

# Supporting Information

## High-throughput analysis of endogenous fruit glycosyl hydrolases using a novel chromogenic hydrogel substrate assay

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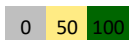
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	Substrate 1	Substrate 2
Assay plate 1	red pullulan	yellow amylopectin
Assay plate 2	red curdlan	yellow arabinan
Assay plate 3	red pachyman	yellow deGal-rhamnogalacturonan I*
Assay plate 4	blue arabinoxylan	yellow $\beta$ -glucan (yeast)
Assay plate 5	blue dextran	yellow casein
Assay plate 6	blue $\beta$ -glucan (oat)	yellow galactomannan
Assay plate 7	green 2-hydroxyethyl-cellulose	yellow amylose
Assay plate 8	green lichenan	yellow pectic galactan
Assay plate 9	green rhamnogalacturonan (soybean)	yellow xylan
Assay plate 10	green xyloglucan	yellow $\beta$ -glucan (barley)

**Table S-1:** Overview of the combination of different substrates in the assay plates, which was used to screen 21 fruits and vegetables. Using two substrates per well effectively doubles the already high-throughput nature of the assay. The highest calculated value was set to 100 and all other data normalized. \* deGal-rhamnogalacturonan I was prepared by enzymatically removing  $\beta$ -1,4-galactan side chains from rhamnogalacturonan I using an *endo*-galactanase as described in reference 18.

	y-CPH-amylose			y-CPH-amylopectin			r-CPH-pachyman			r-CPH-curdian			y-CPH-β-glucan (barley)			g-CPH-lichenan			y-CPH-β-glucan (yeast)			g-CPH-2-HE-cellulose			r-CPH-pullulan			y-CPH-arabinan			y-CPH-xylan			b-CPH-arabinoxylan			y-CPH-galactomannan			y-RGI (potato)			g-CPH-RG (soy bean)			g-CPH-xyloglucan			y-CPH-pectic galactan			y-CPH-casein			b-CPH-dextran					
	4.0	7.0	10.0	4.0	7.0	10.0	4.0	7.0	10.0	4.0	7.0	10.0	4.0	7.0	10.0	4.0	7.0	10.0	4.0	7.0	10.0	4.0	7.0	10.0	4.0	7.0	10.0	4.0	7.0	10.0	4.0	7.0	10.0	4.0	7.0	10.0	4.0	7.0	10.0	4.0	7.0	10.0	4.0	7.0	10.0	4.0	7.0	10.0	4.0	7.0	10.0									
<b>pH</b>	4.0	7.0	10.0	4.0	7.0	10.0	4.0	7.0	10.0	4.0	7.0	10.0	4.0	7.0	10.0	4.0	7.0	10.0	4.0	7.0	10.0	4.0	7.0	10.0	4.0	7.0	10.0	4.0	7.0	10.0	4.0	7.0	10.0	4.0	7.0	10.0	4.0	7.0	10.0	4.0	7.0	10.0	4.0	7.0	10.0	4.0	7.0	10.0	4.0	7.0	10.0	4.0	7.0	10.0	4.0	7.0	10.0			
<i>positive control</i>	0	48	29	0	100	100	54	54	0	90	87	1	100	52	0	59	86	64	64	97	0	31	18	0	0	61	10	0	0	0	26	6	0	38	8	0	0	94	9	47	1	0	0	7	2	0	76	14	31	17	0	1	58	69	17	33	17			
mango	1	37	1	0	61	4	4	1	0	80	11	0	0	2	1	0	0	0	1	1	1	0	0	0	0	1	0	0	0	0	0	1	1	0	1	1	1	1	1	0	0	0	0	1	1	0	1	1	0	0	0	0	0	0						
papaya	1	24	0	0	29	1	2	6	0	9	87	2	0	3	1	0	0	0	1	1	0	1	4	0	0	0	0	0	0	0	56	32	0	1	2	0	0	0	0	0	1	0	0	1	0	0	1	0	1	68	6	0	2	0						
litchi	0	1	6	0	2	10	0	1	2	0	1	2	0	3	7	0	0	0	0	1	5	0	0	0	0	1	2	0	0	0	1	2	6	0	0	1	0	1	0	0	2	1	0	0	0	0	1	0	0	0	0	0	1	4	0	0	0			
avocado	0	10	4	0	5	8	3	24	2	20	29	3	0	1	6	0	1	0	1	5	4	0	0	0	0	0	2	0	0	0	0	1	4	0	0	1	0	1	0	1	0	1	0	0	0	0	0	0	0	0	0	0	1	4	0	0	0			
tomato	0	2	1	1	1	1	20	54	12	94	94	93	0	1	1	0	1	0	6	22	1	0	1	0	1	0	0	0	0	0	1	1	1	0	1	0	1	0	0	1	0	0	0	1	0	0	0	0	0	1	1	0	0	0						
banana	0	2	1	0	1	2	53	3	0	94	16	1	0	1	2	0	0	0	5	2	1	0	0	0	0	0	1	0	0	0	0	1	1	0	0	0	0	1	1	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	1	1	0	0	0
pear	0	1	0	0	2	1	5	3	0	15	6	1	0	1	1	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	1	1	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0			
passion fruit	0	0	0	0	0	0	1	2	3	69	74	2	1	1	1	1	1	3	15	17	27	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7	10	25	3	4	11			
nashi pear	0	0	1	0	0	1	0	0	0	0	0	0	0	1	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0						
clementine	0	13	5	0	10	11	4	5	0	36	38	1	0	4	11	0	0	0	1	2	7	0	1	0	0	0	0	0	0	0	0	2	6	0	0	0	1	3	0	1	0	0	0	0	0	0	1	0	0	0	0	1	1	8	0	0	0			
orange	0	17	6	0	15	14	6	7	1	52	54	1	1	4	15	0	1	0	3	5	8	0	1	0	0	0	0	0	0	0	0	2	9	0	0	0	0	1	0	2	0	1	0	0	0	0	1	0	1	2	0	1	66	5	0	2	0			
red pepper	0	14	1	0	6	1	12	55	3	91	81	47	0	2	1	0	1	0	5	16	1	1	5	0	0	1	1	0	0	0	0	1	1	0	0	0	0	1	2	0	0	2	0	0	0	0	1	0	0	1	1	0	2	1	0	0	0			
green pepper	0	6	1	0	3	2	0	31	1	1	85	5	0	2	2	0	0	0	1	5	1	0	0	0	0	1	0	0	0	0	0	1	1	0	0	0	0	1	2	0	1	0	0	0	0	0	1	0	0	0	0	0	1	1	0	0	0			
yellow pepper	0	16	1	0	7	1	16	57	4	82	85	61	1	2	1	0	1	0	6	17	1	1	4	0	0	0	0	0	0	0	0	1	1	0	0	0	0	1	1	0	1	0	0	0	0	0	1	0	0	1	1	0	2	1	0	0	0			
onion	0	1	2	0	1	5	4	8	1	63	61	3	0	1	5	0	0	0	1	3	4	0	0	0	0	0	0	0	0	0	3	1	3	0	0	0	1	1	0	1	0	1	0	0	0	0	0	0	2	2	0	0	1	4	0	0	0			
white onion	0	0	0	0	0	0	10	9	0	66	61	2	0	0	0	0	0	0	2	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	2	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0						
red onion	0	1	3	0	2	6	14	18	1	80	81	3	0	2	5	0	0	0	3	6	4	0	0	0	0	1	1	0	0	0	1	1	4	0	0	0	0	2	0	0	0	0	0	0	0	0	2	0	1	2	4	0	0	0						
apricot	0	0	0	0	1	1	0	0	0	3	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0						
dry apricot	0	0	1	0	1	2	0	0	0	1	2	0	0	1	2	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	2	0	0	1	0	0	0	0	0	0	0	1	0	0	1	2	0	0	0			
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dry fig	2	3	2	1	2	3	5	13	1	28	95	2	2	3	3	8	5	0	2	5	2	3	5	0	0	0	1	0	0	0	2	2	2	0	1	0	1	2	0	2	4	3	0	0	0	0	0	0	3	3	0	1	37	67	0	1	2			



**Figure S-1:** Selection of 21 different fruits and vegetables were tested towards 20 different CPH substrates at three different conditions (pH 4.0, 7.0 and 10.0). The highest calculated value was set to 100 and all other data normalized.

	pH	CPH-pachyman			CPH-curdian			CPH-β-glucan (barley)			CPH-β-glucan (yeast)			CPH-amylopectin			CPH-amylose			CPH-xylan			CPH-galactomannan			
		4.0	7.0	10.0	4.0	7.0	10.0	4.0	7.0	10.0	4.0	7.0	10.0	4.0	7.0	10.0	4.0	7.0	10.0	4.0	7.0	10.0	4.0	7.0	10.0	
<i>positive control</i>		99	97	0	75	62	1	60	54	0	71	69	0	2	100	95	2	84	83	94	92	0	0	0	79	53
avocado	<i>crude</i>	11	11	1	65	75	5	0	0	1	14	13	1	0	44	3	2	72	3	0	0	1	0	0	1	
	<i>extracted</i>	14	13	1	76	76	4	1	0	0	17	18	1	2	41	2	6	76	1	0	0	0	0	0	0	
orange	<i>crude</i>	1	2	0	68	47	0	0	0	0	2	2	0	0	1	0	0	3	0	0	0	0	0	1	0	
	<i>extracted</i>	1	2	0	51	38	0	0	0	0	2	2	0	0	2	0	0	6	0	0	0	0	0	1	0	
passion fruit	<i>crude</i>	1	6	1	72	77	2	0	0	1	6	9	1	0	0	0	0	0	0	0	0	1	0	0	1	
	<i>extracted</i>	2	7	1	69	76	2	0	0	0	8	10	1	0	0	0	0	0	0	0	1	1	0	0	1	
onion	<i>crude</i>	1	1	0	41	17	1	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	
	<i>extracted</i>	1	1	0	45	17	1	0	0	0	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	
dry fig	<i>crude</i>	4	4	1	72	74	3	1	1	1	8	4	1	0	1	1	0	1	1	0	1	1	0	1	1	
	<i>extracted</i>	5	3	1	78	65	2	2	1	1	8	4	1	0	1	0	0	1	0	1	1	1	0	1	1	

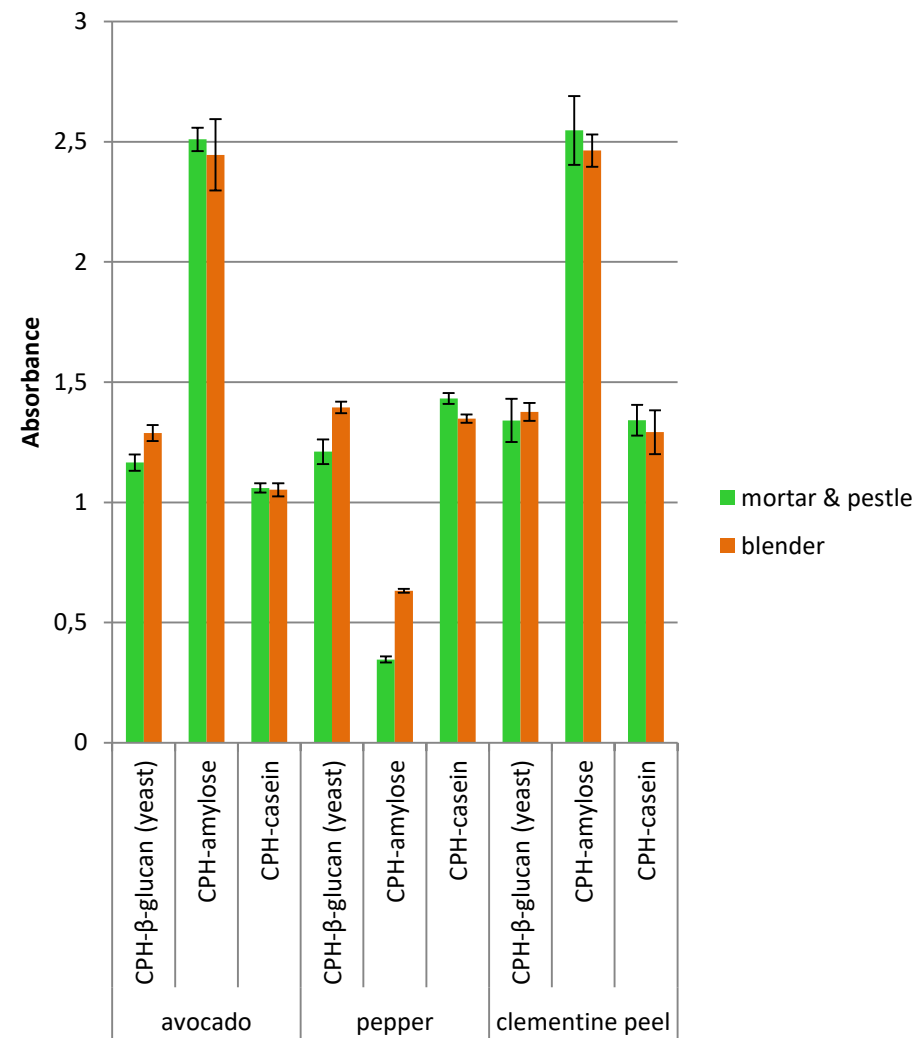
0
50
100

**Figure S-2:** Differences in enzymatic activity in five different fruits when comparing crude material and enzymes extracted from crude material. The highest calculated value was set to 100 and all other data normalized.

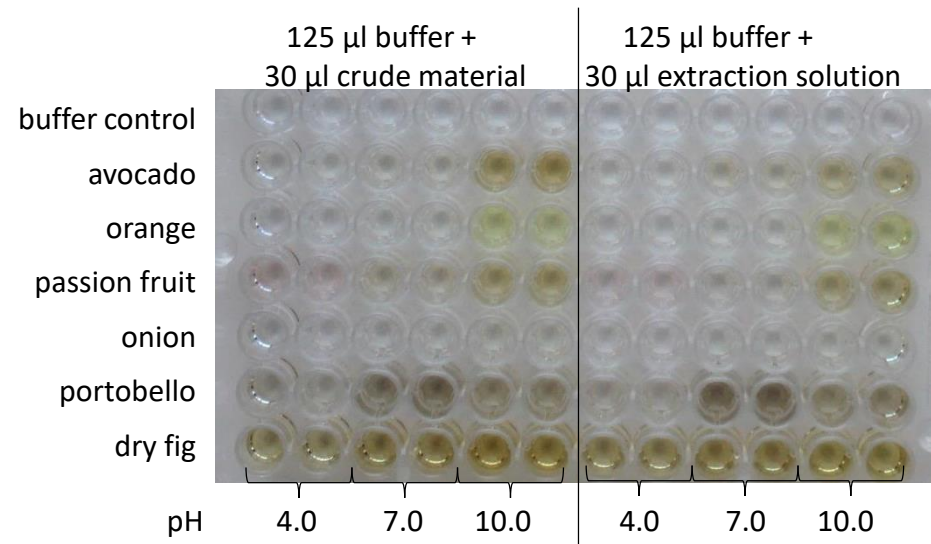
Extraction	Sample	CBM3a (cellulose/xyloglucan)	BS-400-2 ( $\beta$ -1,3-D-glucan)	BS-400-3 ( $\beta$ -1,3/1,4-D-glucan)	BS-400-4 ( $\beta$ -1,4-D-mannan)	INRA-RU1 (backbone of RGI)	INRA-RU2 (backbone of RGI)	LM18 (HG partially methyl-esterified)	LM19 (HG partially methyl-esterified)	JIM5 (HG, low DE)	JIM7 (HG, high DE)	LM5 ( $\beta$ -1,4-D-galactan)	LM6 ( $\alpha$ -1,5-L-arabinan)	LM13 (linear $\alpha$ -1,5-L-arabinan)	LM9 (feruloylated $\beta$ -1,4-D-galactan)	LM15 (xyloglucan, XXXG motif)	LM24 (xyloglucan)	LM25 (xyloglucan)	LM10 ( $\beta$ -1,4-D-xylan)	LM11 ( $\beta$ -1,4-D-xylan/arabinoxylan)	LM23 ( $\beta$ -1,4-D-xylan)	LM28 (glucuronoxylan)	LM3 (extensin)	JIM20 (extensin)	LM2 (AGP, $\beta$ -linked GlcA)	JIM13 (AGP)	JIM14 (AGP)	LM21 ( $\beta$ -1,4-D-galactoglucmannan)	LM22 ( $\beta$ -1,4-D-galactoglucmannan)
CDTA	avocado	1	0	0	0	14	12	3	1	5	45	2	33	4	0	0	0	1	0	1	0	0	0	1	10	0	0	0	0
CDTA	orange	3	0	0	0	27	18	23	31	7	100	33	32	32	0	0	0	1	0	0	0	0	39	51	0	16	0	0	0
CDTA	passion fruit	1	0	0	0	7	3	15	14	11	96	5	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0
CDTA	onion	0	0	0	0	7	5	66	61	83	91	34	1	0	0	0	0	0	0	0	0	0	0	1	3	0	0	0	0
CDTA	dry fig	7	0	0	1	42	48	15	11	25	52	9	47	7	0	1	0	1	0	1	0	1	3	14	0	17	0	7	0
NaOH	avocado	51	9	1	17	50	43	18	21	5	5	26	75	30	0	59	11	61	4	11	0	45	12	24	1	48	0	37	1
NaOH	orange	60	6	0	25	13	3	0	0	0	0	41	14	12	0	71	14	65	2	2	0	21	36	50	1	37	0	36	11
NaOH	passion fruit	56	18	0	23	7	3	4	18	2	0	21	6	1	0	71	16	60	29	40	0	60	6	26	1	9	0	39	1
NaOH	onion	45	7	0	22	11	2	2	6	6	0	43	7	0	0	55	13	53	0	1	0	4	0	5	5	14	0	30	8
NaOH	dry fig	46	13	1	10	51	47	4	16	1	0	21	61	13	0	63	2	52	1	4	0	32	8	26	1	34	0	39	0

0 50 100

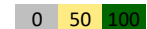
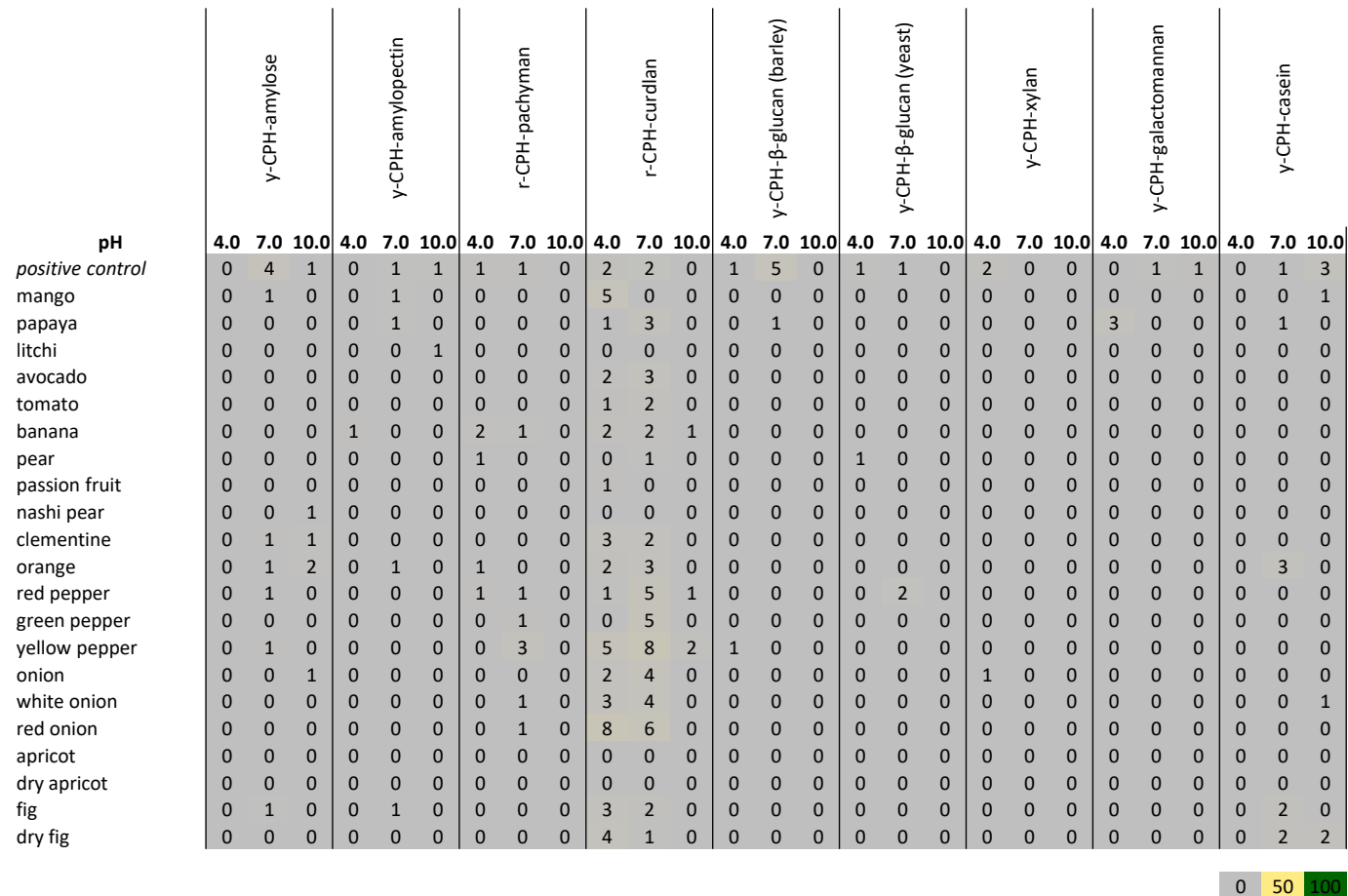
**Figure S-3:** Polysaccharide content analysis of five fruits using CoMPP (Comprehensive Microarray Polymer Profiling). The highest calculated value was set to 100 and all other data normalized.



**Figure S-4:** Comparison of two different sample preparations (using mortar and pestle or a blender) of three different fruits (avocado, pepper and clementine peel).



**Figure S-5:** Inherent color of the crude and extracted fruits material under reaction conditions without any substrate or enzyme.



**Figure S-6:** Standard error (SE) calculations based on the average values of triplicates for data in Figure 1.



pH	γ-CPH-amylose			γ-CPH-amylopectin			r-CPH-pachyman			r-CPH-curdian			γ-CPH-β-glucan (barley)			g-CPH-lichenan			γ-CPH-β-glucan (yeast)			g-CPH-2-HE-cellulose			r-CPH-pullulan			γ-CPH-arabinan			γ-CPH-xylan			b-CPH-arabinoxylan			γ-CPH-galactomannan			γ-RGI (potato)			g-CPH-RG (soy bean)			g-CPH-xyloglucan			γ-CPH-pectic galactan			γ-CPH-casein			b-CPH-dextran		
	4.0	7.0	10.0	4.0	7.0	10.0	4.0	7.0	10.0	4.0	7.0	10.0	4.0	7.0	10.0	4.0	7.0	10.0	4.0	7.0	10.0	4.0	7.0	10.0	4.0	7.0	10.0	4.0	7.0	10.0	4.0	7.0	10.0	4.0	7.0	10.0	4.0	7.0	10.0	4.0	7.0	10.0	4.0	7.0	10.0	4.0	7.0	10.0	4.0	7.0	10.0						
<i>positive control</i>	0	4	1	0	1	1	1	1	0	2	2	0	1	5	0	1	4	4	1	1	0	12	0	0	0	1	0	2	2	0	2	0	0	5	1	0	0	1	1	0	0	0	0	1	0	0	3	1	1	2	0	0	1	3	1	2	1
mango	0	1	0	0	1	0	0	0	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0			
papaya	0	0	0	0	1	0	0	0	0	1	3	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0			
litchi	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0			
avocado	0	0	0	0	0	0	0	0	0	2	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0			
tomato	0	0	0	0	0	0	0	0	0	1	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0			
banana	0	0	0	1	0	0	2	1	0	2	2	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0			
pear	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0			
passion fruit	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0			
nashi pear	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0			
clementine	0	1	1	0	0	0	0	0	0	3	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0			
orange	0	1	2	0	1	0	1	0	0	2	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0			
red pepper	0	1	0	0	0	0	1	1	0	1	5	1	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	1	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0						
green pepper	0	0	0	0	0	0	0	1	0	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0						
yellow pepper	0	1	0	0	0	0	0	3	0	5	8	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	2	3	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0						
onion	0	0	1	0	0	0	0	0	0	2	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0						
white onion	0	0	0	0	0	0	0	1	0	3	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0						
red onion	0	0	0	0	0	0	0	1	0	8	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0						
apricot	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0						
dry apricot	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0						
fig	0	1	0	0	1	0	0	0	0	3	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0						
dry fig	0	0	0	0	0	0	0	0	0	4	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	2	0	0	0						



**Figure S-7:** Standard error (SE) calculations based on the average values of triplicates for data in Figure S-1.

	pH	CPH-pachyman			CPH-curdian			CPH-β-glucan (barley)			CPH-β-glucan (yeast)			CPH-amylopectin			CPH-amylose			CPH-xylan			CPH-galactomannan		
		4.0	7.0	10.0	4.0	7.0	10.0	4.0	7.0	10.0	4.0	7.0	10.0	4.0	7.0	10.0	4.0	7.0	10.0	4.0	7.0	10.0	4.0	7.0	10.0
<i>positive control</i>		1	0	0	2	4	0	4	1	0	1	1	0	0	0	0	0	3	2	2	0	0	0	1	2
avocado	<i>crude</i>	0	0	0	3	1	0	0	0	0	0	1	0	0	2	0	0	1	0	0	0	0	0	0	0
	<i>extracted</i>	1	1	0	2	1	0	0	0	0	1	0	0	0	2	0	0	2	0	0	0	0	0	0	0
orange	<i>crude</i>	0	0	0	1	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	<i>extracted</i>	0	0	0	6	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
passion fruit	<i>crude</i>	0	0	0	2	1	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
	<i>extracted</i>	0	0	0	5	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
onion	<i>crude</i>	0	0	0	6	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	<i>extracted</i>	0	0	0	3	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
dry fig	<i>crude</i>	0	0	0	3	6	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	<i>extracted</i>	0	0	0	3	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0



**Figure S-8:** Standard error (SE) calculations based on the average values of triplicates for data in Figure S-2.