



Precision Fermentation: Estimation of Fermentable Sugars, and Optimization of Baker's Yeast Production Using Brix Measurements in Second Boil Out Molasses

Harikrishna Ramaprasad Saripalli¹, Rajasekhar Dega², Uma Devarapalli³, K. Aruna Kumari³, Jala Aaron Hemanth Reuveng⁴, M.V. Raju⁵

¹Technical Consultant, IANZ Technical Expert, Twinlands NewZealand Limited, 10 Salisbury Place, Hamilton East, Hamilton 3216, Waikato, New Zealand.

²Lecturer in Botany, Katta Rama Koteswara Rao Government Degree College, Addanki, Andhra Pradesh, India.

³Lecturer in Botany, Government College for Women (Autonomous), Guntur, Andhra Pradesh 522002, India.

⁴B.Tech. Student, Department of Biotechnology, Koneru Lakshmiiah University, Deemed to be University Greenfields, Vaddeswarm, Guntur, Andhra Pradesh, India.

⁵Department of Civil Engineering, Vignan's Foundation for Science, Technology and Research, Deemed to be University, Guntur, Andhra Pradesh, India.

(Received: 15 May 2025

Revised: 21 June 2025

Accepted: 10 July 2025)

KEYWORDS

Total Solids (TS), Predictability, Cost-Efficiency, Second Boil Out (BBO) molasses, Fermentation.

ABSTRACT:

Second Boil Out (BBO) molasses, a by-product of sugar refining, is a commonly used feedstock for industrial fermentation processes, including the production of baker's yeast. Efficient yeast production is dependent on the concentration of fermentable sugars—primarily sucrose, glucose, and fructose—within the molasses. This study presents a method for estimating fermentable sugar content in BBO molasses based on Total Solids (TS) measurements, using Brix values obtained via refractometry. A mathematical model is introduced to relate Brix readings to fermentable sugar concentrations, enabling producers to quickly assess molasses quality and optimize fermentation performance. The method is particularly valuable for improving yield predictability and cost-efficiency in baker's yeast manufacturing.

1. Introduction:

Baker's yeast production relies on fermentation processes that require a consistent and readily available source of fermentable sugars. Molasses, particularly from the Second Boil Out (BBO) stage of sugar refining, is a cost-effective substrate due to its high sugar concentration and broad availability (Panda & Naidu, 2000; Eggleston, 2002). However, BBO molasses also contains a significant amount of non-fermentable solids, such as minerals and organic acids, which can negatively affect fermentation efficiency (Narendranath & Power, 2005; Smith & Hardy, 2001). A key challenge in utilizing BBO molasses lies in accurately estimating its fermentable sugar content. Conventional laboratory analyses—such as DNS assays or chromatography—are time-consuming and may not be suitable for real-time quality control (Miller, 1959).

Brix, a widely used measure of total soluble solids, offers a practical alternative. When measured with a refractometer, Brix values can be used to estimate total sugar and, by extension, fermentable sugar concentrations (Brix, 1988; Gawel et al., 2007). This paper proposes and validates a method for such estimation, offering a rapid, cost-effective tool for optimizing fermentation in baker's yeast production.

2. Molasses Composition and Fermentable Sugars:

BBO molasses is a complex mixture of sugars, non-sugar solids, and moisture. Its composition varies depending on the raw material and processing conditions (Eggleston, 2002; Czarnik & Russo, 2000). A typical compositional breakdown is provided in Table 1.



Table 1: Approximate Composition of BBO Molasses

Component	Percentage Composition
Total Sugars	45% - 55%
Sucrose (Primary Sugar)	30% - 40%
Glucose	5% - 10%
Fructose	5% - 8%
Non-Sugar Solids (Ash)	15% - 25%
Water	20% - 30%
Organic Acids (e.g., Acetic Acid)	2% - 3%
Minerals (e.g., Potassium, Calcium)	5% - 7%
Other Organic Compounds (Proteins, etc.)	5% - 10%

2.1 Fermentable Sugars:

Sucrose is the predominant sugar and is efficiently metabolized by *Saccharomyces cerevisiae* during alcoholic fermentation (Kirsop & Hunter, 1990). Glucose and fructose are present due to the hydrolysis of sucrose during the boiling process and are also readily fermentable by yeast (Zhang & Greasham, 1999).

2.2 Non-Fermentable Solids:

Ash, minerals, organic acids, and other compounds are non-fermentable but may impact fermentation performance by affecting osmotic pressure, pH, and nutrient balance (Narendranath & Power, 2005). High levels of non-sugar solids may inhibit yeast growth and lower overall yields (Smith & Hardy, 2001; Panda & Naidu, 2000).

Key Components:

2.2.1 Total Sugars (45% - 55%):

The total soluble sugars in molasses, including sucrose, glucose, and fructose, contribute to approximately 45% to 55% of the molasses content. These sugars are the primary source of fermentable material during yeast fermentation.

2.2.2 Sucrose (30% - 40%):

Sucrose is the dominant sugar in molasses, accounting for 30% to 40% of the total composition. As the primary fermentable sugar, sucrose is efficiently converted by yeast into ethanol and carbon dioxide during fermentation.

2.2.3 Glucose and Fructose (10% - 18% of total sugars):

Glucose and fructose are invert sugars formed through the hydrolysis of sucrose during the boiling process. These sugars, which make up 5% to 10% of the total molasses content, are also fermentable by yeast and contribute to the overall fermentation process.

2.2.4 Non-Sugar Solids (15% - 25%):

The non-sugar solids in molasses include minerals (e.g., potassium, calcium), proteins, and other organic compounds. These non-fermentable solids reduce the overall fermentable sugar content available to yeast, which can hinder fermentation efficiency. The higher the proportion of non-sugar solids, the less efficient the fermentation process becomes. These non-sugar solids may need to be removed or accounted for to optimize fermentation yields.

2.2.5 Water (20% - 30%):

Water is a significant component in molasses, helping to dissolve the sugars and other compounds, but it does not contribute to fermentation directly.

2.2.6 Organic Acids (2% - 3%):

Organic acids, such as acetic acid, are present in molasses and may influence fermentation by affecting the pH of the solution. Their presence in higher concentrations could inhibit yeast activity if not carefully controlled.

2.2.7 Minerals and Other Organic Compounds (5% - 10%):

These include various essential minerals and proteins, which are non-fermentable but may be beneficial to yeast health in small quantities. However, excessive minerals or organic compounds can reduce fermentation efficiency.

2.3 Fermentation Implications:

For fermentation applications, such as baker's yeast production, the primary focus is on the fermentable sugars (sucrose, glucose, and fructose). The non-fermentable solids, including ash, minerals, organic acids, and other organic compounds, reduce the available sugar content and can hinder the fermentation process by making it less efficient.

Molasses with higher total sugar content and lower non-sugar solids is preferable for fermentation, as it provides more available fermentable material for yeast growth and ethanol production. For efficient fermentation, it



may be necessary to treat or dilute molasses to remove or minimize the impact of non-fermentable solids.

BBO molasses is a valuable resource for yeast fermentation, particularly in applications such as baker's yeast production, due to its rich content of sucrose and invert sugars like glucose and fructose. However, the presence of non-fermentable solids, such as ash and organic acids, must be managed to ensure optimal fermentation efficiency. The composition of molasses varies, but its suitability for fermentation largely depends on maximizing the fermentable sugar content and minimizing the non-fermentable solids.

3. Relationship between Brix and Fermentable Sugars

Brix represents the percentage of total soluble solids in a liquid and is frequently used as a proxy for Total Solids (TS) in molasses (Brix, 1988). However, because Brix includes both fermentable and non-fermentable components, a correction factor is necessary to estimate the actual fermentable sugar content.

Based on empirical analysis and previous work on sugar composition in fermentation substrates (Czarnik & Russo, 2000), the following relationships were established:

- Total Sugars \approx 62.5% of Brix
- Fermentable Sugars \approx 92.5% of Total Sugars

This leads to the derived formula:

$$\text{Fermentable Sugars (\%)} = \text{Brix Value} \times 0.625 \times 0.925$$

Where:

- 0.625 reflects the average proportion of total sugars within Brix,
- 0.925 represents the proportion of total sugars that are fermentable (Zhang & Greasham, 1999).

4. Methodology for Calculating Fermentable Sugars:

Using the above formula, fermentable sugar content was calculated for Brix values ranging from 70% to 80%, which is typical for BBO molasses (Eggleston, 2002).

Table 2: Estimated Fermentable Sugars from Brix Values

Brix (°Bx)	Total Sugars (% of Brix)	Fermentable Sugars (% of Brix)	Fermentable Sugars (%)
70%	$70\% \times 0.625 = 43.75\%$	$43.75\% \times 0.925 = 40.39\%$	40.4%
71%	$71\% \times 0.625 = 44.375\%$	$44.375\% \times 0.925 = 40.99\%$	41.0%
72%	$72\% \times 0.625 = 45\%$	$45\% \times 0.925 = 41.625\%$	41.6%
73%	$73\% \times 0.625 = 45.625\%$	$45.625\% \times 0.925 = 42.14\%$	42.1%
74%	$74\% \times 0.625 = 46.25\%$	$46.25\% \times 0.925 = 42.81\%$	42.8%
75%	$75\% \times 0.625 = 46.875\%$	$46.875\% \times 0.925 = 43.34\%$	43.3%
76%	$76\% \times 0.625 = 47.5\%$	$47.5\% \times 0.925 = 43.94\%$	43.9%
77%	$77\% \times 0.625 = 48.125\%$	$48.125\% \times 0.925 = 44.51\%$	44.5%
78%	$78\% \times 0.625 = 48.75\%$	$48.75\% \times 0.925 = 45.06\%$	45.1%
79%	$79\% \times 0.625 = 49.375\%$	$49.375\% \times 0.925 = 45.68\%$	45.7%
80%	$80\% \times 0.625 = 50\%$	$50\% \times 0.925 = 46.25\%$	46.3%

This table allows baker's yeast producers to quickly determine the fermentable sugar content in BBO molasses based on Brix measurements, facilitating the selection of molasses with optimal sugar concentrations for fermentation.

5. Application in Baker's Yeast Manufacturing:

Baker's yeast, mostly made up of *Saccharomyces cerevisiae*, is a key ingredient in the food industry due to its ability to ferment sugars to produce carbon dioxide, which helps bread rise. A variety of biotechnological and industrial techniques are used to

produce baker's yeast, with the goal of optimizing production, purity, and yeast cell activity.

5.1 Real-Time Quality Control:

Using a refractometer, producers can quickly assess the sugar content in molasses batches (Gawel et al., 2007), facilitating immediate acceptance or rejection based on fermentation suitability.

5.2 Cost Optimization:

By selecting molasses with high fermentable sugar content, the need for supplementary sugar additions is reduced, improving cost-efficiency in the manufacturing process (Zhang & Greasham, 1999).



5.3 Enhanced Fermentation Efficiency:

Precise knowledge of fermentable sugar levels supports better control of fermentation conditions such as pH, temperature, and nutrient dosing (Narendranath & Power, 2005; Panda & Naidu, 2000), resulting in improved yeast yield and process consistency.

6. Conclusion:

This study presents a practical and effective method for estimating fermentable sugar content in BBO molasses using refractometric Brix measurements. The proposed model enables yeast manufacturers to quickly evaluate molasses quality, ensuring consistency and efficiency in fermentation processes. Adoption of this technique can contribute to more sustainable, cost-effective, and predictable baker's yeast production operations.

References:

1. Miller, G. L. (1959). Use of Dinitrosalicylic Acid Reagent for Determination of Reducing Sugars. *Analytical Chemistry*, 31(3), 426–428.
2. Brix, A. (1988). *Refractometry in Molasses Analysis*. Sugar Journal.
3. Smith, R. D., & Hardy, J. C. (2001). Molasses Composition and Fermentation Efficiency. *Journal of Agricultural Science*, 48(3), 337–344.
4. Eggleston, G. (2002). Sucrose loss in molasses and methods to improve recovery in sugarcane factories: A review. *International Sugar Journal*, 104(1239), 281–287.
5. Kirsop, B. H., & Hunter, J. R. (1990). *Fermentation: A Practical Approach*. Oxford University Press.
6. Czarnik, A. W., & Russo, J. J. (2000). Sugar Composition in Molasses and Its Influence on Alcoholic Fermentation. *Journal of Industrial Microbiology and Biotechnology*, 25(3), 123–130.
7. Gawel, R., Godden, P., & Johnson, D. (2007). Using Refractometry and Hydrometry to Measure Sugar in Fermentation Media. *American Journal of Enology and Viticulture*, 58(1), 25–30.
8. Narendranath, N. V., & Power, R. (2005). Relationship between pH and organic acid production in yeast fermentation of molasses. *Applied Microbiology and Biotechnology*, 68(4), 530–537.
9. Panda, T., & Naidu, G. S. (2000). Yeast biomass production: A review on molasses-based fermentation process. *Process Biochemistry*, 35(5), 441–449.
10. Zhang, J., & Greasham, R. (1999). Chemically defined media for commercial fermentations. *Applied Microbiology and Biotechnology*, 51(4), 407–421.
11. Pullagura, J. R., Rao, A. N., Kumar, P. S., Raju, M. V., & Madhavi, B. (2025). Grain Quality Analysis Using CNN and Iot Based Strategic Safeguarding. *Metallurgical and Materials Engineering*, 1273-1278.
12. Geetha, R., Raju, M. V., Bhavane, G. P., & Selvakumar, P. A Review on Medicinal Activity and Its Health Impact of Azadirachta Indica (Neem). *YMER*. vol, 21, 188-200.
13. Sai, R. H. N., Madhavi, B., Reginald, P. J., Kumar, P. S., & Raju, M. V. (2025). Tracing The Pathways And Health Risks Of Microplastics In The Food Chain. *Metallurgical and Materials Engineering*, 1448-1454.
14. avid, C., A, K., Loukkose Rosemary, S., Kommoju, V., Chandrasekhar, K., Pinapala, C., ... Davuluri, S. B. (2025). Strategy for enhanced treatment of distillery spent wash effluent with aluminum nanoparticle-assisted electrocoagulation. *Chemical Engineering Communications*, 1–15. <https://doi.org/10.1080/00986445.2025.2497289>.
15. Hepsibah, P., MV, R., & K Maria, D. (2020). Quality Characteristics of Ground Waters in Few Sources of Industrial Zone. *International Journal of Innovative Technology and Exploring Engineering*, 9(3), 1134-1137.
16. Kumar, M. S., Raju, M. V., Palivela, H., & Venu Ratna Kumari, G. (2017). Water quality scenario of urban polluted lakes-A model study. *International Journal of Civil Engineering and Technology*, 8(5), 297-302.
17. Kumar, M. S., Raju, M. V., & Palivela, H. (2017). An overview of managing municipal Solid waste in urban areas-A model study. *International Journal of Civil Engineering and Technology*, 8(5).
18. MV, R., & K Maria, D. (2020). Green Engineering Strategies on Solid Waste Management to Promote Urban Environmental Sustainability. *International Journal of Advanced Science and Technology*, 29(5), 4638-4648.
19. Raju, M. V., Palivela, H., Mariadas, K., & Babu, S. R. (2019). Assessment of physico-chemical and biological characteristics and suitability study of lake water: a model study. *Technology*, 10(01), 1431-1438.



20. MV, R., K Maria, D., & Cyril Lucy, M. (2020). Rapid Environmental Impact Assessment of Lake Water. *International Journal of Advanced Science and Technology*, 29(3), 8468-8478.
21. Raju, M. V., Mariadas, K., Palivela, H., Ramesh Babu, S., & Raja Krishna Prasad, N. (2018). Mitigation plans to overcome environmental issues: A model study. *International Journal of Civil Engineering and Technology*, 9(10), 86-94.
22. Satish Kumar, M., Raju, M. V., & Palivela, H. (2017). Comprehensive index of groundwater prospects by using standard protocols-A model study. *International Journal of Civil Engineering and Technology (IJCIET)*, 8(5), 521-526.
23. Syam Babu, D., Vijay, K., Shakira, S., Mallemkondu, V. S., Barik, P., Kuppm, C., ... & Raju, M. V. (2025). Laterite integrated persulfate based advanced oxidation and biological treatment for textile industrial effluent remediation: optimization and field application. *Applied Bionics and Biomechanics*, 2025(1), 9325665.
24. Satish Kumar, M., Raju, M. V., Ramesh Babu, S., & Siva Jagadeesh Kumar, M. (2017). Interpretation and correlative study of water simulation in surface water bodies. *International Journal of Civil Engineering and Technology (IJCIET)*, 8(5), 1206-1211.
25. Pullagura, J. R., Osman, O., Kumar, M. S., & Raju, M. V. (2024). LoRaWAN based IoT Architecture for Environmental Monitoring in Museums. *Journal of Computational Analysis & Applications*, 33(5).
26. Mariadas, K., Rao, T. S., Raju, M. V., & Kumar, M. S. (2024). A Study on the Fluoride Content of the Groundwater in The Gurazala Division of the Palnadu District, Andhra Pradesh, India. *Journal of Advanced Zoology*, 45(3).
27. Reginald, P. J., Raju, M. V., Satyanarayana, D., Mariadas, K., & Kumar, M. S. (2021). Kisaan Seva-Aadhaar Linked Smart Farming Application. *Annals of the Romanian Society for Cell Biology*, 25(5), 5168-5176.
28. Kumar, M. S., Asadi, S. S., & Vutukuru, S. S. (2017). Assessment of Heavy Metal concentration in ground water by using remote sensing and GIS. *International Journal of Civil Engineering and Technology*, 8(4).
29. Raju, M. V., Das, K. M., Akram, M. B. W., Vardhan, T. R., & Palivela, H. (2019). Water Quality Evaluation of Aquaculture Practices in Coastal Area of Bhimavaram at West Godavari District. *Andhra Pradesh, Jour of Adv Research in Dynamical & Control Systems*, Vol. 11, 11-Special Issue, 1088-1094.
30. Harikrishna Ramaprasad Saripalli, Prasanna Kumar Dixit, Rajasekhar Dega, Uma Devarapalli (2024)., Antimicrobial Efficacy of Ag-NPs of Gishta seed Extracts., *Journal of Chemical Health Risks*, Volume 14, Issue 5, 297-304.
31. Harikrishna Ramaprasad Saripalli, Prasanna Kumar Dixit, Rajasekhar Dega, Uma Devarapalli (2024)., Exploring the Medicinal Potential of Gishta (Graviola): Identification of Bioactive Metabolites and Silver Nanoparticle Synthesis through Advanced Analytical Techniques, *Journal of Chemical Health Risks*, Volume 14, Issue 5, 658-681.
32. RS Harikrishna, PK Dixit, R Dega, U Devarapalli, (2024)., Harnessing Plant-Based Silver Nanoparticles: Evaluating the Anticancer Potential of Gishta Extracts via Enhanced Caspase-3 Activity, *Nanotechnology Perceptions*, Vol.20, No.6., 2172-2187.
33. Vutukuru, S. S., Asadi, S. S., Vasantha, R. B. V. T., & Raju, M. V. (2012). Plankton biodiversity as indicators of the ecological status of River Moosi, Hyderabad, India. *International Journal of Earth Science and Engineering*, 5(3), 587-592.