treatment, scales = "free") +
  scale_fill_viridis_d() +
  theme_bw()

```

Size distribution (in %) within the solutions (top) and samples (bottom):

```

sol_size_pl <- sol_corr %>%
  group_by(solution, starch) %>%
  summarise(across(c(s, m, l, total), mean, na.rm = T)) %>%
  pivot_longer(cols = c(s,m,l, total), values_to = "count", names_to = "size") %>%
  filter(size != "total") %>%

```

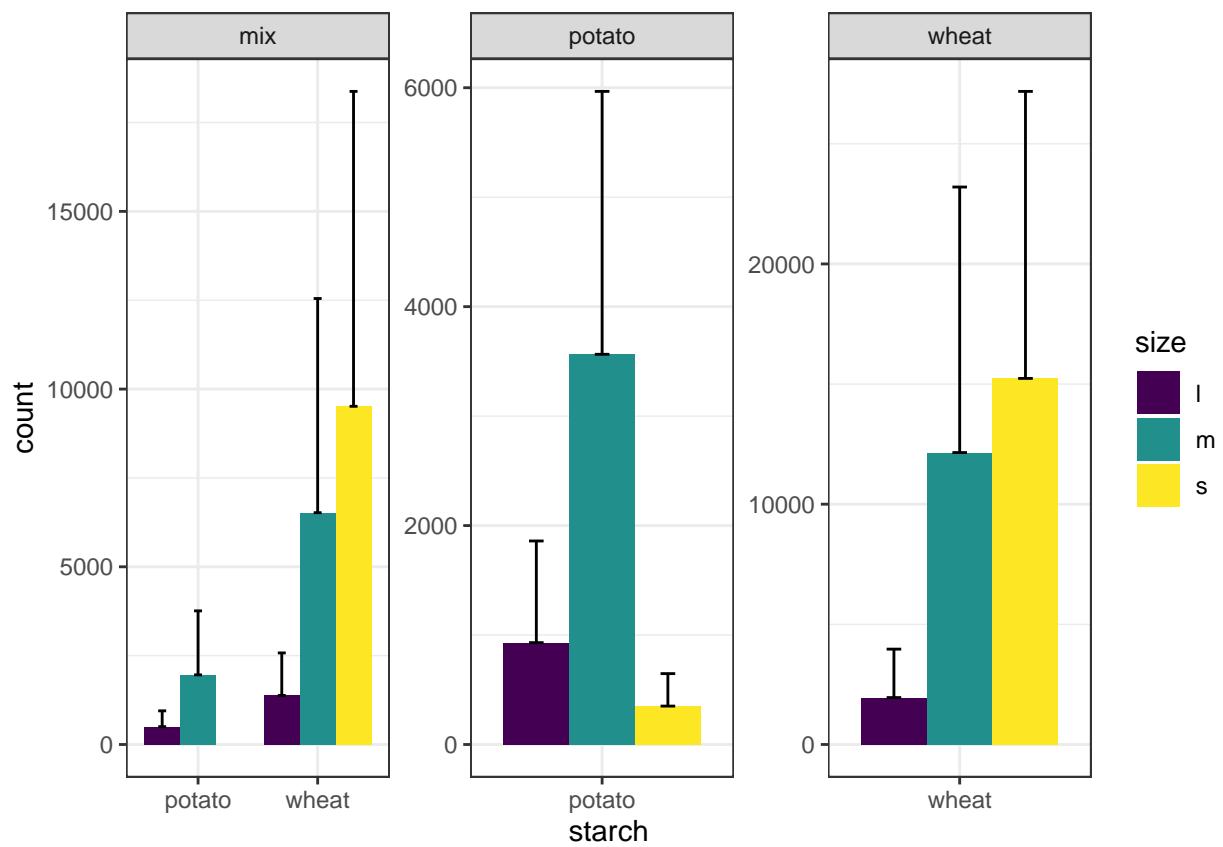


Figure 5: l = large, m = medium, s = small.

```

group_by(solution, starch) %>%
mutate(percent = count / sum(count, na.rm = T) * 100) %>%
ggplot(aes(x = starch, y = percent, fill = size)) +
  geom_col(position = "dodge") +
  facet_wrap(~ solution, scales = "free_x") +
  scale_fill_viridis_d() +
  theme_bw() +
  labs(x = "")

samp_size_pl <- corr_counts_long %>%
  filter(size != "total",
         treatment != "control") %>%
  group_by(treatment, starch, size) %>%
  summarise(count = mean(count, na.rm = T)) %>%
  mutate(percent = count / sum(count, na.rm = T) * 100) %>%
  ggplot(aes(x = starch, y = percent, fill = size)) +
  geom_col(position = "dodge") +
  facet_wrap(~ treatment, scales = "free_x") +
  scale_fill_viridis_d() +
  theme_bw()

sol_size_pl / samp_size_pl + plot_layout(guides = "collect")

```

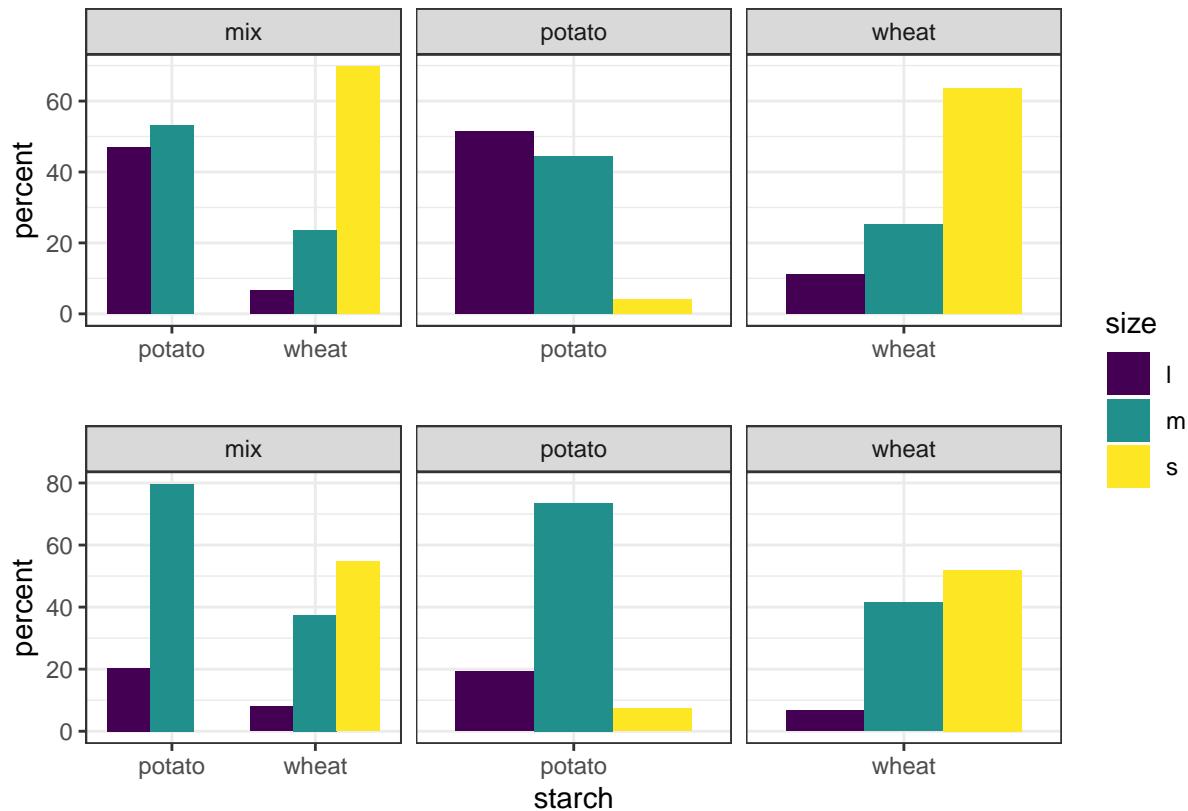


Figure 6: l = large, m = medium, s = small.

Separated correlation plots. These are the same plots as in the main paper, just larger.

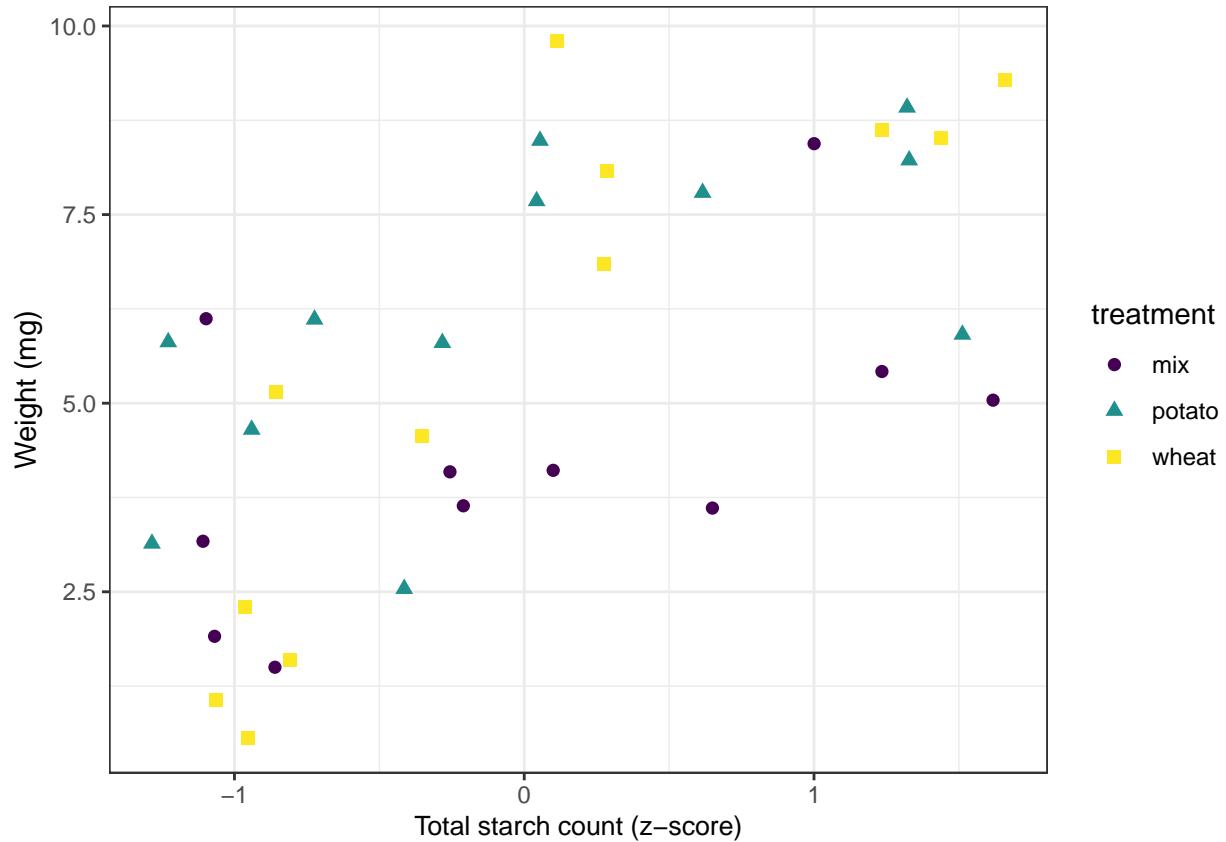


Figure 7: Scatter plot of sample weight and standardised starch count by z-score for separated treatments.

## 9.1 ... and a table

Differences in size ratios (%) of granules between the solutions and the samples. Negative values indicate a loss of granules from solution to sample.

```
size_diff %>%
  mutate(across(where(is.numeric), signif, 3))
```

treatment	starch	s	m	l
mix	potato	NaN	26.5	-26.5000
mix	wheat	-15.00	13.8	1.2900
mix	both	-17.10	17.0	0.0863
potato	potato	3.14	29.2	-32.3000
wheat	wheat	-8.49	18.6	-4.0900

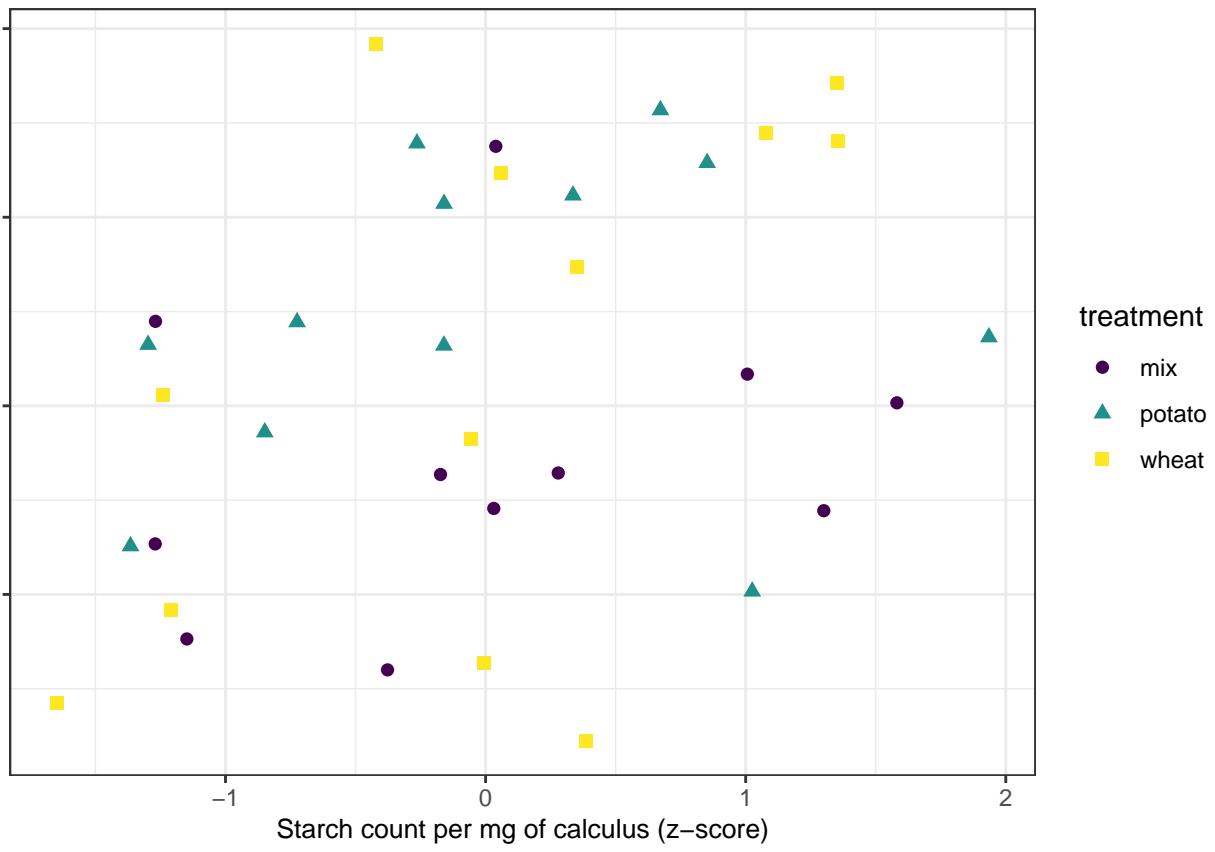


Figure 8: Scatter plot of sample weight in mg and standardised count of starch grains per mg calculus.