Reduction of bamboo shoot cyanide by lactic acid fermentation

Sanchez-Santos Arlette , Mora-Tello Mauricio , Cardoso-Ugarte Gabriel Abraham

MEXICO

1.Introduction

Bamboo shoots?

- A sprout or young bamboo
- Edible: fermented, boiled, canned, dried, etc.

Nutritional value

-Antioxidants
-Carbohydrates
-Dietary fiber

-Vitamins
-Essential amino acids
-Minerals

-Fat content

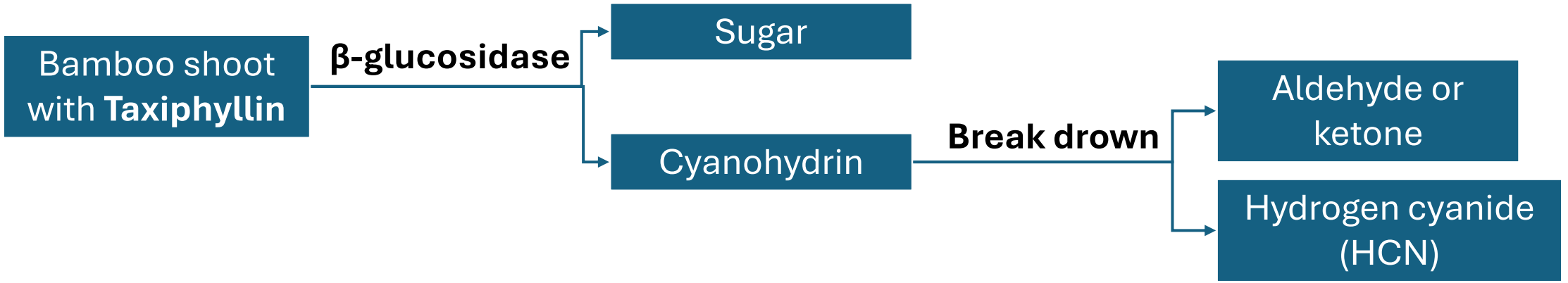
(Das, 2019; Satya et al., 2012)

Social impact

- Helping to reduce malnutrition in poor communities
- **Sustainable Development Goal 2**: Zero hunger (UN)

Drawbacks

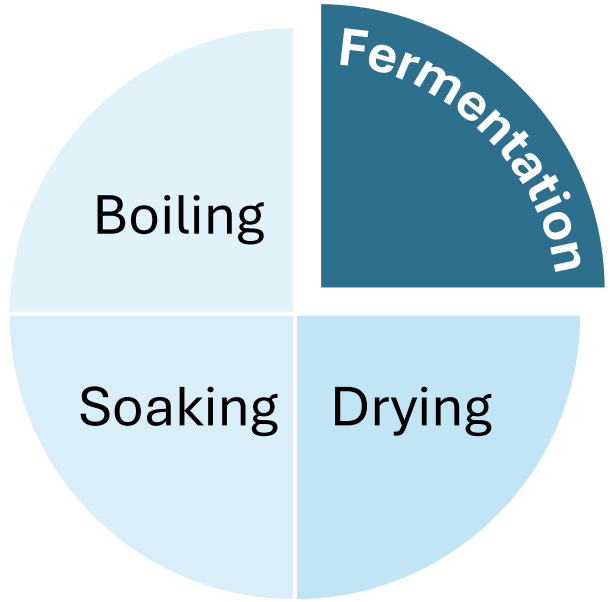
- Presence of cyanogenic glycosides



(Bolarinwa et al., 2016; Satya et al., 2010)

Reduction of HCN

- HCN is toxic at doses from 0.5 to 3.5 mg/kg of body weight
- Pre-treatment is needed to reduce HCN



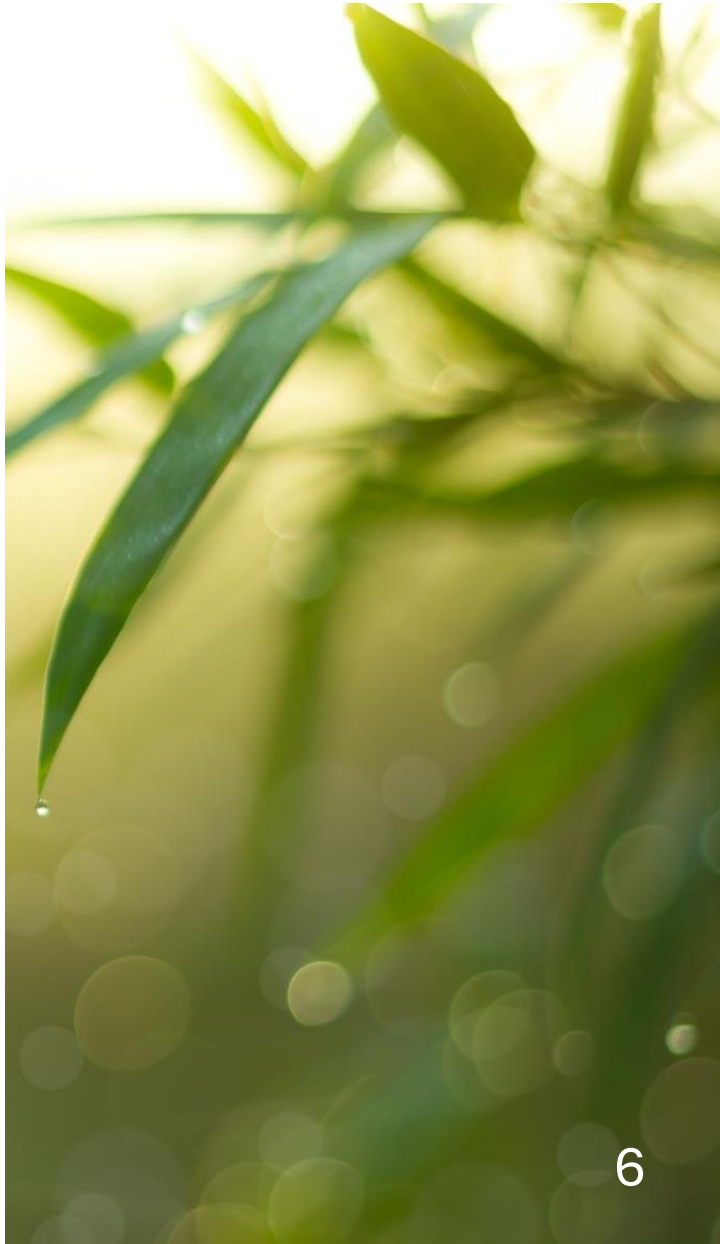
Fermentation

- Aditional benefits (Palatability, shelf-life and health improvement)
- Less studies in reduction of HCN
  - Species belonging to other regions and with native microorganisms

(Marco et al., 2017; Singhal et al., 2021)

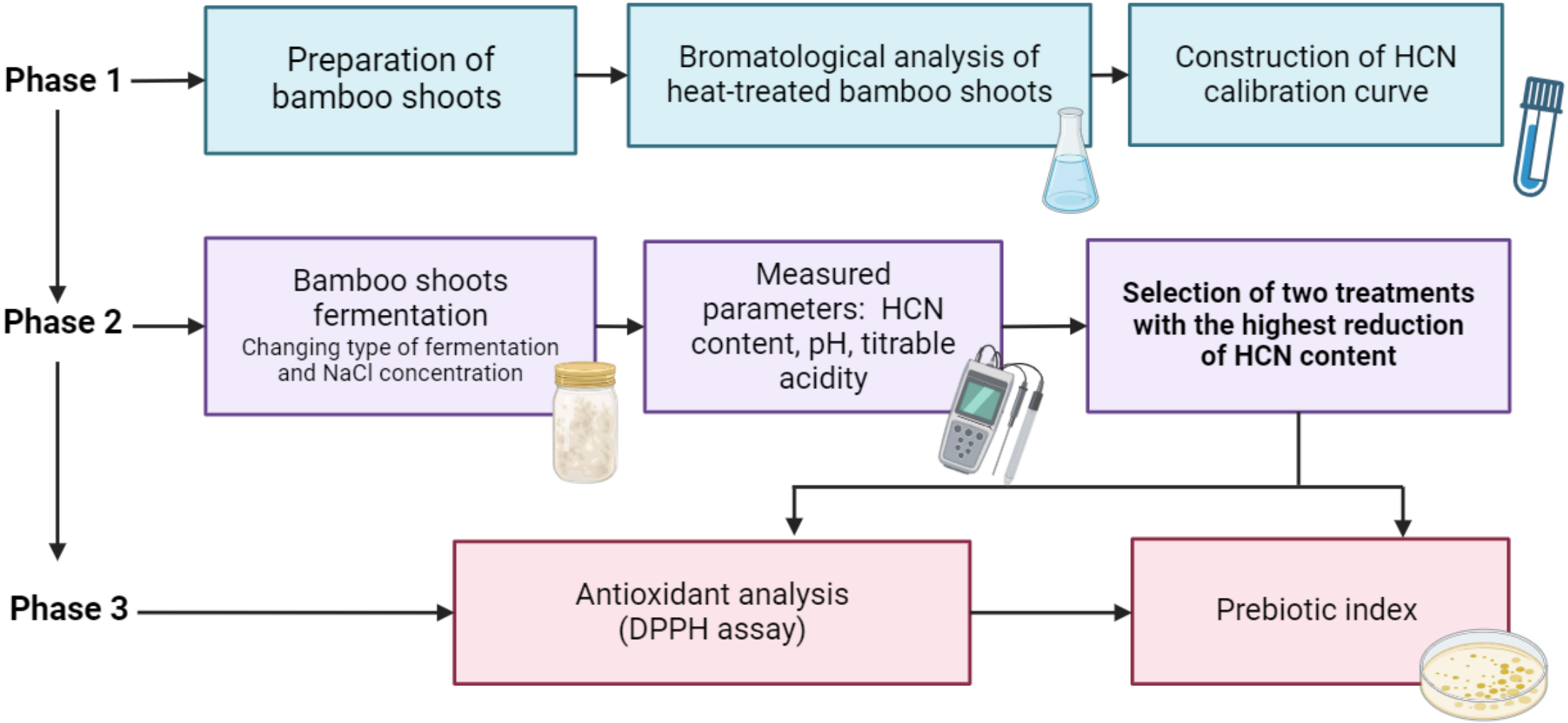
2. General objetive

- To **evaluate** the effect of different **fermentation conditions** of ***Dendrocalamus asper*** bamboo shoots in **cyanide** content, **pH** and **titratable acidity**, in order to **reduce** their **HCN** concentration, **increase** their **antioxidant capacity** and **prebiotic index**, laying the foundations for the development of a product with high nutritional value.



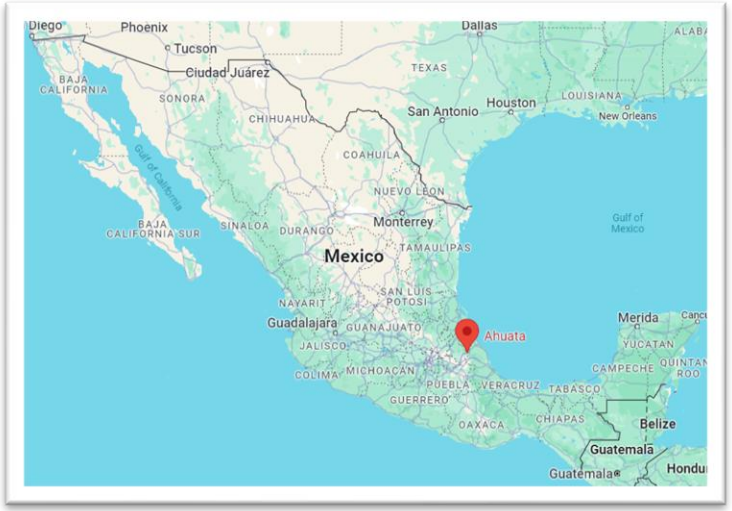
3.Materials and methods

General outline



Bamboo shoots preparation

- Obtained from: the town of Ahuata, in the northeastern highlands of **Puebla, México**; at 930 meters above sea level



Shoots were rinsed

Outer part was removed, soft white parts were used

Heat treatment in boiling water (20 min)

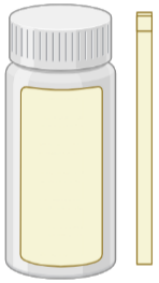
Cooling and freezing for further analysis

Bromatological analysis

Property	Assay	Conditions
Moisture	Oven drying	90°C, 6 hours
Ash	Calcination in muffle	Previous drying, 550°C, 5 hours
Total reducing sugars	Dinitrosalicylic acid (DNS)	Garriga et al., 2017
Lipids	Soxhlet extraction	180g dried sample, petroleum ether, 6 hours extraction time (36 cycles)
Protein content	Spectrophotometric Lowry assay	Waterborg, 2009

HCN content assay

- Spectrophotometric technique of Guignard sodium picrate. (Borja-Zamora et al., 2022)



Sodium picrate strips = Strips of filter paper + 0.05 M picric acid + 10% sodium bicarbonate solution

Sodium picrate strips + 2 g smashed shoot + 1 ml chloroform → Closed, Stand at room temperature for 24 h → Eluted in water (10 ml) → Read at 550 nm

Suspended in the lid of an assay tube

Calibration curve → Stock solution: 0.0024g KCN in 25 ml distilled water → Final concentration 0.1 mg HCN/ ml → Dilutions from 0 to 12 µg/ml HCN

Fermentation of shoots

Shoots were sliced (thickness- 0.5 cm)

Spontaneous

150 g shoot

+

270 g
1% or 10% NaCl

Inoculated

125 g shoot

+

225 g
1% or 10% NaCl

+

5% inoculum
(*Lpb. plantarum*, *L.paracasei*, 1:1 mix)

8 treatments
Room temperature
12 days

Evaluated each 2 days:
-pH
-Titrable acidity
-HCN content

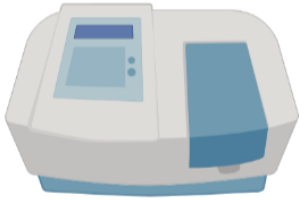
Antioxidant analysis (DPPH assay)

2 ml
Water extract of fermented shoot

+

3.2 ml
DPPH reactant + Methanol
(OD 0.7 at 515 nm)

→ Incubation in shaker at 37°C for 30 min → Read at 515 nm → Correlation with a calibration curve.



(Brand-Williams et al., 1995)

Prebiotic index

Lpb. plantarum 299B was inoculated and incubated in...

- **Fermented shoot + reconstructed MRS**
- **MRS broth (control)**

→ Plated in MRS (10^{-1} to 10^{-8}) Incubated at 37°C, 48 h → UFC were counted

(Figueroa-González et al.,2019)

4. Results and discussion

Bromatological analysis

Similar levels of nutrients than previously reported for *D. asper* and other species

Moisture: 92.61%

Similar to 92.32% in boiled *D. hamiltonii*. (Santosh et al., 2016)

Fat: 0.15% ww, 2.62% dw

Reducing sugar: 0.063% ww

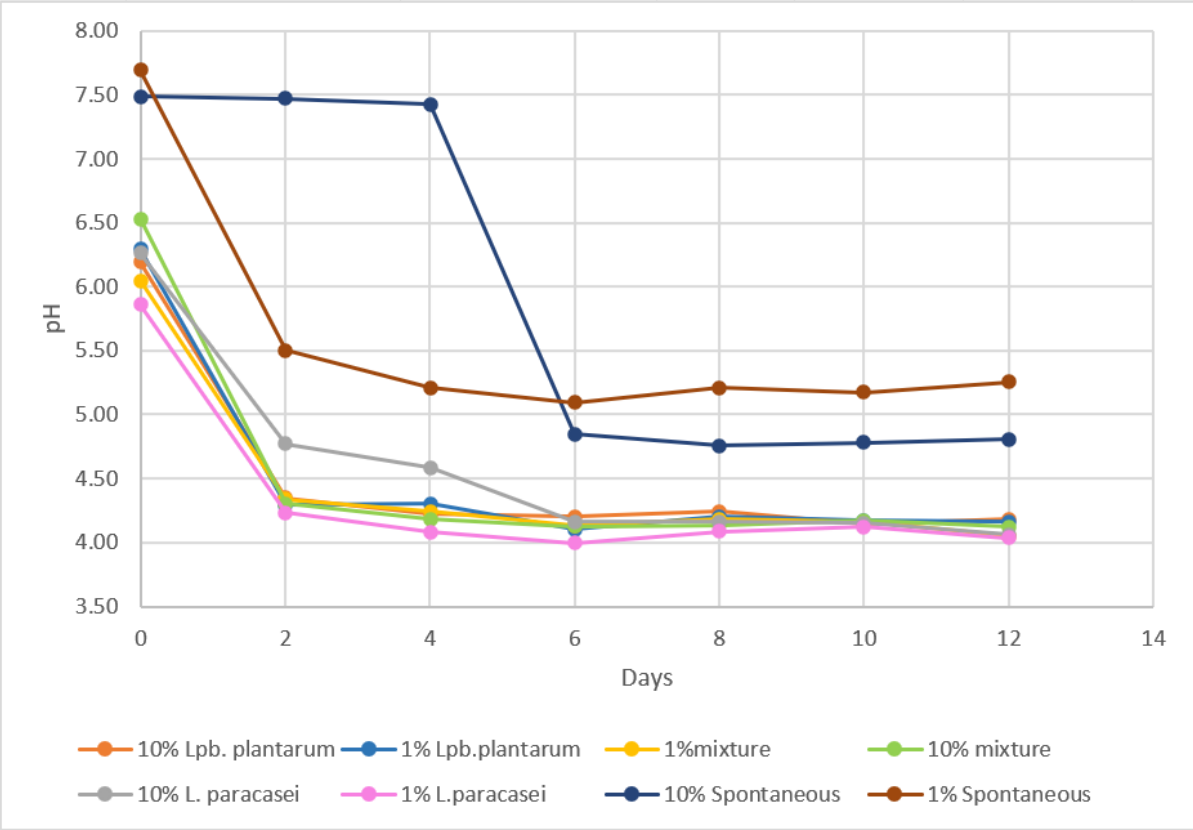
Similar to 0.1% ww in boiled *D. asper*. (Satya et al., 2010)

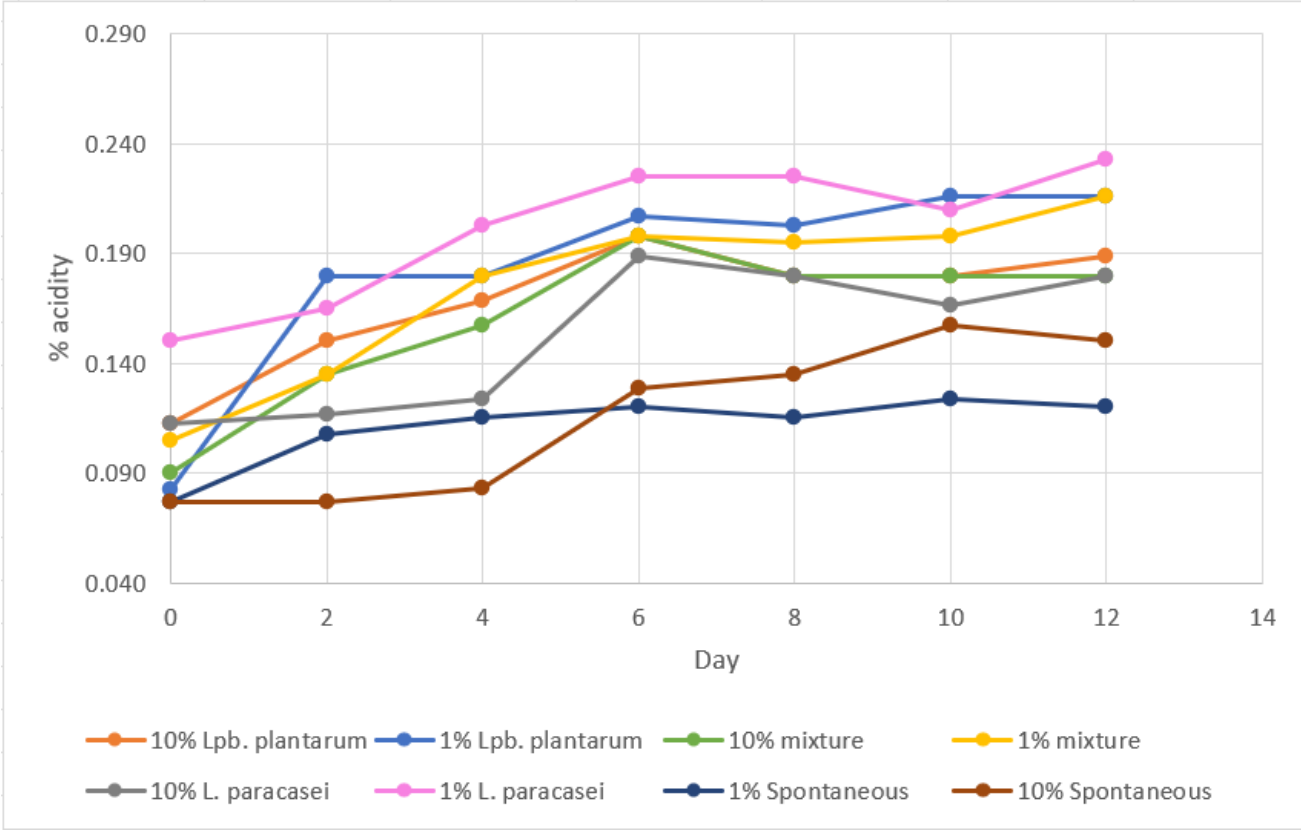
Ash: 0.27% ww

Similar to 0.75% ww in boiled *D. asper*. (Satya et al., 2010)

Protein: 332.66 µg/ml ww

pH and titrable acidity





HCN calibration curve

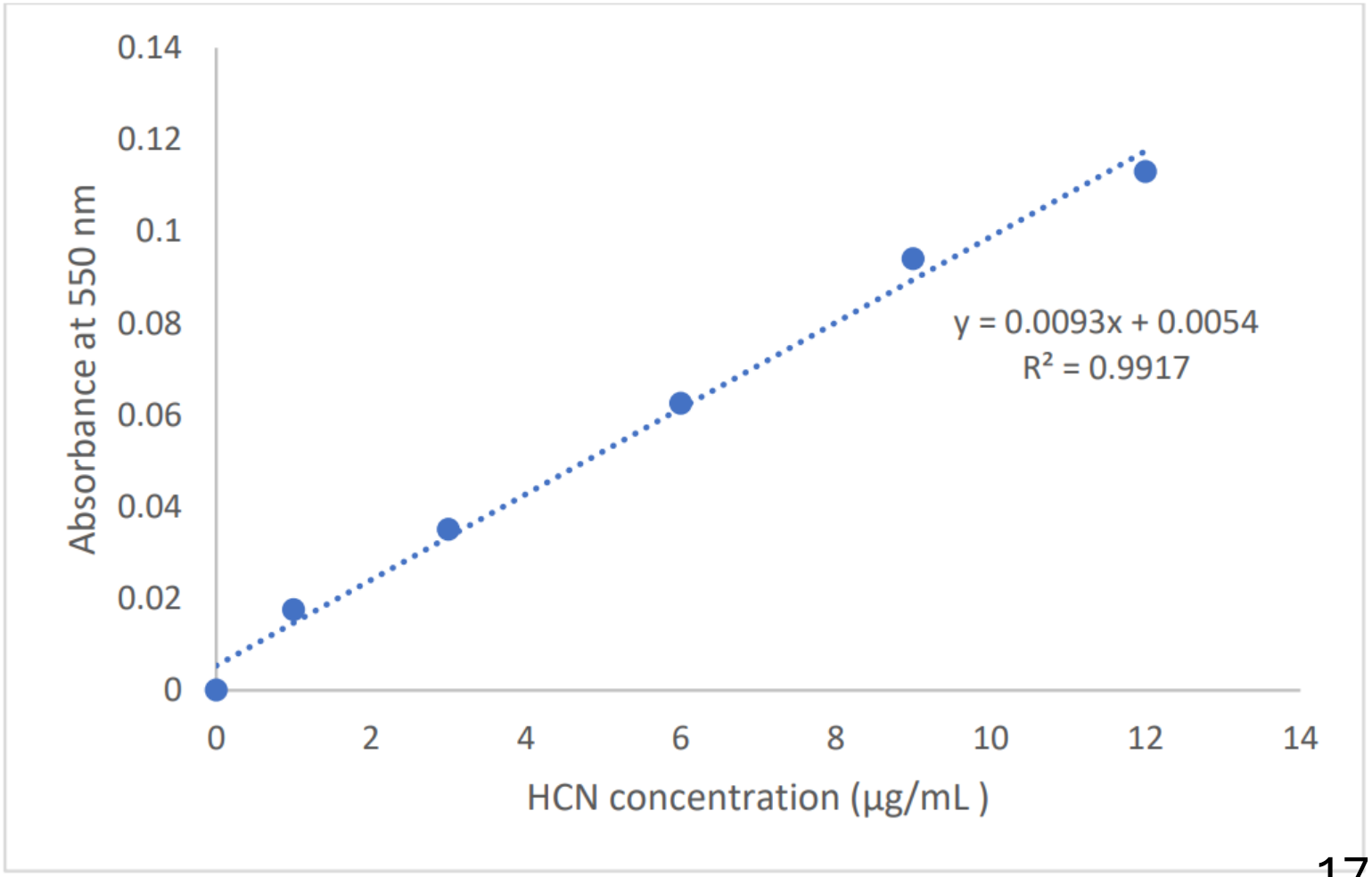
- Correlation of cyanide content with absorbance value

- The inverse equation was obtained

$$x = \frac{(y - 0.0054)}{0.0093}$$

$x = HCN\ concentration$

$y = Absorbance$



Effect of fermentation in HCN content

- Analysis by Tukey test

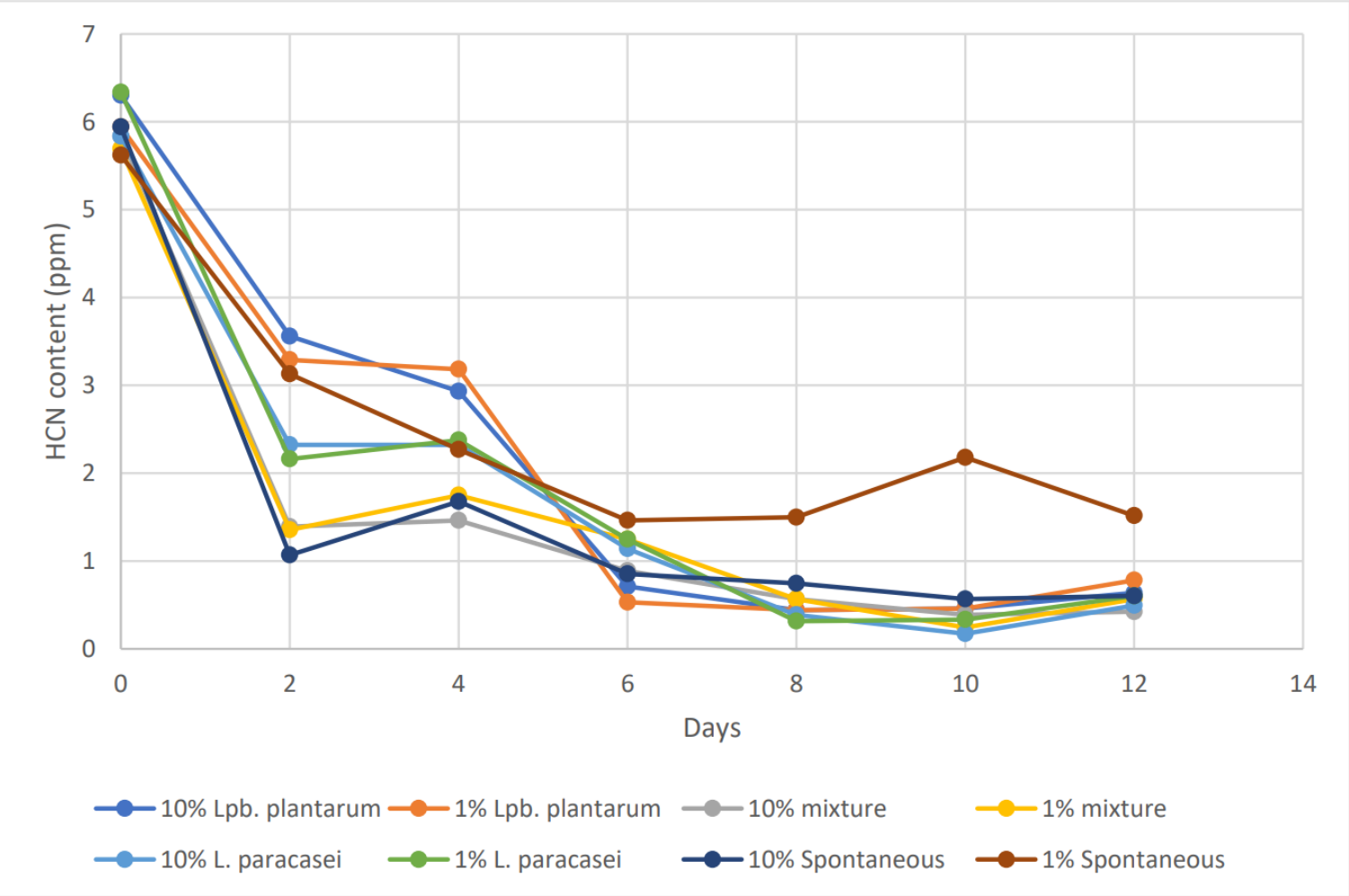
The lowest HCN content reached

No statistically significant change on the following days (95% confidence interval)

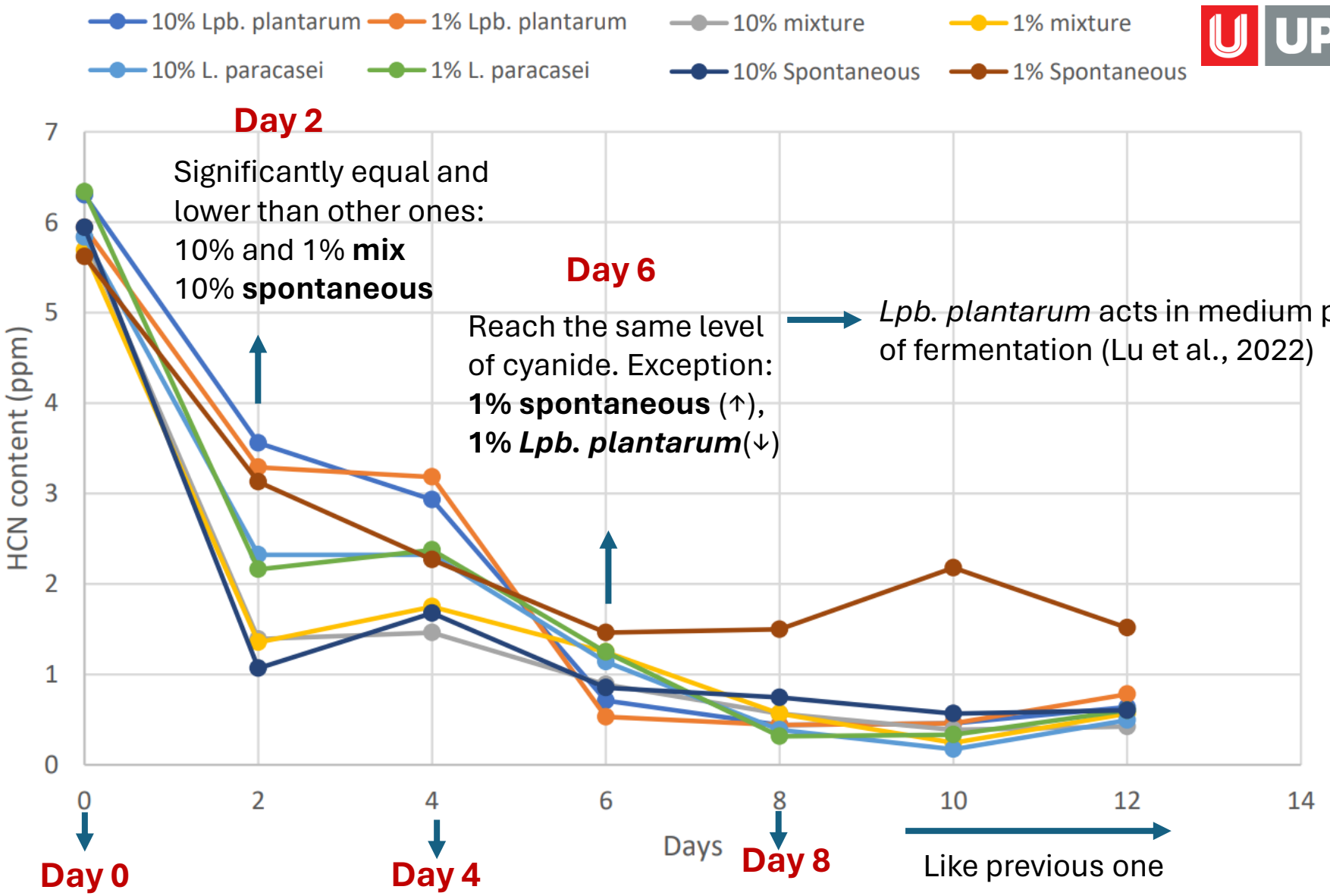
Day 6: **1%** and **10%** spontaneous
1% and **10 %** *Lpb. plantarum*

Day 8: **1 %** mix culture
1% *L. paracasei*

Day 10: **10%** mix culture
10% *L. paracasei*



Differences between treatments on each day



Day 2

Significantly equal and lower than other ones:
10% and 1% **mix**
10% **spontaneous**

Day 6

Reach the same level of cyanide. Exception:
1% spontaneous (↑),
1% *Lpb. plantarum* (↓)

→ *Lpb. plantarum* acts in medium phase of fermentation (Lu et al., 2022)

Day 0

Significantly equal

Day 4

Differences in all treatments

Day 8

Just **1% spontaneous** is different

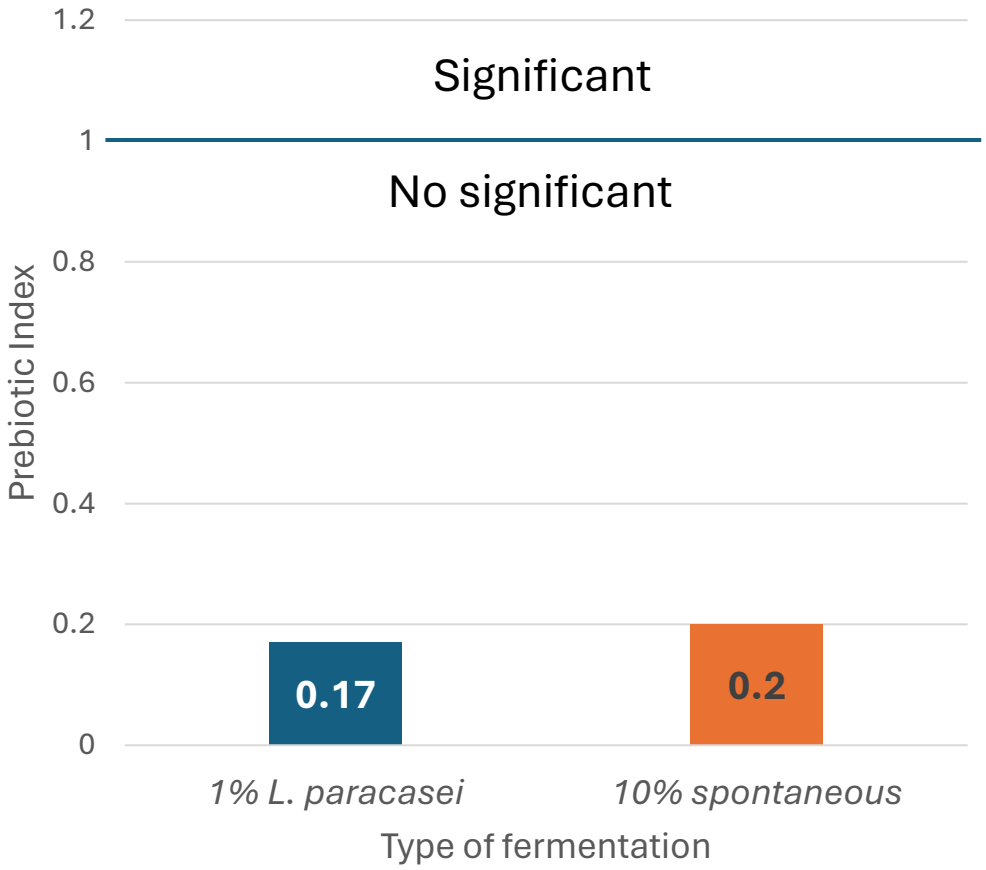
Like previous one

Antioxidant activity of fermented shoots

Type of fermentation	% of inhibition against DPPH radical
10% spontaneous	30.0 %
1% *L. paracasei*	**82.8 %**

- Higher % of inhibition in **1% *L. paracasei***
- Higher than reported by Lu et al.(2022) in fermentation of **soy bean** by ***L. paracasei***
  - **27%** of inhibition against DPPH

Prebiotic index of fermented shoots



- Not a good source of prebiotics
- May be related to previous **use of carbohydrates** by lactic acid bacteria during fermentation

Carbohydrate source	CFU/ml
Control (MRS)	$1.63\ x\ 10^9$
1% L. paracasei (fermented shoot)	$2.8\ x\ 10^8$
10% spontaneous (fermented shoot)	$3.4\ x10^8$

5.Conclusions

- **Best treatments** from each type of fermentation:
    - 10% NaCl spontaneous and 1% NaCl *L. paracasei*
- **Natural fermentation** had variations in HCN levels **depending on % NaCl** used.
    - *The best:* ***10% NaCl*** *– from 5.94 ppm to 0.6 ppm*
- **% NaCl** did **not affect** HCN content in **inoculated fermentation**, but there was differences among microorganism used.
- At **the late phase**, all inoculated treatments reached **similar low cyanide levels** (0.45 ppm)


UPAEP
THANK YOU
Questions?

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