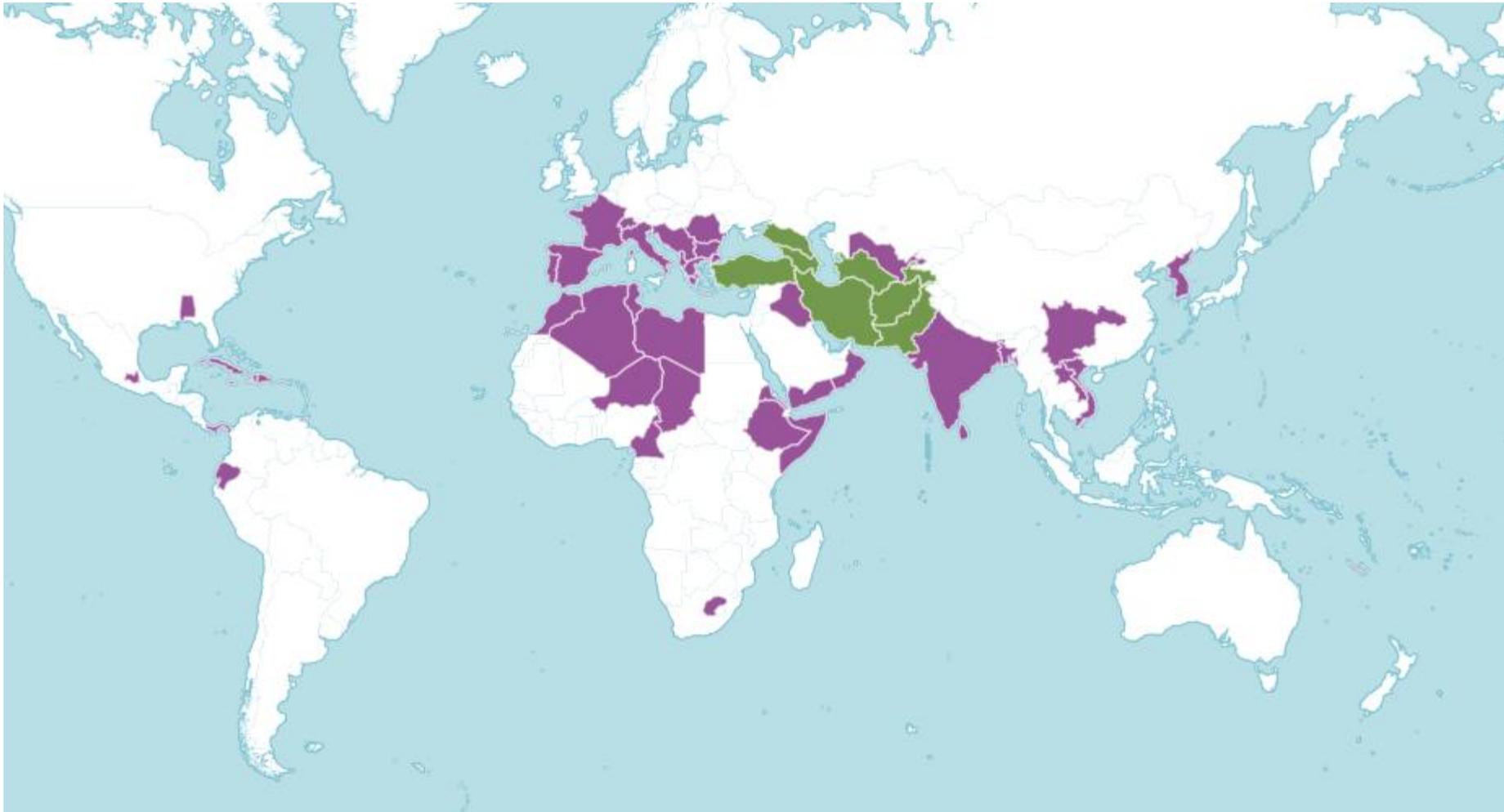


# Turning Pomegranate By- Products into Sustainable Protein Sources

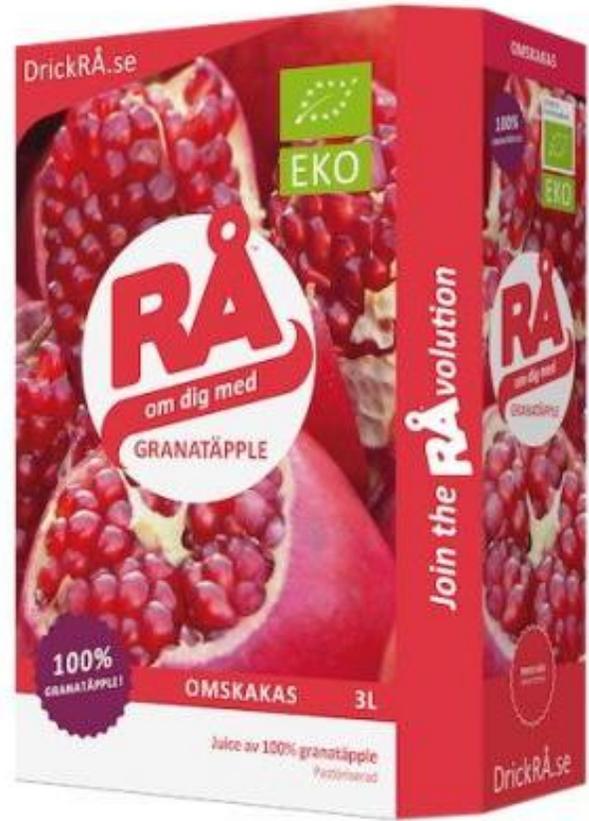
**Taner Sar**







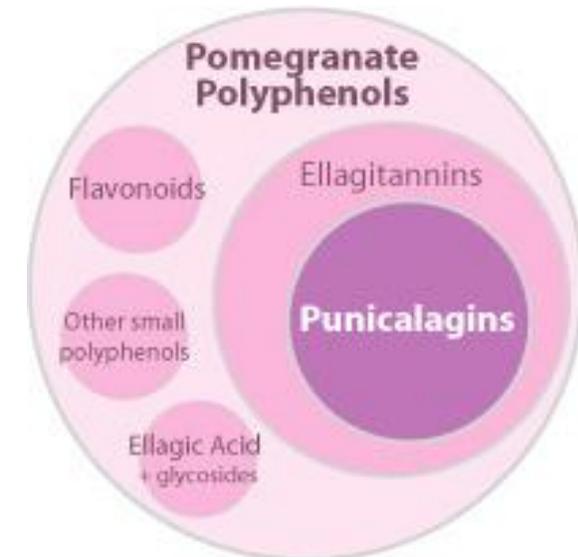
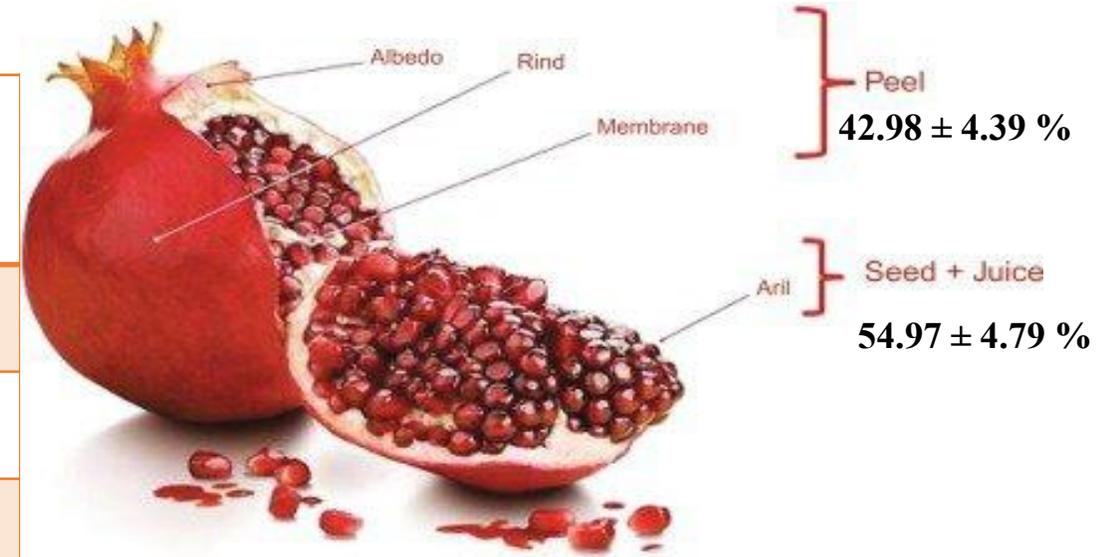
<https://www.kew.org/plants/pomegranate>



# Pomegranate Dried at 50 °C Purification

Dried at  
50 °C

	Unit	Pomegranate Peel	Pomegranate Juice	Pomegranate Aril
<b>pH</b>		3.22 ± 0.01	2.91 ± 0.01	2.96 ± 0.01
<b>TS</b>	(g/kg)	903.69 ± 0.15	156.95 ± 0.53	215.85 ± 4.59
<b>Volatile solid</b>	(g/kg)	864.38 ± 0.08	132.46 ± 0.18	176.06 ± 1.57
<b>Ash</b>	(g/kg)	39.31 ± 0.07	30.20 ± 1.51	39.79 ± 3.02
<b>Protein (*5.8)</b>	(g/kg)	<b>30.89 ± 4.05</b>	3.78 ± 0.33	19.97 ± 1.84
<b>Nitrogen</b>	(g/kg)	5.36 ± 0.69	0.65 ± 0.06	3.44 ± 0.32
<b>Glucose</b>	(g/kg)	<b>114.57 ± 1.95</b>	71.38 ± 0.34	79.21 ± 0.04
<b>Other sugars</b>	(g/kg)	<b>153.74 ± 5.64</b>	78.67 ± 0.38	91.98 ± 0.02



# Filamentous fungi used

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- *Aspergillus oryzae* var. *oryzae* CBS 819.72, isolated from **tané koji** used for sake making,
- *Neurospora intermedia* CBS 131.92, isolated from **oncom**
- *Rhizopus oligosporus* NRRL 2710 is related with soybean **tempeh** fermentation

*Neurospora*



**Oncom**

*Aspergillus*



**Miso**

*Rhizopus*



**Tofu**



**Tempe**

# Experiment design



## Stage 1

### Shake flask experiments

❖ Screening of Pomegranate by-products.

- Peel
- Juice
- Aril

❖ Screening of different fungal strains.

- *A.oryzae*
- *R.oligosporus*
- *N.intermedia*

## Stage 2

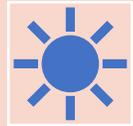
### Bubble-column reactor

- ❖ Scaling up
- ❖ Biomass characterization



# Phase 1

## Shake flask cultivation.



3 days in 35 °C  
pH 5.5

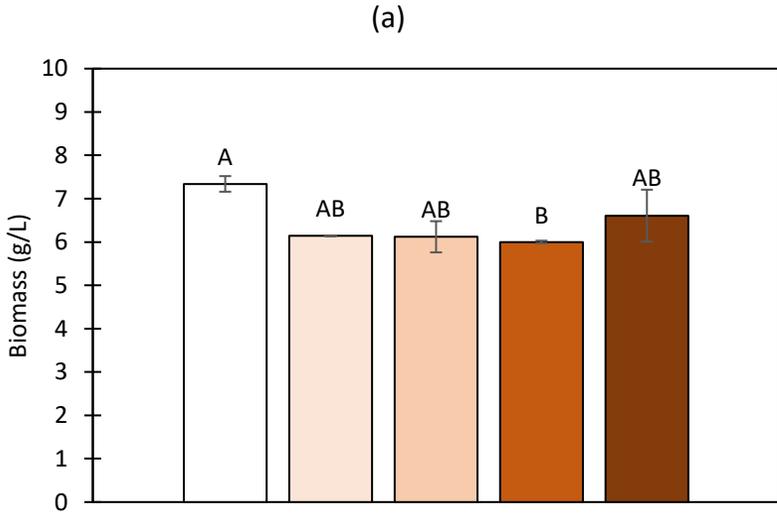


Submerged fermentation

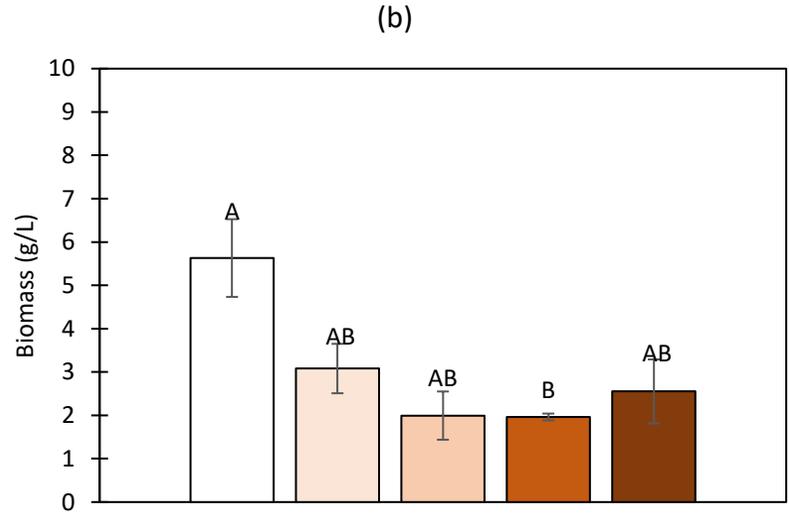
Fungal strain	Glucose g/L	Yeast g/L	Pomegranate gr peel/100 ml substrate	Pomegranate ml juice /100 ml substrate
Ao, Ro, Ni	30	5	0.5	0.5
Ao, Ro, Ni	30	5	1	1
Ao, Ro, Ni	30	5	2	2
Ao, Ro, Ni	30	5	4	3

# Effects of Juice in supplemented media

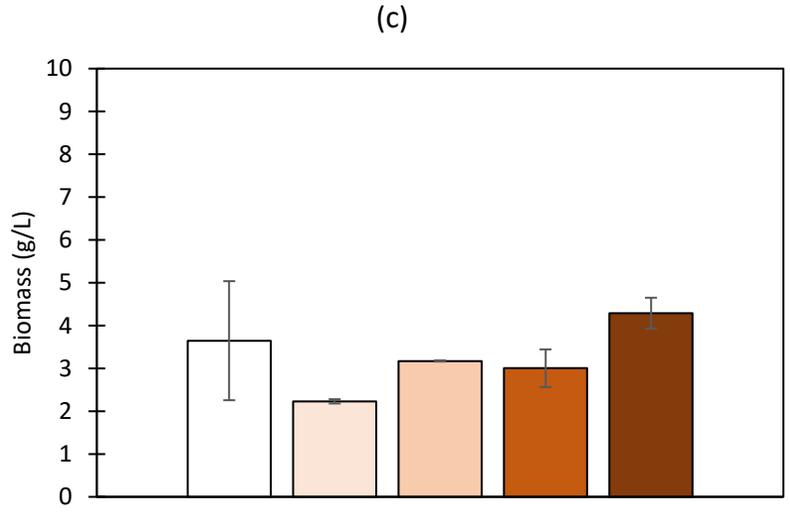
(Glucose+yeast extract)



*A.oryzae*



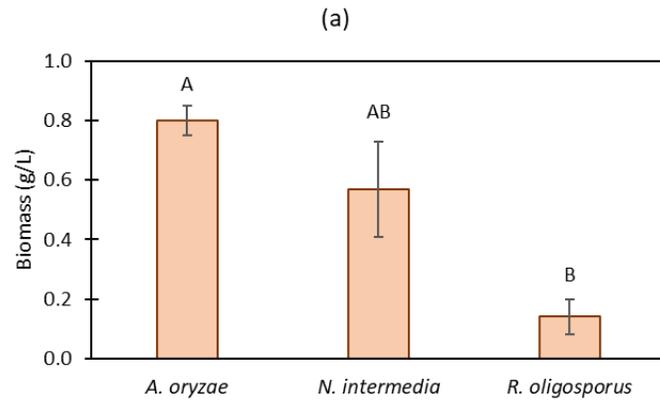
*R.oligosporus*



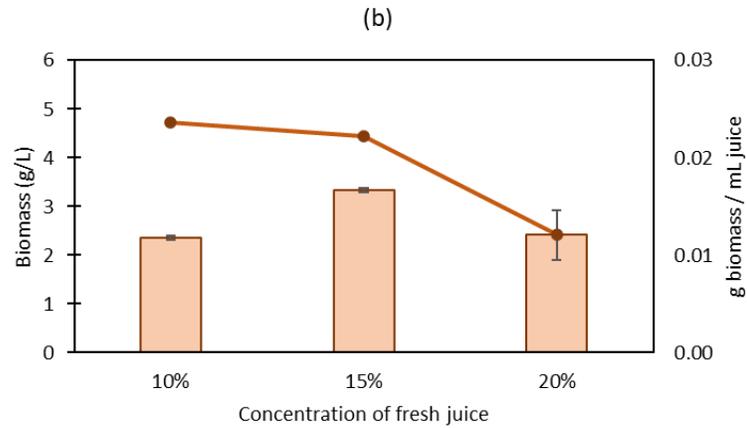
*N.intermedia*

□ 0 □ 0.5% □ 1% ■ 2% ■ 4%

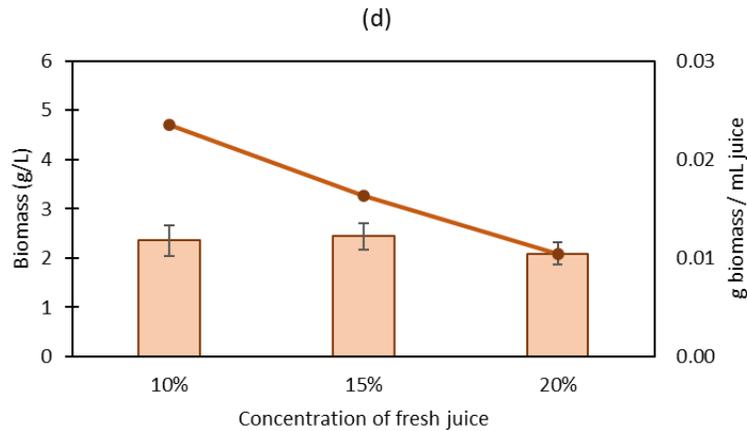
4% Fresh juice



Fresh juice  
*A. oryzae*



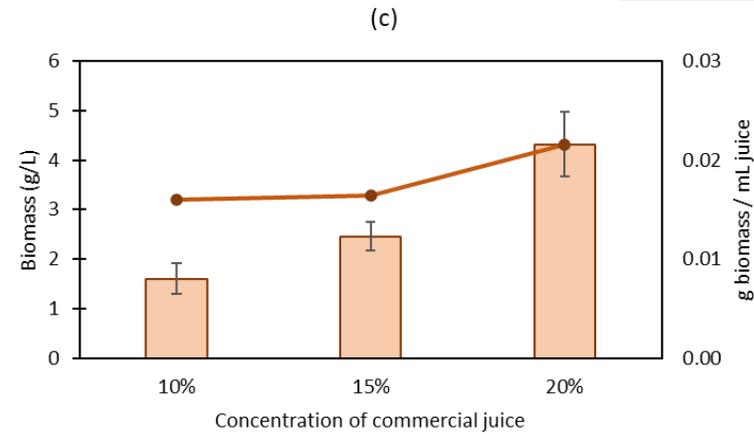
Fresh juice  
*N. intermedia*



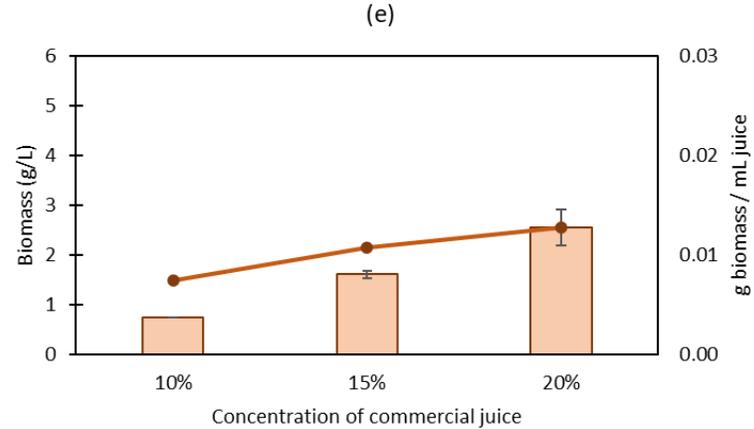
# Effects of Juice



Commercial juice  
*A. oryzae*

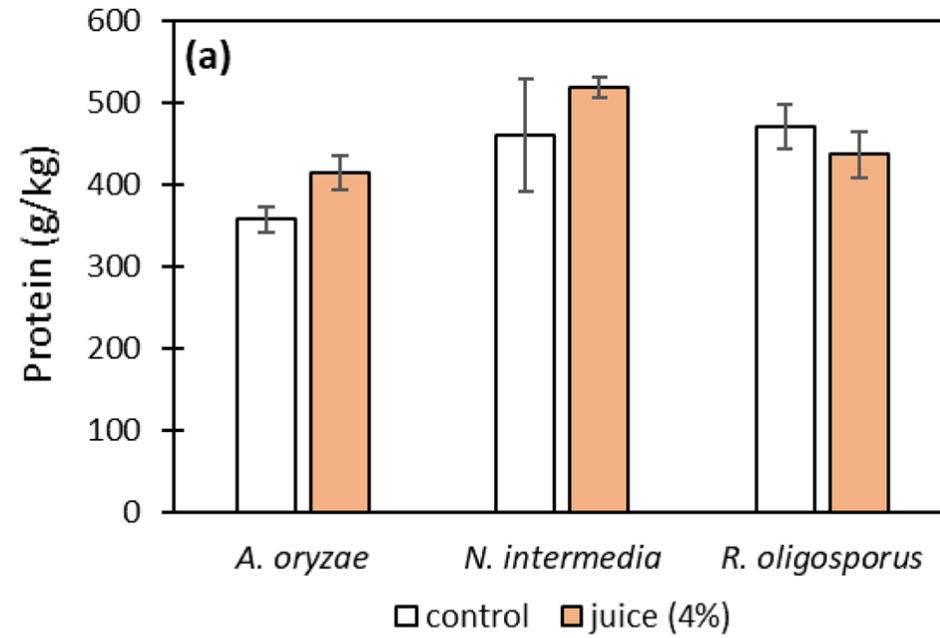


Commercial juice  
*N. intermedia*

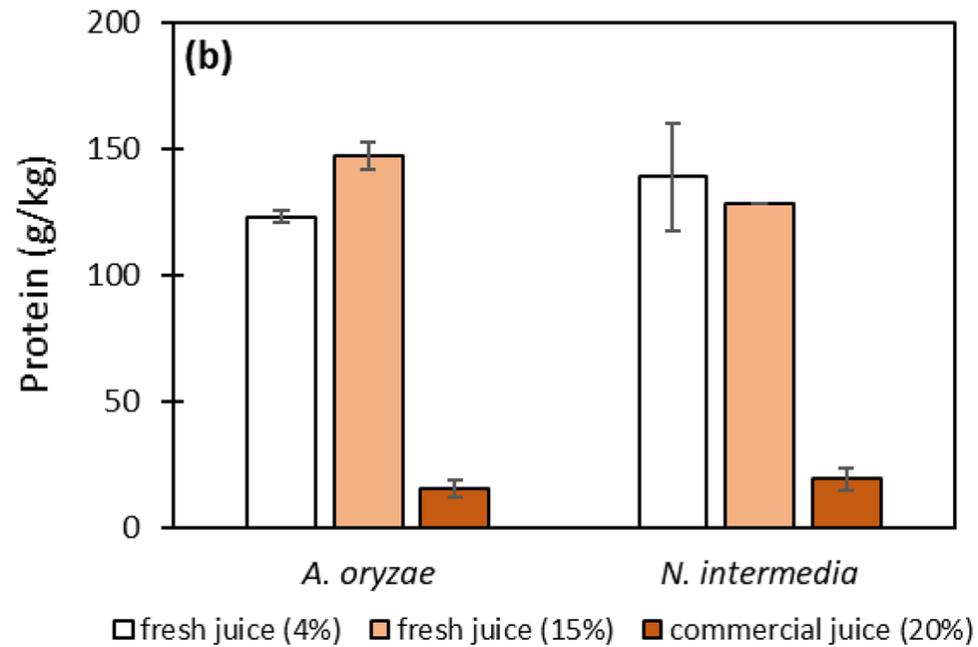


■ Biomass — Yield

## Protein Contents

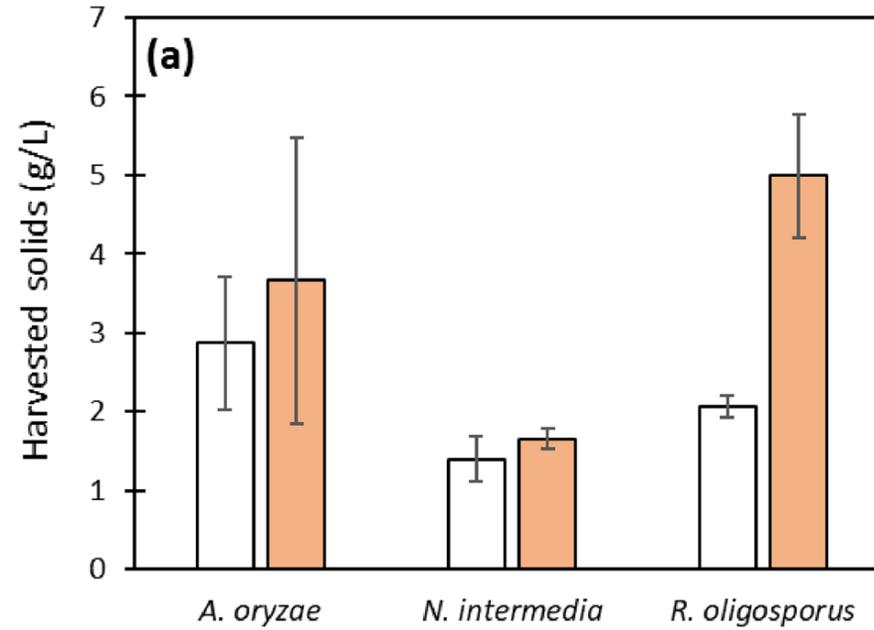


**In glucose and yeast extract media**

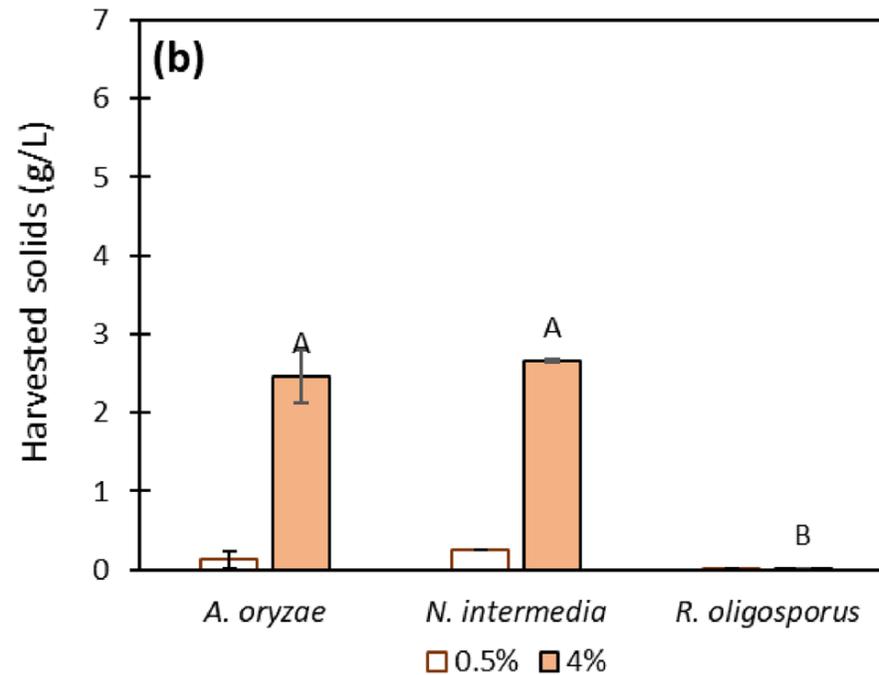


**Without Supplementation**

# Effects of Aril



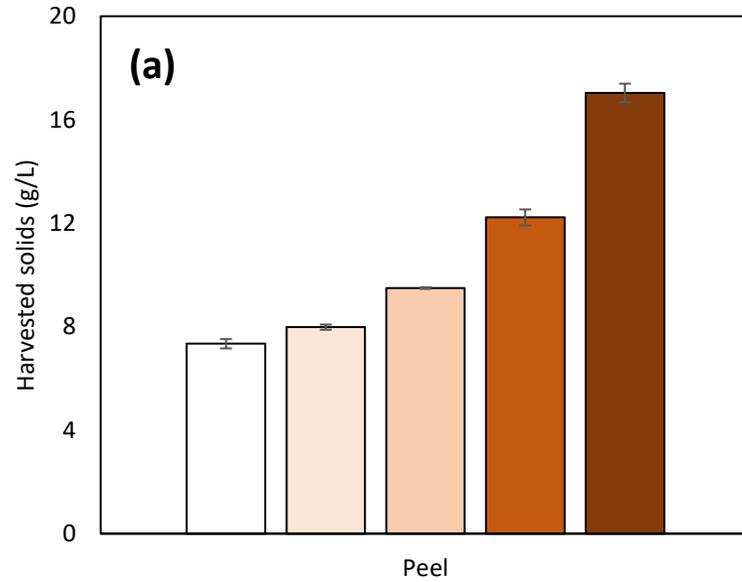
In glucose and yeast extract media



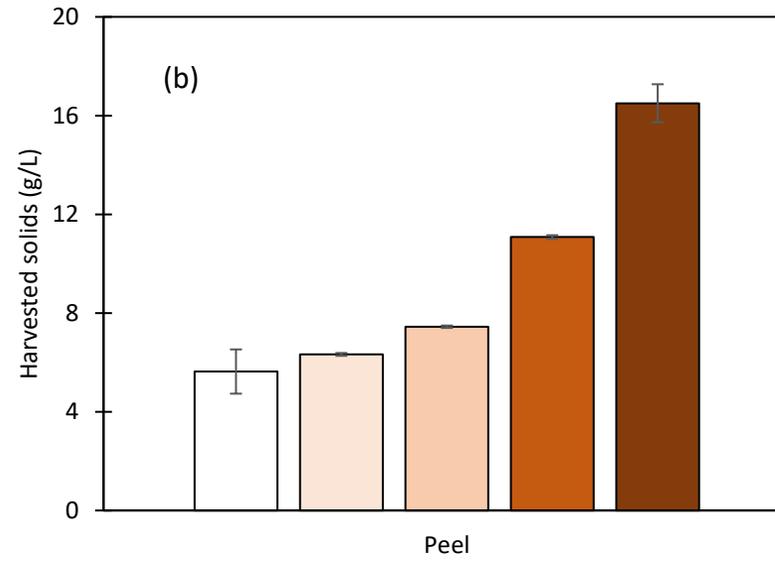
Without Supplementation

# Effects of Peel

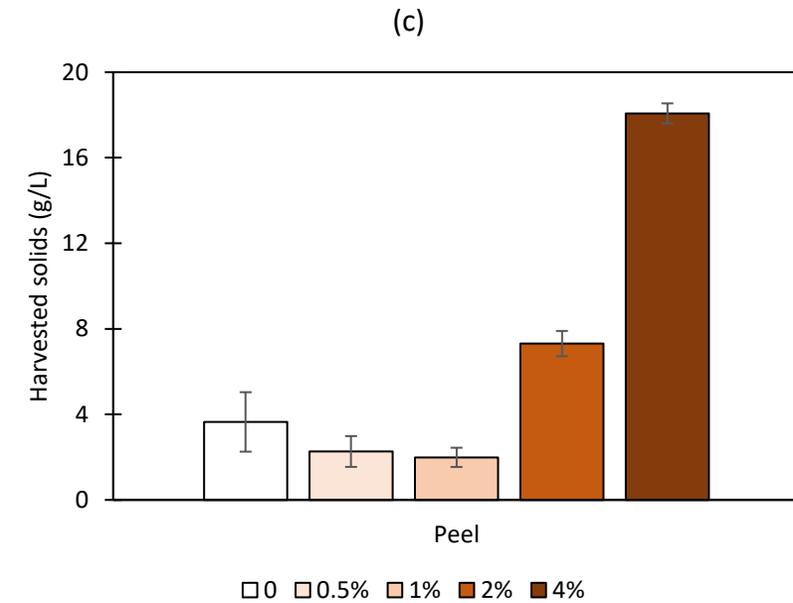
(Glucose+yeast extract)



*A.oryzae*



*R.oligosporus*



*N.intermedia*

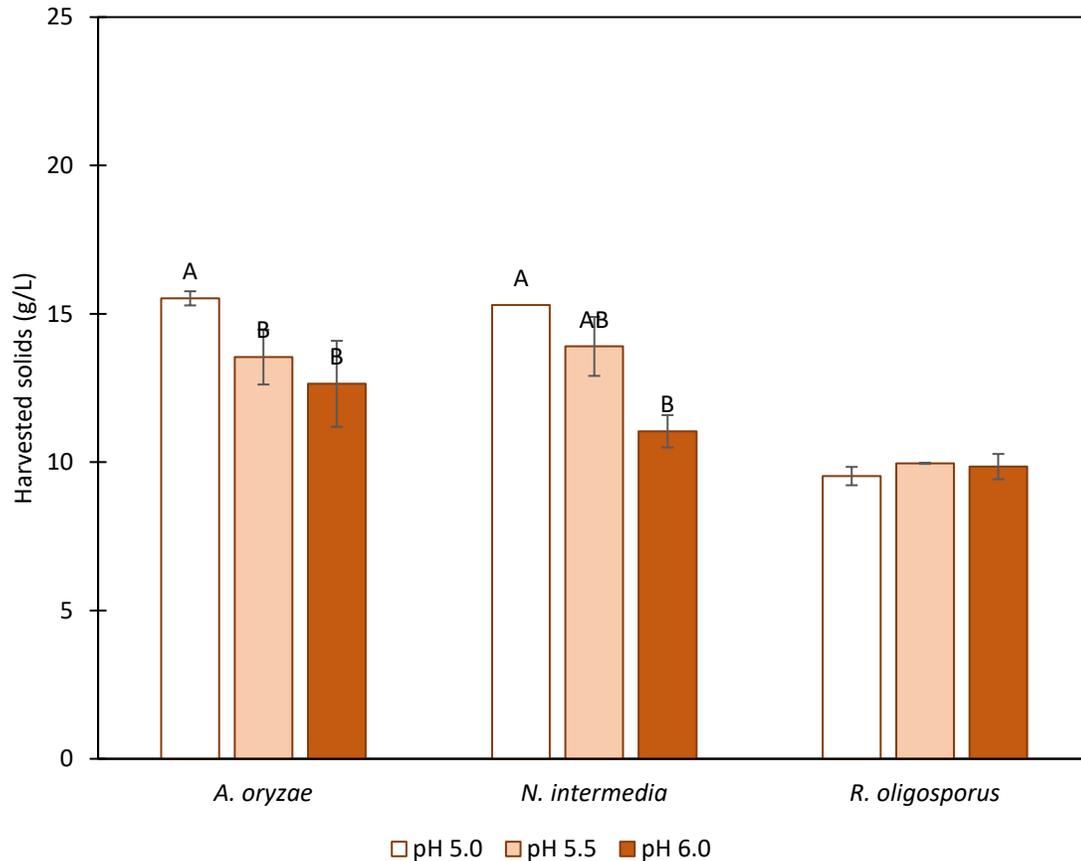
	<b>Protein (g/kg)</b>
<b>Harvested solids</b>	65.56 ± 0.74

## Effects of Peel

Protein (g/kg)	<i>A. oryzae</i>	<i>N. intermedia</i>	<i>R. oligosporus</i>
<b>Dry peel (4%)</b>	85.41 ± 7.56	76.90 ± 0.86	74.54 ± 0.00
<b>Wet peel (16%)</b>	77.44 ± 1.34	68.42 ± 1.66	64.51 ± 2.61

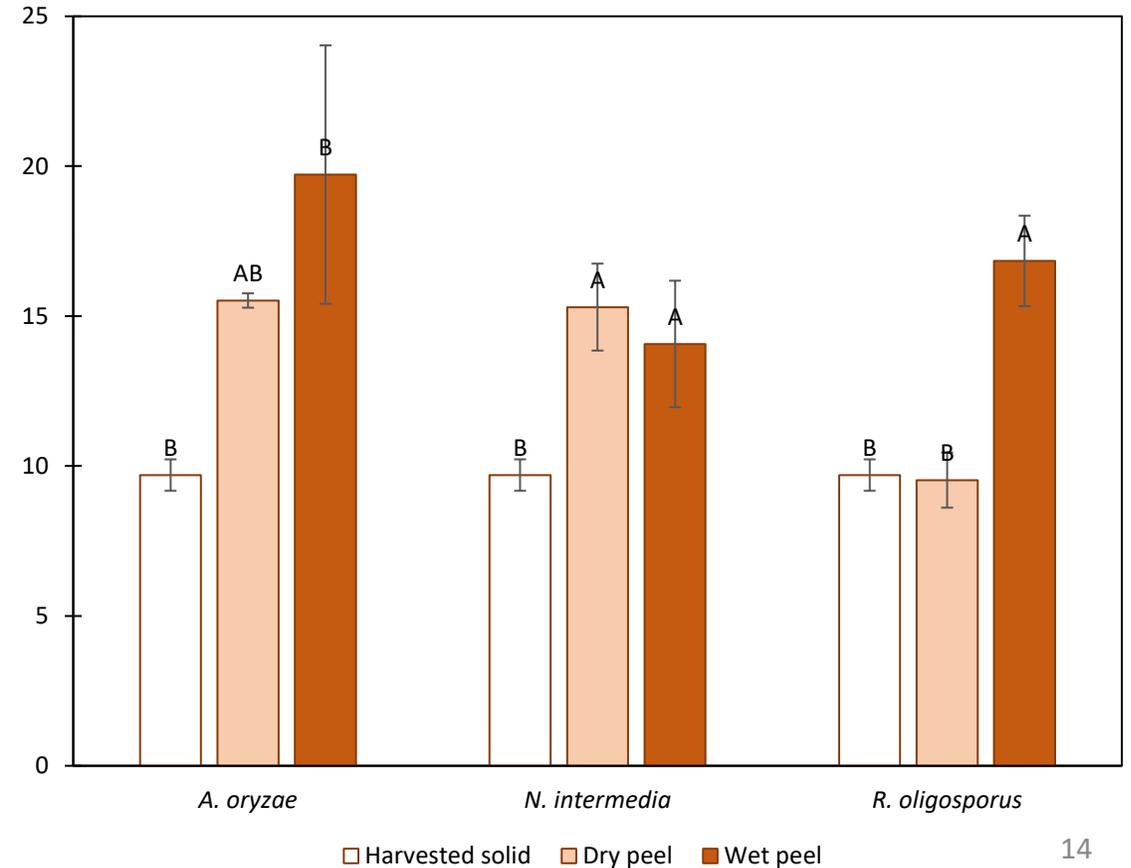
### pH

(a)



### Dry vs. Wet Peel

(b)

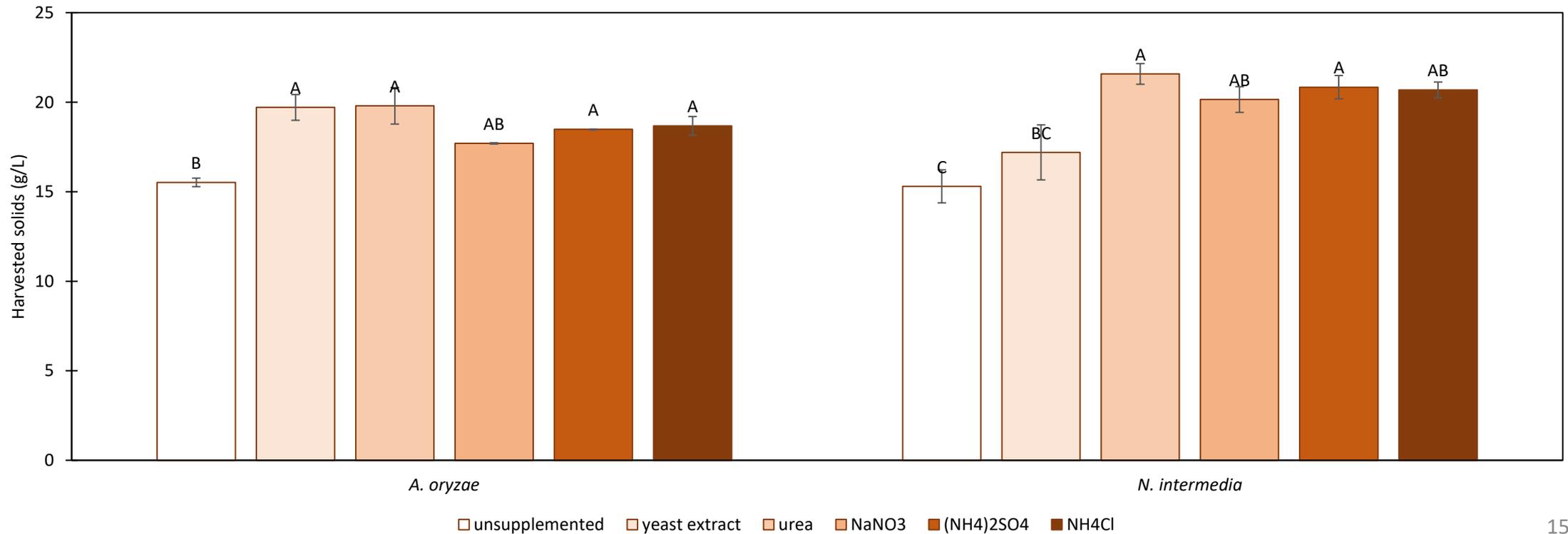


# Effects of Peel

## Nitrogen Supplementation

Protein (g/kg)	<i>A. oryzae</i>	<i>N. intermedia</i>
Yeast extract	<b>198.63 ± 36.37</b>	<b>148.42 ± 12.04</b>
Urea	137.53 ± 2.59	137.81 ± 0.27
NaNO <sub>3</sub>	102.56 ± 0.74	124.22 ± 6.15
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	142.81 ± 3.45	143.63 ± 9.27
NH <sub>4</sub> Cl	130.11 ± 0.76	132.10 ± 1.92

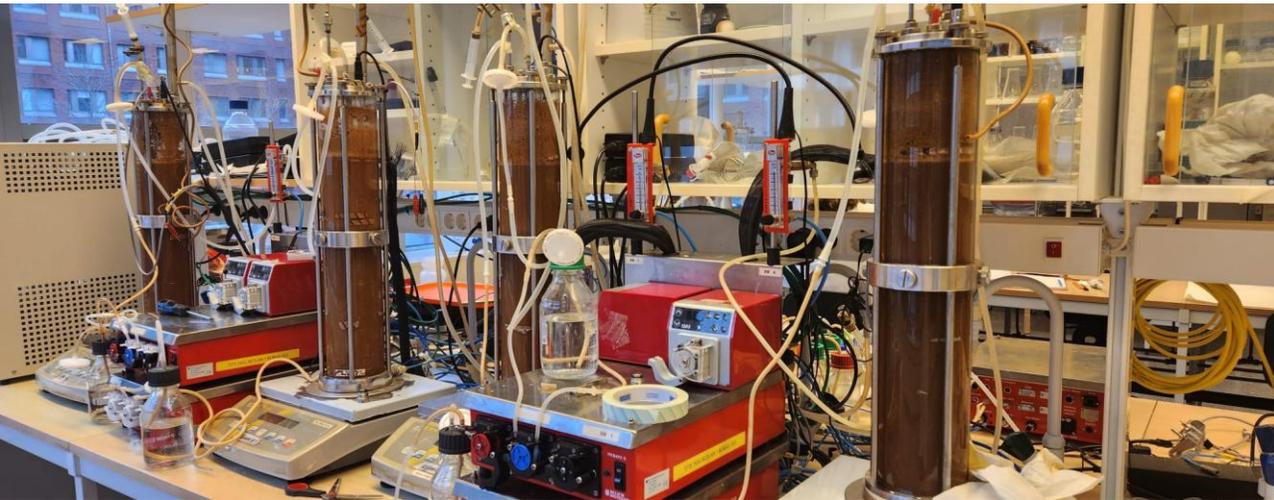
(c)



# Bubble column reactor and biomass characterization

## Stage 2

- pH 5
- Cultivation time: 48h
- Volume: 3700 ml



Fungal strain	Yeast extract g/L	Pomegranate Peel g/L
<i>A.oryzae</i> <i>N.intermedia</i>	5	40

# Biomass production

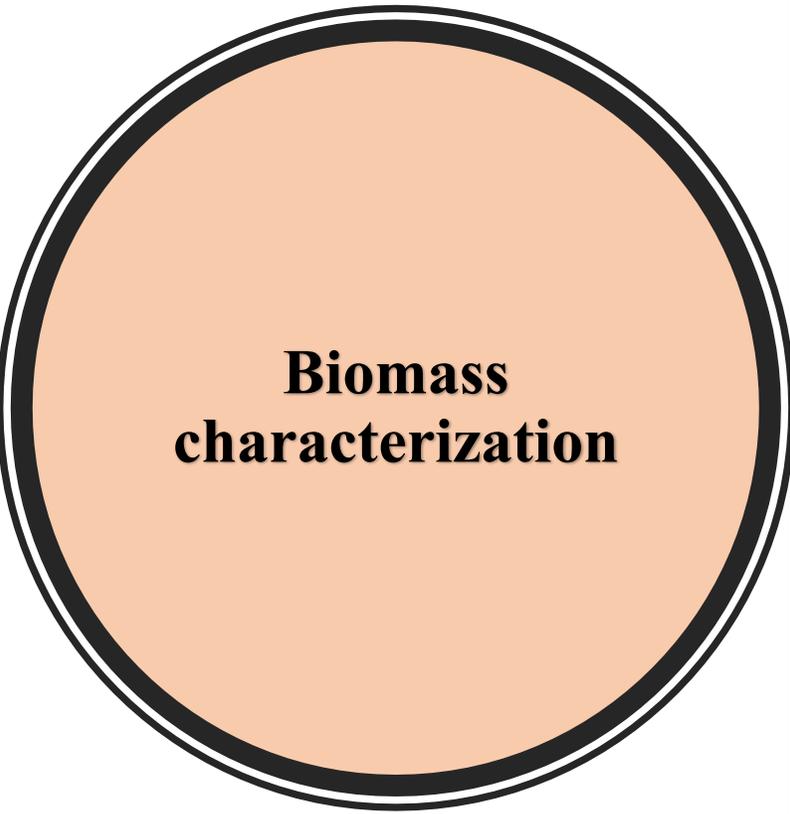
Fungi	Harvested solids g/L	Yield g biomass / g substrate
<i>A. oryzae</i>	20.35 ± 1.15	0.51 ± 0.03
<i>N.intermedia</i>	19.88 ± 1.58	0.50 ± 0.04

*A.oryzae*



*N.intermedia*





**Biomass  
characterization**

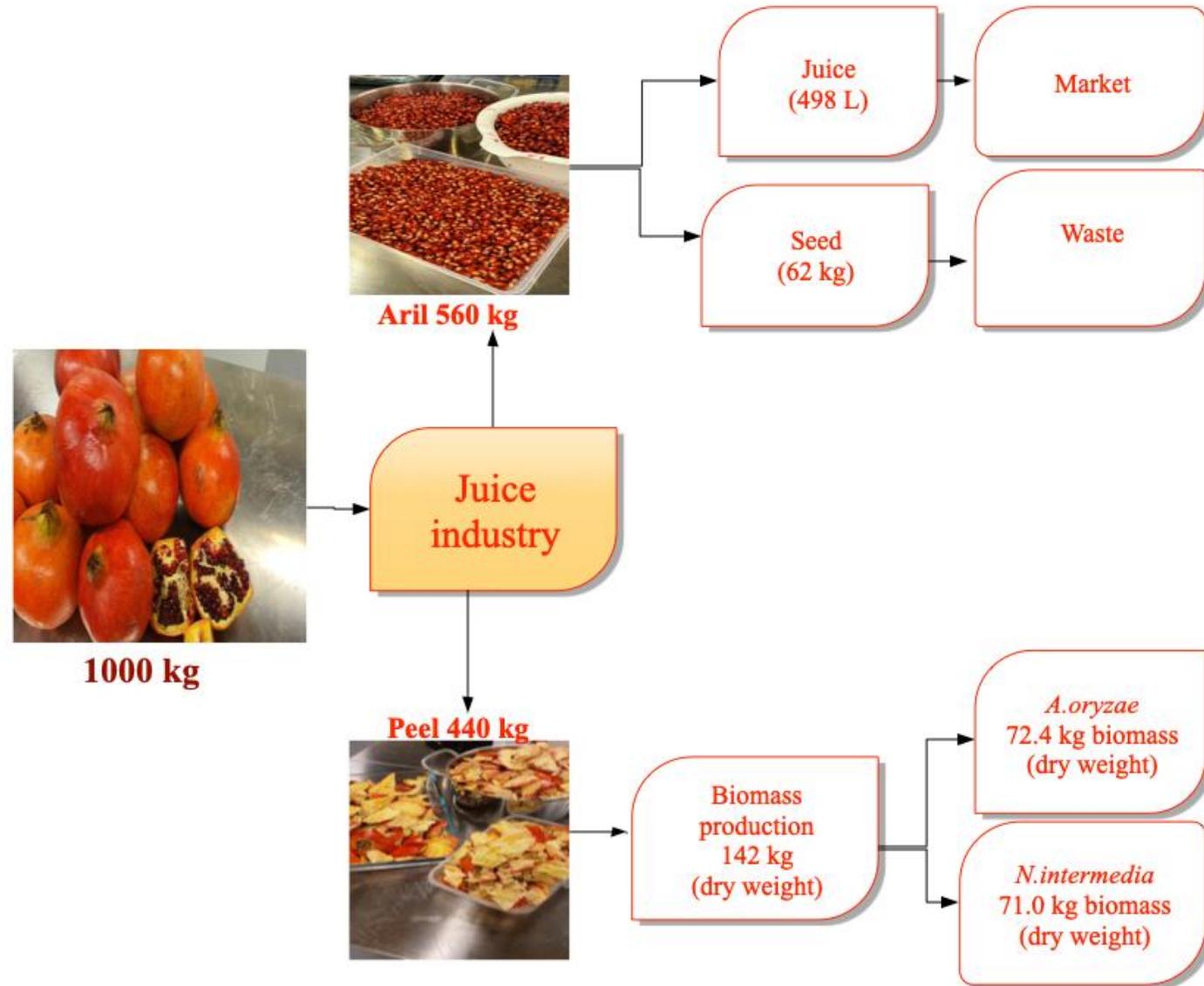
	<b>Unit</b>	<b>Peel</b>	<i>A. oryzae</i>	<i>N. intermedia</i>
<b>TS</b>	(g/kg)	890.41 ± 0.37	952.83 ± 0.38	949.02 ± 0.40
<b>Volatile solid</b>	(g/kg)	847.06 ± 1.60	915.90 ± 4.01	922.03 ± 0.08
<b>Ash</b>	(g/kg)	43.35 ± 1.96	36.93 ± 3.63	26.99 ± 0.32
<b>Total fat</b>	(g/kg)	0.80 ± 0.08	<b>15.31 ± 5.36</b>	<b>13.95 ± 3.31</b>
<b>Protein</b>	(g/kg)	29.89 ± 2.78	<b>179.09 ± 3.41</b>	<b>121.53 ± 3.21</b>
<b>Nitrogen</b>	(g/kg)	5.15 ± 0.48	28.66 ± 0.54	19.44 ± 0.51
<b>D glucose</b>	(g/kg)	130 ± 1.41	12.00 ± 0.00	10.50 ± 0.71
<b>D fructose</b>	(g/kg)	117 ± 4.24	6.00 ± 2.83	8.50 ± 2.12
<b>Sucrose</b>	(g/kg)	0.00	0.00	0.00
<b>Crude Fibers</b>	(%)	16.73 ± 0.10	<b>40.09 ± 0.40</b>	<b>40.49 ± 2.90</b>



## Conclusions

- *A.oryzae* and *N.intermedia* were resistant to the effects of pomegranate juice.
- Aril was not a good substrate for the strains investigated.
- All the strains utilized proved to be resistant at higher concentrations when peel was used as substrate.

# Fungal biomass production from an industrial perspective



Thank you!

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