biology and life sciences forum

MDPI

Proceedings

Physicochemical and Nutritional Analysis of Molasses for Rum Fermentation †

Tinashe W. Mangwanda [1], Janice S. Mani [1], Joel B. Johnson[1], Steve Jackson [3], Tyryn McKeown [3] and Mani Naiker [1,2] *

[1] College of Science and Sustainability, CQUniversity, Bruce Hwy, North Rockhampton, QLD 4701, Australia
[2] Institute for Future Farming Systems, CQUniversity, Bundaberg, QLD 4670, Australia
[3] Bundaberg Rum Distillery, Whitred Street, Bundaberg, QLD 4670, Australia
* Correspondence: m.naiker@cqu.edu.au

† Presented at the 4th International Electronic Conference on Foods, 15-30 October 2023.

Abstract: The production of high-quality rum requires a carefully controlled fermentation process, which is determined by the physicochemical and nutritional properties of the molasses used as a substrate. The flavor, aroma, and alcohol content of the final product are heavily influenced by the composition of molasses. This paper presents the results of an extensive analysis of molasses, a byproduct of sugar production that has various applications in food and feed industries. The physicochemical properties and nutritional composition of molasses were investigated using a range of analytical techniques including Brix, moisture content, pH, total ash content, total dissolved solids, crude protein, sugar content, total nitrogen, free amino acids, amino acids, mineral content, vitamins, and organic acids. The study analyzed molasses samples obtained from three painted concrete constructed storage wells at the Bundaberg Distilling Company. The results indicated that the molasses samples possessed a substantial sugar concentration, with sucrose being the most abundant, ranging from 35.02 – 35.27% w/w. These findings support the traditional use of molasses in rum fermentation and use as an alternative feedstock for the synthesis of various value-added chemicals. The pH of the molasses was slightly acidic, and the total ash content was relatively high ranging from 8.76 – 13.55 % w/w, indicating the presence of mineral salts. Mineral content varied among the samples, but all three contained significant levels of sodium, phosphorus, potassium, calcium, and magnesium. All the samples contained essential amino acids and vitamins, but their levels varied. The molasses also contained varying levels of different organic acids, including acetic, propanoic, aconitic, formic, valeric, lactic, citric, and malic acids. The analysis revealed significant differences in ash content, nitrogen compounds, and minerals between storage wells, indicating inherent variability between batches. However, key fermentable sugars did not significantly differ between samples, and blending or standardization is recommended to minimize batch-to-batch variation prior to fermentation. This study provides valuable information on the composition and quality of molasses, which can be used to optimize its utilization in rum fermentation and various other applications.

Keywords: Molasses analysis; Quality; Physicochemical; Rum; Fermentation; Nutritional; Chemical; Proximate; Minerals; Vitamins; Organic acids

Citation: To be added by editorial staff during production.

Academic Editor: Firstname Lastname

Published: date

1. Introduction

Molasses is a rich source of carbohydrates, minerals, and organic acids, making it an attractive ingredient for fermentation processes [1-4]. The physicochemical and nutritional qualities of molasses can have a considerable effect on the yield and quality of rum [5]. Analysis of the molasses composition would provide crucial information for optimizing the fermentation conditions and enhancing the quality and yield of rum.

The use of molasses as a raw material for rum production is a widely established practice, with many distilleries relying on this ingredient for their operation. Previous studies have found that the quality and composition of molasses can vary significantly depending on factors such as the type of sugarcane, harvesting methods, and processing techniques used [5]. Therefore, a thorough analysis of molasses is necessary to ensure that the fermentation process is optimized and that the quality and consistency of the final product are maintained[1,6,7].

The physicochemical and nutritional analysis of molasses holds significant importance for fermentation in the rum manufacturing industry. It provides crucial information on parameters such as Brix, pH, and Total Dissolved Solids (TDS), allowing for the adjustment and optimization of fermentation conditions. This analysis improves product quality and fermentation yield by guiding adjustments in sugar composition, and informing on the supplies of other major, minor and trace nutrients essential to the fermentation process [8]. Analyzing glucose, fructose, and sucrose contents helps determine fermentability and predict potential flavors. Organic acids affect acidity and flavor profiles, enabling adjustments for the desired characteristics. Nutritional analysis, including total nitrogen, crude protein, and free amino nitrogen (FAN), guides nutrient supplementation for healthy yeast growth and improved fermentation efficiency [6,8,9]. Overall, these analyses contribute to the optimization of fermentation, development of desired flavors, nutrient supplementation, quality control, meeting consumer preferences, and enhancing product consistency, efficiency, and quality in the rum manufacturing industry [1,5-7,10].

Therefore, the present study aimed to evaluate the physicochemical and nutritional composition of molasses for rum fermentation and identify the key factors that influence the fermentation process. Overall, this study aimed to better understand the role of molasses in rum production and to provide insights into how the quality of this ingredient can be optimized to enhance the efficiency and consistency of the fermentation process.

2. Materials and methods

Sampling and reagents:

Molasses samples were collected from three painted concrete constructed storage wells at the Bundaberg Distilling Company in Bundaberg, Queensland, Australia These wells were identified as the front well (Sample 1), middle well (Sample 2), and far well (Sample 3). All reagents used were of analytical grade, purchased from Sigma-Aldrich Australia (Castle Hill, New South Wales). Tests were conducted in triplicate with average results ± 1 standard deviation reported.

Brix, pH and TDS measurement:

The Brix level of the molasses samples was measured using a Brix hydrometer (Carlton Glass, Melbourne, Australia) [11]. The pH and total dissolved solids (TDS) of the molasses samples were measured using a smartCHEM-Lab Multi-Parameter Laboratory Analyzer (SI Analytics GmbH, Mainz, Germany).

Determination of the moisture content:

The moisture content of the molasses samples was determined according to AOAC Official Method 934.01 [12].

Total nitrogen and crude protein analyses:

The total nitrogen content of the molasses samples was determined by the Kjeldahl digestion method according to AOAC Method 984.13 [13]. Crude protein content was calculated from the total nitrogen content using a conversion factor (N × 6.25).

Determination of total ash content:

The total ash content of the molasses samples was determined using AOAC Official Method 942.05 [14].

Sugars and organic acids analysis:

The glucose, fructose, and sucrose contents of the molasses samples were analyzed using a Thermo Scientific™ Dionex™ Integrion™ HPIC™ system with a Dionex AS-AP autosampler [15]. The organic acid contents of the molasses samples were analyzed using a Thermo Scientific™ Trace 1310™.

Vitamins analysis:

The vitamin content of the molasses samples was analyzed using AOAC method 2015.14 with an ACQUITY Premier LC system [16].

Determination of free amino nitrogen content

The free amino nitrogen (FAN) content of the molasses samples was determined using the EBC Ninhydrin method [17].

Minerals analysis:

The mineral content (Sodium, Phosphorus, Potassium, Calcium, and Magnesium) of the molasses samples was analyzed using a Shimadzu ICPMS-2030 inductively coupled plasma mass spectrometer.

Data analysis:

A one-way analysis of variance (ANOVA) was applied to test the null hypothesis of no significant differences between the three samples for each parameter. Subsequently, post-hoc Tukey tests were utilized with a significance level of $p<0.05$.

3. Results

This section provides comprehensive data on the quality and composition of the molasses samples analyzed. Unless otherwise stated, the results are expressed as percentage weight per weight (% w/w) ± 1 standard deviation wet weight basis.

Table 1. Composition and nutritional quality of sugarcane molasses.

Sample	Brix (°)	pH	TDS (% w/w)	Moisture content (%)	Total nitrogen (% w/w)	Crude protein (% w/w)	Total ash (% w/w)	FAN (% w/w)
1	87.40 ± 0.05	5.49 ± 0.01	76.35 ± 0.21	22.56 ± 0.11	0.60 ± 0.00	3.75 ± 0.00	8.76 ± 0.15	0.12 ± 0.00
2	87.54 ± 0.25	5.57 ± 0.13	76.80 ± 0.85	23.01 ± 0.75	0.65 ± 0.07	4.06 ± 0.44	11.00 ± 3.32	0.14 ± 0.03
3	87.84 ± 0.17	5.69 ± 0.04	77.20 ± 0.28	23.26 ± 0.40	0.70 ± 0.00	4.38 ± 0.00	13.55 ± 0.30	0.16 ± 0.00

Table 2. Concentrations of Glucose, fructose, sucrose, and conversion/calculation of Total Sugar as Invert (TSAI).

Sample	Glucose	Fructose	Sucrose	TSAI
1	3.10 ± 0.01	3.83 ± 0.03	35.27 ± 0.04	43.96 ± 0.08
2	3.33 ± 0.34	3.95 ± 0.19	35.12 ± 0.18	44.15 ± 0.34
3	3.45 ± 0.17	4.05 ± 0.05	35.02 ± 0.04	44.26 ± 0.18

Table 3. Organic Acid Profiles in molasses samples using GC-FID.

Sample	Acetic acid	Propanoic acid	Aconitic acid	Formic acid	Valeric acid	Lactic acid	Citric acid	Malic acid
1	0.40 ± 0.01	0.30 ± 0.01	ND	ND	ND	ND	0.37 ± 0.04	ND
2	0.43 ± 0.02	0.34 ± 0.04	ND	ND	ND	ND	0.32 ± 0.10	ND
3	0.43 ± 0.02	0.35 ± 0.01	ND	ND	ND	ND	0.25 ± 0.00	ND

Table 4. Quantification of Vitamins in sugarcane molasses.

Sample	Thiamin (B1)	Riboflavin (B2)	Pantothenic acid (B5)	Pyridoxine (B6)	Biotin (B7)	Folic acid (B9)	Nicotinamide
1	0.60 ± 0.01	2.81 ± 0.02	5.32 ± 0.00	9.94 ± 0.09	0.13 ± 0.00	0.03 ± 0.00	3.26 ± 0.06
2	0.55 ± 0.07	2.72 ± 0.15	5.66 ± 0.49	9.22 ± 1.11	0.13 ± 0.01	0.03 ± 0.00	3.21 ± 0.13
3	0.50 ± 0.00	2.60 ± 0.02	6.01 ± 0.01	8.47 ± 0.05	0.14 ± 0.00	0.03 ± 0.00	3.15 ± 0.05

Table 5. Concentrations of essential minerals (Sodium, Phosphorus, Potassium, Calcium, and Magnesium) in sugar cane molasses measured by ICP-MS.

Sample	Sodium	Phosphorus	Potassium	Calcium	Magnesium
1	0.12 ± 0.00	0.06 ± 0.00	4.16 ± 0.03	0.69 ± 0.00	0.44 ± 0.00
2	0.14 ± 0.02	0.06 ± 0.00	4.20 ± 0.03	0.86 ± 0.25	0.45 ± 0.02
3	0.16 ± 0.00	0.07 ± 0.00	4.24 ± 0.03	1.04 ± 0.00	0.47 ± 0.00

4. Discussion

Molasses is a key ingredient in rum production, and the findings of this study can provide valuable information for rum producers. These results revealed that molasses is a rich source of carbohydrates, minerals, and organic acids, which are essential for the fermentation process in rum production.

Brix is a measure of the sugar content in molasses, and the values for the three samples ranged from 87.40 to 87.84. This indicated a high concentration of dissolved solids, including sugars, in molasses. Measuring the Brix level of molasses is typically common in rum production, as it provides a quick and easy way to monitor the sugar content [1,18]. Various researchers have different views on the significance of Brix measurements in molasses. Baker[19] stated that Brix does not represent anything in an impure sucrose solution such as molasses, other than a number denoting specific gravity, which cannot be related to sucrose or dry matter content. However, some authors [10,20,21] predefined specifications of Brix that produce good, fair, and bad rums. These benchmarks are 87.1 for good quality, 85.4 for fair quality, and 88.2 for poor quality rum, respectively. The pH values ranged from 5.49 to 5.69, with sample 3 having the highest pH value. Slightly acidic levels are within the optimal range for yeast growth and fermentation [5,7]. This means that yeast in the fermentation process can thrive in this environment, leading to successful fermentation and potentially producing high-quality rum [8]. Additionally, slightly acidic conditions can inhibit the growth of undesirable microorganisms that can otherwise compete with yeast during fermentation [1].

Saccharomyces cerevisiae, the yeast used in rum production requires nitrogen for growth and development, and inadequate nitrogen can result in poor fermentation and poor quality of the final product [1,5,8]. The crude protein content in the molasses samples was approximately 3.75%–4.38 % w/w, which is not particularly high for molasses, but is in line with results obtained by other scholars ranging from 0.50 to 5.50 % w/w [18,22]. Molasses is not considered to be a significant source of proteins during rum fermentation. In this study, the total nitrogen values ranged from 0.60 to 0.70 % w/w, which is a reasonable quantity for molasses used for rum fermentation [3]. The FAN values were in the range of 0.12 to 0.16 % w/w, which is slightly lower than the expected range of 0.20 to 0.45 % w/w [1-4].

Molasses is a valuable substrate in rum production because of its high carbohydrate content, which is primarily composed of glucose, fructose, and sucrose [1,5,7]. These sugars serve as the primary substrates for yeast metabolism during fermentation, leading to the production of ethanol, the primary alcohol in the rum. The results of this study showed that the sugar content of molasses was reasonably high, containing 42.20 to 42.52 % total sugars composed of approximately 35 % sucrose, with the remainder being almost equal portions of glucose and fructose. These values are slightly lower than reported by other scholars who have found molasses contains approximately 55% of fermentable sugars composed of about 35 % of sucrose and 20% of a mixture of glucose and fructose

[7,23]. In rum production, knowing the sugar content allows the distiller to predict the fermentation performance of a batch of molasses [1].

The importance of vitamins in fermentation cannot be understated as they are essential for the growth and metabolism of microorganisms, including the yeasts used in rum fermentation. The concentrations obtained in this study for the vitamins Thiamin (0.50-0.60 mg/kg), Riboflavin (2.60 – 2.81 mg/kg), Pyridoxine (8.47 – 9.94), Nicotinamide (3.15 – 3.26 mg/kg), Pantothenic acid (5.32 – 6.01 mg/kg), Folic acid (0.03 mg/kg), and Biotin (0.13 – 0.14 mg/kg) are relatively lower than those reported by other scholars [3,5,24]. [24] reported Nicotinamide and Pantothenic acid concentrations of as high as 25 mg/kg. Thus, vitamin supplementation can be explored in cases where the observed quantities cannot support optimal yeast growth during rum fermentation.

This study found that molasses has a relatively high total ash content, which indicates the presence of mineral salts. The results obtained from the analysis of the mineral content in sugar cane molasses showed that potassium had the highest percentage concentration weight per weight in the three samples (4.16 ± 0.03, 4.20 ± 0.03, 4.24 ± 0.03), followed by phosphorus, sodium, calcium, and magnesium. The type and concentration of metal ions provided in growth media can greatly affect yeast fermentation [8]. Potassium is an important mineral in rum production, as it plays a significant role in the flavor development of the final product. It also influences the rate of fermentation and the growth of yeast cells [8].

Organic acids in molasses, such as acetic, propanoic, aconitic, formic, valeric, lactic, citric, and malic acids, can also play a role in rum production. These organic acids can contribute to the acidity of the fermentation mixture and affect the flavor and aroma of the final product. The study found that molasses had a significant amount of organic acids, which suggests that it can produce high-quality rum. Acetic acid was the most abundant organic acid, followed by propanoic acid and citric acid, whereas aconitic acid, formic acid, valeric acid, lactic acid, and malic acid were not detected, as they were below the detection limit. Organic acids are important for pH regulation during fermentation. However, the absence (or very low levels) of aconitic acid, formic acid, and valeric acid is desirable because these organic acids can have negative effects on the fermentation process and the quality of the final product [1].

No significant difference ($p>0.05$) was found between samples for total sugars (TSAI), sucrose, glucose, fructose, moisture content and pH. All three samples had statistically similar high levels of fermentable sugars and pH values in the optimal range for rum fermentation. Ash content was significantly different between samples ($p=0.012$), with Sample 3 showing the highest mean value. Total nitrogen differed significantly ($p=0.031$), again with Sample 3 having the highest mean. Sodium, calcium, acetic acid and propanoic acid also exhibited significant between-sample variation ($p<0.05$). Post-hoc Tukey test indicated Sample 3 had statistically higher means for ash, nitrogen, sodium, calcium, acetic and propanoic acid than Samples 1 and 2. In conclusion, molasses is an essential ingredient in rum production and contains high levels of sugars, amino acids, vitamins, minerals, and organic acids, which provide all the necessary nutrients for yeast growth and metabolism. The findings of this comprehensive analysis provide basic insight into the role of molasses as a key ingredient in rum production.

Author Contributions: Conceptualization, M. N. and J. J.; methodology, T.M. and J.J.; software, J.J. and T.M.; validation, J.J., K.W., J.M., and M.N.; formal analysis, T.M.; investigation, T.M.; resources, M.N., T.M., S. J.; data curation, T.M.; writing—original draft preparation, T.M.; writing—review and editing, T.M. and J.M..; visualization, T.M. and J.M.; supervision, K.W., S.J., T.M., and M.N.; project administration, M. N. and K.W.; funding acquisition, M.N. All the authors have read and agreed to the published version of the manuscript.

Funding: One of the Authors, T.M., acknowledges funding from a Research Elevate Scholarship sponsored by CQUniversity and Bundaberg Distilling Company.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data are available from the authors upon request.

Acknowledgments: The authors gratefully acknowledge technical assistance provided by Allison Stirrat, Karen Chadwick and Prof. Kerry Walsh.

Conflicts of Interest: Two authors (T.M.). (Tyryn McKeown), and S.J.) are affiliated with the Bundaberg Distilling Company. No other conflict of interest exists.

References

1. Piggot: R. Treatment and fermentation of molasses when making rum-type spirits. *The alcohol textbook* **2003**, 75-84.
2. CURTIN, L.V. Molasses in Animal Nutrition. National Feed Ingredients Association, West Des Moines, Iowa **1982**, 1.
3. Olbrich, H. The molasses. *Biotechnologie-Kempe GmbH* **2006**, *128*.
4. Palmonari, A.; Cavallini, D.; Sniffen, C.; Fernandes, L.; Holder, P.; Fagioli, L.; Fusaro, I.; Biagi, G.; Formigoni, A.; Mammi, L. Characterization of molasses chemical composition. *Journal of dairy science* **2020**, *103*, 6244-6249.
5. Mangwanda, T.; Johnson, J.B.; Mani, J.S.; Jackson, S.; Chandra, S.; McKeown, T.; White, S.; Naiker, M. Processes, challenges and optimisation of rum production from molasses—a contemporary review. *Fermentation* **2021**, *7*, 21.
6. Fleet, G.H.; Green, V. The microbiology and biotechnology of rum production. *Distilled spirits* **2010**, *3*, 203.
7. Medeiros, A.; de Matos, M.; de Pinho Monteiro, A.; de Carvalho, J.; Soccol, C. Cachaça and rum. In *Current developments in biotechnology and bioengineering*; Elsevier: 2017; pp. 451-468.
8. Walker, G.M.; Stewart, G.G. Saccharomyces cerevisiae in the production of fermented beverages. *Beverages* **2016**, *2*, 30.
9. Stewart, G.G.; Hill, A.; Lekkas, C. Wort FAN–its characteristics and importance during fermentation. *Journal of the American Society of Brewing Chemists* **2013**, *71*, 179-185.
10. Nicol, D.A. Rum. In *Fermented beverage production*; Springer: 2003; pp. 263-287.
11. Xu, W.; Liang, L.; Zhu, M. Determination of sugars in molasses by HPLC following solid-phase extraction. *International Journal of Food Properties* **2015**, *18*, 547-557.
12. AOAC. Official method 925.10: Solids (total) and moisture in foodstuffs (air oven method). *Official methods of analysis of the association of official analytical chemists.* **2000**.
13. AOAC. Official methods of analysis of AOAC International: Method 984.13. **2000**.
14. International, A. AOAC Official Method 942.05, Ash of Animal Feed. *Official Methods of Analysis of AOAC International, 18th ed. Chapter 4* **2005**, *8*.
15. Patil, S.; Narani, A.; Coffman, P.; Pray, T.; Tanjore, D.; Voelker, P.; Rohrer, J. Rapid and sensitive determination of biofuel sugars by ion chromatography.
16. McClure, S. Simultaneous Determination of Total Vitamins B1, B2, B3, and B6 in Infant Formula and Related Nutritionals by Enzymatic Digestion and LC-MS/MS—A Multi-Laboratory Testing Study Final Action: AOAC Method 2015.14. *Journal of AOAC International* **2020**, *103*, 1060-1072.
17. Lie, S. The EBC-ninhydrin method for determination of free alpha amino nitrogen. *Journal of the Institute of Brewing* **1973**, *79*, 37-41.
18. Mordenti, A.L.; Giaretta, E.; Campidonico, L.; Parazza, P.; Formigoni, A. A review regarding the use of molasses in animal nutrition. *Animals* **2021**, *11*, 115.
19. Baker, P. In Proceedings of the Proc. AFMA Ninth Ann. Liquid Feed Symp. Amer. Feed Manufacturers Assoc, Arlington, VA 1979.
20. Arroyo, R.; CH, E.; SE, F. The economics of rum production. *International Sugar Journal* **1947**, *49*, 292-294.
21. Jamir, L.; Kumar, V.; Kaur, J.; Kumar, S.; Singh, H. Composition, valorization and therapeutical potential of molasses: a critical review. *Environmental Technology Reviews* **2021**, *10*, 131-142.
22. Gasmalla, M.; Yang, R.; Nikoo, M.; Man, S. Production of ethanol from Sudanese sugar cane molasses and evaluation of its quality. *J Food Process Technol* **2012**, *3*, 163-165.
23. Clarke, M. Syrups. In *Encyclopedia of Food Science*; Food Technology and Nutrition; Sugar Processing Research Institute Incorporated, New Orleans: LA, USA, 1993; pp. 5711-5717.