Abstract

Wainwright Baquedano, Kathya Nicole. Process Optimization and Characterization of a Novel Mannose Syrup from Palm Kernel Cake Using Enzymatic Hydrolysis and Response Surface Methodology (Under the direction of Dr. Marvin L. Moncada).

The revalorization of agricultural by-products offers an important aspect in the production of sustainable foods and ingredients. Palm kernel cake (PKC) is a byproduct from the oil industry known for its high cellulose, hemicellulose and lignin content. The hemicellulose section offers an opportunity to study it a source of mannose, a multifunctional sugar with applications in the pharmaceuticals, prebiotics, and food ingredients industry. However, PKC has a complex composition, as the mannans present are intertwined in the lignin-cellulose matrix that makes it up. This makes the efficiency of mannose extraction from this by-product a challenge. In this study, enzymatic hydrolysis using β-mannanase was evaluated as a sustainable alternative to chemical hydrolysis for mannose recovery. Using response surface methodology, the hydrolysis parameters were optimized to maximize the mannose yield while minimizing the other parameters considered (% enzymes, % solids, and hydrolysis time). Box-Behnken design was used to evaluate the effects of hydrolysis time, enzyme concentration and solids content on mannose extraction efficiency. By statistical analysis, it was determined that the optimum conditions for enzymatic hydrolysis were 16 hours of reaction, 5% (w/v) solids and 10% (w/w) β-mannanase giving a concentration of 4.981 g/L, corresponding to a mannose extraction efficiency of 26.67%. The palm kernel mannose syrup was characterized by its physicochemical properties, having a moisture content of 15.85±0.07%, a water activity (aw) of 0.6918±0.003, and a pH of 4.05±0.282, showing a shelf stability. This research established an optimized enzymatic hydrolysis pathway for producing a functional mannose syrup and explored its potential applications in the food industry.

Process Optimization and Characterization of a Novel Mannose Syrup from Palm Kernel Cake Using Enzymatic Hydrolysis and Response Surface Methodology

by
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DEDICATION

This achievement is a tribute to my parents for their endless love, sacrifices , and unmatched support. To my sister and nephew, thank you for your support and companionship on this journey. Your confidence in me is demonstrated by this achievement.

To my grandparents, whose love and wisdom have always motivated me. To my dear grandmother Lidia, whose unwavering love and support has given me courage and joy all my life. Special thanks to my dear grandfather José, whose love and courage continue to guide me from heaven.

BIOGRAPHY

Kathya Nicole Wainwright Baquedano was born and raised in Choluteca, Honduras. In 2022, she earned her Bachelor's degree in Food Science and Technology from Zamorano University in Honduras. In the same year, she completed an internship at Plant for Human Health Institute, where she worked in product formulation, food analysis and food processing. Subsequently, she pursued a Master's degree in Food Science at North Carolina State University (NC State University), where she worked as a research assistant in the Department of Food Science. During her master's she worked in the revalorization of industry by-products, product development projects, and optimization of food processes, collaborating with the food industry. She had the opportunity to do an internship at Nutreo in Colombia in 2025.

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CHAPTER 1

UPCYCLING OF PALM KERNEL CAKE: PRODUCTION, COMPOSITION, AND METHODS FOR MANNOSE EXTRACTION

Abstract

The oil palm (*Elaeis guineensis*) is an economically important crop in the edible oil industry due to its high oil yield. The palm oil industry generates multiple by-products. One is palm kernel cake (PKC), rich in lignocellulosic biomass. PKC is composed of significant amounts of cellulose, hemicellulose, and lignin, which makes it a potential source of compounds, particularly D-mannose, derived from the mannans that make up hemicellulose. The hemicellulosic fraction of PKC is mainly composed of β-mannans, galactomannans, and glucomannans, structurally linked by β-1,4- and β-1,6-glycosidic bonds. These polysaccharides can be hydrolyzed into mannooligosaccharides (MOS) and subsequently transformed into D-mannose, a sugar widely used in pharmaceuticals, food, and biofuels. Mannose has been widely studied over the years for its prebiotic properties, its role in glycoprotein biosynthesis, and its applications in the treatment of urinary tract infections (UTIs). This chapter explores the composition of PKC, particularly focusing on its hemicellulose-rich fraction and the different approaches to D-mannose extraction.

1. Introduction

Native to West Africa, the African oil palm (*Elaeis guineensis*) is the most important palm species in the world due to its high palm oil production (Murphy et al., 2021). It is considered a perennial monocot plant that belongs to the genus *Elaeis*. It consists of two major species: *E. oleifera*, which is native of South and Central America, and *E. guineensis*, which originated on the coasts of West and Central Africa and eventually spread to Southeast Asia (Gupta, 2012). The African oil palm has been utilized for centuries as a source mainly for oil but also for animal feed and biofuel. Consequently, over the last 50 years, this crop has expanded worldwide, turning palm oil into one of the main commodities in international trade (Henson, 2012).

Oil palm cultivation has gained popularity because of its high oil production and adaptability to various climates and conditions. This crop is vital to Indonesia and Malaysia's economies, which together account for approximately 85% of the global production of palm oil, exporting oil and biomass cake (Tabe-Ojong et al., 2023). Indonesia is the world’s leading producer and exporter of crude palm oil, with an annual output of 256 million tons of fresh fruit bunches in 2020 (*FAOSTAT*, 2022). Additionally, oil palm also represents a source of income for the humid tropical regions of Asia, Africa and the Americas, where its products are exported to world markets (Murphy, 2019; Murphy et al., 2021).

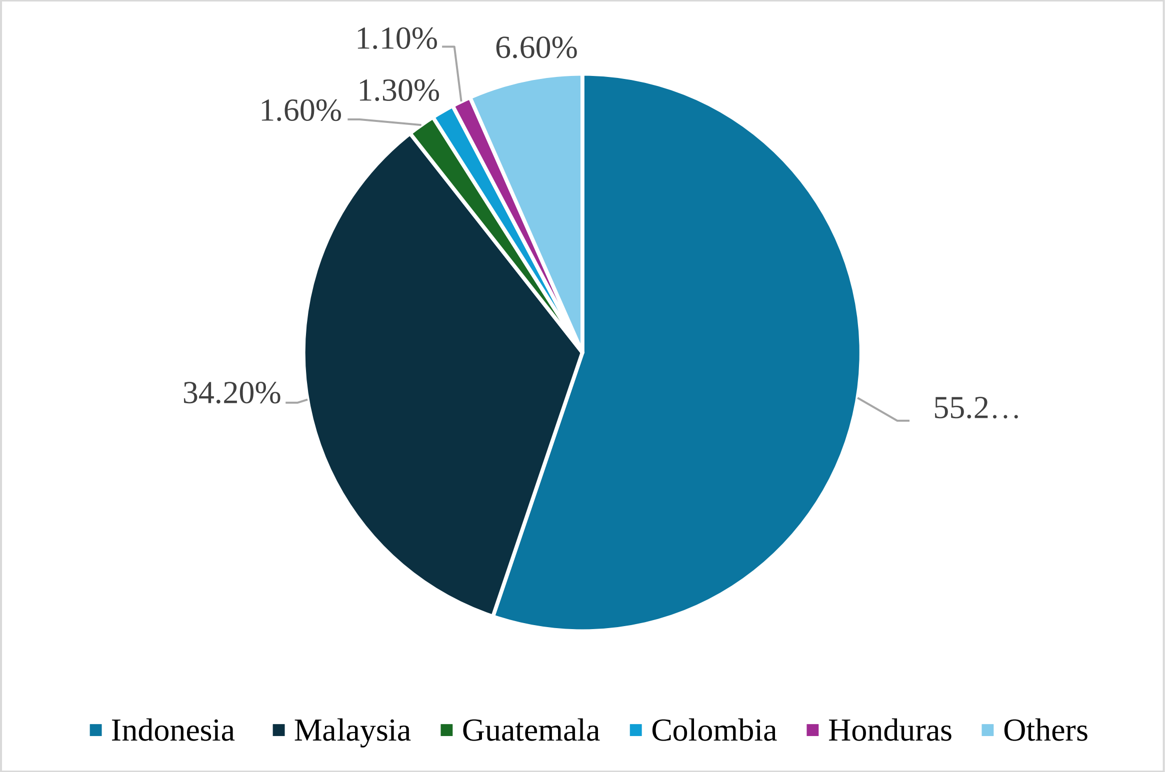

Figure 1. Worldwide palm oil production. Adapted from (De Almeida Rios et al., 2018)

The palm oil extraction plant produces two types of oils: palm oil, which is extracted from the mesocarp and used for human food, and palm kernel oil, which is extracted from the kernels and mainly used in the oleochemical industry (Véronique Gibon, 2012; Sundram et al., 2003). However, the palm oil industry, which is the basis of many economies, generates enormous quantities of by-products, including palm kernel cake. After extracting the oil from oil palm fruits, the high-fiber biomass residue is called palm kernel cake (Buong Woei Chieng et al., 2017). In 2017, worldwide production of palm kernel cake surpassed 9.5 million metric tons (René Renato Balandrán-Quintana et al., 2019). Traditionally, the African palm oil industry has used this abundant by-product as livestock feed as a source of energy and fatty acids (Hashim et al., 2012; Abdelrahim Abubakr et al., 2015). However, economic and environmental issues have led to an increased industrial interest in developing sustainable production protocols that allow for the rational use of agriculture by-products. A promising approach is adding value to palm kernel cake as a potential source of mannose. Mannose ($C6H12O6$) is a hexose sugar used in a variety of commercial products, including food, pharmaceuticals, feed additives, and biofuels. (Hu et al., 2016). D-mannose possesses remarkable physiological qualities and biological activity, characterized by low caloric content and minimal taste (Sharma et al., 2018). Multiple human studies have demonstrated that mannose is effective in preventing urinary tract infections (Wagenlehner et al., 2022). This has awakened the interest of the pharmaceutical industry in finding new sources of mannose. In addition, because it is water-soluble and an inert molecule, it is commonly used to manufacture chewable pills and granulated powder (Pan et al., 2021; Saha & F. Michael Racine, 2010). Recent studies highlighting its biological effects on the human body have further driven this interest. For example, the body uses it for glycoprotein synthesis and plays a role in immune control. (Wei et al., 2020). D-mannose occurs naturally in several plants and fruits, including cranberries. Moreover, mannose exists as galactomannans (indigestible plant polysaccharides) in coffee beans, fenugreek, and guar gum (Srivastava & Kapoor, 2005; Ala-Jaakkola et al., 2022). This chapter reviews the potential that palm kernel cake has as a source of mannose. Incorporating this by-product as a source of mannose allows companies to generate more income while eliminating the problem of disposal.

2. Oil Palm

Individual species of the palm family (*Arecaceae)* can be found in a variety of tropical ecosystems, such as arid regions, dry forests, alpine regions, tropical forests, and desert oases (Tomlinson Pb, 2006). Because of their distinct monopodial development type, palms can respond to changes in their surroundings very differently from dicotyledonous trees, which exhibit secondary growth traits, making oil palms very resistant and adaptable to different climatic conditions (Renninger & Phillips, 2016). In the tropics, from an economic point of view, palms are even more important than legumes because they provide a wide range of valuable goods, such as oil, dates, and coconuts (Henderson, 2009).

The oil palm fruit is an oval drupe that clusters in big bunches (Ali et al., 2014). Typically, it is 2-5 cm long, weighing 3 to 30 g, and consists of the seed and pericarp. As oil palm trees age, the weight of fruit bunches increases, ranging from 10 to 50 kg, with 500-4,000 fruits per bunch. (Ngando-Ebongue et al., 2011) The pericarp of the oil palm fruit is separated into the exterior exocarp, the fleshy mesocarp, and the endocarp, referred to as the shell in oil palm. The shell surrounds the seed or kernel, the embryo, and the endosperm (Barcelos et al., 2015).

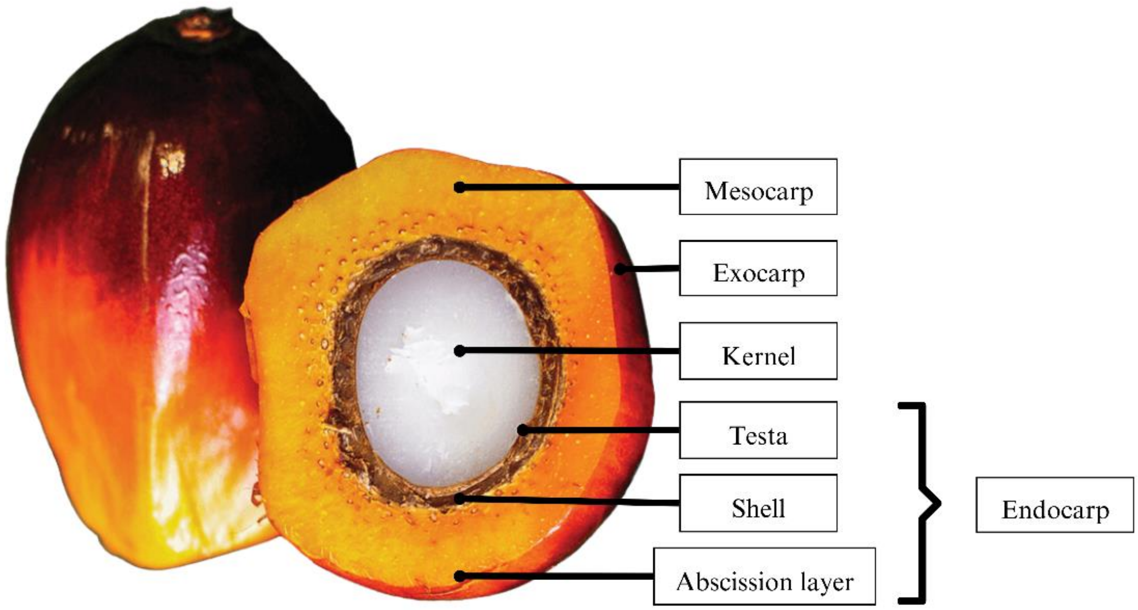

Figure 2. Palm fruit composition. Adapted from (Harun et al., 2016)

There are two products of high commercial value for industry: palm oil and palm kernel oil, representing approximately 22% and 4-6% of the fresh weight of the fruit (Boateng, 2008). African palm oil is extracted from the mesocarp of the fruit, which contains 56-70% oil when ripe (Ogan et al., 2015). As the largest oil-producing plant globally, it provides 40% of the world's vegetable oil and is a crucial source of essential nutritional components (Murphy et al., 2021). The oil palm produces fruits throughout the year, which allows producers to have a higher oil production and profitability (Murphy et al., 2021). As a result, it is considered the crop with the highest yield in vegetable oil production (Ogan et al., 2015). For almost 7,000 years, indigenous populations have relied on oil palm fruits as a reliable source of food that is available year-round (Murphy et al., 2021). Palm oil is mainly composed of palmitic and oleic acid and palm kernel oil is mainly composed of lauric and myristic, like the coconut oil composition (Jones & Hughes, 1989). Palm oil is used in a wide range of culinary applications thanks to its distinctive composition of fatty acids and triacylglycerols, this increases its commercial value and demand in the food industry. In addition, it has a peculiar composition, which makes it a vegetable oil that has an almost equal distribution of saturated (50%) and unsaturated (50%) fatty acids, which allows this oil to have multiple uses in different processing industries. (Mba et al., 2015) Half of the fatty acids found in the E. guineensis fruit mesocarp are present in the crude palm oil, which is composed primarily of 44% palmitic acid (C16:0), 5% stearic acid (C18:0), and traces of myristic acid (C14:0) (Sambanthamurthi et al., 2000). An important point to highlight is that the richest plant source of pro-vitamin A and pro-vitamin E is oil palm fruits (Barcelos et al., 2015). This provides a source of vitamin A to the countries in the tropics suffering from vitamin A deficiency and prevents possible health problems associated with this deficiency.

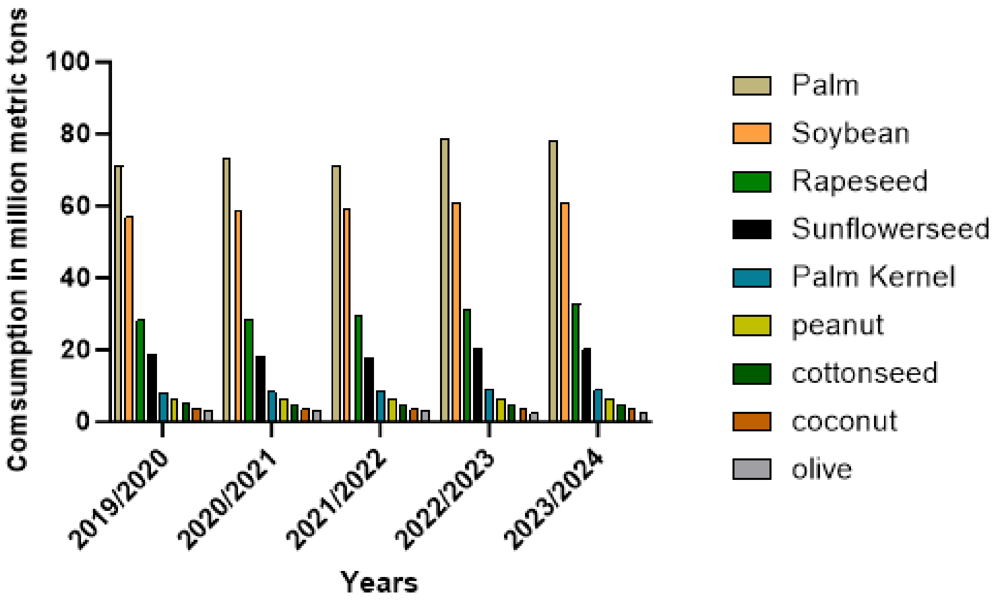

Figure 3. Worldwide annual consumption of vegetable oils. Adapted from (Statista, 2024)

3. Palm oil

3.1. Palm oil production process

The production of African palm oil has changed in recent years, primarily focused on enhancing efficiency, reducing costs, and minimizing environmental impact. However, the principle has remained the same over the years. Repined oil palm fruit's mesocarp can contain up to 70% edible oil, making it valuable for the oil industry. The first step of the oil processing is the reception of the fresh fruit bunches, in this step the fruits are classified and separated to ensure the quality of the final product (Hashim et al., 2012). The quality of the fruit determines the quality of the oil, so while processing cannot enhance the oil's quality, it can prevent its decline (Poku, 2002). Subsequently, fruits go through a pre-treatment process known as sterilization. The industry uses steam sterilization with temperatures around 130–160 °C and a duration of 60–90 minutes at high pressure (A.K. Mohd Omar et al., 2018). This process enables the fruit to soften and better absorb moisture, leading to the fruit reaching high temperatures inside. This, in turn, makes the detachment of fruit easier and reduces the activity of lipolytic enzymes in the fruit mesocarp and the production of free fatty acids (Mba et al., 2015; Hassan et al., 2021).

To perform the peeling and separation process, the sterilized fruits are placed in a rotary drum strip, where\by means of rotation, they will be beaten to facilitate the separation of oil fruits or pulps from the bunches. The fruits then go through a digestion process that uses a steam jacket to soften them at 80–90 °C. This process will break down the oil cells, releasing the palm oil (Shamsuddin et al., 2021). There are two known methods for extracting oil from the oil palm fruits: the dry and wet methods. During the oil extraction, the dry method entails subjecting the fruit to high temperatures and mechanical pressure. It is important to emphasize that there are different ways to perform dry extraction, such as hydraulic systems with pressure or screw threads (Small-Scale Palm Oil Processing in Africa, 2024). On the other hand, the wet method includes the use of water during the extrusion to produce the crude palm oil (Shamsuddin et al., 2021). After extraction, the oil undergoes a clarification process to remove the hydrolyzed and coagulated products (Mba et al., 2015). This allows the final product to be free of impurities and of better quality. Once the oil is clean, it goes through a vacuum drying process to remove moisture (Hashim et al., 2012).

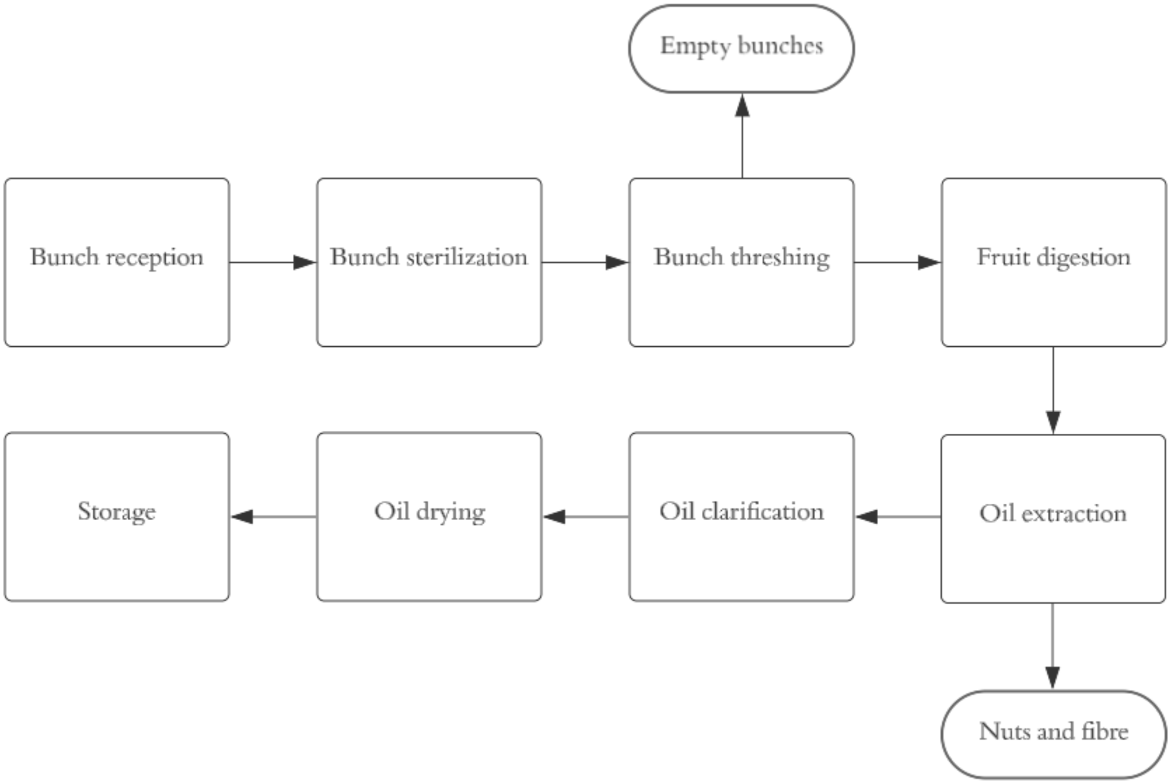

Figure 4 . Palm oil processing. Adapted from (Poku, 2002)

3.2. Palm kernel oil production process

Palm kernel oil has gained popularity in recent years, as it is a major source of lauric acid, which is in worldwide demand due to the growth of the oleochemical industry and the decline in the production of lauric acid from coconut. (B.S. Jalani et al., 2003) After the palm kernels are separated, they go through a drying step to preserve their qualities, and then they are stored in silos until processing (Muhammad Johan Iskandar et al., 2018). Following the kernel pretreatment process, the palm kernels move on to palm kernel oil extraction. The extraction process could be carried out by multiple processes: traditional, chemical, and mechanical extraction. Given its adaptability to both small- and large-capacity operations, this chapter will focus on mechanical extraction (Small-Scale Palm Oil Processing in Africa, 2024). The mechanical process includes three basic steps: kernel pretreatment, screw-pressing and oil clarification (Thin Sue Tang & Pek

Kooi Teoh, 1985). In the pretreatment, to prevent equipment damage or contamination, kernels are cleaned to remove any foreign materials. Next, kernels are ground to reduce the particle size and improve the oil extraction efficiency (Small-Scale Palm Oil Processing in Africa, 2024). Subsequently, the ground kernels go through a series of pressing screws to remove as much oil as possible. The final palm kernel cake will be between 6-7% fat. Then the oil passes through a clarification process, using filters and membranes, to remove any impurities from the final product (Hashim et al., 2012).

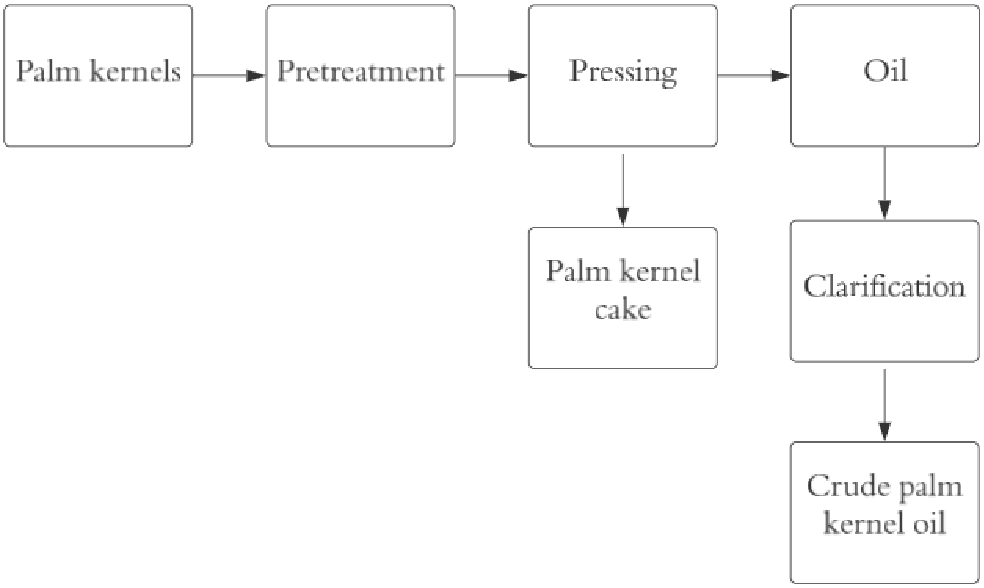

Figure 5. Palm kernel oil processing. Adapted from (Poku, 2002)

3.3. Generation of by-products

After the palm and palm kernel oil is extracted, the palm oil industry generates around 169.72 million metric tons of solid waste (Khan et al., 2011). During the palm oil extraction process, byproducts and wastes are produced, some of which are empty fruit bunches, palm kernel shells, and palm kernel cake (E Hambali & M Rivai, 2017). As the name suggests, empty fruit bunches are the biomass that remain after removing the oil palm fruit. This is one of the highest volume by-products in the palm oil extraction industry. This industry produces about one-third of its biomass as empty fruit bunches (Michael Osei Adu et al., 2022). After extracting the kernel nut, the industry refers to the remaining shell portions as palm kernel shells (Hashim et al., 2012). Due to their high calorific value, palm kernel shells serve as an energy source to produce biofuel (Uche Paul, 2015).

This chapter will focus on palm kernel cake, a by-product of palm oil extraction that primarily serves as a source of fat, protein, and carbohydrates in animal feeding supplements (Mohammad Naeem Azizi et al., 2021). Although its primary purpose is as an animal feed supplement, palm kernel cake remains one of the most widely available and affordable agricultural products in certain regions (Hamide Filiz Ayyildiz et al., 2023). It is considered a good choice as an ingredient in the feed formulation industry, but its high fiber content limits the amount that can be used. (Wan Zahari Mohamed & Abdul Razak Alimon, 2003) Palm kernel cake contains high quantities of acid-detergent fiber, which limits feed intake, degradability, and animal performance, making it a feed not suitable for all animals (Ahmed M. Abdeltawab and Mostafa S.A. Khattab, 2018). The chemical composition of palm kernel cake is highly dependent on the method used and the efficiency of oil extraction (Onuh S.O et al., 2010). The palm kernel cake is composed of approximately 14–18% crude protein, 47.71% carbohydrates, 12–20% crude fiber (CF), 3–9% ether extract, and minerals (Mohammad Naeem Azizi et al., 2021). According to Daud and Jarvis (1992), the palm kernel cake carbohydrate section contains 58% mannan, 12% cellulose, and 4% xylan. The main component of palm kernel cake is β–mannan (Sundu et al., 2006).

3.3.1 Lignocellulosic biomass

It is estimated that the production of total dry matter (TDM) per hectare of land used by the oil palm sector is approximately 55 tons per year (Nur et al., 2021). The lignocellulosic part of the oil palm biomass is mainly composed of three principal components: cellulose (30-60%), hemicellulose (17-23%) and lignin (18-23%) (S.S. Mohtar et al., 2015). Over 75% of PKC consists of cell-wall constituents, comprising 35.2% mannose, 2.6% xylose, 1.1% arabinose, 1.9% galactose, 15.1% lignin, and 5.0% ash (Cerveró et al., 2010).

The enormous amount of leftover plant biomass considered waste may be utilized to make a variety of goods with additional value, including chemicals, enzymes, biofuels, and prebiotics (Nosipho Hlalukana et al., 2021). However, the process of transforming lignocellulosic biomass into a commercially valuable product involves a long process of pretreatments and bio-compounds extractions. As a result, alternative methods for valorizing these by-products are currently being explored (S.S. Mohtar et al., 2015).

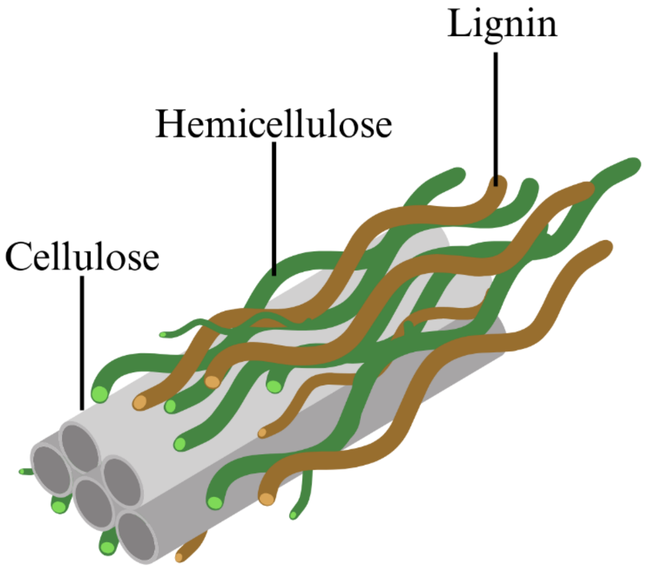

Figure 6. Biomass composition. Adapted from (Chai et al., 2022)

4. Palm kernel cake composition

4.1. Cellulose

Cellulose, the primary constituent of oil palm biomass, is a hard, solid polymer composed of glucose (Said et al., 2021). Plants, animals, algae, fungus, and minerals are all natural sources of cellulose (Heinze, 2015). Made up of linear chains of glucopyranose units connected by β-1,4 glycosidic bonds, cellulose is an insoluble polymer with a degree of polymerization of around 10,000. The general formula of cellulose is $C_6H_{10}O_5$ (Buong Woei Chieng et al., 2017). Each cellulose unit is composed of approximately 500 glucoses linked together by hydrogen bonds to form a ribbonlike structure (Alberts et al., 2024). Cellulose, the most abundant polysaccharide in the world since it is found in nearly all plant cell walls. It provides structural support and strength to plants by forming small crystals that organize into various structures depending on the plant (Parker et al., 2023).

4.2. Hemicellulose

After cellulose, hemicellulose components are likely the second most common renewable component of lignocellulosic biomass. These are hetero polysaccharides found in lignocellulosic biomass that are considerably underutilized (Farhat et al., 2017). A range of polysaccharides having linear or branching polymers formed from sugars including xylose, galactose, mannose, glucose, and arabinose are collectively referred to as hemicelluloses (Xu et al., 2011). Mokhtar et al. (2011) reported that the dry basis of palm kernel fruit is composed of 26-29% hemicellulose.

4.2.1. Mannan

Mannans are a type of hemicellulose mainly composed of long chains of mannose. Mannans make over 75% of all non-starch polysaccharides, making it the most prevalent kind

(E.M. Düsterhöft et al., 1992). Although mannan polysaccharides are found across the kingdom of plants, there are significant differences in their structure and quantity among species, tissues, and developmental stages (Catalin Voiniciuc, 2022). Four distinct types of mannans are found in nature: glucomannan, galactomannan, linear mannan, and galactoglucomannan, which are extensively distributed in nature. Researchers worldwide have discovered that they can be isolated from a variety of sources (Samkelo Malgas et al., 2015, Singh et al., 2018). Mannose coupled by β-1,4 linkages containing galactose substituents form the primary backbone of mannan (Nosipho Hlalukana et al., 2021). The primary fiber components of palm kernel cake are β-galactomannan and non-starch polysaccharides, which contribute to the cake's high fiber content (Almaguer et al., 2014). β-galactomannan is a polysaccharide composed of repeated units of mannose, together with galactose, glucose, or both (Hsiao et al., 2006). According to Jackson et al., 2004, the β-mannan content of palm kernel cake ranges from 300 to 350 g/kg.

4.2.2. Mannoligosacharides

Mannose oligosaccharides (MOS) can be synthesized by the breakdown of mannan. Different MOS products are made when endo-β-1,4-mannanases cut the mannan backbone at specific cleavage sites determined by the substitution pattern (Nosipho Hlalukana et al., 2021).

Since hemicellulose, which represents a high percentage of crude fiber in palm kernel cake and contains approximately 58% mannan, one of the main components of palm kernel cake, it presents a source of MOS (Daud & Jarvis, 1992) Also, because it includes a high quantity of β-mannan, which may be further hydrolyzed to produce MOS, palm kernel cake (PKC) is a prospective source of prebiotics. (W. Utami et al., 2013).

4.2.3. Mannose

The compound D-mannose is a C-2 epimer of d-glucose and is classified as a natural monosaccharide. Categorized as hexose, mannose belongs to the aldohexose group because of the aldehyde group in its open-chain structure (Adair, 2007). It may be derived from both vegetation and microorganisms (Hu et al., 2016). D-mannose is naturally present in some human cells and has a role in immune cell modulation as a prebiotic (Wang et al., 2021).

5. Mannose production methods

5.1 Enzymatic hydrolysis

Enzymatic hydrolysis is an eco-friendly, non-toxic, and energy-efficient technique (Liu et al.,2023). It is a technology that employs enzymes to decompose molecules into simpler chemicals (T. Wang & Lü, 2021). By enzymatic hydrolysis polysaccharides like mannan, glucomannan, and galactomannan can break down into mannooligosaccharides or D-mannose. The most common enzymes used to degrade these polysaccharides are β-mannanase and β-mannosidase (Wang et al., 2021). Endo-β-1,4-mannanase breaks the β-1,4 linkages, generating mannooligosaccharides that will be hydrolyzed to mannose by β-mannosidase action (Monteiro et al., 2019).

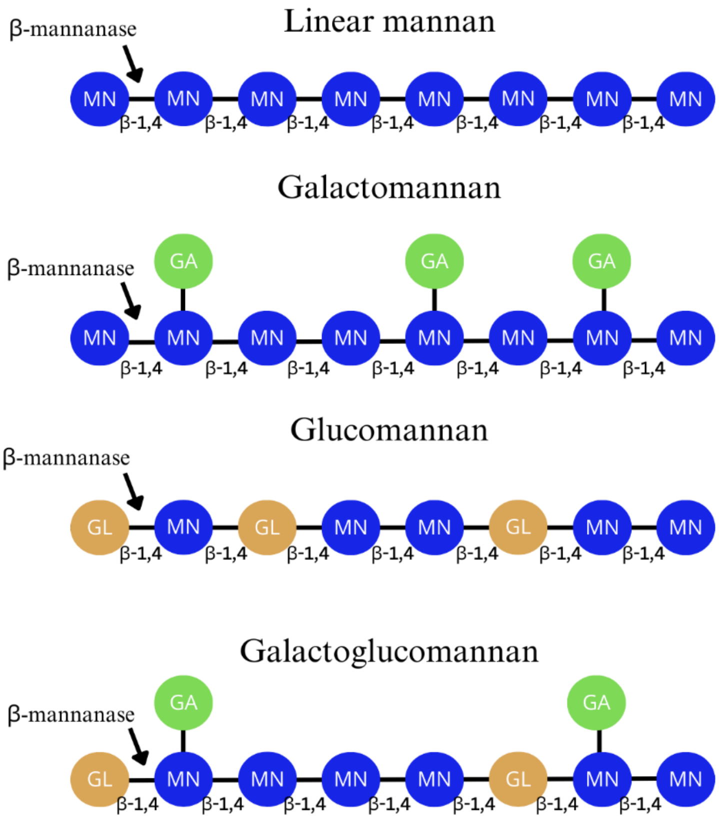

Figure 7. Structures of different types of mannans and the action of β-mannanase (β-1,4 glycosidic bonds hydrolysis). Adapted from (La Rosa et al., 2019).

5.2 Chemical catalysis

Catalysis is the technique of accelerating a chemical reaction by the addition of a catalyst. Acid or alkaline catalysts are used to accelerate the decomposition of substances (L. Wang & Yu, 2023). Extraction of mannose using chemical catalysis works by breaking glycosidic bonds (β-1,4 and β-1,6 bonds) which results in the release of mannose (Wan et al., 2024). An example is the chemical synthesis using molybdic acid or molybdate as catalyst to isomerize D-glucose into D-mannose (Hu et al., 2016).

5.3 Acid hydrolysis

Acid hydrolysis is a chemical process that employs acids to break down elements of food or other substances into smaller molecules. It may be employed to alter the structure and characteristics of materials such as starch, proteins, and cellulose (Punia et al., 2021; S. Wang & Copeland, 2013). Hemicellulose and cellulose undergo hydrolysis during dilute acid treatment, resulting in the production of soluble sugars (Świątek et al., 2020). Dilute acid hydrolysis is a highly successful technique for liberating hemicellulose sugars by breaking glycosidic bonds; nevertheless, a significant drawback is the simultaneous disintegration of monosaccharides generated during the process alongside the hydrolysis of polysaccharides (Steinbach et al., 2017; Wang et al., 2021).

6. Mannose applications

D-Mannose is a hexose with extensive applications in the pharmaceutical sector, the food and feed sectors, and biological research (Zhang et al., 2009). In the pharmaceutical industry D-mannose is frequently recommended as a dietary supplement for urinary tract health. Studies indicate that free D-mannose in urine may saturate E. coli FimH structures, inhibiting E. coli attachment to urinary tract epithelial cells (Ala-Jaakkola et al., 2022). Also, it has being demonstrated that mannose can significantly inhibit the proliferation of tumor cells and is crucial in the chemical modification of anti-cancer agents and nano-vaccines (Gonzalez et al., 2018; Dong et al., 2019). Mannose is utilized as a texture modifier in ice creams, salad dressings, and processed fruits within the food sector (De Mello Capetti et al., 2023).

7. Conclusions

The demand for African palm oil continues to rise, leading to an increase in the production of palm oil by-products. Traditionally, PKC has been considered a low commercial-value ingredient used in the livestock feed industry. The possibility of using PKC for mannose extraction allows the oil industry to make sustainable use of agricultural by-products and generate income, while moving towards a circular economy. Due to its high content of hemicellulosic material, particularly β-mannans, PKC presents a promising source of D-mannose. However, since it is composed of a high percentage of lignin, the extraction of compounds is challenging. Therefore, future research should focus on developing methods for the sustainable and efficient extraction of mannose to transform the commercial value of this by-product.

REFERENCES

1. USDA. “Indonesia: Oilseeds and Products Annual.” USDA Foreign Agricultural Service, 15 May 2024, fas.usda.gov/data/indonesia-oilseeds-and-products-annual-8.
2. Gupta, S.K. “Technological Innovations in Major World Oil Crops, Volume 1.” SpringerLink, Springer New York, 2012, link.springer.com/book/10.1007/978-1-4614-0356-2.
3. USDA FAS. “Production - Palm Oil.” Palm Oil | USDA Foreign Agricultural Service, 2024, fas.usda.gov/data/production/commodity/4243000.
4. René Renato Balandrán-Quintana, Ana María Mendoza-Wilson, Gabriela Ramos-Clamont Montfort, & José Ángel Huerta-Ocampo. (2019). “Chapter 4 - Plant-Based Proteins.” Elsevier EBooks, 97–130. https://doi.org/10.1016/b978-0-12-816695-6.00004-0
5. Murphy D. Oil palm value chain management. In: Allan T, Bromwich B, Keulertz M, Colman A, editors. The oxford handbook of food, water, and society. Oxford: Oxford University Press; 2019. p. 630–51.

6. Murphy, D. J., Goggin, K., & Paterson, R. M. (2021). Oil palm in the 2020s and beyond: challenges and solutions. CABI Agriculture and Bioscience, 2(1). https://doi.org/10.1186/s43170-021-00058-3
7. Pan, Y., Qi, Y., Li, X., Luan, S., & Huang, Y. (2021). Application of Mannose-Functionalized Microgel as a Novel Vaccine Delivery Platform for Subunit Vaccines. Advanced Functional Materials, 31(45). https://doi.org/10.1002/adfm.202105742
8. Véronique Gibon. (2012). Palm Oil and Palm Kernel Oil Refining and Fractionation Technology. Elsevier EBooks, 329–375. https://doi.org/10.1016/b978-0-9818936-9-3.50015-0

9. Wei, Z., Huang, L., Cui, L., & Zhu, X. (2020). Mannose: Good player and assister in pharmacotherapy. Biomedicine & Pharmacotherapy, 129, 110420–110420. https://doi.org/10.1016/j.biopha.2020.110420

10. Ngando-Ebongue, G. F., Ajambang, W. N., P. Koona, B. Lalu Firman, & V. Arondel. (2011). Oil Palm. Springer EBooks, 165–200. https://doi.org/10.1007/978-1-4614-0356-2_7

11. Abdelrahim Abubakr, Abdul Razak Alimon, Halimatun Yaakub, Abdullah, N., & Ivan, M. (2015). Effect of Feeding Palm Oil By-Products Based Diets on Muscle Fatty Acid Composition in Goats. PloS One, 10(3), e0119756–e0119756. https://doi.org/10.1371/journal.pone.0119756

12. Mba, O. I., Dumont, M.-J., & Ngadi, M. (2015). Palm oil: Processing, characterization and utilization in the food industry – A review. Food Bioscience, 10, 26–41. https://doi.org/10.1016/j.fbio.2015.01.003

13. Poku, K. (2002). Small-scale palm oil processing in Africa. Food and Agriculture Organization of the United Nations.

14. Hashim, K., Shawaluddin Tahiruddin, & Ahmad Jaril Asis. (2012). Palm and Palm Kernel Oil Production and Processing in Malaysia and Indonesia. Elsevier EBooks, 235–250. https://doi.org/10.1016/b978-0-9818936-9-3.50011-3

15. A.K. Mohd Omar, T.L Tengku Norsalwani, M.S. Asmah, Z.Y. Badrulhisham, Azhar Mat Easa, Fatehah Mohd Omar, Md. Sohrab Hossain, M.H. Zuknik, & N.A. Nik Norulaini. (2018). Implementation of the supercritical carbon dioxide technology in oil palm fresh fruits bunch sterilization: A review. Journal of CO2 Utilization, 25, 205–215. https://doi.org/10.1016/j.jcou.2018.03.021

16. Mba, O. I., Dumont, M.-J., & Ngadi, M. (2015). Palm oil: Processing, characterization and utilization in the food industry – A review. Food Bioscience, 10, 26–41. https://doi.org/10.1016/j.fbio.2015.01.003
17. Hassan, Md. Sohrab Hossain, Balakrishnan, V., Mark Harris Zuknik, Muliadi Mustaner, Azhar Mat Easa, Adel Al-Gheethi, & Naim, A. (2021). Influence of Fresh Palm Fruit Sterilization in the Production of Carotenoid-Rich Virgin Palm Oil. Foods, 10(11), 2838–2838. https://doi.org/10.3390/foods10112838
18. Shamsuddin, R., Singh, G., Kok, H., Rosli, M., Dawi Cahyono, N., Lam, M., Lim, J., & Low, A. (2021). Palm Oil Industry—Processes, By-Product Treatment and Value Addition. In Sustainable Bioconversion of Waste to Value Added Products (pp. 121–143). essay, Springer. Retrieved 2024, from https://link-springer-com.prox.lib.ncsu.edu/book/10.1007/978-3-030-61837-7.
19. Small-Scale Palm Oil Processing in Africa. (2024). Fao.org. https://www.fao.org/4/Y4355E/y4355e00.htm#Contents
20. Hashim, K., Shawaluddin Tahiruddin, & Ahmad Jaril Asis. (2012). Palm and Palm Kernel Oil Production and Processing in Malaysia and Indonesia. Elsevier EBooks, 235–250. https://doi.org/10.1016/b978-0-9818936-9-3.50011-3
21. Mba, O. I., Dumont, M.-J., & Ngadi, M. (2015). Palm oil: Processing, characterization and utilization in the food industry – A review. Food Bioscience, 10, 26–41. https://doi.org/10.1016/j.fbio.2015.01.003
22. E Hambali, & M Rivai. (2017). The Potential of Palm Oil Waste Biomass in Indonesia in 2020 and 2030. IOP Conference Series. Earth and Environmental Science, 65, 012050–012050. https://doi.org/10.1088/1755-1315/65/1/012050

23. Thin Sue Tang, & Pek Kooi Teoh. (1985). Palm kernel oil extraction - The malaysian experience. Journal of the American Oil Chemists' Society, 62(2), 254–258. https://doi.org/10.1007/bf02541387

24. Harun, M. Y., Azizi, M., M.H. Shah Ismail, & N. Azian Morad. (2016). A comparative investigation on the effect of thermal treatments on the mechanical properties of oil palm fruitlet components. Journal of the Taiwan Institute of Chemical Engineers, 60, 582–587. https://doi.org/10.1016/j.jtice.2015.10.030

25. Muhammad Johan Iskandar, Azizah Baharum, Farah Hannan Anuar, & Rizafizah Othaman. (2018). Palm oil industry in South East Asia and the effluent treatment technology—A review. Environmental Technology & Innovation, 9, 169–185. https://doi.org/10.1016/j.eti.2017.11.003

26. Michael Osei Adu, Atia, K., Arthur, E., Paul Agu Asare, Peter Bilson Obour, Eric Oppong Danso, Kwame Agyei Frimpong, Kwabena Azure Sanleri, Asare-Larbi, S., Adjei, R., Mensah, G., & Mathias Neumann Andersen. (2022). The use of oil palm empty fruit bunches as a soil amendmentto improve growth and yield of crops. A meta-analysis. Agronomy for Sustainable Development, 42(2). https://doi.org/10.1007/s13593-022-00753-z

27. Uche Paul, O., et al. (2015). Calorific value of palm oil residues for energy utilisation.

28. Ahmed M. Abdeltawab and Mostafa S.A. Khattab, 2018. Utilization of palm kernel cake as ruminant feed: A Review. Asian journal of biological sciences, 11: 157-164.

29. Daud, M. J., & Jarvis, M. C. (1992). Mannan of oil palm kernel. Phytochemistry, 31(2), 463–464. https://doi.org/10.1016/0031-9422(92)90017-k

30. Sundu, B., Kumar, A., & Dingle, J. (2006). Palm kernel meal in broiler diets: Effect on chicken performance and health. World's Poultry Science Journal, 62(2), 316–325. https://doi.org/10.1079/wps2005100
31. Mohammad Naeem Azizi, Teck Chwen Loh, Hooi Ling Foo, & Lim, E. (2021). Is Palm Kernel Cake a Suitable Alternative Feed Ingredient for Poultry? Animals, 11(2), 338–338. https://doi.org/10.3390/ani11020338
32. Hsiao, H.-Y., Anderson, D. M., & D, N. M. (2006). Levels of B-mannan in soybean meal. Poultry Science, 85(8), 1430-1432. https://doi.org/10.1093/PS/85.8.1430
33. Nosipho Hlalukana, Mihle Magengelele, Samkelo Malgas y Brett Ivan Pletschke. (2021). Enzymatic conversion of mannan-rich plant waste biomass into prebiotic mannooligosaccharides. Alimentos, 10(9),2010-2010. https://DOI.ORG/10.3390/FOODS10092010
34. Henson, I. E. (2012). A Brief History of the Oil Palm. In Elsevier eBooks (pp. 1–29). https://doi.org/10.1016/b978-0-9818936-9-3.50004-6
35. Wagenlehner, F., Lorenz, H., Ewald, O., & Gerke, P. (2022). Why d-Mannose May Be as Efficient as Antibiotics in the Treatment of Acute Uncomplicated Lower Urinary Tract Infections—Preliminary Considerations and Conclusions from a Non-Interventional Study. Antibiotics, 11(3), 314. https://doi.org/10.3390/antibiotics11030314
36. Tabe-Ojong, M. P. J., Alamsyah, Z., & Sibhatu, K. T. (2023). Oil palm expansion, food security and diets: Comparative evidence from Cameroon and Indonesia. Journal of Cleaner Production, 418, 138085. https://doi.org/10.1016/j.jclepro.2023.138085
37. FAOSTAT. (2022). Retrieved December 28, 2022, from https://www.fao.org/faostat/en/#data/QCL

38. Ali, F. S., Shamsudin, R., & Yunus, R. (2014). The Effect of Storage Time of Chopped Oil Palm Fruit Bunches on the Palm Oil Quality. Agriculture and Agricultural Science Procedia, 2, 165–172. https://doi.org/10.1016/j.aaspro.2014.11.024
39. Barcelos, E., De Almeida Rios, S., Cunha, R. N. V., Lopes, R., Motoike, S. Y., Babiychuk, E., Skirycz, A., & Kushnir, S. (2015). Oil palm natural diversity and the potential for yield improvement. Frontiers in Plant Science, 6. https://doi.org/10.3389/fpls.2015.00190
40. Hu, X., Shi, Y., Zhang, P., Miao, M., Zhang, T., & Jiang, B. (2016). d-Mannose: Properties, Production, and Applications: An Overview. Comprehensive Reviews in Food Science and Food Safety, 15(4), 773–785. https://doi.org/10.1111/1541-4337.12211
41. Shamsuddin, R., Singh, G., Kok, H. Y., Rosli, M. H., Cahyono, N. a. D., Lam, M. K., Lim, J. W., & Low, A. (2021). Palm Oil Industry—Processes, By-Product Treatment and Value Addition. In Advances in Science, Technology & Innovation/Advances in science, technology & innovation (pp. 121–143). https://doi.org/10.1007/978-3-030-61837-7_8
42. De Almeida Rios, S., Da Cunha, R. N. V., Lopes, R., Barcelos, E., Da Rocha, N. R. N. C., & De Lima, W. a. A. (2018). Correlation and Path analysis for yield components in Dura oil palm germplasm. Industrial Crops and Products, 112, 724–733. https://doi.org/10.1016/j.indcrop.2017.12.054
43. Poku, K. (2002). Small-scale palm oil processing in Africa (F. and A. O. of the U. Nations, Ed.). Food & Agriculture Org.
44. Statista. (2024, February 13). Vegetable oils: global consumption 2013/14 to 2023/24, by oil type. https://www.statista.com/statistics/263937/vegetable-oils-global-consumption/

45. Said, F. M., Hamid, N. F., Razali, M. A., & Daud, N. F. S. (2021). Lignocellulosic of oil palm biomass to chemical product via fermentation. In IntechOpen eBooks. https://doi.org/10.5772/intechopen.99312
46. Cerveró, J. M., Skovgaard, P. A., Felby, C., Sørensen, H. R., & Jørgensen, H. (2010). Enzymatic hydrolysis and fermentation of palm kernel press cake for production of bioethanol. In Enzyme and Microbial Technology (Vol. 46, pp. 177–184). https://doi.org/10.1016/j.enzmictec.2009.10.012
47. Sharma, V., Smolin, J., Nayak, J., Ayala, J. E., Scott, D. A., Peterson, S. N., & Freeze, H. H. (2018). Mannose alters gut microbiome, prevents Diet-Induced obesity, and improves host metabolism. Cell Reports, 24(12), 3087–3098. https://doi.org/10.1016/j.celrep.2018.08.064
48. Srivastava, M., & Kapoor, V. P. (2005). Seed Galactomannans: An Overview. Chemistry & Biodiversity, 2(3), 295–317. https://doi.org/10.1002/cbdv.200590013
49. Wang, P., Zheng, Y., Li, Y., Shen, J., Dan, M., & Wang, D. (2021). Recent advances in biotransformation, extraction and green production of D-mannose. Current Research in Food Science, 5, 49–56. https://doi.org/10.1016/j.crfs.2021.12.002
50. Zhang, T., Pan, Z., Qian, C., & Chen, X. (2009). Isolation and purification of d-mannose from palm kernel. Carbohydrate Research, 344(13), 1687–1689. https://doi.org/10.1016/j.carres.2009.06.018
51. Adair, W. L. (2007). Carbohydrates. In Elsevier eBooks (pp. 1–12). https://doi.org/10.1016/b978-008055232-3.60056-x
52. Ala-Jaakkola, R., Laitila, A., Ouwehand, A. C., & Lehtoranta, L. (2022b). Role of D-mannose in urinary tract infections – a narrative review. Nutrition Journal, 21(1). https://doi.org/10.1186/s12937-022-00769-x

53. Gonzalez, P. S., O’Prey, J., Cardaci, S., Barthet, V. J. A., Sakamaki, J., Beaumatin, F., Roseweir, A., Gay, D. M., Mackay, G., Malviya, G., Kania, E., Ritchie, S., Baudot, A. D., Zunino, B., Mrowinska, A., Nixon, C., Ennis, D., Hoyle, A., Millan, D., . . . Ryan, K. M. (2018). Mannose impairs tumour growth and enhances chemotherapy. Nature, 563(7733), 719–723. https://doi.org/10.1038/s41586-018-0729-3
54. Dong, Z., Wang, Q., Huo, M., Zhang, N., Li, B., Li, H., Xu, Y., Chen, M., Hong, H., & Wang, Y. (2019). Mannose-Modified Multi-Walled carbon nanotubes as a delivery nanovector optimizing the antigen presentation of dendritic cells. ChemistryOpen, 8(7), 915–921. https://doi.org/10.1002/open.201900126
55. De Mello Capetti, C. C., Dabul, A. N. G., De Oliveira Arnoldi Pellegrini, V., & Polikarpov, I. (2023). Mannanases and other mannan-degrading enzymes. In Elsevier eBooks (pp. 279–293). https://doi.org/10.1016/b978-0-323-91805-3.00013-7
56. Liu, A., Wu, H., Naeem, A., Du, Q., Ni, B., Liu, H., Li, Z., & Ming, L. (2023). Cellulose nanocrystalline from biomass wastes: An overview of extraction, functionalization and applications in drug delivery. International Journal of Biological Macromolecules, 241, 124557. https://doi.org/10.1016/j.ijbiomac.2023.124557
57. Wang, T., & Lü, X. (2021). Overcome saccharification barrier. In Elsevier eBooks (pp. 137–159). https://doi.org/10.1016/b978-0-12-818862-0.00005-4
58. Monteiro, A. F., Miguez, I. S., Silva, J. P. R. B., & Da Silva, A. S. (2019). High concentration and yield production of mannose from açaí (Euterpe oleracea Mart.) seeds via mannanase-catalyzed hydrolysis. Scientific Reports, 9(1). https://doi.org/10.1038/s41598-019-47401-3

59. Wang, L., & Yu, J. (2023). Principles of photocatalysis. In Interface science and technology (pp. 1–52). https://doi.org/10.1016/b978-0-443-18786-5.00002-0
60. Wan, J., Chu, P., Wang, X., Sun, Z., Zhang, W., Wang, Y., Zhang, Q., Yan, W., Jin, X., & Yang, C. (2024). Catalytic epimerization of glucose to mannose. ACS Sustainable Chemistry & Engineering. https://doi.org/10.1021/acssuschemeng.4c03893
61. Hu, X., Shi, Y., Zhang, P., Miao, M., Zhang, T., & Jiang, B. (2016). d-Mannose: Properties, Production, and Applications: An Overview. Comprehensive Reviews in Food Science and Food Safety, 15(4), 773–785. https://doi.org/10.1111/1541-4337.12211
62. Świątek, K., Gaag, S., Klier, A., Kruse, A., Sauer, J., & Steinbach, D. (2020). Acid hydrolysis of lignocellulosic biomass: sugars and furfurals formation. Catalysts, 10(4), 437. https://doi.org/10.3390/catal10040437
63. Punia, S., Kumar, M., Siroha, A. K., Kennedy, J. F., Dhull, S. B., & Whiteside, W. S. (2021). Pearl millet grain as an emerging source of starch: A review on its structure, physicochemical properties, functionalization, and industrial applications. Carbohydrate Polymers, 260, 117776. https://doi.org/10.1016/j.carbpol.2021.117776
64. Steinbach, D., Kruse, A., & Sauer, J. (2017). Pretreatment technologies of lignocellulosic biomass in water in view of furfural and 5-hydroxymethylfurfural production- A review. Biomass Conversion and Biorefinery, 7(2), 247–274. https://doi.org/10.1007/s13399-017-0243-0
65. Chai, Y. D., Pang, Y. L., Lim, S., Chong, W. C., Lai, C. W., & Abdullah, A. Z. (2022). Recent progress on tailoring the Biomass-Derived cellulose hybrid composite photocatalysts. Polymers, 14(23), 5244. https://doi.org/10.3390/polym14235244

66. La Rosa, S. L., Leth, M. L., Michalak, L., Hansen, M. E., Pudlo, N. A., Glowacki, R., Pereira, G., Workman, C. T., Arntzen, M. Ø., Pope, P. B., Martens, E. C., Hachem, M. A., & Westereng, B. (2019). The human gut Firmicute Roseburia intestinalis is a primary degrader of dietary β-mannans. Nature Communications, 10(1). https://doi.org/10.1038/s41467-019-08812-y

CHAPTER 2

PROCESS OPTIMIZATION AND CHARACTERIZATION OF A NOVEL MANNOSE SYRUP FROM PALM KERNEL CAKE USING ENZYMATIC HYDROLYSIS AND RESPONSE SURFACE METHODOLOGY

Abstract

Global industries are persistently seeking to integrate sustainable practices. Revalorization of by-products presents a promising opportunity to minimize environmental impacts and yield profits for businesses. Mannose is an important monosaccharide that can be extracted from the palm kernel cake (PKC), a by-product from the palm oil industry rich in mannans. Mannose has gained popularity over time because it is highly used in the pharmaceutical and food industry. It is mainly extracted from wood, legumes and seeds. In this study, an optimized enzymatic hydrolysis method using mannanase was developed to maximize mannose extraction from PKC. To optimize the enzymatic treatments and maximize the response (mannose content) a Box-Behnken experimental design was used. The optimum parameters were 16 hours, 5% solids and 10% enzymes (mannanase). With these conditions an extraction efficiency of 26.67% was achieved. The optimized treatment was subsequently used to elaborate a syrup that underwent physicochemical characterization. The syrup had low moisture content (15.85±0.07), water activity (0.6918±0.003), and pH (4.05±0.282) which ensure an extended shelf and microbiological stability.

1. Introduction

Over the years, the demand for food products has been constantly growing. This growth has also led to an increase in the production of by-products. The oil industry is no exception, with a daily demand of more than 104 million barrels (Statista, 2025). Responsible by-product management has become an important concern for the industry. Consumers now demand to be informed about the processing of their food and the impact it has on the planet. The industry is looking for new ways to revalorize these by-products, starting with making products or ingredients from them. African palm oil is the largest oil crop produced with an annual production of 76.26 million metric tons (Statista, 2024). With this growth also comes a higher production of by-products, such as palm kernel cake (PKC). This is the solid residue from the extraction of palm kernel cake oil (Olukomaiya et al., 2019).

Mannose is a naturally occurring monosaccharide that has attracted considerable interest in food science and biotechnology, attributed to its functional properties and prospective health advantages (Capetti et al., 2023). It is an epimer of glucose essential in glycosylation activities in human metabolism (Sharma et al., 2014). Mannose is commonly found in complex polysaccharides such as mannan, made up of β-1,4-linked mannose (linear mannan) or glucose (glucomannan) and alternating side chains of α-1,6-linked galactose residues (galactomannan/galactoglucomannan) (Berglund et al., 2019). The hydrolysis of these polysaccharides into free mannose is an opportunity for producing a mannose-rich syrup that can serve as a functional ingredient in food, pharmaceuticals, and nutraceuticals.

PKC contains about 30 to 35% β-mannan, which allows it to be considered a source of mannose. However, the composition of PKC makes this a challenge. PKC contains 50.3% carbohydrates, of which cellulose constitutes 11.6% and hemicellulose 61.5% (Balandrán-

Quintana et al., 2019; Azman et al., 2016). Cellulose, being strongly coupled to hemicellulose and lignin in the PKC, presents considerable obstacles in the isolation of mannose (Abolore et al., 2023). The complex morphology of lignocellulosic biomass composed of a network of cellulose, lignin, and hemicellulose makes the processing and isolation of compounds from them difficult (Asim et al., 2020). There are different delignification methods, such as the kraft method, alkali method, and acid hydrolysis; however, all generate a high impact on the planet (Kaur et al., 2020). In this study, a more environmentally friendly delignification method was used, such as the Wise method, which uses acetic acid and sodium chlorite.

In this study, the enzyme mannanase was used for the extraction of mannose from delignified PKC. Mannanase hydrolyzes the β-1,4 glycosidic bond and converts long-chain mannans into smaller oligosaccharides and free mannose (Linton, 2019; X. Zhang et al., 2024). The main objective was to develop an efficient methodology for mannose production from enzymatic hydrolysis. For this purpose, an optimization of enzymatic hydrolysis was performed by assessing the effect of operational parameters on mannose content using the response surface methodology (RSM). The physicochemical characteristics (Brix, pH, water activity, moisture content, and color) of the optimized treatment were evaluated. The results of this research provide key information in the optimization of this process that will allow reducing processing costs in mannose extraction through enzymatic hydrolysis.

2. Materials and methods

2.1 Materials

Palm kernel cake used was donated by (Palmas del Ixcan, Guatemala and UUMBAL AgroIndustrial, México). Chemicals and reagents used were purchased from Sigma Aldrich (Saint Louis, MO), unless otherwise specified. Sodium chlorite ($NaClO_2$; 80%), acetic acid

(CH_3CO_2H; 99.5%), hydrochloric acid (HCl; 37%), n-hexane ($CH_3(CH_2)_4CH_3$; $\geq$97.0%), Laccase (Laccase; CAS No. 80498-15-3; Amano Enzyme Inc.), Aromase (Beta-glucosidase; CAS No. 9001-22-3; Amano Enzyme Inc.), Mannanase BGM “Amano” 10 (Mannanase; CAS No. 37288-54-3; Amano Enzyme Inc.).

2.2 Palm kernel meal preparation

The sample was ground (high-speed multifunction Grinder HC-2000, Goldenwall, Glendale, CA, USA) and sieved (80 mesh) to obtain a homogeneous flour. The sample was defatted using n-hexane; a mix of PKC in hexane (25 w/v) was prepared. The sample was subjected to ultrasonication (YUCHENGTECH Ultrasonic Homogenizer Sonicator) (20 kHz, 900 W) for 30 minutes. Subsequently, the sample was stirred using a 5-inch LED magnetic hotplate stirrer at room temperature for an hour at 800 rpm. After, the sample was centrifuged to separate the supernatant from the solids. Finally, the supernatant was roto evaporated to remove the solvent (n-hexane). The solids and the oil were left in the hood for 24 hours to evaporate any solvent residue in the samples.

The sample was delignified using the Wise method following the methodology described by Kumar et al. (2012); 20 g of defatted palm kernel meal was mixed with 500 ml of double deionized water (DDI) in a 1,000 ml Erlenmeyer. Next, 4 ml of acetic acid and 6.66 g of sodium chlorite were added to the mixture. It was then covered and stirred in a water bath at 70°C for 2 hours. The addition of acetic acid and sodium chlorite was repeated 2 more times to complete a total of 6 hours of delignification. Once the delignification was finished, the sample was centrifuged; the solids were recovered and washed until a neutral pH was reached. The sample was dried in the oven (Isotemp 285A vacuum oven, Fisher Scientific, Hampton, NH, EUA) at

50°C; it was ground (high-speed multifunction Grinder HC-2000, Goldenwall, Glendale, CA, USA) and sieved (80 mesh) to obtain homogeneous powder.

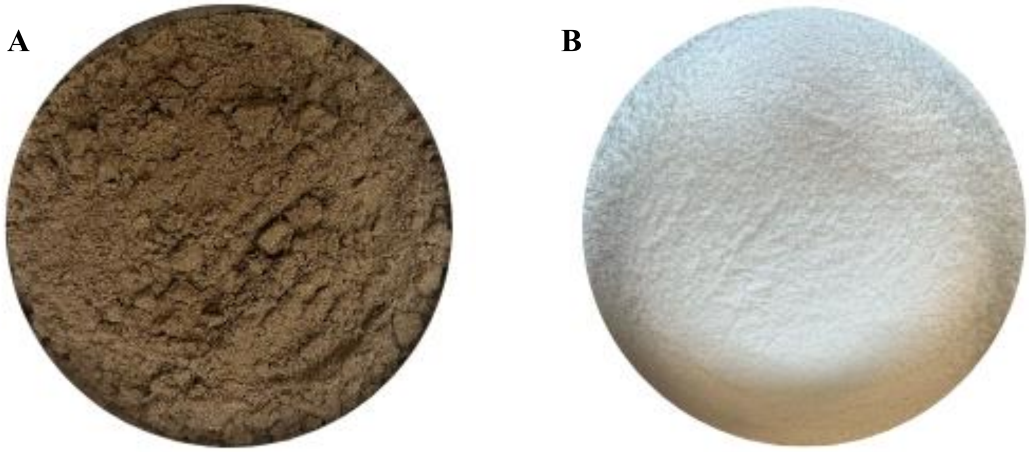

Figure 1. Palm kernel cake before and after the delignification process. A: Palm kernel cake. B: Delignified palm kernel cake.

2.3 Enzymatic hydrolysis of palm kernel cake

2.3.1 Experimental design

To determine optimized conditions for palm kernel meal hydrolysis, a 3^3 Box-Behnken (BBD) design for the response surface methodology (RSM) was used. For the optimization, fifteen different tests were run (Table 1). These were determined by the combination of three independent variables with three different levels: time (16, 32, 48 hours), enzyme content (5, 7.5, 10%), and solid content (5, 10, 15%). The dependent variable analyzed was mannose recovery. The objective was to determine the best hydrolysis conditions that maximize mannose recovery.

To prepare the treatment, 3 grams of sample were mixed with DDI water in 50 ml centrifuge tubes to prepare the different solid treatments (Table 1). The pH of the solutions was adjusted to 4.0 with a pH meter (model Orion Star A211, Thermo Fisher Scientific, Waltham, MA, USA) using an HCl solution. The samples were pretreated with laccase (10% w/w) and aromase (10% w/w) using the methodology described by Singh et al. (2023) and Shukor et al. (2015) with slight modifications. The samples were placed in an incubator under constant mixing (200 rpm) for 6 hours. Subsequently, mannanase was added in different percentages and was incubated for various periods (Table 1). Once the hydrolysis was finished, the samples were placed in a water bath at 90°C for 7 minutes to deactivate the enzyme. The samples were kept in cold storage (-20°C) until the mannose content was measured.

Table 1. BBD experimental design for the hydrolysis optimization of palm kernel meal using Mannanase BGM "Amano" 10.

Test	Time (hrs.)	Solid (% w/v)	Enzyme (% w/v)
1	16	5	7.5
2	48	5	7.5
3	16	15	7.5
4	48	15	7.5
5	16	10	5
6	48	10	5
7	16	10	10
8	48	10	10
9	32	5	5
10	32	15	5
11	32	5	10
12	32	15	10
13*	32	10	7.5
14*	32	10	7.5
15*	32	10	7.5

Legend: *Central points

2.4 Mannose content

Mannose content of the hydrolysis was measured with the D-Mannose, D-Fructose, D-Glucose Assay kit (K-MANGL) (Megazyme International, Wicklow, Ireland) according to the manufacturer's instructions.

2.5 Characterization of palm kernel mannose syrup

To produce palm kernel mannose syrup, the supernatant from the hydrolysis was collected and the water evaporated in a rotary evaporator (BUCHI Rotovapor R-300) at 55°C. The treatment that was identified as the best in the optimization process was characterized.

2.5.1 Moisture content and water activity

Moisture content of the sample was measured using a moisture analyzer (Mettler Toledo HE53, Columbus, OH, USA). An Aqualab water activity meter (model 4TE, Meter, Palo Alto, CA, USA) was used to measure the water activity (a_w) of the sample.

2.5.2 Brix (°Bx) and pH

Brix (°Bx) of the syrup was measured using a digital refractometer (model MA871, Milwaukee). A pH meter (model Orion Star A211, Thermo Fisher Scientific, Waltham, MA, USA) was used to measure the pH of the syrup.

2.5.3 Color analysis

Color analysis was carried out using a colorimeter (CR-5, Konica, Minolta, Japan) previously calibrated with white and black standards to ensure accuracy. To measure the color the CIELAB L* (lightness), a* (redness-greenness), and b* (yellowness-blueness) parameters were recorded. The values were input into an online color visualization tool (ColorDesigner website) to generate an approximate visual representation.

3. Statistical analysis

The experimental design and data analysis of the BBD were executed using the DesignExpert (version 13; Stat-Ease, Minneapolis, MN) software. The Regression model was adjusted to experimental data and response surfaces were elaborated.

4. Results and discussion

4.1 Optimization of mannose hydrolysis extraction

One independent regression model was constructed using the mannose content (g/L). For the model coded factors were used: A) hours, B) solid (% w/v), and C) enzyme (% w/v) (Eq. 1):

Mannose content (g/L) = $2.00 + 0.7620A - 1.32B + 0.4490C + 0.4467AB + 0.3583AC + 1.14B^2$ [Eq. 1]

Mannose content was influenced by: A) hours, B) solids , and C) enzyme, and the linear interaction between AB and AC. The quadratic term B^2 (solid %) dictates the downward curvature in the 3D response surface images (Fig. 2).←no. Fig 1 is the picture of the powder cakes. Do you mean something else here? Do you mean Fig. 2? A downward curvature in the graphs indicates that factors (B) had an influence in the mannose production in a non-linear manner. This indicates that there is an optimal region where the mannose yield is maximized before it decreases. This optimization was important because it showed the importance of identifying the optimal conditions to prevent efficiency losses in the process.

Table 2. Experimental results of sugar content by BBD experimental design.

Test	**Time (hrs.)**	**Solid (% w/v)**	**Enzyme (% w/w)**	**Mannose content (g/L)**
1	16	5	7.5	4.334
2	48	5	7.5	4.87
3	16	15	7.5	1.02
4	48	15	7.5	3.343
5	16	10	5	1.508
6	48	10	5	2.41
7	16	10	10	1.749
8	48	10	10	4.084
9	32	5	5	4.533
10	32	15	5	1.3
11	32	5	10	4.981
12	32	15	10	2.475
13*	32	10	7.5	1.982
14*	32	10	7.5	1.798
15*	32	10	7.5	2.214

Legend: *Central points

The ANOVA results for the BBD model indicate that the model is significant for predicting the mannose content, showing an F-value of (68.26) and a p-value of 0.0001. The model sum of squares (SS= 26.57) accounts for almost all the total variability (SS= 26.78) which demonstrates that the model correctly calculates mannose yield. Furthermore, the lack-of-fit (LoF) test is not significant (p = 0.5375), confirming that the model adequately fits the data. The R^2 of 0.9919 indicates that the model correctly explains the variability of the variable measured (mannose content).

Table 3. Analysis of variance (ANOVA) of BBD model to maximize mannose content.

Factor	**SS**	**df**	**MS**	**F-value**	**p-value**
Mannose content (g/L)					
Model	26.57	9	2.95	68.26	0.0001
Residual	0.2162	5	0.0432		
LoF	0.1293	3	0.0431	0.9920	0.5375
PE	0.0869	2	0.0435		
Total	26.78	14			
R^2	0.9919				

Legend: SS: Sum of squares; df: Degree of freedom, MS: Means Square, LoF: Lack of fit, PE: Pure error; R^2 : correlation coefficient.

In Table 2 it can be observed that the highest mannose content (4.981 g/L) was reached at 32 hours, 5% w/v solids, and 10% w/w enzyme achieving a yield of 27.67% mannose extraction. This data can be compared with what José María Cerveró et al. (2009) reported, a yield of 30% using 5% solids and 10% (v/w) enzyme (mannanase). This result was obtained from the extraction of mannose from palm kernel cake by enzymatic hydrolysis. On the other hand, Monteiro et al (2019) had a mannose recovery of 98.6% from açaí seeds by pretreating the sample with sulfuric acid at 121 °C followed by an enzymatic hydrolysis using mannanase. However, the unique recalcitrant lignocellulosic composition of PKC makes it more resistant to enzymatic hydrolysis, meaning that the enzyme cannot access the mannans as easily as in other biomass products (Shukor et al., 2016). This indicates that the methodology used in this research for lignin removal should be improved; looking for pretreatment options that help to better remove lignin and degrade cellulose should be the next step in this research. In this way, mannanase will be able to act efficiently by accessing mannans and produce a larger amount of mannose. Chang and Holtzapple (2000) and Kumar et al. (2012) used peracetic acid at 25°C, 5 wt.% solids and peracetic acid loadings of 0.75 g/g dry solids, 2.0 g/g dry solids, 3.5 g/g dry solids, and 5.5 g/g dry solids for 24–48 h. Achieving a delignification of >90% in switchgrass, poplar, corn stover, and pine sawdust. These materials have a similar composition to PKC, giving a promising solution for the delignification step. If these results are achieved in PKC, the efficiency of hydrolysis should increase because the enzyme will have easier access to the hemicellulose, resulting in more mannose conversion.

Based on the results obtained from 15 hydrolysis treatments, the optimized enzymatic hydrolysis treatment conditions were calculated using a desirability function with the goal of maximizing the solids and minimizing the enzyme content and hours while maximizing mannose content. The predicted value for mannose content was (4.682 g/L) under the following conditions: 16 hours, 5% solids (w/v) and 5% enzyme (w/w). The actual experiment value was 4.325 g/L.

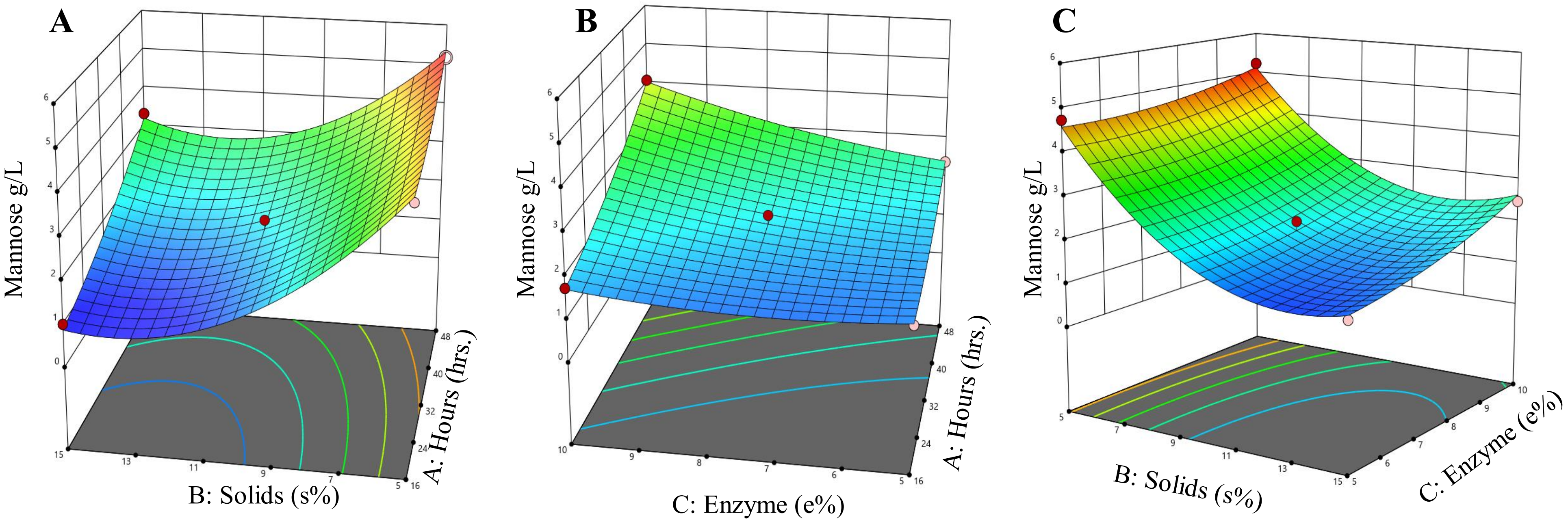

Figure 2. Three-dimensional response surface for mannose content. A: solid (%) and hours (hrs.). B: enzyme (e%) and hours. C: solid (%) and hours (hrs.).

4.2 Palm kernel mannose syrup characterization

To assess the stability and suitability for storage of the palm kernel mannose syrup the physicochemical properties were analyzed (Table 4). It was found that the optimized treatment had a low moisture content (15.85±0.07%), which is similar to what BeMiller (2018) found, which was 15% moisture content in corn syrup. Moisture content is an important parameter to consider in syrups production because it affects the stickiness, stability and shelf life of the final product (Wang & Hartel, 2020). The water activity ($a_{w\,=}$ 0.6918±0.003) shows that the syrup has limited water available limiting the microbial growth risk. The sensorial properties of foods, including aroma, flavor, and texture are significantly influenced by water activity (Rahman, 2007, Maneffa et al., 2017). The syrup has a °Brix value of 70.1±0.424, which compares with that reported by Willis et al. (2013), who reported 74 °Brix in a sorghum syrup. Additionally, the pH (4.05±0.282) indicates an acidic value. Which will help in the microbial growth inhibition and enhance shelf-life stability. Food items fall into two categories: low-acid (pH over 4.6) and high-acid (pH below 4.6). The basis for this differentiation is the capacity of the foodborne pathogen Clostridium *botulinum* to proliferate at pH 4.6, which is the lower limit (Rolfe & Daryaei, 2020). The physicochemical characteristics measured suggest that the palm kernel mannose syrup is a stable product with a reduced risk of spoilage.

Color is an important attribute in the food industry, as it allows a product to be used in different food matrices. The CIELAB color space parameters were measured (Table 5); the *L value* (lightness) was 47.92±0.198 indicating moderate brightness (Figure 3). Figure 3 should be cited here --- I do not see anywhere the internal citation for Fig 3 The *a value* (34.35±0.565) represents an inclination for the red hue, while the *b value* (64.81±0.261) inclines towards a yellow component in the color. The color can be compared to syrups available in the market.

Table 4. Physicochemical properties of palm kernel mannose syrup obtained under optimized conditions**.**

Parameter	**Palm kernel mannose syrup**
Moisture (%)	15.85±0.07
a_w	0.6918±0.003
°Brix	70.1±0.424
pH	4.05±0.282

Legend: Results are shown as mean ± standard deviation (n=2).

Table 5. Color parameters of palm kernel mannose syrup obtained under optimized conditions**.**

Parameter	**Palm kernel mannose syrup**
L* (Lightness)	47.92±0.198
a* (Red-Green)	34.35±0.565
b* (Yellow-Blue)	64.81±0.261

Legend: Results are shown as mean ± standard deviation (n=2).

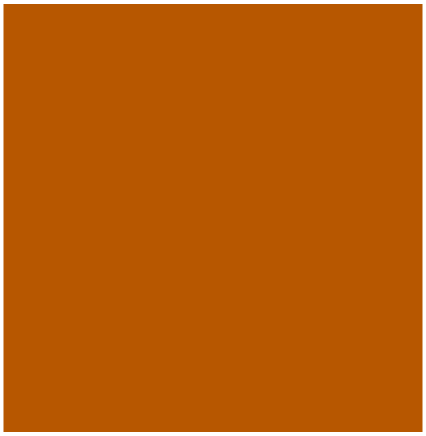

Figure 3. Visual representation of palm kernel mannose syrup color.

5. Conclusions

This study successfully optimized the hydrolysis of PKC using response surface methodology, identifying ideal conditions of 5% (w/v) solids and 10% (w/w) enzyme over 16 hours. The resulting mannose-rich syrup exhibited low moisture content, water activity, and pH, enhancing its shelf stability and expanding its potential applications across various industries. By leveraging enzymatic hydrolysis and physicochemical characterization, this research highlights PKC as a valuable source of mannose for industrial use. Further advancements in extraction efficiency and sensory evaluation could drive innovation in the development of high-quality, sustainable ingredients.

REFERENCES

1. Olukomaiya, O., Fernando, C., Mereddy, R., Li, X., & Sultanbawa, Y. (2019). Solid-state fermented plant protein sources in the diets of broiler chickens: A review. Animal Nutrition, 5(4), 319–330. https://doi.org/10.1016/j.aninu.2019.05.005
2. Kumar, R., Hu, F., Hubbell, C. A., Ragauskas, A. J., & Wyman, C. E. (2012). Comparison of laboratory delignification methods, their selectivity, and impacts on physiochemical characteristics of cellulosic biomass. Bioresource Technology, 130, 372–381. https://doi.org/10.1016/j.biortech.2012.12.028
3. Singh, G., Kumar, S., Afreen, S., Bhalla, A., Khurana, J., Chandel, S., Aggarwal, A., & Arya, S. K. (2023). Laccase mediated delignification of wasted and non-food agricultural biomass: Recent developments and challenges. International Journal of Biological Macromolecules, 235, 123840. https://doi.org/10.1016/j.ijbiomac.2023.123840
4. Shukor, H., Abdeshahian, P., Al-Shorgani, N. K. N., Hamid, A. A., Rahman, N. A., & Kalil, M. S. (2015). Saccharification of polysaccharide content of palm kernel cake using enzymatic catalysis for production of biobutanol in acetone–butanol–ethanol fermentation. Bioresource Technology, 202, 206–213. https://doi.org/10.1016/j.biortech.2015.11.078
5. Balandrán-Quintana, R. R., Mendoza-Wilson, A. M., Montfort, G. R., & Huerta-Ocampo, J. Á. (2019). Plant-Based proteins. In Elsevier eBooks (pp. 97–130). https://doi.org/10.1016/b978-0-12-816695-6.00004-0
6. Statista. (2025, February 4). Global oil industry. Statista. https://www.statista.com/topics/1783/global-oil-industry-and-market/

7. Statista. (2024, February 13). Vegetable oils: global consumption 2013/14 to 2023/24, by oil type. https://www.statista.com/statistics/263937/vegetable-oils-global-consumption/
8. BeMiller, J. N. (2018). Carbohydrate and Noncarbohydrate Sweeteners. Elsevier EBooks, 371–399. https://doi.org/10.1016/b978-0-12-812069-9.00019-4
9. Willis, O. O., Mouti, M. E., Sila, D. N., Mwanjala Mwasaru, Thiongo, G., Hunja Murage, & Ojijo, N. O. (2013). Physico-Chemical Properties and Antioxidant Potential of Syrup Prepared from “Madhura” Sweet Sorghum (Sorghum bicolor L. Moench) Cultivar Grown at Different Locations in Kenya. Sugar Tech, 15(3), 263–270. https://doi.org/10.1007/s12355-013-0222-0
10. Capetti, C. C. de M., Dabul, A. N. G., Pellegrini, V. de O. A., & Polikarpov, I. (2023). Mannanases and other mannan-degrading enzymes. Glycoside Hydrolases, 279–293. https://doi.org/10.1016/b978-0-323-91805-3.00013-7
11. Sharma, V., Ichikawa, M., & Freeze, H. H. (2014). Mannose metabolism: More than meets the eye. Biochemical and Biophysical Research Communications, 453(2), 220–228. https://doi.org/10.1016/j.bbrc.2014.06.021
12. Berglund, J., Azhar, S., Lawoko, M., Mikael Lindström, Vilaplana, F., Wohlert, J., & Henriksson, G. (2018). The structure of galactoglucomannan impacts the degradation under alkaline conditions. Cellulose, 26(3), 2155–2175. https://doi.org/10.1007/s10570-018-1737-z
13. José María Cerveró, Pernille Anastasia Skovgaard, Claus Felby, Hanne Risbjerg Sørensen, & Henning Jørgensen. (2009). Enzymatic hydrolysis and fermentation of palm kernel press cake for production of bioethanol. Enzyme and Microbial Technology, 46(3-4), 177–184. https://doi.org/10.1016/j.enzmictec.2009.10.012

14. Azman, N. F., Abdeshahian, P., Kadier, A., Shukor, H., Al-Shorgani, N. K. N., Hamid, A. A., & Kalil, M. S. (2016). Utilization of palm kernel cake as a renewable feedstock for fermentative hydrogen production. Renewable Energy, 93, 700–708. https://doi.org/10.1016/j.renene.2016.03.046
15. Abolore, R. S., Jaiswal, S., & Jaiswal, A. K. (2023). Green and sustainable pretreatment methods for cellulose extraction from lignocellulosic biomass and its applications: A review. Carbohydrate Polymer Technologies and Applications, 7, 100396. https://doi.org/10.1016/j.carpta.2023.100396
16. Asim, A. M., Uroos, M., & Muhammad, N. (2020). Extraction of lignin and quantitative sugar release from biomass using efficient and cost-effective pyridinium protic ionic liquids. RSC Advances, 10(72), 44003–44014. https://doi.org/10.1039/d0ra09098k
17. Kaur, N., Singh, G., Khatri, M., & Arya, S. K. (2020). Review on neoteric biorefinery systems from detritus lignocellulosic biomass: A profitable approach. Journal of Cleaner Production, 256, 120607–120607. https://doi.org/10.1016/j.jclepro.2020.120607
18. Linton, S. M. (2019). Review: The structure and function of cellulase (endo-β-1,4-glucanase) and hemicellulase (β-1,3-glucanase and endo-β-1,4-mannase) enzymes in invertebrates that consume materials ranging from microbes, algae to leaf litter. Comparative Biochemistry and Physiology Part B Biochemistry and Molecular Biology, 240, 110354. https://doi.org/10.1016/j.cbpb.2019.110354
19. Zhang, X., Chen, Y., Lv, Z., Zhou, L., & Guo, Y. (2024). Analysis of the effects of β-mannanase on immune function and intestinal flora in broilers fed the low energy diet based on 16S rRNA sequencing and metagenomic sequencing. Poultry Science, 103(5), 103581. https://doi.org/10.1016/j.psj.2024.103581

20. Monteiro, A. F., Miguez, I. S., Silva, J. P. R. B., & Da Silva, A. S. (2019). High concentration and yield production of mannose from açaí (Euterpe oleracea Mart.) seeds via mannanase-catalyzed hydrolysis. Scientific Reports, 9(1). https://doi.org/10.1038/s41598-019-47401-3
21. Wang, R., & Hartel, R. W. (2020). Effects of moisture content and saccharide distribution on the stickiness of syrups. Journal of Food Engineering, 284, 110067–110067. https://doi.org/10.1016/j.jfoodeng.2020.110067
22. Rahman, M. S. (2007). Handbook of Food Preservation (3rd ed.). Taylor & Francis Group. https://doi.org/10.1201/9781420017373
23. Maneffa, A. J., Stenner, R., Matharu, A. S., Clark, J. H., Matubayasi, N., & Shimizu, S. (2017). Water activity in liquid food systems: A molecular scale interpretation. Food Chemistry, 237, 1133–1138. https://doi.org/10.1016/j.foodchem.2017.06.046
24. Shukor, H., Abdeshahian, P., Al-Shorgani, N. K. N., Hamid, A. A., Rahman, N. A., & Kalil, M. S. (2016). Enhanced mannan-derived fermentable sugars of palm kernel cake by mannanase-catalyzed hydrolysis for production of biobutanol. Bioresource Technology, 218, 257–264. https://doi.org/10.1016/j.biortech.2016.06.084