TEXAS TECH UNIVERSITY

Extraction and Characterization of Galactomannan from Guar Seeds

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Outline

- Introduction
- Objectives
- Extraction of Guar Gum
- Characterization of Guar Gum
- Conclusions

Introduction

- **Guar gum is a natural water dispersible hydrocolloid that has great thickening power when dispersed in water**

- **It is extracted from the seeds of Cluster Bean/Guar**

  (*Cyamopsis tetragonoloba (L)*)

- **Growing regions: India**

  Pakistan

  USA - Texas and Oklahoma

Introduction

Major Commercial Uses of Guar Gum

- **Hydraulic fracturing in oil well drilling:**
  - **Mixed with the fracturing fluid to harvest shale gas and oil**
- **Food industry**
  - **Stabilizer in frozen (ice cream) and baked foods**
  - **Thickener for salad dressing due to high viscosity, acid stability & cold water dispersibility**
- **Pharmaceutical, textile, and paper industries**





Picture source: http://2.imimg.com/data2/QR/DQ/MY-1031926/we-offer-250x250.jpg, http://www.therecklessoptimist.com/wp-content/uploads/2010/10/Milkshake-12447500.jpg, http://momentumbooks.com.au/wp-content/uploads/2013/10/squash-and-apple-soup.jpg, http://images2.alphacoders.com/130/130230.jpg, http://www.pumpkinseedbulkfood.com/upload/images/Troyer_Pictures/donna_sharp_images/BREAD_PHOTO.jpg

Introduction

Hydraulic Fracturing

- Hydraulic fracturing uses high pressure fluid to crack open hydrocarbon bearing zones in shale rock formations
- Guar gum is one of the most popular polymer used in aqueous based fracturing fluids since it has a great viscosifying property
- Guar gum thicken the fracturing fluid to retain the graded sand/ proppant in suspension and prevent the settling of the proppant
- The proppant helps to keep the fracture open, creating a permeable route for the oil or gas to flow to the well bore

Picture source: https://student.societyforscience.org/article/fracking-fuels-energy-debate

http://www.halliburton.com/public/pe/contents/Brochures/Web/H09347.pdf

Introduction

Hydraulic Fracturing

- Guar gum increases the efficiency of the process by reducing the friction of the system
- ~ 9000 kg of guar gum is required per oil well
- Hydroxypropyl guar (HPG) and carboxymethyl-hydroxypropyl guar (CMHPG) are also used in hydraulic fracturing
- The organometallic cross-linked guar, HPG and CMHPG are used when there is a need for extended fracture length
- Borate cross-linked guar and HPG provide good proppant transportation and high temperature stability

Introduction

Guar Plant

- A leguminous plant that grows up to 3 – 6 feet
- Produces many 5 – 12.5 cm long bean-like pods in clusters with 6 – 9 small seeds per pod
- Extremely drought tolerant annual crop that can be cultivated with very limited supply of resources

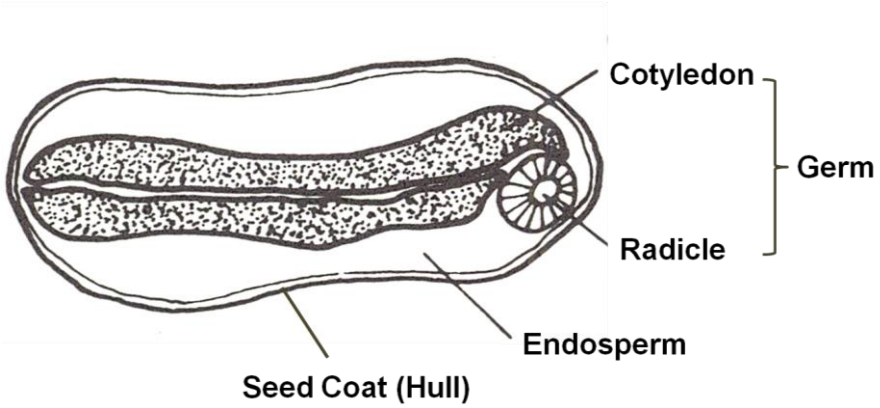
Guar Plants

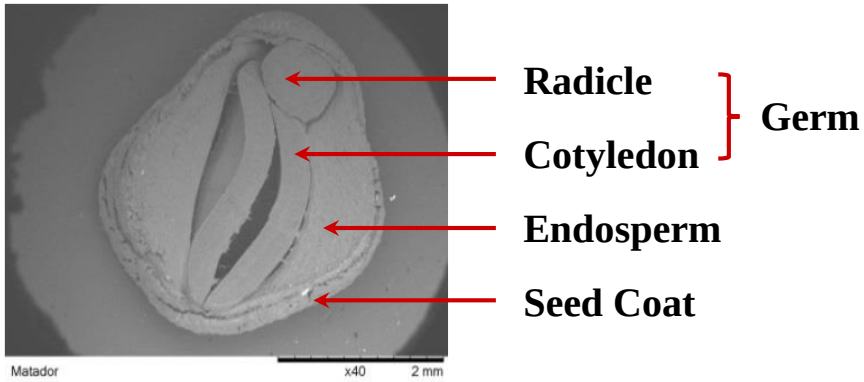
Picture Source: Dr. D. L. Auld – Quaker Farm, Texas Tech University

Introduction

Guar Seed

Guar Seed - 40 - 46 % - Germ
38 - 45 % **- Endosperm**
14 - 16 % - Hull





Guar Seed Cross Section

Picture source: Whistler, R.L., Hymowitz, T., (1979), Guar: Agronomy, Production, Industrial use and Nutrition (left)

Introduction

Composition of Guar Endosperm

Source: Chudzikowski, (1971).

Compound	Percentage
Nitrogen	0.67
Phosphorus	0.06
Ash	1.07
Water soluble polysaccharide	86.50
Water insoluble fraction	7.75
Alcohol soluble fraction	1.50

- **Water soluble polysaccharide fraction consists of**

D - galactose - 36.6%

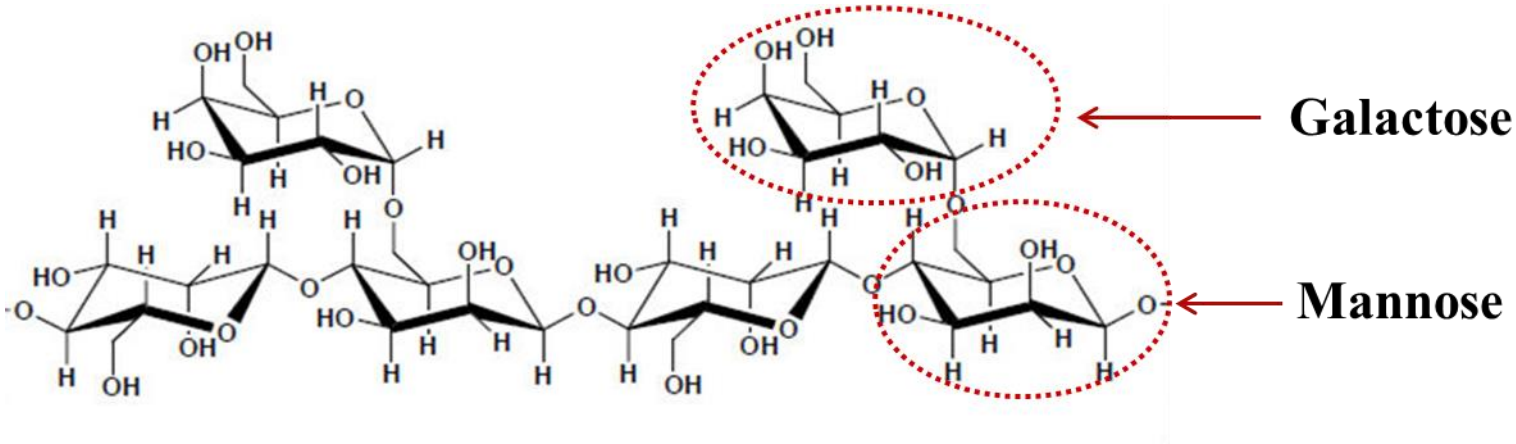
D - mannose - 63.1 %

} **Galactomannan = Pure guar gum**

Introduction

Chemical Structure of Guar Galactomannan

- Linear chain of D-mannose units linked together via a β(1 – 4) acetal linkage and having approximately one D galactose unit for every alternate mannose unit linked via an α(1-6) acetal linkage



Picture source : http://www.fao.org/fileadmin/templates/agns/pdf/jecfa/cta/69/Guar_gum.pdf.

- Molecular weight ~ 220 KDa

Introduction

- There is an increased interest in guar gum and its application, particularly in the food and hydraulic fracturing industries
- Therefore, characterizing guar gum using different analytical tools is very important to understanding the chemistry of guar galactomannan
- However, a limited number of studies have been conducted to characterize the gum extracted from the currently available guar germplasm

Objectives

1. **Optimize the extraction method to minimize contamination of galactomannan**

2. **Characterize guar gum using different analytical tools**

3. **Elucidate the physical and chemical differences between guar cultivars**

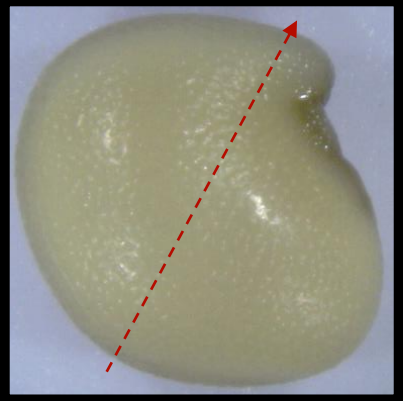
Morphology of Guar Seed

Scanning Electron Microscope

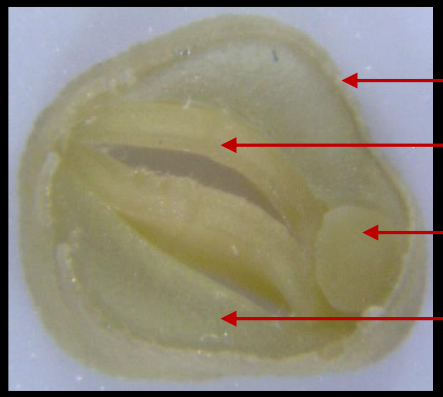
Pictures:
Fiber and Biopolymer Research Institute - Texas Tech University

Morphology of Guar Seed

Guar Seed (dry)



Guar Seed (wet)



Cross Section

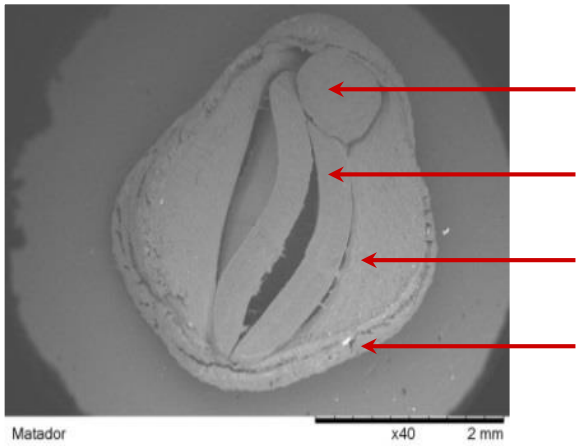
Longitudinal Section

Guar Endosperm

Guar Germ

Cross Sections of Guar Seeds

Matador



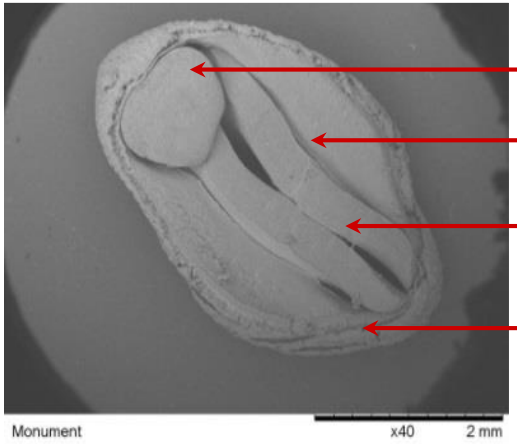
Radicle

Cotyledon

Endosperm

Seed Coat

Monument



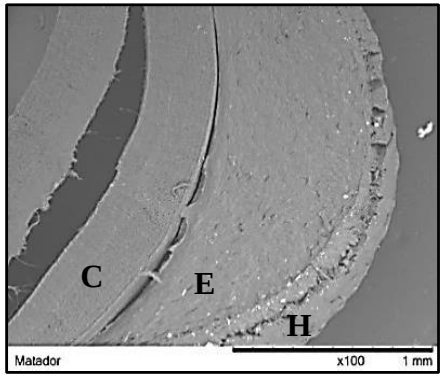
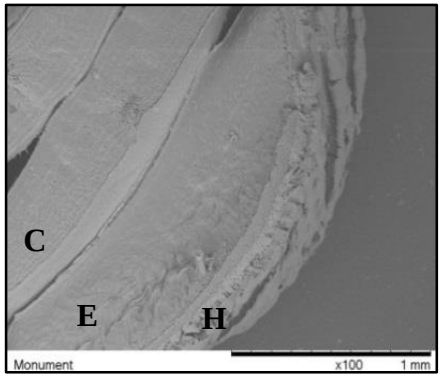
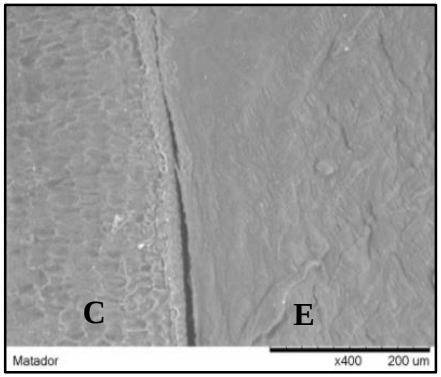
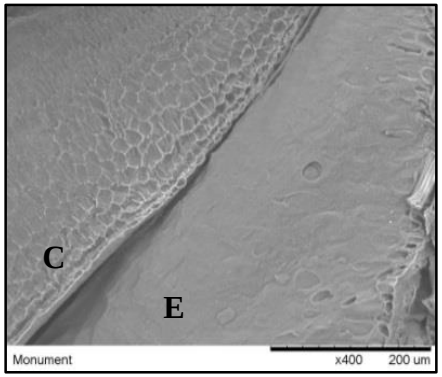
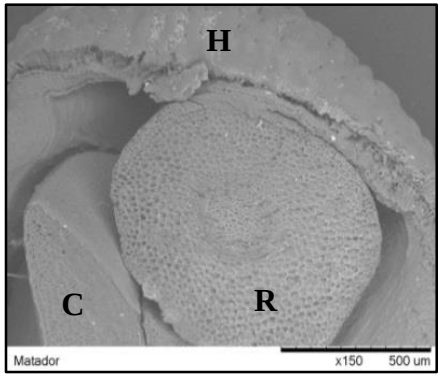
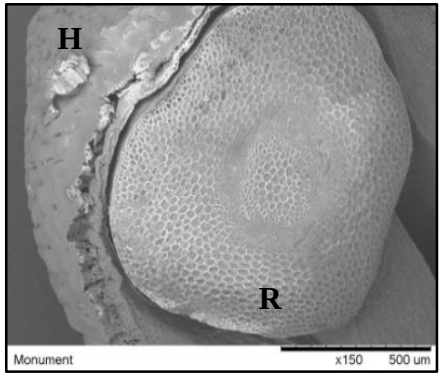
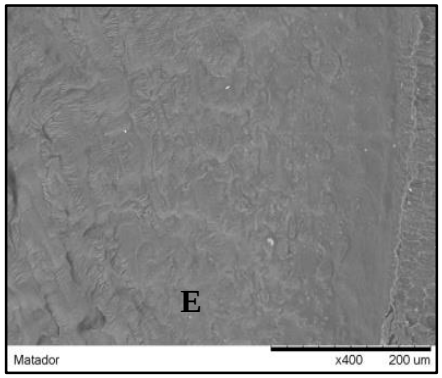
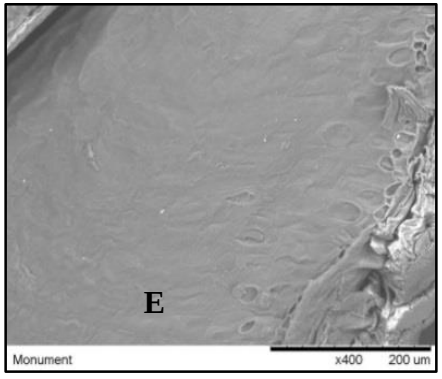
Radicle

Endosperm

Cotyledon

Seed Coat

Cross Sections of Guar Seed

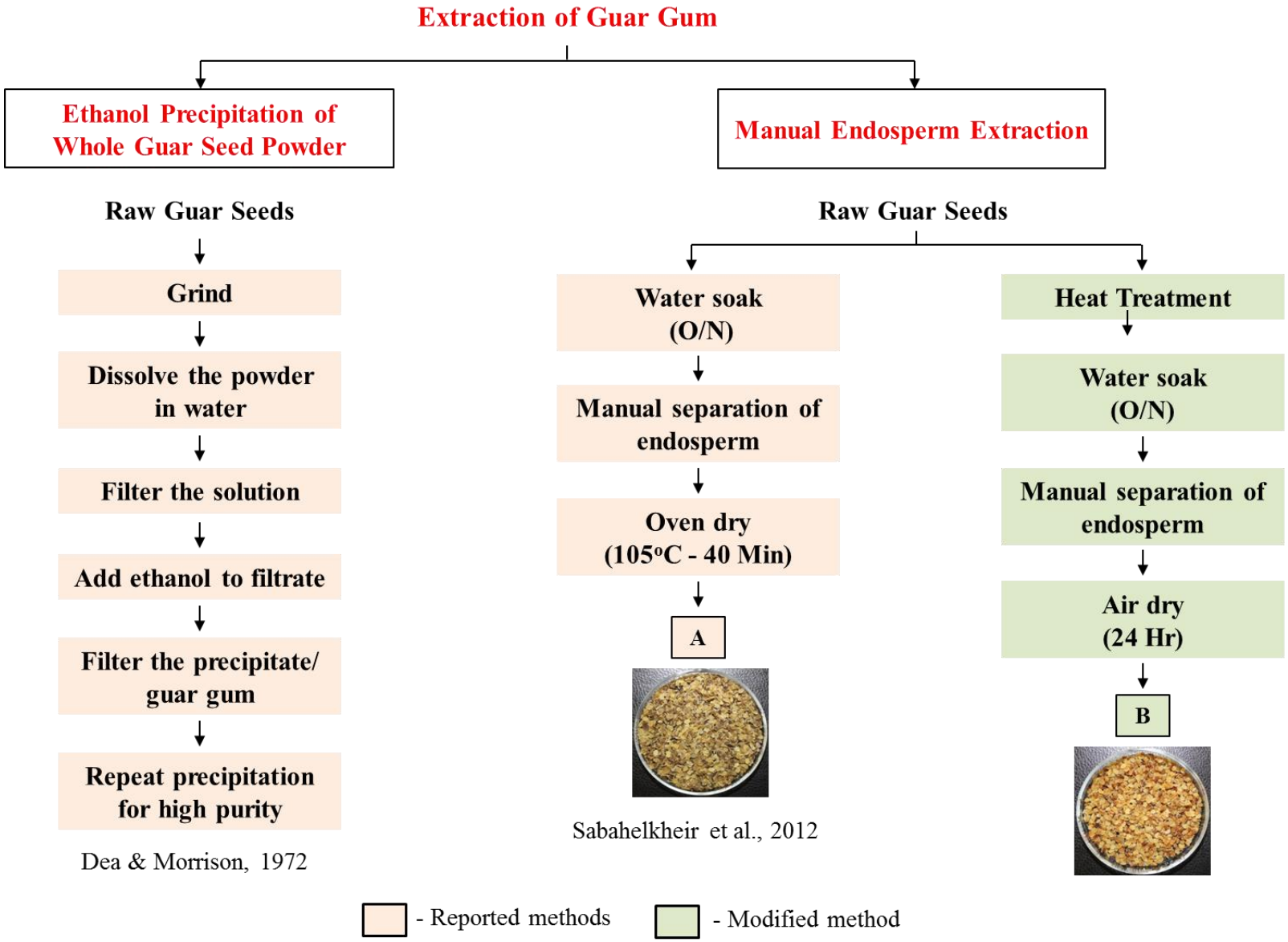
Matador	Monument	Matador	Monument
			
x40		**x400**	
			
x100		**x1000**	

C- Cotyledon, E- Endosperm, R- Radicle, H- Seed Coat/Hull

Extraction of Gum from Guar Seeds

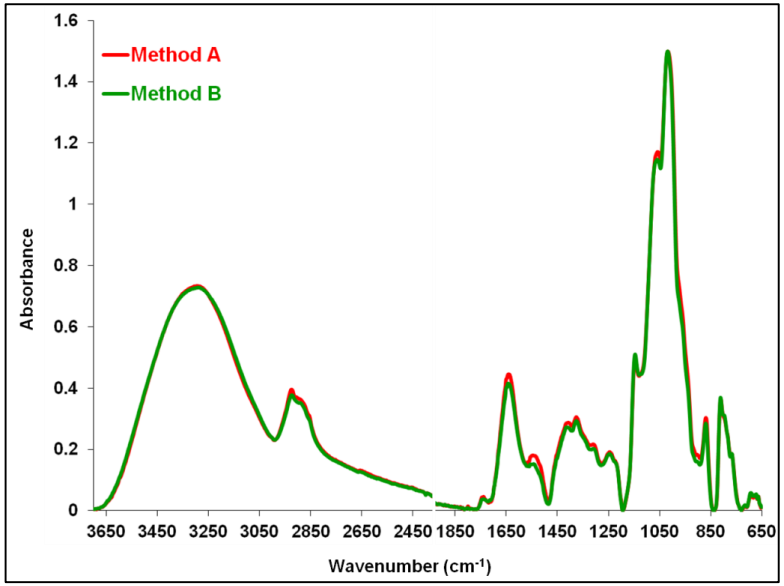
1. Manual Extraction of Endosperm
2. Ethanol Precipitation of Whole Seed Powder

Extraction of Guar Gum



Extraction of Guar Gum

1. Manual Extraction of Guar Gum

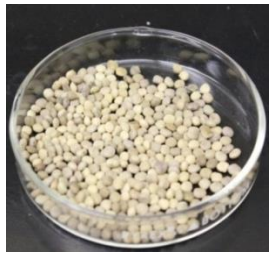


FTIR spectra of Guar endosperm powder separated by different methods

- **FTIR spectra were collected from sample A and B to investigate the impact of heat treatment on galactomannan**
- **Both spectra are identical and therefore initial heat treatment can be used before the manual extraction to prevent germination during water soaking and endosperm extraction**

Extraction of Guar Gum

1. Manual Extraction of Guar Gum



Matador & Monument Seeds

↓

Heat Treatment at 100°C for 30 min

↓

Water soak overnight (80°C)

↓

Manually separate endosperm

↓

Dry at 105°C for 40 min

↓

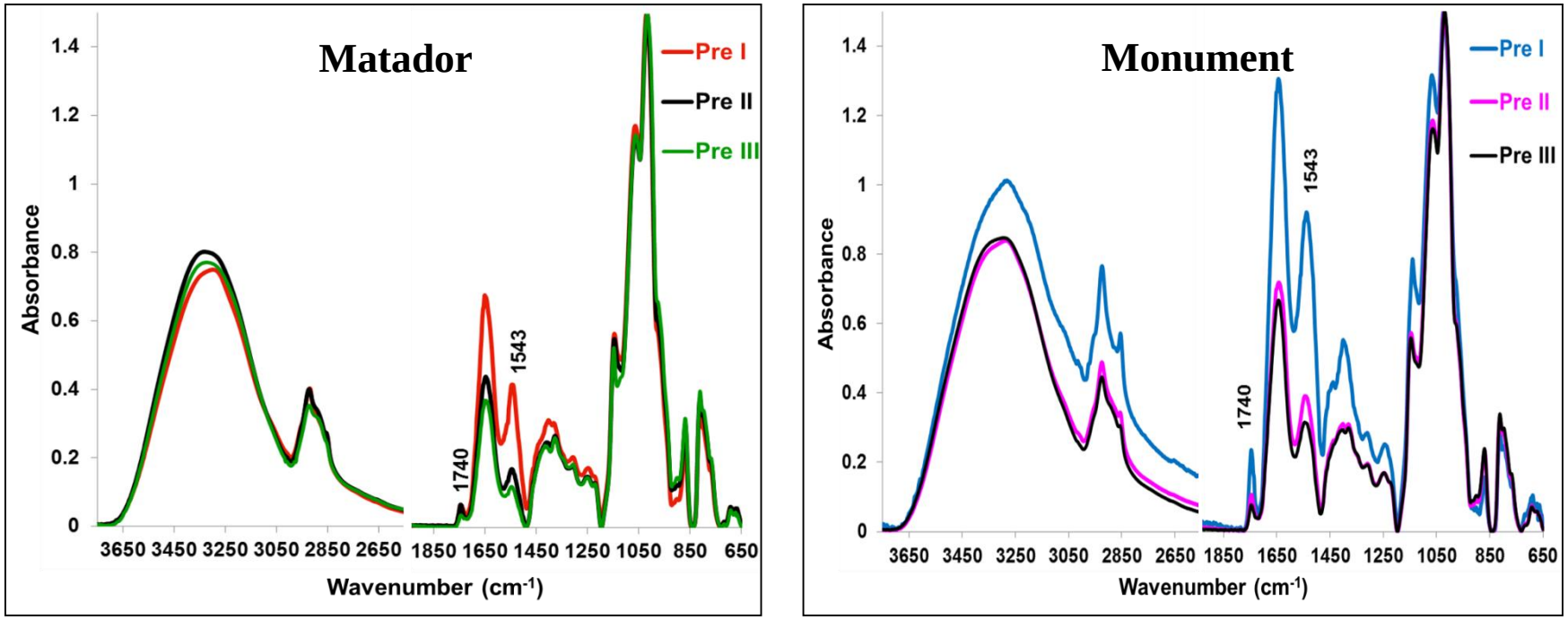
Grind endosperms and sieve the powder

↓

Store the endosperm powder in refrigerator at 4°C

Extraction of Guar Gum

2. Ethanol Precipitation of Whole Guar Seed Powder



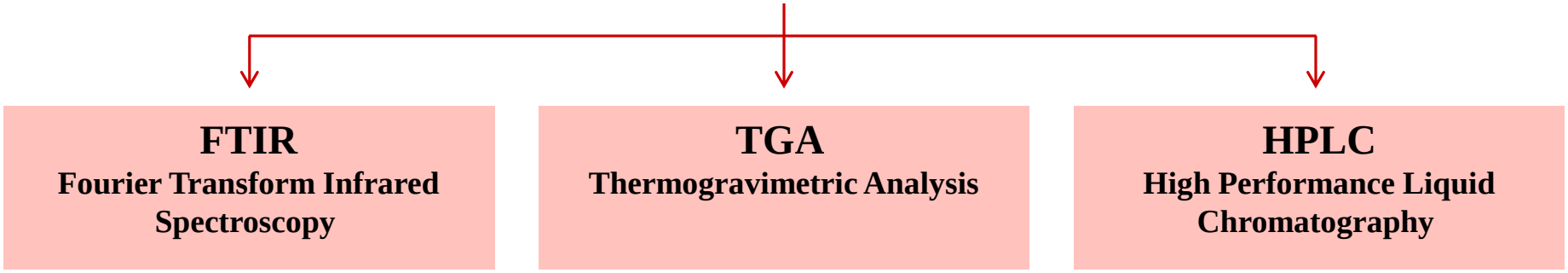
FTIR Spectra of Ethanol precipitated Matador and Monument whole seed powder

- **Level of contamination is high with this method**
- **1740 and 1543 cm^{-1} are attributed to C=O and NH_2 functional groups respectively (from ester, amino acids or proteins)**
- **Repetitive ethanol precipitation reduces the level of contamination**

Characterization of Guar Gum

Characterization of Guar Gum

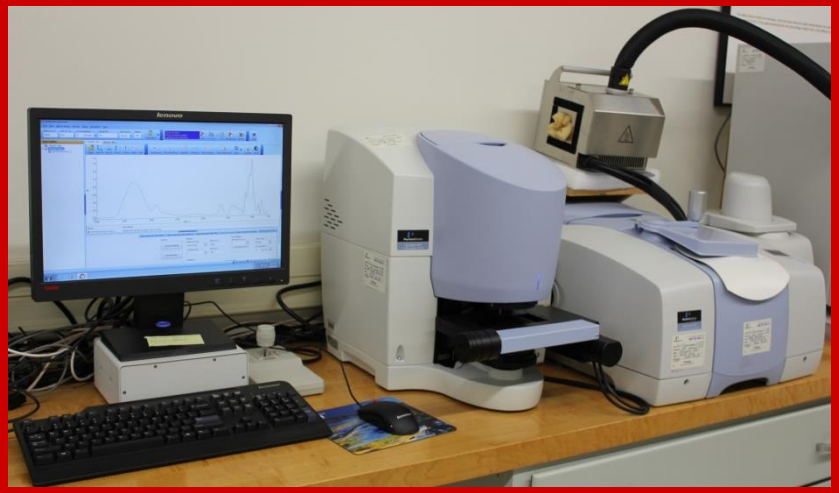
1. Matador endosperm powder **(MAT)**
2. Monument endosperm powder **(MON)**
3. Guar galactomannan **(GGM)**
4. Food grade guar gum **(FGG)**



* The galactomannan samples extracted by ethanol precipitation of whole seed powder were not further characterized in this study

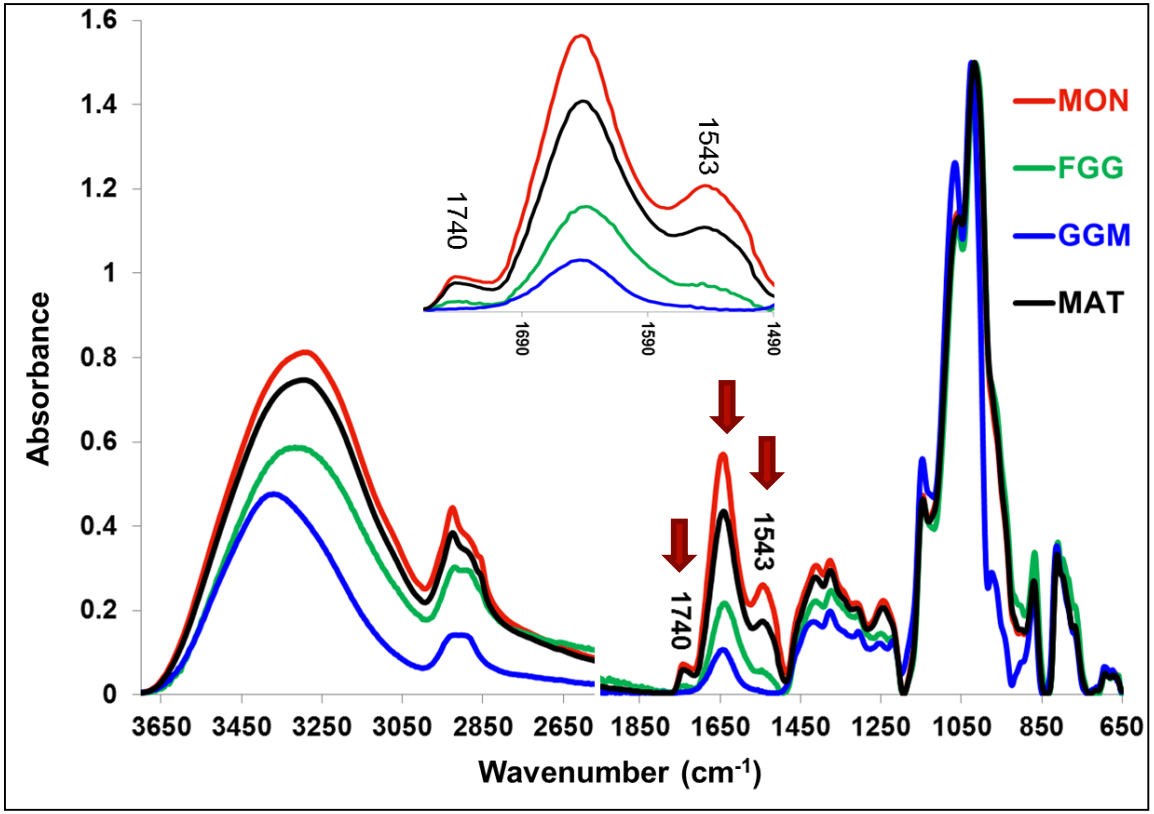
FTIR Study:

Purity of guar gum



Pictures:
Fiber and Biopolymer Research Institute - Texas Tech University

FTIR Study



FTIR Spectra of Guar Gum

- **FTIR spectra were collected for FGG, MAT, MON, GGM**
- **Vibrations 1740 and 1543 cm^{-1} are attributed to C=O and NH_2 functional groups respectively (from ester, amino acids or proteins)**

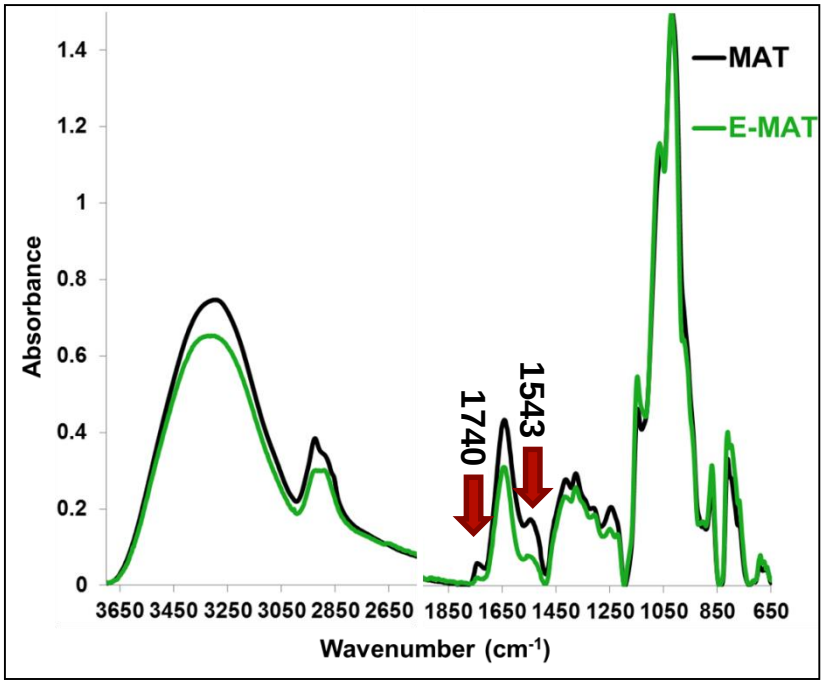
FTIR Study

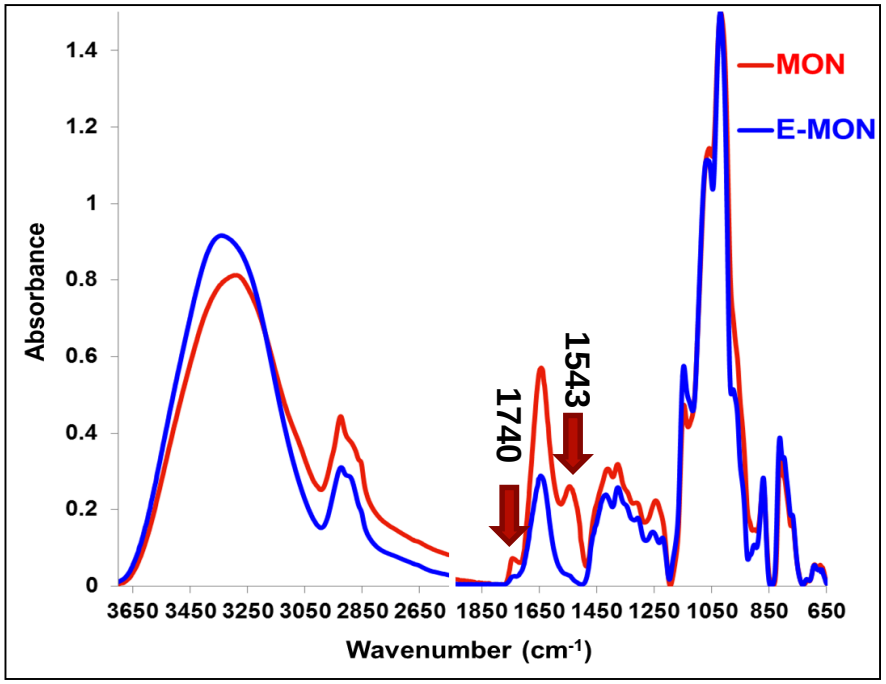
(MAT, MON)

+ MilliQ Water (80°C) → Filter → Ethanol →

→

(E-MAT, E-MON)





FTIR spectra of MAT & E-MAT (left), MON & E-MON (right)

TGA Study:

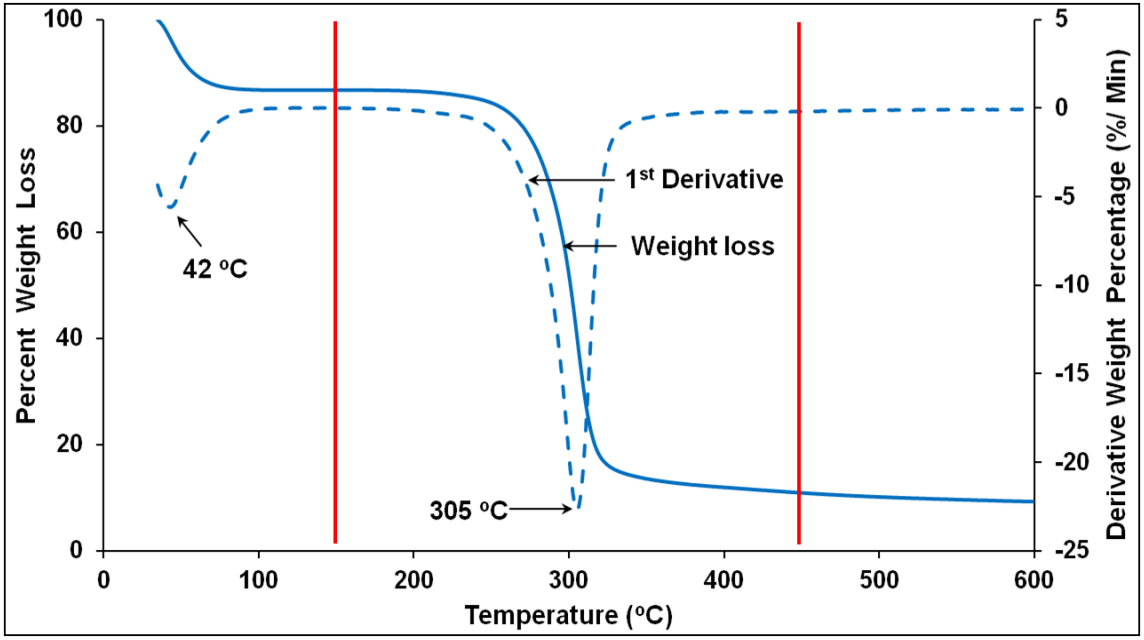
Thermal Behavior of Guar Gum

Pictures:
Fiber and Biopolymer Research Institute - Texas Tech University

Thermal Behavior of Guar Gum

- All samples were conditioned at 21±1°C and 65±2% RH for at least 24 hrs
- Temperature range: 40 to 600°C
- Heating rate : 10°C/ min
- Atmosphere : Nitrogen
- Weight losses of materials were recorded
- Number of replicates: 3

Thermal Behavior of Guar Gum



TGA Thermogram of GGM

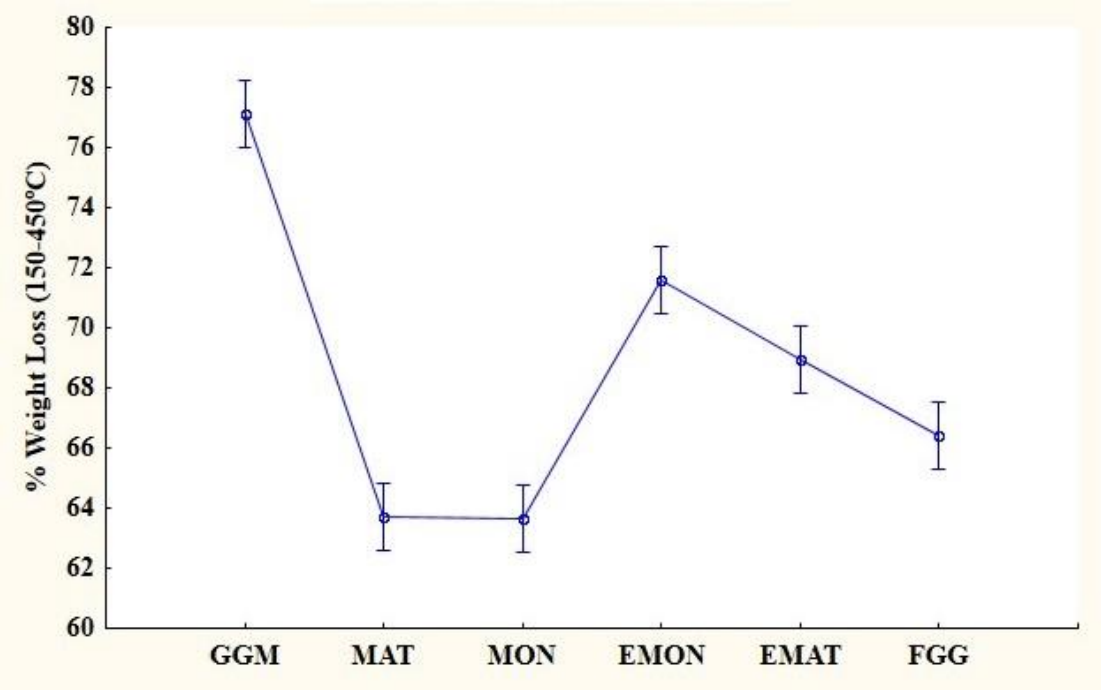
- **Weight loss at ~ 42°C and ~ 305°C are attributed to evaporation of water and degradation of galactomannan respectively**

Percent weight loss between 150 - 450°C

Galactomannan degradation temperature

} **ANOVA**

Weight Loss Between 150- 450°C
(Degradation of Galactomannan)



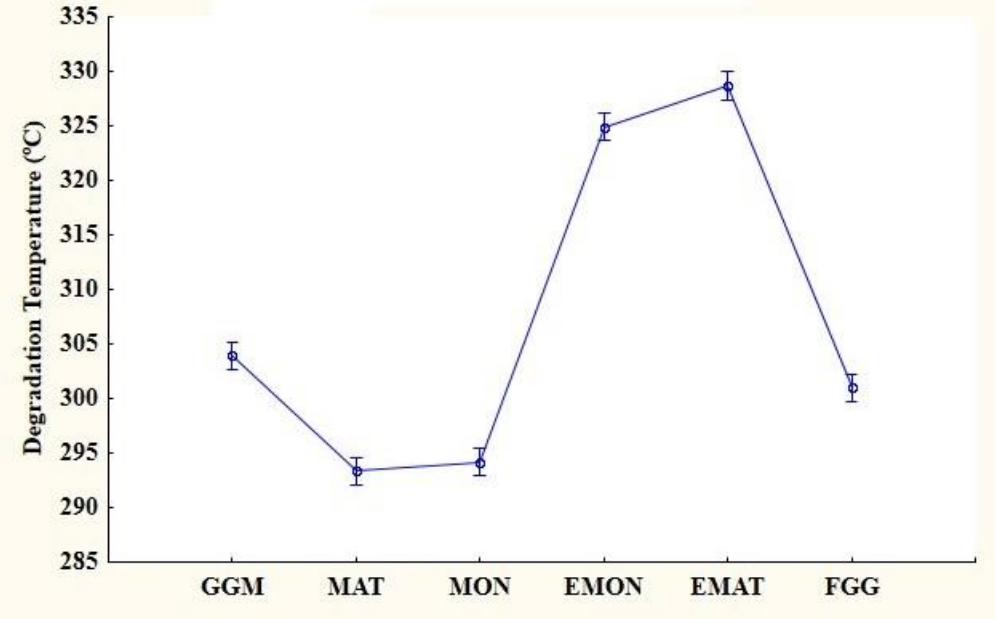
Sample ID	Mean weight loss (%)
GGM	77.1 a
E-MON	72.6 b
E-MAT	68.9 c
FGG	66.4 d
MON	63.7 e
MAT	63.6 e

Values not followed by the same letter are significantly different with α = 0.05

- **The percent weight losses in the temperature range of 150- 450°C are significantly different (α = 0.05)**
- **The percent weight loss in the second region could be an indication of the galactomannan content (purity) of the material**

  (GGM > E-MON > E-MAT > FGG > MAT/MON)

Galactomannan Degradation Temperature



Parameter	Temperature (°C)
E-MAT	328.7 a
E-MON	324.9 b
GGM	303.9 c
FGG	300.9 d
MON	294.1 e
MAT	293.3 e

Values not followed by the same letter are significantly different with α = 0.05

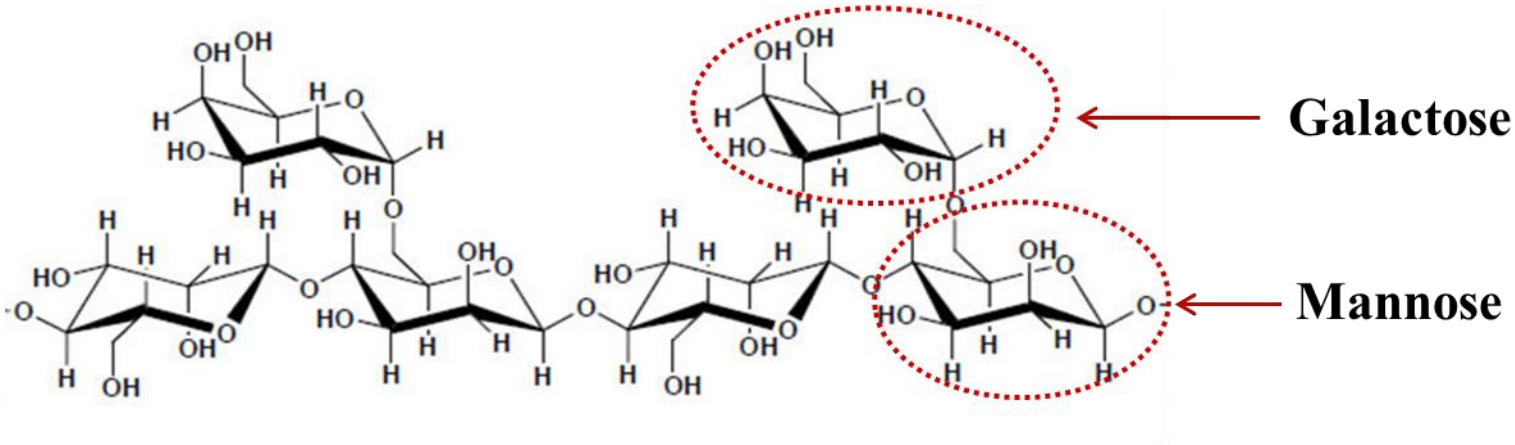
- **The degradation temperatures of the samples are significantly different (α=0.05)**
- **Impurities with a lower degradation temperature may induce galactomannan to degrade at an early temperature**
- **Ethanol precipitation has significantly increased the thermal stability of endosperm samples in both cultivars**
- **Removal of impurities as well as the mannose to galactose ratio of the samples may have great impact on the improved thermal stability**

HPLC Study:

Mannose to Galactose Ratio (M/G) of Guar Gum

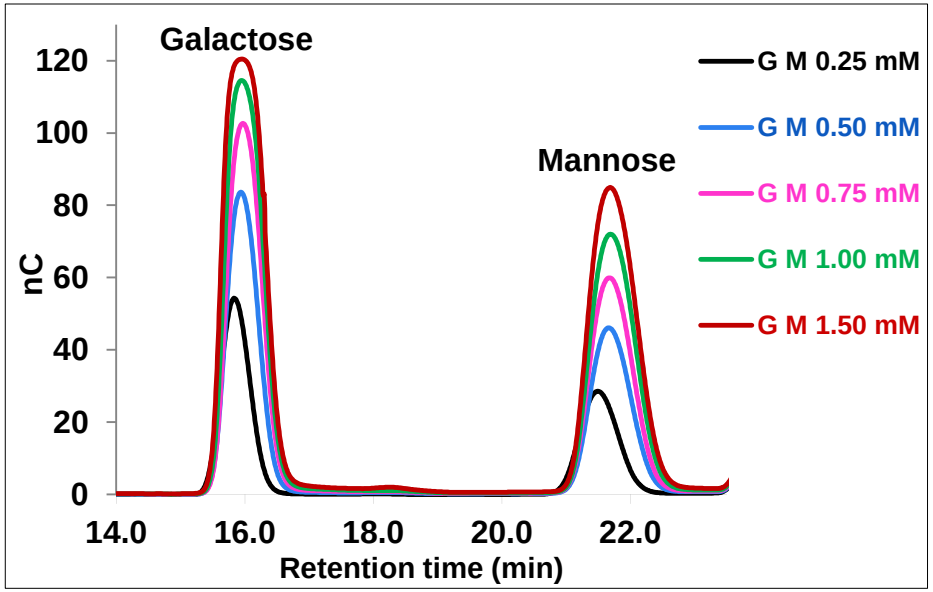
Pictures:
Fiber and Biopolymer Research Institute - Texas Tech University

Chemical Structure of Guar Galactomannan

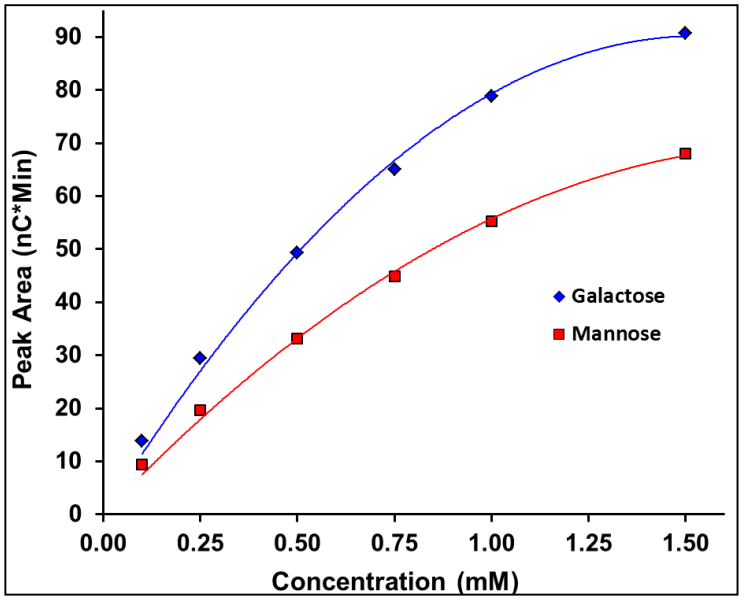


Picture source : http://www.fao.org/fileadmin/templates/agns/pdf/jecfa/cta/69/Guar_gum.pdf.

M/G Ratio of Guar Gum



Chromatogram of Mannose and Galactose with different concentrations



Calibration Curves for Mannose and Galactose

- Calibration curves for galactose and mannose were developed using different concentrations of both sugars
- Well-separated peaks with different retention times indicate:
  - CarboPac PA10 column is suitable for identification of galactose and mannose
  - The detector is suitable for quantification of galactose and mannose

M/G Ratio of Guar Gum

- **10 mg of GGM was hydrolyzed using Trifluoroacetic Acid (TFA) and Sulfuric Acid (H_2SO_4)**

  A. **2N TFA at 120°C for 2 hrs:** **Wide overloaded peaks**

     M/G ratio: 2.46

  B. **1N TFA at 120°C for 2 hrs:** **Wide overloaded peaks**

     M/G ratio: 1.72

  C. **0.5M H_2SO_4 at 100°C for 3 hrs:** **Well-separated peaks**

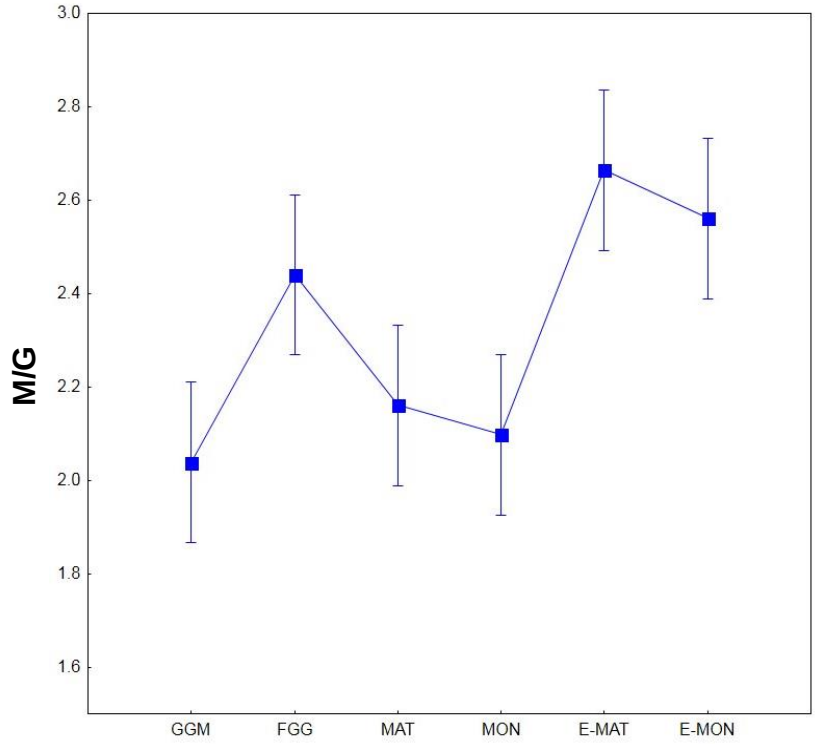
     M/G ratio: 1.94

- **Number of replicates: 3**

M/G Ratio of Guar Gum

- **10 mg of GGM, FGG, MAT, MON, E-MAT and E-MON samples were hydrolyzed using 500 µL of 0.5M H_2SO_4 at 100°C for 3hrs : 3 replicates**
- **Samples were dried in SpeedVac at 40°C**
- **Samples were dissolved in 300µL of methanol and dried in SpeedVac**
  - **To remove methanol soluble compounds**
  - **To prevent microbial degradation of sugars**
- **Samples were dissolved in 500µL of milliQ water**
- **Samples were analyzed using HPLC**

M/G Ratio of Guar Gum



Parameter	*df*	*F*	P	M/G
Intercept	1	5248.7	0.000001	
ID	5	11.2	0.000354	
GGM				2.04 b
MON				2.10 b
MAT				2.16 b
FGG				2.44 a
E-MON				2.56 a
E-MAT				2.66 a
Error	12			

Values not followed by the same letter are significantly different with α = 5% (according to Newman- Keuls test)

- **The M/G ratios of the materials are significantly different ($\alpha = 0.05$)**
- **Varietal differences and purification method could change the M/G ratio**
- **Ethanol purification may have removed galactose side branches which may result in significantly high M/G ratios in E- MAT and E-MON**

Conclusions

- **Guar gum is extracted with a high level of purity with two methods**
  1. **Manual endosperm extraction followed by ethanol precipitation**
  2. **Repetitive ethanol precipitation of aqueous solution of whole guar seed powder**
- **FTIR can be used as a fast and non-destructive method to investigate the purity of guar gum**
- **TGA study confirms that the thermal behavior of MAT and MON are not significantly different**

Conclusions

- **Guar gum can be hydrolyzed using 0.5M H_2SO_4 at 100^oC for 3 hours**
- **HPLC study confirms that the M/G ratios of MAT and MON are not significantly different**
- **Method of purification has a significant influence on the M/G ratio and thermal stability of guar gum;**

  Ethanol precipitation has significantly increased the M/G ratio and the thermal stability of both MAT and MON

Thank you

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