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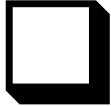
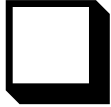
# **Potential of Near Infrared Spectroscopy (NIR) Using Quantitative Analysis of Sugars in Molasses and High Test Molasses**

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# Outline

-  **Introduction**
-  **Objective**
-  **Methodology**
-  **Results**
-  **Conclusion**

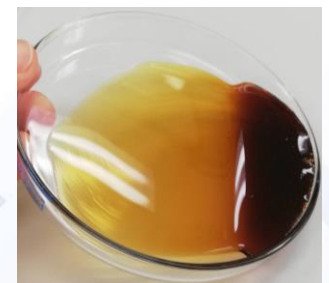
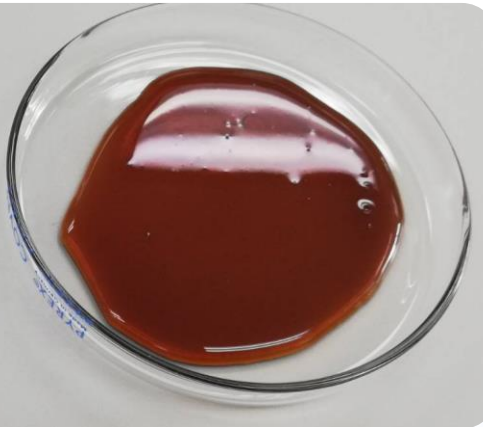
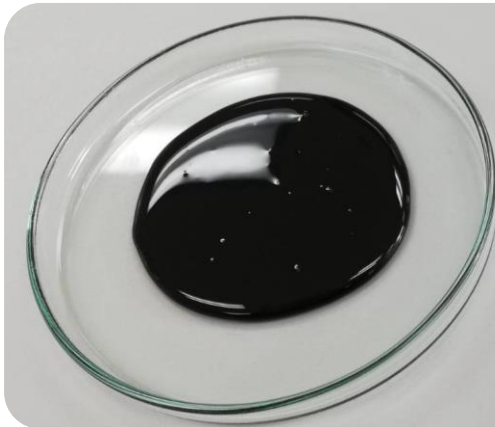


# Introduction

**Molasses and High Test Molasses (HTM) are used as raw material for ethanol production by fermentation process of fermentable sugars.**

**Molasses** is by product from sugar production

- Brix > 75
- Fermentable Sugars (Sucrose, Glucose, Fructose) 35-55%



**High Test Molasses (HTM)** is produced directly from sugarcane juice by enzymatic process

- Brix 65-75
- Fermentable Sugars (Sucrose, Glucose, Fructose) 45-75%



# Introduction

- The variation of molasses quality was found throughout the season depend on storage conditions.



- Storage Tank 2 Cubic Meters
- Average Molasses Temperature 30 °C
- FS Reduction rate = 0.78 % / Month
- Storage Tank 10,000 Cubic Meters
- Average Molasses Temperature 38°C
- FS Reduction rate = 0.96% / Month

Ref: Nootas . (2016)

- Amount of fermentable sugars is very important for fermentation process optimization.
- The rapid determination method of fermentable sugar in molasses is need.



# Introduction

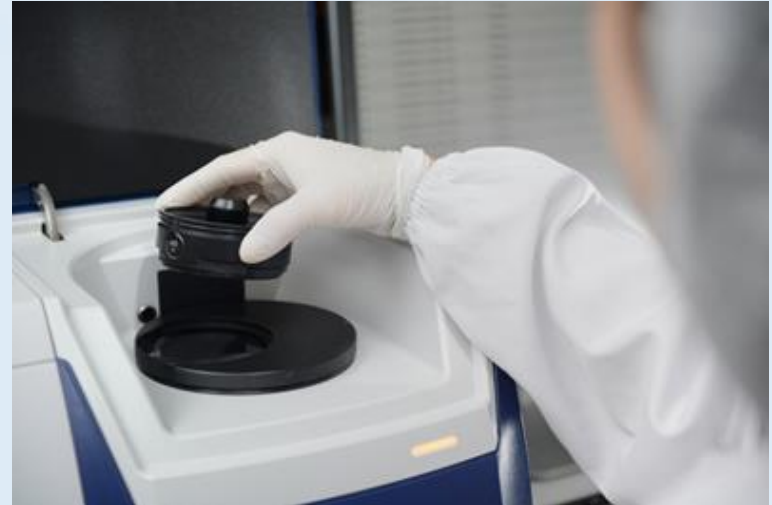


## Reference Method

Using High Performance Liquid  
Chromatography (HPLC)



Need About 1 Hour  
for FS Analysis



## NIR Method

Using NIR Equation which developed  
from Spectrum and HPLC Result

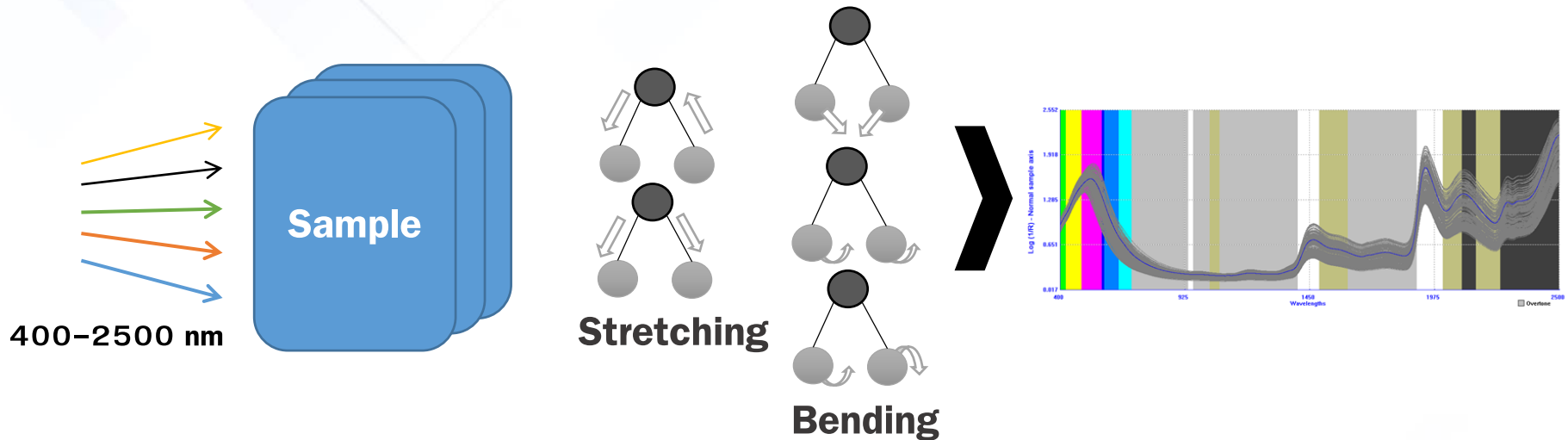


**Less than 1 Minute**  
for FS Analysis



# Introduction

- NIR spectroscopy is a rapid technique which has been increasingly used for the analysis of various products and been widely applied in many fields.



**Difference absorbance depend on different components of sample**

**Spectra for sample**

Using correlation of NIR spectra and components in molasses to develop NIR equations for prediction of molasses quality.



# Introduction

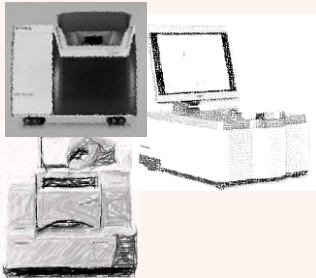
Standard Analysis Method

**HPLC**



**Start of NIR**

1. Develop Equation



**HPLC**



**NIR**



2. Apply to normal analysis

3. Update

**NIR**



**HPLC**

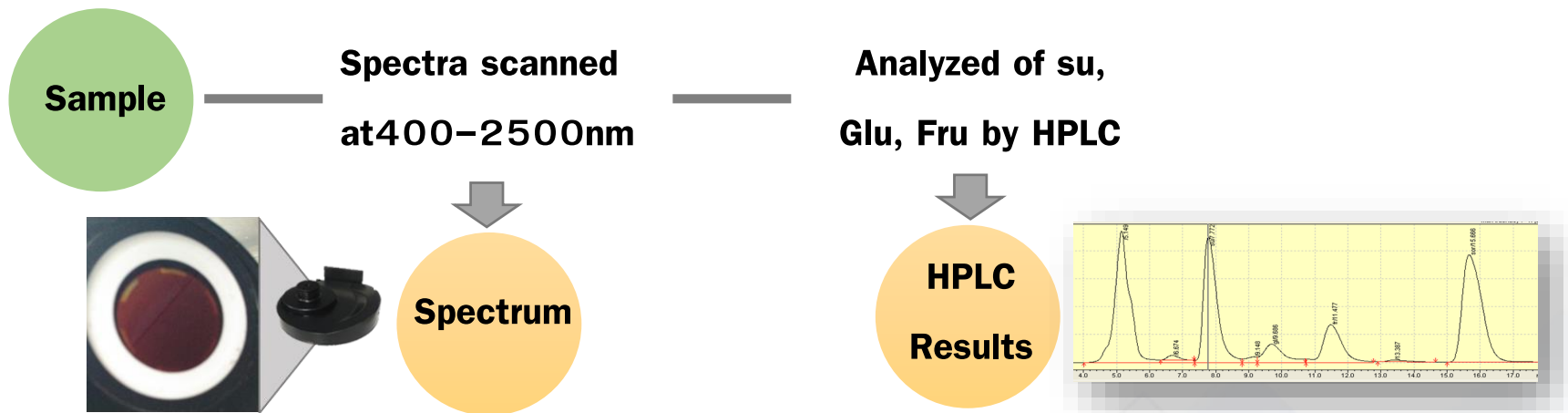




# Objective

To develop near infrared spectroscopy (NIR) for quantitative analysis of fermentable sugars in molasses and high test molasses.

# Methodology

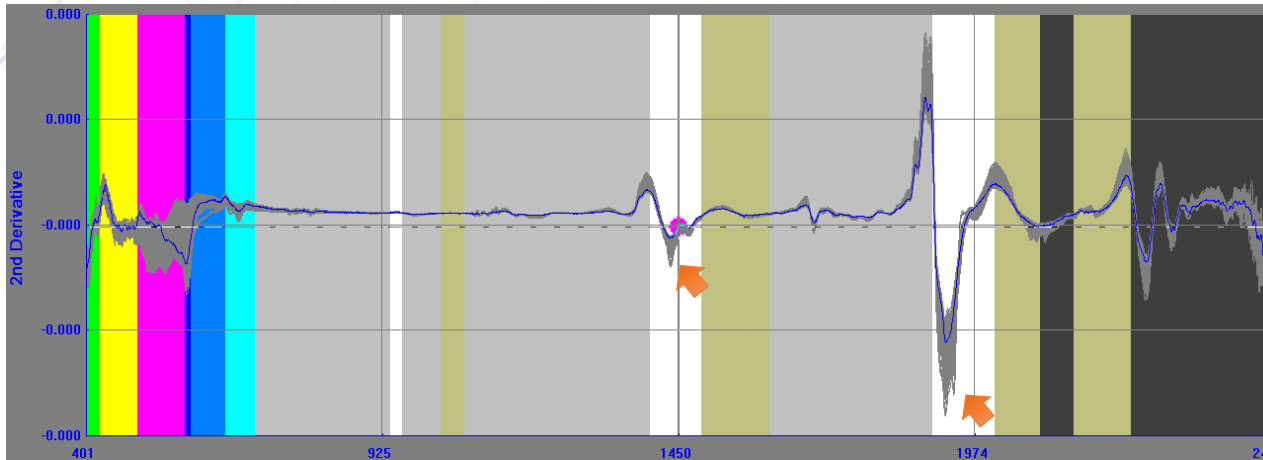


70% of Datas (Spectrum & HPLC Results) used for NIR equation.

30% of Datas (Spectrum & HPLC Results) used for equation validation.

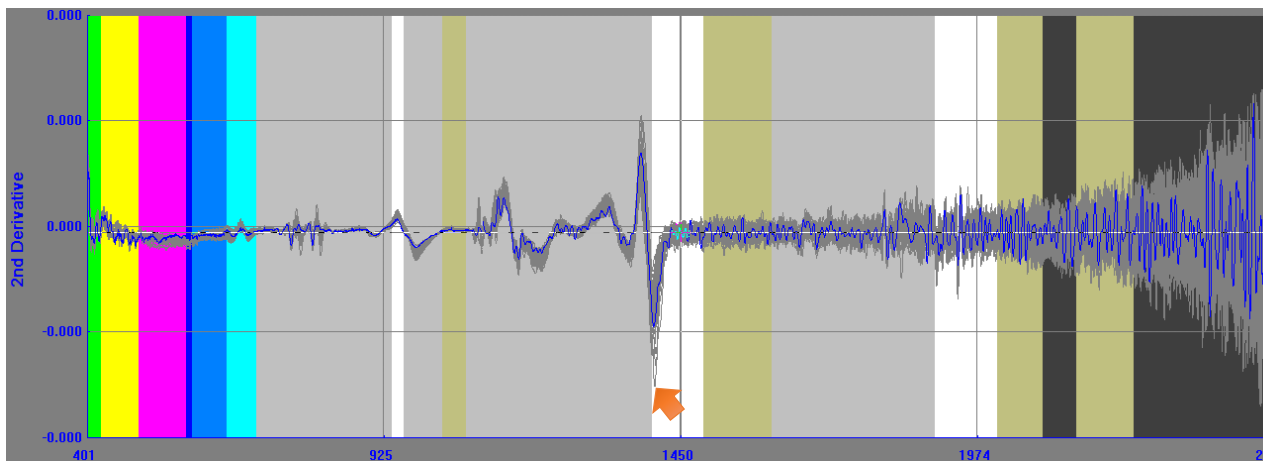


## 2<sup>nd</sup> derivative spectrum of molasses and high test molasses.



### Molasses

Found dominate at 1450 , 1940 nm, related to the first overtone of O-H stretching, and the combination O-H stretch and bending.

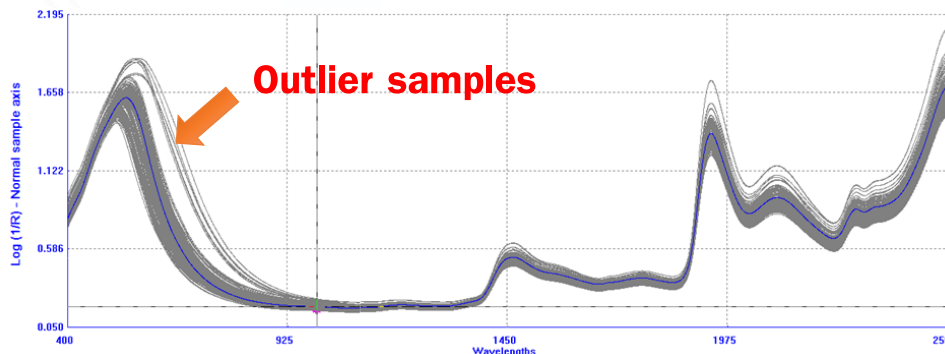


### High Test Molasses

Found dominate at 1402 (first overtone of O-H stretching and the combination C-H stretching)



NIR was used for **molasses** quality analysis in Mitr Phol since 2017, focusing on fermentable sugars in molasses for ethanol production.



Annually update is processed to improve the NIR equation.

**Outlier samples** were analyzed by HPLC

70% of **outlier samples** were merged to the recent NIR equations the updated.

Validate equation.

30% of **outlier samples** were merged to the recent validation set



**Table 1. External validation results of NIR for sucrose glucose and fructose after updated comparing to before update. (N = 100 validation samples)**

Items	Sucrose		Glucose		Fructose	
	Update	Recent	Update	Recent	Update	Recent
<b>Bias</b>	0.121	0.179	-0.003	0.152	-0.029	0.030
<b>SEP</b>	1.601	1.622	0.844	0.893	0.727	0.766
<b>RSQ</b>	0.642	0.636	0.712	0.684	0.636	0.594
<b>p-value</b>	0.45	0.27	0.98	0.086	0.69	0.70

- The updated equation showed good trends by SEP and Bias were lower than results of recent equations.
- There were not found statically different between HPLC results and NIR results both from recent equations and update equations for sucrose, glucose and fructose.



# Results

Fig1. Showed Residual (HPLC – NIR) of sucrose in 100 external validation samples.



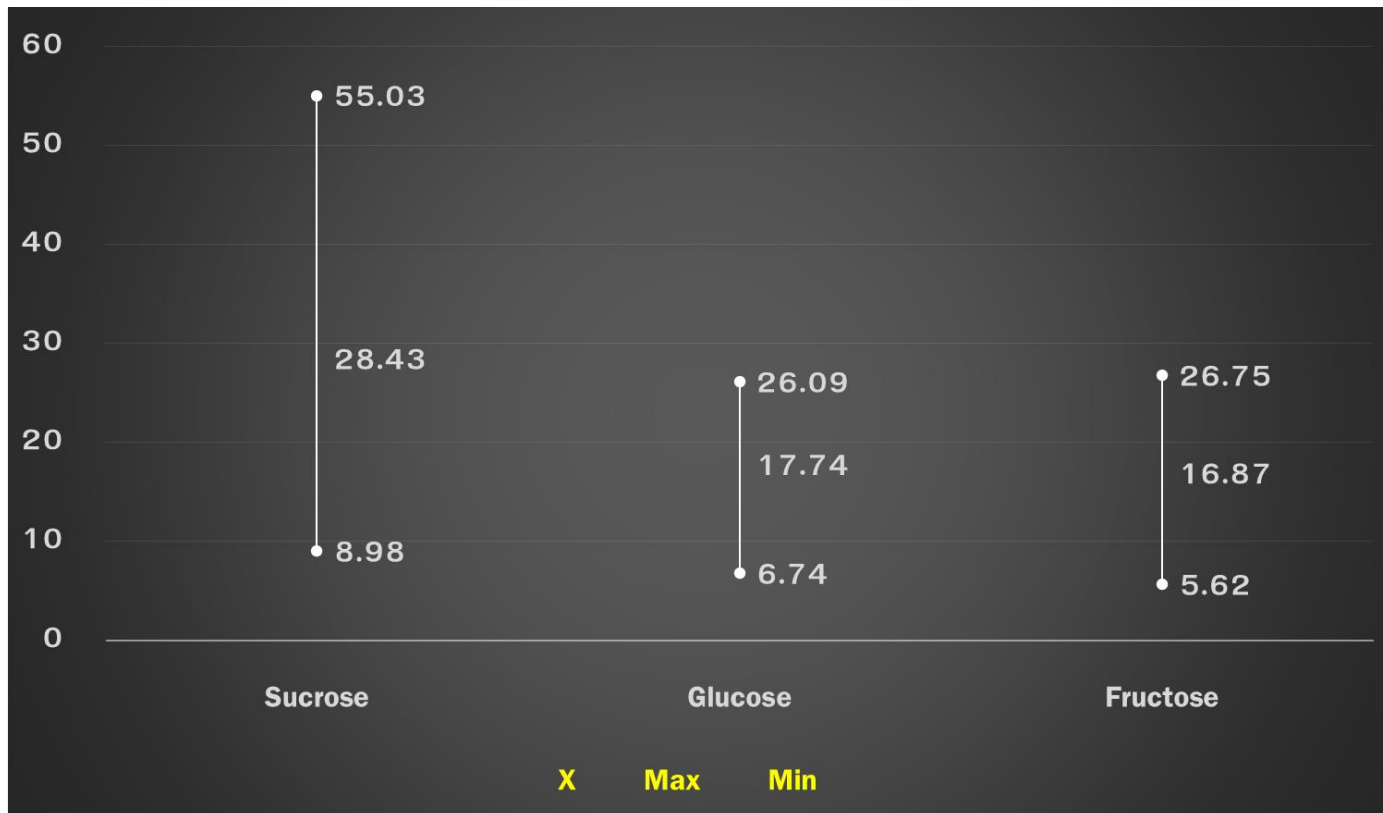
**Acceptable range of residual for sucrose is  $\pm 1.5$  as yellow lines.**

- Results of update equation showed 13% out of acceptable range.
- Results of recent equation showed 23% out of acceptable range.



NIR equations for high test molasses (HTM) were developed by the same procedure as NIR for molasses

Fig2. Showed average (x), maximum and minimum value of calibration set (N =68 samples)





**Table 2. External validation results of sucrose glucose and fructose.**

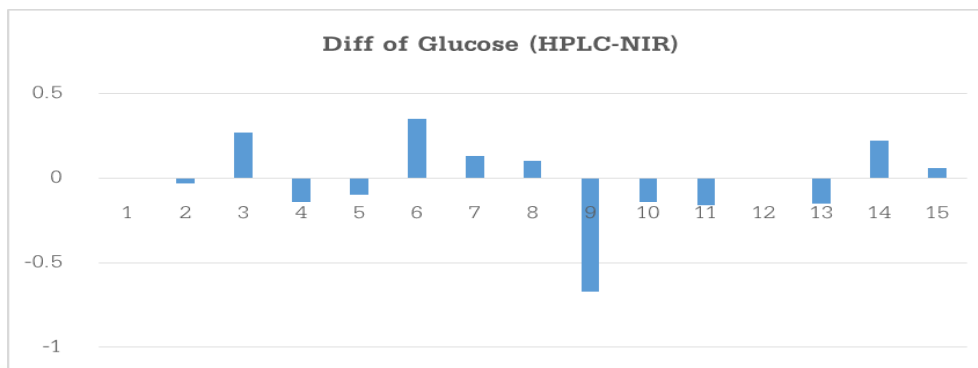
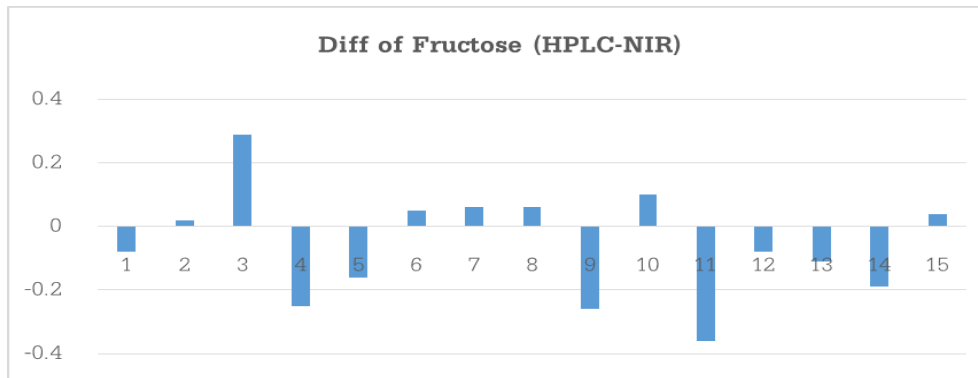
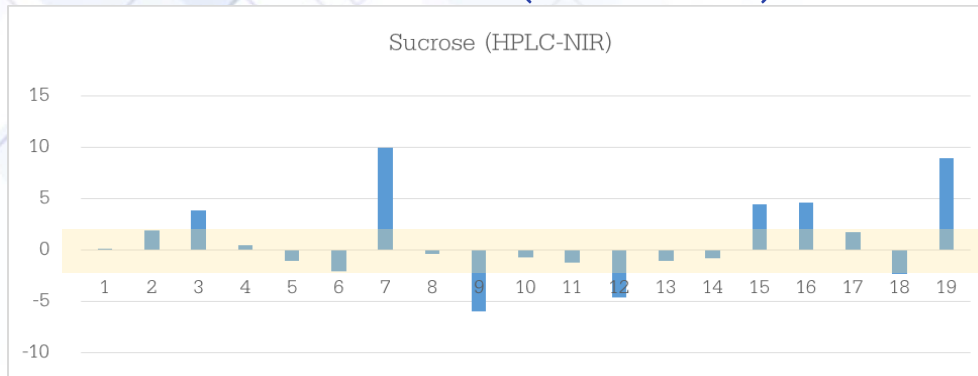
<b>High Test Molasses</b>	<b>N</b>	<b>HPLC (Average)</b>	<b>NIR (Average)</b>	<b>R<sup>2</sup></b>	<b>Bias</b>	<b>SEP</b>	<b>SEL</b>	<b>p-value</b>
<b>Sucrose (%)</b>	19	50.326	51.069	0.865	-0.744	1.734	1.16	0.22
<b>Glucose (%)</b>	15	4.050	4.174	0.964	-0.059	0.171	0.13	0.13
<b>Fructose (%)</b>	15	3.177	3.194	0.929	-0.018	0.235	0.09	0.78

There were not found statically different between HPLC results and NIR for sucrose, glucose and fructose.



# Results

**Fig3. Showed Residual (HPLC – NIR) of sucrose, glucose and fructose in external validation of HTM samples.**



❑ Residual of sucrose is out of acceptable range by 7/19 (Acceptable range  $\pm 1.5$ )

❑ Residual of fructose and glucose showed in acceptable range.

❑ More samples are need for NIR equation improvement.



# Conclusion

- ❑ NIR spectroscopy is a feasible method for the rapid analysis of sucrose, glucose, fructose in molasses.
  
- ❑ NIR spectroscopy have possibility for the rapid analysis of sucrose, glucose, fructose in high test molasses. More samples are collecting for further NIR equation improvement.
  
- ❑ The update is technically need for NIR equation improvement.



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Thank you for  
your attention.