BACTERIAL CELLULASE PRODUCTION USING GRAPE POMACE HYDROLYSATE AS SOLE CARBON SOURCE BY SHAKE-FLASK SUBMERGED FERMENTATION

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OUTLINE

- INTRODUCTION
- AIM OF THE STUDY
- MATERIALS & METHODS
- RESULTS & DISCUSSION
- CONCLUSION

GRAPE

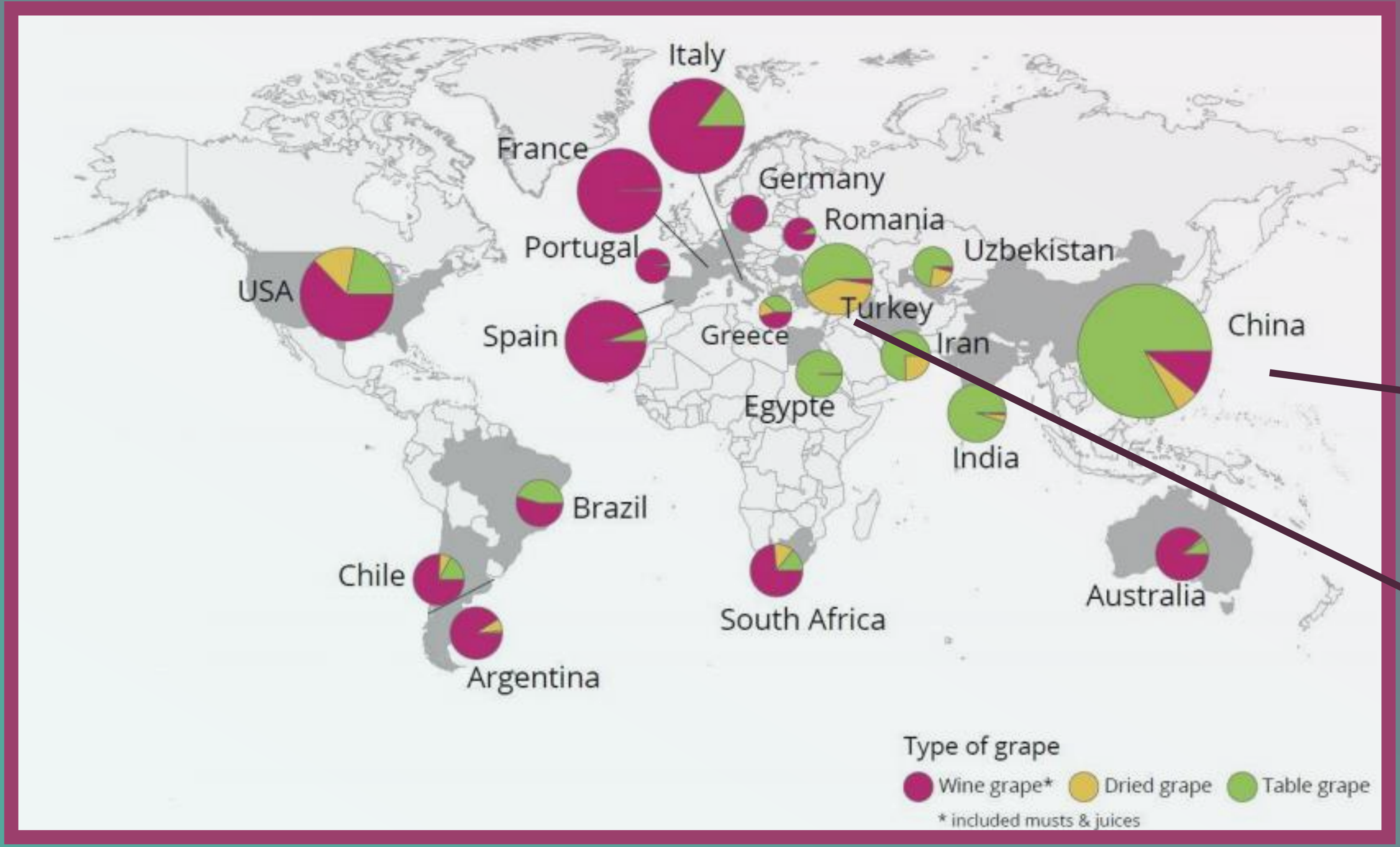
mostly grown and consumed fruit

nearly 50% of the grown grapes → wine industry

human health

vinification process

economic aspects



74.28 million tons

13.7 million tons

4.2 million tons

Skins

Seeds

Stems

Grape pomace:
13.5-30% of the total volume of grapes crushed

- Grape species
- Equipment used for pressing
- Process applied for pressing

Approximately 10 million tons

- Surface and groundwater pollution
- Unpleasant odor
- Oxygen reduction in soil

Composition and quality of grape pomace;

- ✓ Type of grape
- ✓ Stage of ripeness
- ✓ Fertilization conditions
- ✓ Harvesting and processing conditions
- ✓ Location
- ✓ Climate

- ✓ Protein Content: 8.49%
- ✓ Fat Content: 8.16%
- ✓ Carbohydrates: 29.2%
- ✓ High concentration of iron, potassium, zinc, manganese, and calcium

Glucose
Xylose
Fructose
Mannose
Arabinose
Galactose

Cellulose
Hemicellulose
Starch
Pectin

Animal Feed

Restricted
- Lignified fiber
- Tannin component

Composting

- Aerobic microbial decomposition
- Carbon & Nitrogen
- Used in vineyards

Antioxidants

Good source to prevent lipid oxidation
- Meat products
- Dairy products

Biosurfactants

- Lower toxicity
- Higher biodegradability

Grape Seed Oil

- Source of lipophilic compounds
- High smoke point
- Enhances the nutritional properties of meat products

Edible Film Formation

- Pectin, cellulose, sugar
- Natural pigments, flavors, and polyphenols
- Enhance the chitosan film capability

Animal Feed

Composting

Antioxidants

Biosurfactants

ENZYME PRODUCTION

- Amylases
- Pectinases
- Cellulases

Grape Seed Oil

Edible Film Formation

Cellulase;

β-1,4 linkages in cellulose chain

amino acid sequences
crystal structures

Endoglucanases
Exoglucanases
β-glucosidases

Cleave internal β-1,4 bonds
Produce; soluble cellodextrin,
insoluble cellulose fragment,
cellobiose

Cleavage at non-reducing end;
Glucose or cellobiose

Attack to soluble cellodextrin and cellobiose
Generate glucose
Inactive against insoluble cellulose chains

Application area;

- Textile
- Pulp and Paper
- Food
- Detergent
- Agriculture

Food →

- Starch processing
- Grain alcohol fermentation
- Malting and brewing
- Fruit and vegetable juice processing

! COST of cellulase

agricultural waste → low-cost cellulose source for cellulase production

Secreted by fungi, bacteria, protozoans, plants, and animals

Aerobic
Anaerobic
Mesophilic
Thermophilic

Fungi vs Bacteria

- Higher growth rate
- Shorter generation time
- Genetically varied
- High adaptability
- Highly amendable against genetic manipulation

Cellulomonas
Cellvibrio
Pseudomonas sp.
Bacillus sp.
Micrococcus
Paenibacillus sp.

Bacillus subtilis

- Rod shape
- Produces spores under extreme conditions
- Facultative anaerobic
- Sense the oxygen concentration
- Gram-positive

High amount of extracellular enzymes

Amylases, Proteases, Lipases, Xylanases, Pectinases, Cellulases

To produce **bacterial cellulase**
using grape pomace as sole carbon source

Bacillus subtilis Natto DSM 17766
(at 37°C, 130 rpm)

pH
- 5.0
- 7.0
- 9.0

Solid Loading
- 5%
- 12.5%
- 20%

Incubation Time
- 3 days
- 5 days
- 7 days

Dried Grape Pomace

+

Dilute H_2SO_4 at concentration of 3% (w/v)

Grape Pomace Hydrolysate

Submerged Fermentation

2% inoculum

DNS Method

Filter Paper Assay

Absorbance at 575 nm

Glucose concentration

Max. cellulase activity

- 0.196 IU/mL
- pH 7.0, 12.5%, after 5 days

Initial reducing sugar conc. 12.56 g/L
Final reducing sugar conc. 5.71 g/L

Min. cellulase activity

- 0.045 IU/mL
- pH 9.0, 5%, after 5 days

Initial reducing sugar conc. 5.33 g/L
Final reducing sugar conc. 1.78 g/L

- Incubation time ↑ sugar consumption ↑
- pH ↑ sugar consumption ↑

Optimum conditions;

pH	Solid Loading (%w/v)	Incubation Time
6.0	15%	7 days

Predicted: 0.178 IU/mL

Experimented: 0.176 ± 0.0127 IU/mL

Grape pomace
Lignocellulosic waste material
Enzyme production

- ✓ Max cellulase activity 0.196 IU/mL (12.56 g/L)

  12.5%, pH 7.0, 5 days

- ✓ The optimum conditions; 15%, pH 6.0, 7 days (15.87 g/L)

  0.176 ± 0.0127 IU/mL
  67% saccharification

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THANK YOU!