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ALIMENTOS**

LÚCIO BELO

**QUALITY PARAMETERS, MARKET CHALLENGES, AND CONSUMPTION
TRENDS OF TRADITIONAL PALM BRANDY**

**PARÂMETROS DE QUALIDADE, DESAFIOS DE MERCADO E TENDÊNCIAS
DE CONSUMO DA AGUARDENTE TRADICIONAL DE PALMA**

GOIÂNIA, GO, 2025



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LÚCIO BELO

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Area of concentration: Food Science and Technology

Advisor: Prof. Dr. Julião Pereira

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Ata Nº **55/2025** da sessão de Defesa de Tese, de Lúcio Belo, que confere o título de Doutor em **Ciência e Tecnologia de Alimentos**, na área de concentração em **Ciência e Tecnologia de Alimentos**.

Aos **vinte e sete dias do mês de outubro de dois mil e vinte e cinco**, a partir das **14h**, por videoconferência, realizou-se a sessão pública de Defesa de Tese intitulada "QUALITY PARAMETERS, MARKET CHALLENGES, AND CONSUMPTION TRENDS OF TRADITIONAL PALM BRANDY". Os trabalhos foram instalados pelo Professor Doutor Julião Pereira (IQ/UFV), com a participação dos demais membros da Banca Examinadora: Professora Doutora Láisa Gomes Dias (UFT), membro titular externo; Professor Doutor Igor Savioli Flores (IFG), membro titular externo; Doutora Taís Aragão Ishizawa (EA/UFV), membro titular interno e Doutora Prof.ª Maria Carolina de Almeida - PPGCTA-UFV, membro titular interno. Durante a arguição os membros da banca **não fizeram** sugestão de alteração do título do trabalho. A Banca Examinadora reuniu-se em sessão secreta a fim de concluir o julgamento da Tese tendo sido o candidato **aprovado** pelos seus membros. Proclamados os resultados pelo Professor Doutor Julião Pereira, Presidente da Banca Examinadora, foram encerrados os trabalhos e, para constar, lavrou-se a presente ata que é assinada pelos Membros da Banca Examinadora, aos **vinte e sete dias do mês de outubro de dois mil e vinte e cinco**.

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DEDICATORY

I dedicate this thesis first and foremost to God, who has always blessed my academic journey and given me strength every day, allowing me to reach this stage. I dedicate it to my family, especially my wife and sons, who have always been by my side throughout this journey. I dedicate it to my parents, especially my mother and father, my siblings, nephews and nieces, all my relatives, and also to the friends and colleagues who supported me along this journey.

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“Believe in what you dream”

Ignore everything that doesn't belong to you, trust your potential, and proceed with determination.

“Lúcio Belo”

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ABSTRACT

Tua-sabu is a traditional alcoholic beverage with a long-standing history dating back to ancient times, recognized and valued as part of the sociocultural and economic heritage of Timor-Leste. It is produced from the sap of various palm species, such as *akadiru* (*Borassus flabellifer* L.), *tua-metan* (*Arenga pinnata* (Wurmb) Merr), *nuu* (*Cocos nucifera* L.), and *tua-tali* (*Corypha utan* Lam.), through sap extraction, spontaneous fermentation, and simple distillation. This study aimed to determine the alcohol content (%ABV) and identify volatile organic compounds (VOCs) in each distillation fraction of *tua-sabu* across all palm species, produced and commercialized in various regions of Timor-Leste. A total of 98 fractions were analyzed, comprising *tua-ulun* (head), *tua-klaran* (heart), and *tua-ikun* (tail), collected from multiple municipalities and representing three different collection points: directly at production sites, small grocery stores, and local markets or fairs. Ethanol content was determined by liquid chromatography (LC), VOCs were identified using gas chromatography (GC), and data analysis was performed using principal component analysis (PCA). The results showed that the *tua-ulun* fractions from all palm species, regions, and collection points had ethanol concentrations ranging from 22.3% to 61.0% v/v, while *tua-klaran* ranged from 10.1% to 30.5% v/v, and *tua-ikun* from 6.7% to 24.0% v/v. Notably, some *tua-ulun* fractions exhibited lower alcohol content than certain *tua-klaran* or even *tua-ikun* fractions. Despite significant variation in ethanol concentrations among fractions, many samples exhibited similar levels, even when collected from different regions, producers, palm species, or points of sale. These differences and similarities are primarily attributed to factors such as the origin of the raw materials, the types and hygienic conditions of storage containers, and variations in processing methods across the production and supply chain, including the mixing of different fractions prior to sale. The volatile compound analysis revealed that each distillation fraction contained a distinct profile of VOCs, which may reflect differences in geographical origin, palm species, or distillation processes. The identified chemical groups included alcohols, acids, aldehydes, esters, ethers, ketones, and other organic compounds that contribute to the beverage's aroma and flavor. However, when present in excess, these compounds can negatively alter the chemical profile, leading to consumer rejection and potential health risks. The absence of national quality standards and the lack of implementation of Good Manufacturing Practices (GMP) further contribute to the potential unsafety of the beverage for human consumption. Therefore, strict control over the production and distribution chain, particularly avoiding the mixing of distillation fractions, is essential to improve the quality of *tua-sabu* and reduce associated risks to public health.

Keywords: Palm sap. Spontaneous fermentation. Traditional beverage. Quality aspect. Sociocultural identity

RESUMO

Tua-sabu é uma bebida alcoólica tradicional com uma longa história que remonta à antiguidade, sendo reconhecida e valorizada como parte do patrimônio sociocultural e econômico de Timor-Leste. É produzida a partir da seiva de diversas espécies de palmeiras, como *akadiru* (*Borassus flabellifer* L.), *tua-metan* (*Arenga pinnata* (Wurmb) Merr), *nuu* (*Cocos nucifera* L.) e *tua-tali* (*Corypha utan* Lam.), por meio da extração da seiva, fermentação espontânea e destilação simples. Este estudo teve como objetivo determinar o teor alcoólico (%ABV) e identificar os compostos orgânicos voláteis (VOCs) em cada fração da destilação da *tua-sabu*, considerando todas as espécies de palmeiras utilizadas, com amostras produzidas e comercializadas em diferentes regiões de Timor-Leste. Foram analisadas 98 frações, compostas por *tua-ulun* (cabeça), *tua-klaran* (coração) e *tua-ikun* (cauda), coletadas em diversos municípios e provenientes de três tipos de pontos de coleta: diretamente nos locais de produção, em pequenas mercearias e em feiras ou mercados locais. O teor de etanol foi determinado por cromatografia líquida (LC), os compostos voláteis foram identificados por cromatografia gasosa (GC), e a análise estatística foi realizada por meio de análise de componentes principais (PCA). Os resultados mostraram que as frações *tua-ulun* apresentaram teores alcoólicos variando de 22,3% a 61,0% v/v, *tua-klaran* de 10,1% a 30,5% v/v, e *tua-ikun* de 6,7% a 24,0% v/v. Notavelmente, algumas frações *tua-ulun* apresentaram teores inferiores aos observados em certas frações *tua-klaran* ou até mesmo *tua-ikun*. Apesar das variações significativas, muitas amostras apresentaram concentrações semelhantes de etanol, mesmo quando provenientes de diferentes regiões, espécies de palmeiras, produtores ou pontos de venda. Essas variações e semelhanças são atribuídas principalmente à origem da matéria-prima, aos materiais e condições higiênicas de armazenamento, e às variações nos processos de produção e na cadeia de distribuição, incluindo a prática comum de mistura de frações antes da comercialização. A análise dos compostos voláteis revelou que cada fração apresenta um perfil distinto, o que pode refletir diferenças de origem geográfica, espécies de palmeiras e processos de destilação. Os grupos químicos identificados incluíram álcoois, ácidos, aldeídos, ésteres, éteres, cetonas e outros compostos orgânicos que contribuem para o aroma e sabor da bebida. No entanto, quando presentes em excesso, esses compostos podem comprometer o perfil químico, levar à rejeição sensorial pelos consumidores e representar riscos à saúde humana. A ausência de padrões nacionais de qualidade e a falta de implementação de Boas Práticas de Fabricação (BPF) contribuem ainda mais para a potencial insegurança da bebida para o consumo humano. Portanto, o controle rigoroso de todas as etapas da produção e distribuição, especialmente evitando a mistura de frações, é essencial para melhorar a qualidade da *tua-sabu* e reduzir os riscos associados à saúde pública.

Palavras-chave: Seiva de palmeira. Fermentação espontânea. Bebida tradicional. Aspecto da qualidade. Identidade sociocultural.

CHAPTER I: INTRODUCTION

1. 1. Global context on distilled beverages

Distilled beverages can be defined as alcoholic drinks obtained from the distillation of fermented agricultural products, which are alcoholic beverages manufactured and sold for human consumption worldwide. They are fermented and distilled from a variety of raw materials, additional ingredients, and processing methods that vary depending on the category of beverage produced (CARRELLO; FARIA, 1998).

The peculiar characteristics of the products may vary according to the type of raw material and the production processes involved, which will determine the yield, color, flavor, and aroma of the products (SERAFIM; FRANCO, 2015).

Distilled alcoholic beverages are typically produced from a variety of agricultural sources, including grains, seeds, fruits, herbs, roots, tubers, and vegetables (MORAES, 2021). In Brazil, cachaça is the sugarcane distillate (ALCARDE *et al.*, 2012). Other fruit-based spirits include jabuticaba (ASQUERI, SILVA, & CÂNDIDO, 2009), apple (MADRERA *et al.*, 2013), passion fruit (OLIVEIRA *et al.*, 2020), banana (SILVA *et al.*, 2019), pineapple (OLIVEIRA *et al.*, 2015), seriguela (SANTOS *et al.*, 2021), and star fruit (MOREIRA *et al.*, 2019).

Brandy or spirits from diverse substrates are reported worldwide, honey (CAMPOS, 2011), sweet potato (ABUJAMRA, 2009), cassava (FERREIRA *et al.*, 2023), corn bran (DIQUE *et al.*, 2025), rice (MORAES, 2021), as well as palm-sap distillates (NAKNEAN *et al.*, 2015).

Brandy can be obtained from the sap of different species of palm trees, among them, known as taberna in Mexico, derived from the thorn coconut (*Acrocomia aculeata*) or the *koyol* palm tree (SANTIAGO-URBINA; VERDUGO-VALDEZ; RUIZ-TERÁN, 2013; ASTUDILLO-MELGAR *et al.*, 2019).

Across Africa and Asia, palm-sap brandies are known as ogogoro (Nigeria), bandji (Burkina Faso), pyuwa (Kenya), toddy/arrack (India/Sri Lanka), lambanog (Philippines), and *arak* (Indonesia) (CHANDRASEKHAR *et al.*, 2012; ZONGO *et al.*, 2020; PRIANTARA, 2019).

In Timor-Leste, *tua-sabu*¹ is distilled from the sap of several palms: lontar (*Borassus flabellifer* L.; Tetum: *akadiru*) (SIRAJUDDIN *et al.*, 2016; Figure 1e); sugar

¹ Name given in Tetum, one of the official languages of Timor-Leste, to the brandy or distilled product obtained from the sap of palm trees in that country.

palm (*Arenga pinnata* (Wurmb) Merr.; Tetum: *tua-metan*) (ANSAR *et al.*, 2021; SANYANG *et al.*, 2016; Figure 1f); coconut palm (*Cocos nucifera* L.; Tetum: *nuu*) (XIA *et al.*, 2011; Figure 1g); and *Corypha utan* Lam. (Tetum: *tua-tali*) (WITONO, KUSUMA, & NAIOLA, 2018; Figure 1h).

1. 2. History and relevance of *tua-sabu* production in Timor-Leste

Tua-sabu is the best-known local distilled beverage in Timor-Leste and has been produced by traditional, small-scale methods for generations. Historically, production was primarily embedded in cultural exchange networks and the beverage was often obtained by barter-especially for food-rather than purchased with money (BELO *et al.*, 2023).

Timorese producers continue to apply inherited traditional knowledge and methods. Over time, techniques and equipment have evolved, and gradual changes have occurred in increasing production volume, supporting greater availability and market access; nevertheless, the producer base remains dominated by micro and small-scale operations.

1. 3. Economic, cultural, and scientific justification

Currently, *tua-sabu* is being increasingly developed, becoming a product of greater interest to the agricultural and economic development sector, contributing to the diversification of income and the livelihood of families directly and indirectly involved in its value chain. Its current prominence in the market represents its competitiveness in relation to other imported/industrial spirits in the country, in terms of accessibility, prices, and market movement.

Moreover, this beverage is still considered a sociocultural heritage of the Timorese and is widely recognized and appreciated in traditional ritual ceremonies and other important celebrations (BELO *et al.*, 2023).

Scientifically, *tua-sabu* is still little explored or studied, in addition to the absence of national quality standards and regulations, which allows the beverage to continue to be produced without adequate conditions of quality and hygiene involved in production steps using several plastic materials as of disposable plastic bottles of mineral water or cooked oil mineral water bottles (PET) and recycled cooking oil (HDPE), with the frequent use of low-density plastic (LDPE) as a seal to prevent product leaks during the production and distribution process, marketed without restrictions, and consumed freely in the country (BELO *et al.*, 2025).

It is worth highlighting that some of the processes involved can be considered factors that possibly compromise the quality of the beverage, causing unwanted contamination, altering its chemical characteristics, such as alcohol content, colors, aromas, and flavors, making it low-yielding and less competitive in the market, in addition to generating major and serious problems for human health.

Consumers frequently prefer high-strength (high-ABV) cuts, notably the head (*tua-ulun*) fraction, sometimes blending them with *tua-klaran* (heart) or *tua-ikun* (tail) without ABV verification, which are also generally intended for regular consumption (BELO *et al.*, 2023). These fractions may contain undesirable compounds, such as methanol, acetaldehyde, and ethyl acetate, at elevated levels, which are harmful to human health.

Quality is an important aspect in the production of food and beverages, which must involve the entire production chain and is strongly linked to total consumer satisfaction, covering intrinsic attributes, organoleptic properties, packaging and storage, price, and form of marketing (ANDRADE; LIMA; MEIRELLES, 2016).

Food and beverage safety remains a major public-health concern as supply chains and manufacturing become more complex. Food safety is one of the most important public health issues, and although in both developing countries that still lack rigorous surveillance by safety authorities and in developed countries that have several advanced surveillance and monitoring systems, outbreaks of food and beverage-borne diseases continue to be common (KING *et al.*, 2017).

These observations underscore the need for quality-control and safety standards for foods and beverages in Timor-Leste, particularly governing production, marketing, and consumption.

1. 4. Research problem and guiding question

Tua-sabu in Timor-Leste is gaining economic relevance; however, it is still far from achieving the quality required to guarantee consumer health. These problems are primarily associated with manufacturing and storage processes, including the use of inappropriate materials, inadequate sanitation, improper management during storage, distribution, unregulated marketing, and consumption. Furthermore, consumers often prefer the *tua-ulun* fraction, which may blend with *tua-klaran* and *tua-ikun* fractions.

This highlights a significant gap that motivates greater interest in developing this research proposal. Therefore, this study focuses on *tua-sabu* to quantify alcoholic strength

(ABV) and characterize volatile organic compounds (VOCs) in head, heart, and tail fractions, given the limited fraction-level evidence in the literature.

The results obtained can serve to contribute to the establishment of standards for quality control, to value this product of cultural identity with quality for its competition in the domestic market and its expansion for export, ensuring direct consequences in the economic and food security of the local populations involved in its production, commercialization, and consumption. In addition, it will also open a step towards intensifying scientific research related to other quality parameters.

Given these assumptions, we hope to answer the following question: What are the quality profiles of *tua-sabu* fractions? To answer the research problem, the general and specific objectives of this thesis are addressed below.

1.5. Objectives

1.5.1. General objective

To our knowledge, this is the first study in Timor-Leste to evaluate the physicochemical profile and volatile compounds of *tua-sabu*, analyzing the distilled fractions of different palm species, produced and sold in various locations, from the production site to kiosks/grocery stores and markets/fairs in several municipalities of the country.

1.5.2. Specific objective

- 1). Describe the *tua-sabu* production processes and their sociocultural importance in Timor-Leste, including the identity of the products and their controls.
- 2). To determine the alcoholic strength (ABV) of *tua-sabu* fractions derived from palm sap produced, sold, and consumed in Timor-Leste.
- 3). Profile the volatile organic compounds of *tua-sabu* fractions derived from palm sap produced, sold, and consumed in Timor-Leste.

1.6. Thesis organization

The thesis consists of five chapters. The first chapter is an introduction, presenting the global context of distilled alcoholic beverages, including the historical importance of *tua-sabu* production and issues related to its marketing and local consumption in Timor-Leste.

The second chapter provides the theoretical foundation, consisting of two published review articles. These articles serve as the basis for the analyses and address the problems and gaps related primarily to *tua-sabu* production, marketing, and consumption in Timor-Leste.

The third chapter addresses the Materials and Methods, while the fourth chapter presents the results and discussion of the experimental sections on determining ethyl alcohol concentrations and identifying volatile compounds. This chapter is organized into three articles: two articles on the determination of ethyl alcohol based on sap extraction methods and a single article on the identification of volatile organic compounds and their traceability. Finally, the fifth chapter presents the concluding remarks on the work developed.

CHAPTER II: THEORETICAL FRAMEWORK

2. 1. Overview of distilled beverages

Distilled alcoholic beverages are among the most consumed in the world, with the most dynamic and diverse markets, encompassing a wide range of brandies of different origins and characteristics (SERAFIM; FRANCO, 2015).

Distilled beverages are produced from a variety of raw materials or basic ingredients found worldwide, each with distinct characteristics (Table 1).

Table 1. Types of alcoholic beverages and the basic ingredients for their production.

Base Ingredients	Types beverages	
Cereals	Barley, wheat, corn, rye, rice, corn bran, and other grains.	Whiskey, scotch whisky, vodka, arrack, shochu, baijiu, and soju, among others
Fruits	Grapes, apples, pears, plums, prunes, strawberries, raspberries, bananas, passion fruit, pineapple, cherries, apricot pits, almonds, oranges, and many others.	Brandy, cognac, vodka, gin, armagnac, calvados, poire, schnapps, mirabelle, slivovitz, arrack, framboise, pisco, fraise, shochu, kirsch, maraschino, amaretto, and cointreau, among many others.
Vegetables, Molasses, Herbs, roots, or tubers	Sugar cane, palm trees, beets, molasses, potatoes, agave, herbs and spices, cassava, sweet potatoes, and others.	Sugar cane spirit, rum, gin, vodka, cachaça, pinga, cachao, tequila, absinthe, lambanog, chang'aa, ogogoro, blanca, aquavit, benedictine, chartreuse, Jägermeister, soju, shochu, kahlúa, wine, arrack, <i>arak</i> , <i>tua-sabu</i> , taberna, and toddy, among many others.

Sources: ALCARDE *et al.*, 2012; ASQUERI; SILVA; CÂNDIDO, 2009; MADRERA *et al.*, 2013; OLIVEIRA *et al.*, 2020; SILVA *et al.*, 2019; OLIVEIRA *et al.*, 2015; SANTIAGO-URBINA; VERDUGO-VALDEZ; RUIZ-TERÁN, 2013; ASTUDILLO-MELGAR *et al.*, 2019; BELO *et al.*, 2023; ROSENTRATER; EVERS, 2018; AYLLOTT; AYLLOTT, 2022; FERREIRA *et al.*, 2023; DIQUE *et al.*, 2025.

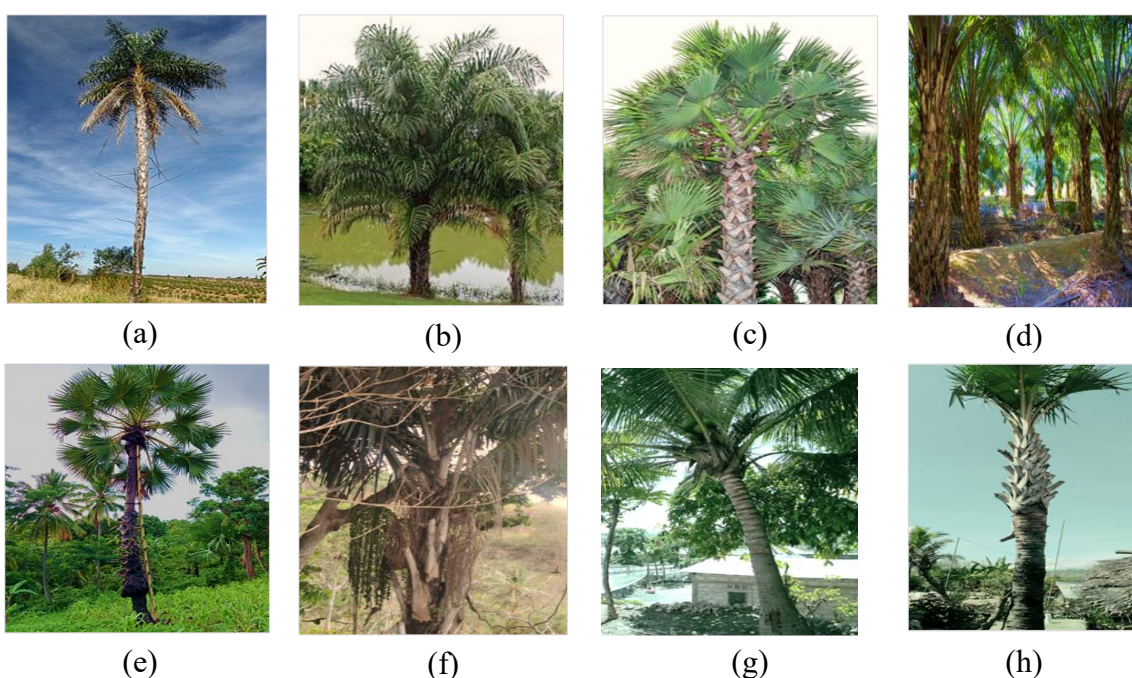
Brazil is one of the world's largest producers of aguardente and cachaça, obtained by distilling fermented sugarcane juice, with an alcoholic strength (ABV) between 38% and 54% v/v and 38% and 48% v/v at 20°C, respectively (PORTUGAL *et al.*, 2017; SILVA *et al.*, 2020). These beverages may contain sugars with an initial sucrose concentration of 6 g/L, according to the 2005 regulation of the Ministry of Agriculture, Livestock and Supply/*Ministério da Agricultura, Pecuária e Abastecimento* - MAPA (BRASIL, 2005).

These beverages have a long history dating back to the colonial period. Cachaça production has evolved over the years, being carried out industrially and artisanally in all Brazilian states, with each region presenting distinct characteristics and cultural

influences in its production (PINOTTI; VERDI; JERONIMO, 2018; PORTUGAL *et al.*, 2017).

In the production of palm-derived alcoholic beverages, each region has its techniques and production methods; the extraction of palm sap and its natural fermentation are common elements in this process.

Figure 1. Plants that produce sap or juice. (a) *Coco-de-espinho* palm (*Acrocomia aculeata*), (b) *Raphia* palm (*Raphia hookeri*), (c) *Borassus aethiopum* Mart. (*Areceaceae*), (d) *Oil palm* (*Elaeis guineensis*), (e) *Akadiru* (*Borassus flabellifer* L.), (f) *Tua-metan* (*Arenga pinnata* (Wurmb) Merr.), (g) *Nuu* (*Cocos nucifera* L.), and (h) *Tua-tali* (*Coryphatan* Lam.).



Sources: *Coco-de-espinho* palm (CORRÊA *et al.*, 2019); *Raphis* palm (KAMGA *et al.*, 2020); *Borassus aethiopum* Mart. palm (SARKODIE *et al.*, 2015); Oil palm (DESCALS *et al.*, 2019), and (e) *Akadiru*, (f) *Tua-metan*, (g) *Nuu*, and (h) *Tua-tali* palm (authors, 2025).

In southern Mexico and other parts of Central America, the production of alcoholic beverages stands, such as taberna, obtained from palm trees, especially the *coco-de-espinho* palm (*Acrocomia aculeata*), also known as the *coyol* palm (Figure 1a), is produced by natural fermentation, and its production is often carried out under non-aseptic conditions. Its presence is a fundamental sociocultural and economic activity in countries, both historically and in contemporary times (SANTIAGO-URBINA; VERDUGO-VALDEZ; RUIZ-TERÁN, 2013).

In West Africa, sap extraction is common from several palm species, such as the raffia palm (*Raphia hookeri*) (ERUKAINURE; CHUKWUMA; ISLAM, 2019) (Figure

1b), the oil palm (*Elaeis guineensis*) (Figure 1c), the date palm (*Phoenix dactylifera*), the fan palm (*Borassus aethiopum* Mart.) (Figure 1d), and the coconut palm (*Cocos nucifera*) (Figure 1g). The naturally fermented sap is known as palm wine, a collective name for a group of traditional alcoholic beverages from the region (DJENI *et al.*, 2020).

Ogogoro is a traditional Nigerian distilled beverage, similar to local gin, obtained from the fermentation and distillation of palm wine. It has deep cultural and spiritual importance in the country, being used in several ceremonies, including social, religious, and spiritual, e.g., libations and offerings to the gods (TONBRA; IKHIDE; LOVEDAY, 2018).

In Kenya, a traditional distilled alcoholic beverage known as *Chang'aa* is obtained from various raw materials such as palm sap, agave, and sugarcane, and is a beverage that plays a high socioeconomic role in low-income local communities in both urban and rural areas (MENYA *et al.*, 2019).

The production of alcoholic beverages from palm trees is also common in Southeast Asian countries, e.g., Sri Lanka, the Philippines, and Indonesia, including Timor-Leste. The beverages can have distinct characteristics, influenced by traditional production techniques that vary from region to region and by local culture.

In Sri Lanka, the brandy is known as *arrack*, a traditional distilled beverage made exclusively from fermented coconut sap. It is the most popular local alcoholic beverage, consumed at celebrations and social gatherings, and its production is an important part of the country's culture and economy, with a history dating back centuries (SAMARAJEEWA; ADAMS; ROBINSON, 1981).

Lambanog, known as coconut vodka in the Philippines, is a traditional artisanal distilled spirit derived from coconut sap. Available in a variety of flavors, including coconut and tropical fruits, it typically contains 40–45% ABV (Alcohol Content by Volume). Although it faces challenges related to safety and standardization in production, lambanog continues to grow in popularity in the region and is recognized internationally (BONINA; HERRERA, 2022; CALEJA-BALLESTEROS; BALLESTEROS, 2025).

Arak is a distilled alcoholic beverage derived from coconut or sugarcane sap, with an alcohol content ranging from 30% to 45%. It originates from Indonesia and is highly appreciated by the local community. Its production and consumption are important aspects of culture, spirituality, and economy, with significant potential to boost sustainable tourism and the prosperity of local communities (CANDRANEGARA; SURYANA; PUTRI, 2021).

2. 2. *Tua-sabu* in Timor-Leste

In Timor-Leste, *tua-sabu* is considered an essential element in various ceremonial and ritual occasions, valued as an integral part of their culture and tradition. Its presence remains vital to most Timorese. It is widely offered to guests at sociocultural events, including *barlaques* or dowry ceremonies, weddings, birthdays, inaugurations of traditional houses, wakes, and other celebrations.

For example, communities in the municipality of Lautém still maintain *tua-sabu* as a central and highly valued part of ceremonial traditions. Although it remains a cottage industry with limited commercial reach, demand for the product is relatively high due to the sociocultural demands of the region. For this reason, local producers continue to produce the distillate seasonally, keeping tradition and culture alive in local communities (MC WILLIAM, 2022).

As in the municipality of Baucau, much of society still uses it in ritual ceremonies such as the *barlaque* and *sau-batar*², as well as in the construction of sacred houses (*umalulik* in Tetum), offering it as a tribute to ancestors. Additionally, *tua-sabu* also plays an important role in conflict mediation and is often shared with local leaders, parents, or even the local community as a symbol of reconciliation, strengthening decisions, and dispute resolution between communities, family members, or neighbors (BELO *et al.*, 2023). These cultural practices are fundamental to the preservation of the society's traditions and cultural identity.

2. 3. Palm species used for sap extraction

In the territory of Timor-Leste, a variety of palm trees are found, each with distinct characteristics that make them suitable for different purposes. These plants are used in a variety of ways, including extracting sap (*tua-mutin* in Tetum) to produce brandy, sugar, and for other uses.

2.3.1. *Lontar palm/Akadiru (Borassus flabellifer L.)*

The *lontar/akadiru* palm (Figure 1e) is a species of the palm family *Aceraceae* (SUKAMALUDDIN *et al.*, 2016). A plant originating in Africa, it has spread to India, Malaysia, Cambodia, Thailand, Indonesia, and other regions (SANCAVANINGSIH;

² Celebration of ritual ceremonies consisting of offerings to ancestors before harvesting and consuming green corn.

SANTOSA; UTAMI, 2016; SARMA *et al.*, 2022), including Timor-Leste. Higher palm species measuring between 15 and 40 m in height (NASRI *et al.*, 2017) are suitable for cultivation in tropical, temperate, and dry climates at an altitude of 0 to 800 m above sea level and can survive in soil containing areas with an ideal temperature of 30 °C (KONAY; PAKAN; KARERI, 2019).

The *Borassus flabellifer* palm, locally known as *akadiru* in Tetum and referred to as ental/siwalan in Indonesia, has multiple vernacular names across Asia, Africa, and Europe (NASRI *et al.*, 2017; SUKAMALUDDIN *et al.*, 2019). Most of this plant has numerous uses, including as a construction material, utensils, crafts, and as the main source of food for both human and animal consumption, as shown in Table 2a.

2.3.2. Sugar palm/*tua-metan* (*Arenga pinnata* (Wurmb) Merr.)

The *tua-metan* palm tree (Figure 1f) is a plant native to tropical Asia and has spread naturally from eastern India, Laos, Cambodia, Sri Lanka, Thailand, Vietnam, Malaysia, the Philippines, Indonesia, and Australia (SAHARI *et al.*, 2012; ISHAK, 2013; SARMA *et al.*, 2022).

The plant generally survives and grows in various types of soil at altitudes ranging from 0 to 1,500 m above sea level, with an average temperature of 25°C and 1,200 mm of precipitation per year (FERITA; TAWARATI; SYARIF, 2015). The plant can reach heights of up to 25 m and exhibits high adaptability, having developed in marginal lands and making it very suitable for water and soil conservation purposes (SARI *et al.*, 2019). In addition, plants have multipurpose potential, serving as construction materials, crafts, and food sources (Table 2b).

2.3.3. Coconut tree/*Nuu* (*Cocos nucifera* L.).

Coconut tree or *nuu* (Figure 1g) is a tropical species known as the “tree of life or tree of abundance”, which has spread widely around the world, even on distant islands from Asia to America, by migration and trade between countries of the world (NAMPOOTHIRI *et al.*, 2019).

The species exhibits great phenotypic variability, including existing varieties of giant (var. typical) and dwarf (var. nana), which are composed of three dwarf varieties distinguished by colors such as green, yellow, and dwarf red. The giant variety also presents variations in its characteristics and can be divided into populations identified by

the name of the country where the variety grew (DAHER *et al.*, 2002). The plant has multipurpose potential, primarily as a construction material, in crafts, and as a food source for human consumption and animal feeding, as shown in Table 2c.

2.3.4. *Tua-tali palm (Corypha utan Lam.)*

The *tua-tali* palm (Figure 1h) is from the Palmae family, class monocotyledon, a subdivision of angiosperms, is a large tree, grows between 10 and 30 m in height, preferably in hot climates, survives and grows in low and mountainous areas (SANCAVANINGSIH; MAHARANI; SANTOAS, 2012; AMODY; ANGGREANI, 2017).

Tua-tali is cultivated in a vast region from Bengal-India, the Philippines, Maluku, Indonesia, and the Malacca peninsula to the north of Australia and the regions of Timor-Leste, mainly in the coastal areas (SARMA *et al.*, 2022). It is a type of plant that is resistant to drought and adapts well in open areas as a constituent of the savanna ecosystem community (WITONO; KUSUMA; NAIOLA, 2018).

In the Philippines, cultivation of *tua-tali* has potential as an export product (PARTOMIHARDJO; NAIOLA, 2009), whereas in Timor-Leste, the plants are wild. Most of the plant has numerous uses, including as a construction material, utensils, crafts, and as the main source of food for human consumption and animal feeding (Table 2d).

Table 2. The multiple uses of *akadiru*, *tua-metan*, *nuu*, and *tua-tali* palm species

Plant species	Plant Uses
[a] <i>Akadiru (Borassus flabellifer L.)</i>	<ul style="list-style-type: none"> • The stems and leaves, among others, are used by communities for construction (shelter and house walls, among others), domestic and artistic or craft furniture (rugs, hats, mats) [1] • The pulp of the youngest green fruit, between five and six months, is tasty, resembling the green coconut fruit, with a soft texture [2], widely consumed by Timorese and Indonesians as food, while the ripe or dried fruit is used for pig feed [3-4]. • The sap is a sweet liquid extracted by female or male flowers of the plant [5], known as “<i>Neera</i>” in Bengali is frequently used to produce brown sugar, chocolate, cake, sweet soy sauce, food toppings, and typical Bengali ice creams [6], and in Nusa Tenggara Timur (NTT) and Maluku provinces of Indonesia sap fresh can immediate consumption or used to produce liquid sugar, brown sugar, and ant sugar, among others. • In addition, the sap can undergo natural fermentation, capable of being used as raw material to produce brandy and vinegar [7-9].
[b] <i>Tua-metan (Arenga pinnata (Wurmb) Merr.)</i>	<ul style="list-style-type: none"> • The leaves are often used to build roofs of houses and huts, while the roots have been used in the production of handicrafts (flowerpots and baskets, among others). The fibers (brooms, brushes, mats, rugs, cushions for chairs or sofas, house roofs, ropes, water filters, and others) [10-12].

	<ul style="list-style-type: none"> • The stems are resistant and have palm fiber; the inner part of the stem contains starch, being a plant matrix with high potential for use in starch and sugar production [13-14]. • Palm flour obtained from the inside of the plant is used as an ingredient in the formulation of cakes, breads, biscuits, and other food products [12]. • The flowers are good sources of sap production [14-15]. • The fresh sap can be consumed immediately or used as raw material to produce brandies, vinegar, cream, and sugar, among others [7,15,16].
<p>[c]</p> <p><i>Nuu (Cocos nucifera L.).</i></p>	<ul style="list-style-type: none"> • The plant is widely cultivated for its multiple uses, mainly for its nutritional, medicinal, and cosmetic values throughout the world [17,18]. • The trunks, stems, stalks, the outer layer of the rump, peels, nuts, and fruit fibers and leaves are used as wood, construction, domestic, and craft materials such as brooms, carpets, flowerpots, brushes, and ropes, among others [19]. • Coconut water and pulp are consumed as beverages and foods, containing essential nutrients, minerals (iron, zinc, manganese) [20]. • Coconut water has numerous medicinal properties (antibacterial, antifungal, antiviral, antiparasitic, antidermatophyte, antioxidant, hypoglycemic, hepatoprotective, and immunostimulant) [20]. In addition, coconut water is also widely used to produce brown sugar [21-22]. • Copra or coconut milk, extracted from the pulp of old/dried coconuts, is normally used for cooking rice and sauces. Additionally, as the main source to produce cooking oil via RBD (Refining, Bleaching, and Deodorization), and unlike refined oil via VCO (Virgin Coconut Oil), produced through a wet method or aqueous processing [18,20,23]. • The flower is composed of male and female parts that can develop into fruits or be used to extract sap [24]. • The sap is a transparent, sweet, and translucent liquid, rich in nutrients. It can be consumed fresh and/or used as raw materials to produce brandy, and vinegar (<i>tua-siin</i> in Tetum), among others [18,24-26].
<p>[d]</p> <p><i>Tua-tali (Corypha utan Lam.)</i></p>	<ul style="list-style-type: none"> • The major part of the plant is used as raw materials for construction (to make shelters or shade, building houses, etc.), fences, ropes, handicrafts (rugs, mats, drums, baskets), among others [27,28]. • The inner rib fiber can be used for polymer composites production, with economic value, and can be easily obtained in large quantities [4,29]. • Starch is the main food source for the population of Nusa Tenggara Timur (NTT) province and Timor-Leste, being an alternative food known as "<i>akar-bilan</i>" in Tetum or "<i>puta</i>" or "<i>laka</i>" as a substitute for rice. In addition, it is also used in animal feed [30]. • Furthermore, the plant has great potential as a source of sap production, and the sap is the main raw material to produce <i>tua-sabu</i>, brown sugar, and vinegar [31].

Sources: [1] (BAIHAQI; WISANTI; PUTRI, 2022), [2] (SUKAMALUDDIN *et al.*, 2019), [3] (SALUDUNG; YAHYA, 2008), [4] (KURNIAWAN; YUNIATI, 2015), [5] (HAWA; LUTFI; MAKHFUDHI, 2019), [6] (HEBBAR *et al.*, 2018), [7], (SOLO *et al.*, 2019), [8] (CEUNFIN *et al.*, 2021), [9] (BASCHALI, 2017), [10] ((TICOALU; THIRU; CARDONA, 2014), [11] (YUNITA *et al.*, 2015), [12] (WULANTIKA, 2019), [13] ((SAHARI *et al.*, 2012), [14] (ISHAK, 2013), [15] (SUKA; UN; RAMMANG, 2020), [16] (SARMA *et al.*, 2022), [17] (DAHER *et al.*, 2002), [18] (DEBMANDAL; MANDAL, 2011), [19] (WEARN; MONTAGNA; PASSADOR, 2020), [20]; EYRES *et al.*, 2016), [21] (YONG *et al.*, 2009), [22] (SANTOSA *et al.*, 2020), [23] (MARINA; MAN, 2009), [24] (NAMPOOTHIRI *et al.*, 2019), [25] (HEBBAR, 2015), [26] (TAPSOBA *et al.*, 2021), [27] (PARTOMIHARDJO; NAIOLA, 2009), [28] (NAIOLA, 2007), [29] AMODY; ANGGREANI, 2017), [30] (WITONO *et al.*, 2018), [31] (NAHAK *et al.*, 2021).

2. 4. Characteristics and chemical composition of sap

The sap extracted from palm trees is an aqueous solution containing 10 to 20% sugar, and fresh sap can be consumed immediately after extraction, a common practice in countries in the intertropical regions of Asia, Africa, and the Americas. Furthermore, a large portion of the harvest is also frequently used for various purposes, as it contains a higher amount of sugars, considered essential for the efficient production of alcoholic beverages, sugar, and vinegar (ABDEL-BANAT *et al.*, 2010; VARELA, 2010; FRANCISCO-ORTEGA; ZONA, 2013; ASTUDILLO-MELGAR *et al.*, 2019).

The composition and quality of sap vary primarily depending on the plant species, location, season, and the time or duration of extraction (BORSE *et al.*, 2007; NAKNEAN; MEENUNE; ROUDAUT, 2010; LIMTONG *et al.*, 2020). Although the sap is collected in the same production area, the difference in quality is primarily due to the fermentation of sugars by microorganisms during sap collection (NAKNEAN *et al.*, 2019).

LALEL & RUBAK (2024) carried out a study on the chemical composition of fresh sap *or tua-mutin* of *tua-tali* in Kupang Regency, East Nusa Tenggara province of Indonesia, reported a total soluble solids content ranging from 8.5 to 14.1 °Brix, demonstrating its viability as the main source of raw material to produce brandies.

EZE *et al.* (2019) reported that fresh palm sap contains sucrose as the main ingredient, about 12–15% by weight, with some reducing sugars, including proteins, fats, and minerals, and SARMA *et al.* (2022) reported that palm sap mainly consists of sugars such as sucrose, glucose, fructose, maltose, and raffinose, which are susceptible to fermentation by the microflora present in the sap.

According to HEBBAR *et al.* (2018), fresh sap contains the main compounds, total and reducing sugars, including free amino acids, proteins, phenolic compounds, essential elements (nitrogen, sodium, phosphorus, potassium, calcium, and magnesium), and micronutrients (zinc, iron, and copper).

Furthermore, the sap is also rich in other compounds such as flavonoids, phenolic compounds, organic acids, minerals, vitamins (B, C), soluble proteins, amino acids, and amides, serving as a rich substrate for the growth of various types of microorganisms such as bacteria and yeast (SANTIAGO-URBINA *et al.*, 2013; ZONGO *et al.*, 2020).

As illustrated in Table 3, previous studies present the levels of the main sugars found in the sap produced by different species of palm trees from various locations.

Table 3. Sugar content of sap from different palm species in previous studies.

Plant Species	Sugar Content						Source
	Total sugars (%)	Reducing sugar (%)	Sucrose (%)	Glucose (%)	Fructose (%)	Soluble solids (°Brix)	
<i>Acronym aculeate</i> “coyol”	na	na	0.22-11.36	0.05-2.15	0.27-3.54	na	[a]
<i>Arenga pinnata</i>	na	na	50.37-61.20	3.83-6.53	3.27-6.77	na	[b]
<i>Arenga pinnata</i>	15.73	na	10.88	1.08	1.46	13.00	[c]
<i>Borassus flabellifer</i> Linn.	10.36-18.94	0.88-3.56	9.29- 17.44	0.50-1.85	0.48-1.81	10.67-17.33	[d]
<i>Borassus aethiopum</i> Mart.	9.65-12.62	2.51-2.37	5.05-10.02	3.23-7.03	6.72-7.86	na	[e]
<i>Cocos nucifera</i>	6.57-7.50	0.44-0.65	na	na	na	13.0-18.0	[f]
<i>Cocos nucifera</i>	9-11	na	na	na	na	14.0-15.6	[g]
<i>Cocos nucifera</i>	18.77-20.00	na	9.47-13.71	2.76-4.96	3.53-4.37	16.15-16.55	[h]
<i>Cocos nucifera</i>	na	na	1.76-5.76	2.25-4.46	3.23-5.76	na	[i]
<i>Cocos nucifera</i>	12.92	na	6.91	2.53	3.48	12.40	[c]
<i>Cocos nucifera</i>	15.56	4.76	10.80	2.65	2.11	na	[j]
<i>Nipa (Nypa fruticans)</i>	15.9-18.9	na	9.3-11.1	5.1-6.5	1.4-1.6	na	[k]
<i>Phoenix dactylifera</i>	na	na	95.27	2.5 1	1.61	na	[l]
<i>Phoenix canariensis</i>	66.0	na	37.8	9.50	4.80	na	[m]
<i>Phoenix dactylifera</i> L.	70.40-85.58	na	19.96-63.38	16.88-48.56	1.14-3.55	10.0-16.7	[n]

Note: na (not analyzed), [a] (SANTIAGO-URBINA *et al.*, 2013), [b] (YONUS *et al.*, 2018), [c] (ASGHAR *et al.*, 2019), [d] (NAKNEAN *et al.*, 2019), [e] (ZONGO *et al.*, 2020), [f] (HEBAR *et al.*, 2015), [g] (RAMASWAMY; RAMASWAMY, 2017), [h] (PATHIRANA *et al.*, 2023), [i] (SOMAWIHARJA *et al.*, 2018), [j] (SHETTY *et al.*, 2017), [k] (TAMUNAIDU *et al.*, 2013), [l] (THABET *et al.*, 2009), [m] (LUIS *et al.*, 2012), and [n] (MAKHLOUF-GAFSI *et al.*, 2016).

Reducing sugars act as a substrate for the *Maillard* reaction that occurs during the production of palm sap. The high presence of these sugars in the sap can also cause the juice to darken later, due to the same non-enzymatic browning reaction as the *Maillard* reaction (NAKNEAN *et al.*, 2019).

KARAMOKO *et al.* (2016) reported that wild or indigenous microorganisms found in the sap have been present since the extraction process began, responsible for the spontaneous fermentation process. In their studies, microorganisms found were aerobic mesophilic, lactic acid bacteria, yeasts, *Enterococci*, and coliforms, including total and fecal and sulfite-reducing bacteria (KARAMOKO *et al.*, 2016). Furthermore, some of the main players involved in the fermentation of palm saps are lactic acid bacteria (LAB) and yeasts (SHETTY *et al.*, 2017).

The sap flow occurs slowly during extraction, making it highly susceptible to fermentation (HEBBAR *et al.*, 2015); as it has a pH close to neutral that can drop rapidly as the sap undergoes spontaneous fermentation during the collection period in the presence of microorganisms (THABET *et al.*, 2009; FRANCISCO-ORTEGA; ZONA, 2013).

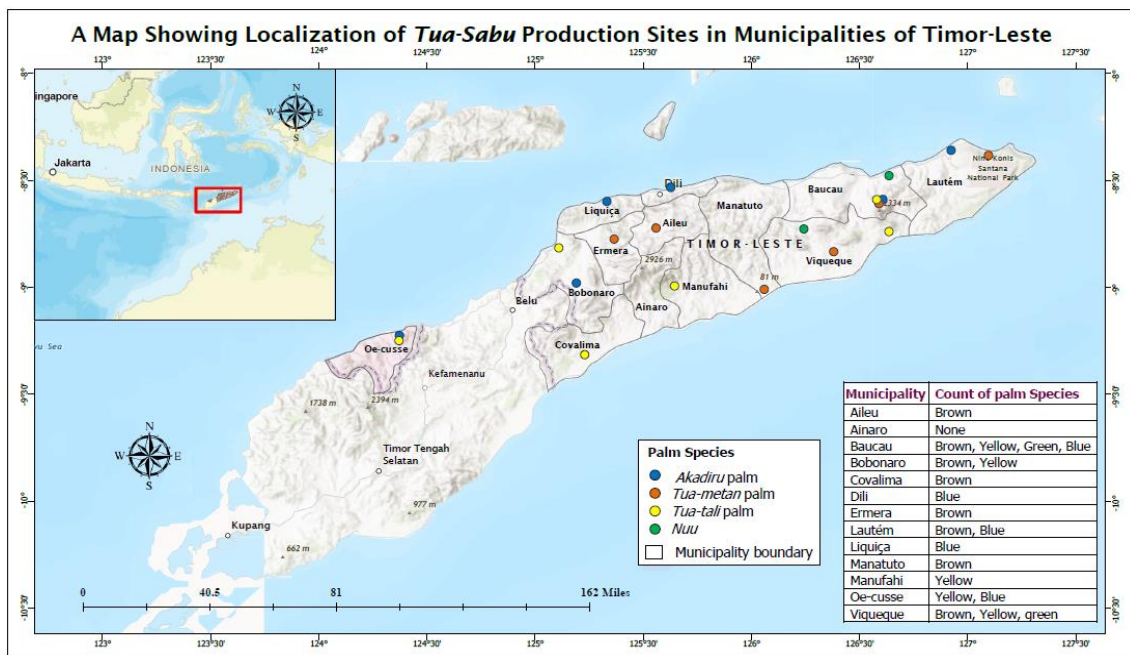
2. 5. Traditional production process of *tua-sabu*

For generations, Timorese ancestors produced a distinctive beverage known as *tua-sabu*, a distilled beverage derived from the sap extracted from palm trees, which is widely recognized and used in Timor-Leste. Today, Timorese producers still follow the traditional knowledge and production methods that their ancestors created and passed down. Over time, there have been some changes in the techniques and instruments used at all stages of production to increase the quantity produced (BELO *et al.*, 2023).

2.5.1. *Tua-sabu* producing regions or municipalities in Timor-Leste

Tua-sabu is now available in all regions of Timor-Leste, primarily in markets. However, regarding production, data collected during a 2021 field visit revealed that not all regions produce all species, as shown in Figure 2.

Figure 2: The production of *tua-sabu* in the regions of Timor-Leste represents the origin of the palm species.



Source: authors, 2025

Some municipalities produce only one plant species, while others may have two or more. This variation depends on the availability of plant species and the experience of producers, especially in sap extraction.

2.5.2. *Tua-sabu* production process

The process of producing *tua-sabu* from palm sap in Timorese producers consists of several main stages, including palm selection and sap extraction, fermentation, distillation, and bottling.

2.5.2.1. *Extraction process*

The palm sap extraction process in Timor-Leste consists of two traditional methods: extraction through palm inflorescences, known as a non-destructive method, especially in the *akadiru*, *tua-metan*, and *nuu* palms (Figure 3), and extraction performed by drilling the cavity of the soft meristem or soft apical stem, known as a destructive method, in the case of the *tua-tali* palm (Figure 4).

2.5.2.1.1. *Extraction of palm sap by non-destructive method (inflorescences)*

The sap extraction process consists of four main steps: selection, preparation, extraction, and collection. Local producers reported that the palm tree selection process is conducted through direct observation of the plants, primarily based on their age, maturity, size, and productivity in terms of both quantity and quality of inflorescences, as well as other factors. Therefore, these measurements are considered fundamental factors in determining the amounts of sap produced.

Figure 3. The indigenous process of extracting palm sap from the (non-destructive) inflorescence of palm trees, (a) *akadiru* palm (*Borassus flabellifer* L.), (b) *tua-metan* palm (*Arenga pinnata* (Wurmb) Merr.), and (c) *nuu* (*Cocos nucifera* L.).



Source: authors, 2025.

The preparation stage begins with climbing the palm tree trunk until reaching its dense crown, where it produces a series of fibrous inflorescences. For *akadiru* palms, non-fruiting male stems are preferred. The chosen flowers are subsequently cleaned and sanitized, and the flower tips are tied and sealed (Figure 3a). The lower part of the stem of the tied flowers is beaten or pressed, and the ends of the stems are cut into thin slices twice a week over about a month.

The release of clear droplets or sweet liquid (sap) can be observed in the presence of bees, and at this point, the flower tips are continuously cut in the order of harvest days, and the resulting sap flow is collected in a plastic bottle or bamboo cylinder attached to the rod. The presence of bees not only indicates the release of sap but is also vital for flower pollination, which can potentially impact the yield. The bees' activity can influence the sugar concentration in the sap, potentially improving the quality of the harvested sap.

This is a common process that applies, where the sap is harvested every 12 to 24 hours, usually in the early morning or late afternoon. With each collection, a thin slice of the stem is cut to encourage more flow, and this process continues for several months until the stem is reduced to a stump against the trunk and abandoned. Then, a new inflorescence will be selected at another point on the trunk, and the process will be repeated. A similar form of extraction is also carried out in regions of Indonesia (HAWA *et al.*, 2019).

On the other hand, the extraction of sap from *tua-metan* involves bruising and cutting the young inflorescences that produce the seed head from the flowering stems (inflorescence) that emerge from its trunk (Figure 3b). This extraction process requires experienced hands to deliver great results. As it matures, the grower may strip and peel the tough coverings from the stems or stalks and using a wooden stick, begin a twice-weekly process of beating the stalk causing bruising along its entire length for approximately a month, then look for signs of sap flow at the yellow end of the seeds, the presence of bees, or assess the release of sap in the stem through a prick with thorns. At this stage, the fruiting head of the inflorescence is cut off, and the resulting sap flow is collected in a bamboo cylinder or plastic bottle attached or tied to the stem (see BELO; BRICENO; PEREIRA, in press).

For the extraction of coconut/*nuu* sap, clusters of young flowers are preferred and must be cleaned and sanitized. After this, processes similar to those used with the *akadiru* palm may be employed, or the first cut can be made directly at the tip, approximately 10

centimeters, to facilitate the release of the sap. This eliminates the need to hit or press the lower part of the flower stem (Figure 3c).

According to local producers, the estimated volume of sap extracted from the *akadiru* palm tree ranges from 3 to 5 liters per flower/day over a period of 2 to 3 months. In the *tua-metan* palm, the estimated daily sap flow sustains an average of 10 liters. It can last approximately 2 to 3 months, where the amount of sap produced is twice that of *akadiru* production. Meanwhile, a cluster of coconut flowers/*nuu* can make an average amount of sap between 2 and 3 liters per day.

According to MC WILLIAM (2022), a well-managed palm tree can produce approximately 10 liters (for *akadiru*) and 20 to 24 liters (for *tua-metan*) of sap every day during the process, until the flow is reduced. Variations in the amount of sap produced depend on several factors, including the preparation stage, plant productivity, soil fertility, age, and size of the plant, as well as climatic and seasonal factors (MC WILLIAM, 2022).

In southern Tunisia, primarily in the Tunisian Sahara, a traditional practice exists for producing date palm sap, which is used to make a popular juice known as “*Lagmi*”. Consumers highly appreciate this beverage due to its sweet and distinctive flavor. It is generally harvested in the spring for approximately four months, from March to June, with a production quantity of 8 to 10 liters per day per palm tree (THEBAT, 2009). There are also several methods of extracting sap using non-destructive methods that are applied to several palms in some countries around the world (Table 4).

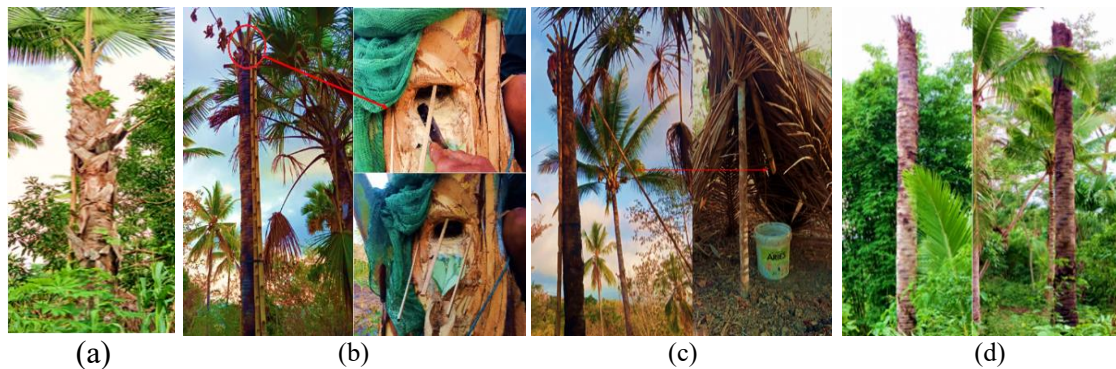
Table 4. Sap extraction with the non-destructive method (Inflorescence threading) of different types of palms.

Scientific name	Type of extraction	Sources
<i>Elaeis guineensis</i> (Oil palm)	Inflorescence extraction	FRANCISCO-ORTEGA; ZONA, 2013.
<i>Phoenix sylvestris</i> (Wild date palm)	Inflorescence extraction	FRANCISCO-ORTEGA; ZONA, 2013.
<i>Arenga pinnata</i> (Sugar palm)	Inflorescence extraction	KURNIAWAN; KUSTININGSIH; FIRDAUS, 2020.
<i>Borassus akeassii</i>	Inflorescence extraction	ZONGO et al., 2017
<i>Phoenix dactylifera</i> (Date palm)	Inflorescence extraction	NGUYEN; HARIFARA; SHIRO, 2016.
Coconut (<i>Cocos nucifera</i> L.)	Inflorescence extraction	NGUYEN; HARIFARA; SHIRO, 2016.

2.5.2.1.2. Extraction by a destructive method of the tua-tali palm (*Corypha utan* Lam.)

Obtaining the sap begins by selecting the plant before flowering, primarily due to its maturation. For extraction, the leaves, stems, and top cleaning are initially removed, followed by cutting or excavating the cavity in the soft apical meristem (Figure 4) twice a day for 1 to 2 weeks. Once formation has begun and the liquid starts bubbling, collection can commence. The sap is directed into a container using a rope or wood to position it.

Figure 4. Palm sap extraction process using the destructive method of the *tua-tali* palm tree (*Corypha utan* Lam.), (a) before extraction, (b) cleaning, excavation in the cavity or drilling of the apical meristem, (c) sap extraction and collection, and (d) the Tree trunk after extraction.



Source: authors, 2025.

According to local producers (interviewed), reported that the volume of sap extracted per tree/day can reach 30 to 40 liters for a period of two to three months; however, the quantity produced depends on several factors such as the stages of preparation, production season, climatic factors, soil fertility, and productivity, including the age and size of the plant. This form of extraction is similar to the process carried out in African and Western countries, where extraction is done by felling or incising the apical meristem of the palm tree.

The collection of sap in this process involves drilling or excavating the cavity in the soft apical meristem of the tree trunk and inserting a tube or opening directed into traditional containers such as a calabash pot or terracotta clay pot, or containers such as plastic bottles (FRANCISO-ORTEGA; ZONA, 2013; DJENI *et al.*, 2020).

Table 5. Extraction of sap by destructive method (Cavity cutting and extraction) from different types of palm trees.

Scientific name	Type of extraction	Sources
<i>Acrocomia aculeata</i> (Macawpalm, Coyolpalm)	Tipping (Cutting and excavating the cavity in the soft apical meristem).	FRANCISCO-ORTEGA; ZONA, 2013.
<i>Jubaea chilensis</i> (Chilean palm)	Tipping (Cutting and excavating the cavity in the soft apical meristem).	FRANCISCO-ORTEGA; ZONA, 2013.
<i>Pseudophoenix Ekmanii</i> (Dominican cherry Palm)	Perforation or excavation of the cavity in the soft apical meristem.	FRANCISCO-ORTEGA; ZONA, 2013.
<i>Phoenix canariensis</i> (Canary Island date)	Tipping (Cutting and excavating the cavity in the soft apical meristem).	FRANCISCO-ORTEGA; ZONA, 2013.
<i>Borassus aethiopum</i> (African fan palm)	Perforation or excavation of the cavity in the soft apical meristem.	DJENI <i>et al.</i> , 2020
<i>Raphiahookeri</i> (Raffia palm, Wine palm)	Perforation or excavation of the cavity in the soft apical meristem.	DJENI <i>et al.</i> , 2020
<i>Hyphaene coriacea</i> (Lalapalm)	Tipping (Cutting and excavating the cavity in the soft apical meristem).	MARTINS; SHACKLETON, 2017.
<i>Hyphaene petersiana</i> (Realfanpalm, Ivorypalm)	Tipping (Cutting and excavating the cavity in the soft apical meristem).	BABITSENG; TEKETAY, 2013.

2.5.2.2. Fermentation process

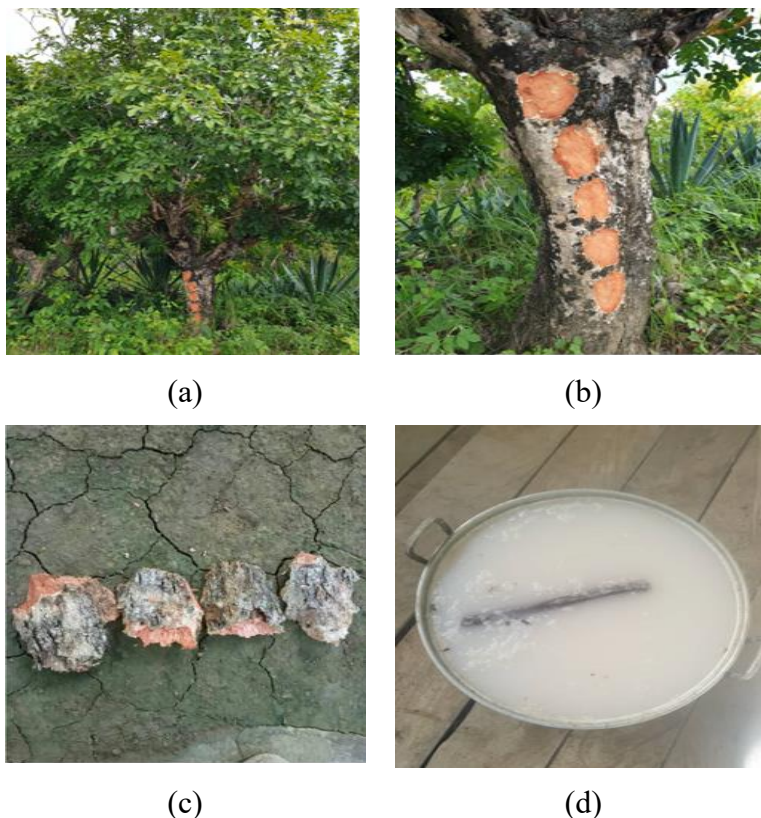
In tropical countries under environmental conditions that include the use of non-sterilized or unsanitized materials, fermentation occurs easily, as the sap is rich in nutrients, and contains carbohydrates and proteins that harbor a complex microbiota, which includes native and invasive flora carried by insects, and materials and tappers used for sap collection (KARAMOKO *et al.*, 2016).

The natural presence of bacteria in the sap easily ferments sugar due to exposure to light and/or increased temperature (HEBBAR *et al.*, 2018). Therefore, in some cases, it is necessary to add natural antimicrobial ingredients or agents, such as lime, ground bark, or leaves from a specific tree, to avoid the rapid fermentation process. Furthermore, the hygiene of the containers is crucial; they are regularly sterilized and sanitized, sometimes using lime or smoke (FRANCISCO-ORTEGA; ZONA, 2013).

According to some producers interviewed by the author, mainly from the eastern region of Timor-Leste, producing brandies from palm trees such as *akadiru*, *tua-metan*, and *nuu* requires gathering a volume of sap of around 50 liters (BELO *et al.*, 2023). For palm sap, *tua-tali* requires approximately 200 liters, depending on the capacity of the vats

used, and it is left to ferment for a period of two to five days, or in some cases, fermented immediately after extraction.

Figure 5. *Kusum* plant (*Schleichera oleosa*). (a) Tree. (b). Peels. (c) Bark. (d) Bark is added to the sap in the fermentation stage



Source: authors' 2025

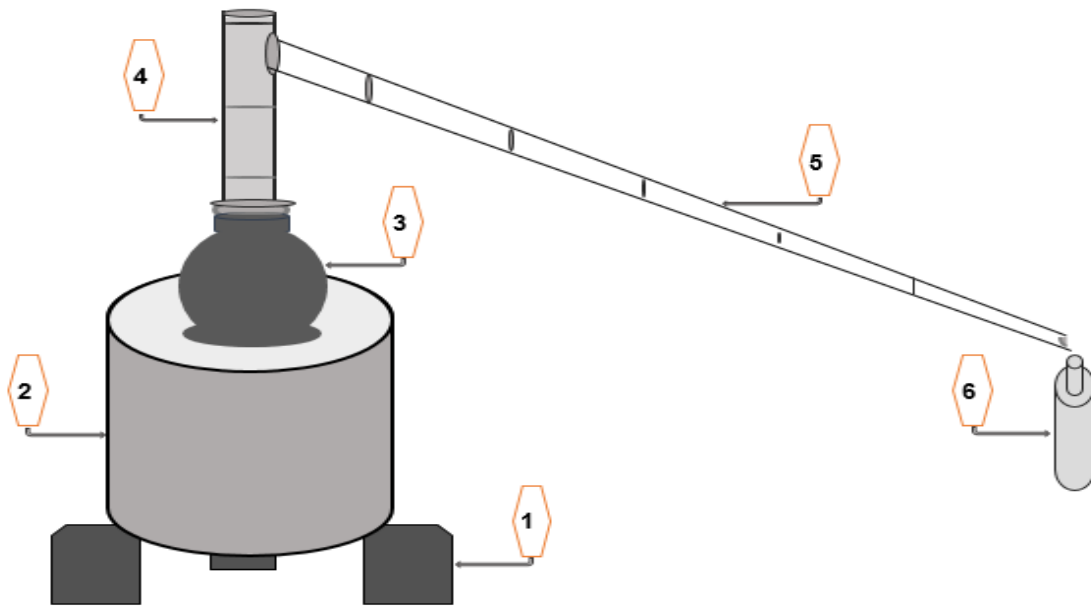
The fermentation process is mostly carried out in open spaces, where variations in environmental conditions and consequent changes in the process of converting sugar into alcohol can occur. The fermented broth is then qualitatively evaluated based on the manufacturer's experience, considering its appearance, including the desired volume, color, and the number of days of rest before proceeding to the next stage. Additionally, according to producers in the region, some use the green peels of certain plants, such as *Kusum* (*Ai-daak*) (Figure 5), in the process, aiming to fortify the flavor, increase alcohol concentration, and enhance the color of the final product (BELO *et al.*, 2023).

It is worth mentioning that the presence of peels in the extract can contribute to the formation of undesired secondary compounds in the product and also affect the yield of the final distillate, as the peel can act as a natural antimicrobial agent capable of inhibiting the fermentation process (FRANCISCO-ORTEGA; ZONE, 2013).

2.5.2.3. Distillation process

Distillation is a separation process based on the phenomenon of liquid-vapor equilibrium of a mixture. Thus, the alcoholic fraction can be separated through the condensation of alcohol vapor from the liquid when it is heated, because the boiling point of alcohol is lower than that of water in the must (BELO *et al.*, 2023).

Figure 6. Distillation system: (1) tripod, (2) iron or aluminum still, (3) hood, (4) bamboo gooseneck, (5) bamboo condenser tube, and (6) distillate collection bottle or container



Source: prepared by the authors

Timorese producers use different systems for distilling *tua-sabu*. As illustrated in Figure 6, in some regions of the east of the country, the distillation system consists of: (1) a tripod, usually made of three stones to support the still; (2) the alembic or still, commonly made of iron (barrel/*bidon*³) or aluminum (pan/*sanan*⁴); (3) the exhauster – a clay container that connects the still to a bamboo evaporator tube (4), known as a swan neck, whose length can vary between 1.5 meters and 2.0 meters; (5) the condenser tube, with a length of approximately 6 m to 15 meters; and (6) a container for the distillate, such as a glass or plastic bottle.

The distillers used by producers in the respective municipalities of Bobonaro, Covalima, and Liquiça are made of iron called 'Bidon,' with a capacity of 220 liters, and

³ Bidon is an iron barrel.

⁴ Sanan is a pot made of aluminum or clay.

are placed in a vertical position (Figure 7a, b & c). The same stills are also used by producers in the municipalities of Viqueque and Covalima; however, these distillers are positioned horizontally (Figure 7d & e).

The swan neck tube, made of bamboo-based material, varies in height between 1.5 and 3 meters. It is placed in a vertical position and connected directly to the mouth of the still. The type of condenser tube used also varies by region; bamboo tubes are most commonly used, although some producers in the municipality of Bobonaro opt for PVC (polyvinyl chloride) tubes. These tubes, measuring over 15 meters in length, are mounted in various configurations, often shaped like the letters 'C,' 'V,' or 'U.' At the end of the process, the distillate is collected from these tubes.

Figure 7. The techniques and uses of different types of distillers by *tua-sabu* producers in the regions of Timor-Leste are from the municipalities of (a) Bobonaro; (b) Covalima; (c) Liquiça; (d) Covalima; (e) Viqueque; (f) Lautém; (g) Baucau; (h) Baucau; (i) Manatuto; (j) Liquiça.



Source: authors' 2025

Some producers of Baucau and Lautém municipalities also use the “*Bidon*” still, with a maximum capacity of 110 liters or half of the remaining capacity (Figure 7f & g). In this system, the still is placed in a vertical position, with two clay or aluminum-based pots placed one on top of the other, functioning as a swan neck evaporator tube. Once this is done, the bamboo condenser tube, measuring between 10 and 15 meters in length, is

connected directly to the pots, which are placed in a straight position and inclined towards the ground. The distillate is collected at the end of it. In some municipalities, including Baucau, Manatuto, and Liquiça, an aluminum-based distiller still is used, with a maximum capacity of 80 liters (Figure 7h, I & j).

The system features a pot-based connection in the form of a hood placed between the distiller pot and the swan neck tube, typically made of bamboo and measuring between 1.5 and 2 meters in height when positioned vertically. A condenser tube is connected directly to the hood and extends approximately 10 to 15 meters in length, inclined towards the ground, where the distillate is collected at its end. However, some operational systems used by producers in the municipalities of Manatuto and Liquiça incorporate a condenser tube refrigeration system throughout the process (Figure 7I & j).

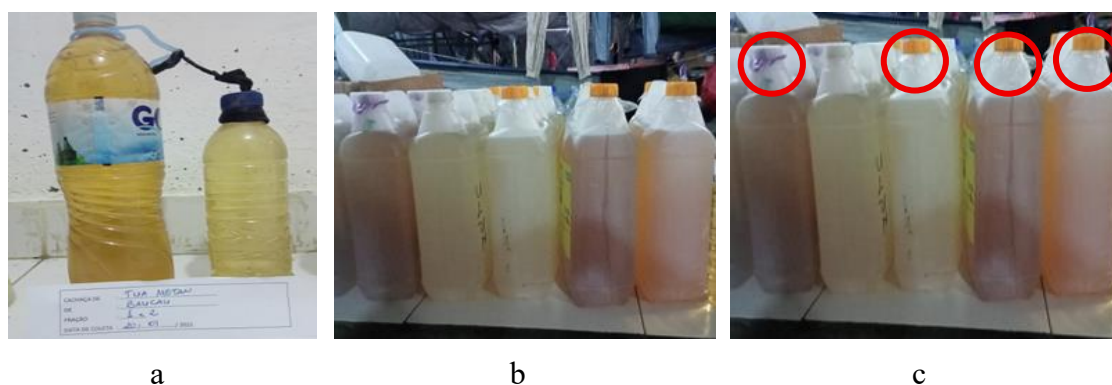
In general, the process initially involves pouring the fermented sap into a still, followed by heating it using a wood stove for 60 to 90 minutes. The separation of distillate fractions varies among producers. Most producers separate the fractions into three categories: first, second, and third, like the head, heart, and tail. In some cases, only two fractions are separated: the first (*tua-ulun*) and the second fraction (*tua-ikun*). The final distillate volume collected corresponds to 10-15% of the total boiler volume.

The first distilled fraction, classified as *tua-ulun*, has a considerably high alcohol content compared to the subsequent fractions. In contrast, the second fraction, namely *tua-klaran*, contains a moderate alcohol content. The third fraction, called "*tua-ikun*", has a significantly lower alcohol content compared to the *tua-ulun* and *tua-klaran* fractions, and is also referred to as weak water (BELO *et al.*, 2023).

2.5.2.4. *Tua-sabu* bottling process

Packaging a product is a step that requires careful attention and thorough knowledge of container use and materials. In this context, packaging is crucial for preventing health issues and maintaining the product's identity and characteristics during storage. In Timor-Leste, products ready for sale are mostly packaged in plastic bottles, including PET (Polyethylene Terephthalate) (Figure 8a) and HDPE (High-Density Polyethylene) (Figure 8b). Additionally, these products are often lined with LDPE (low-density polyethylene) (Figure 8c) plastic materials, such as plastic bags, to prevent alcoholic liquids from leaking (BELO *et al.*, 2023).

Figure 8. The common process of bottling *tua-sabu* material by the Timorese



Source: authors, 2025.

A concern regarding the use of petroleum-derived containers is the potential contamination through direct contact or the migration of plasticizers and other substances into beverages, which can impact consumer health (ALMEIDA *et al.*, 2023). Therefore, using glass bottles or containers would be more suitable for storing the product, thereby reducing the risk of health problems.

In the context of *tua-sabu* production, Timorese producers do not apply any type of maturation or aging to their products, due to limitations of knowledge and resources. As a result, the products are sold directly for consumption. Therefore, it is worth highlighting that the process could be a crucial step in improving the quality of *tua-sabu* in Timor-Leste in the future.

2. 6. Market and consumption aspects

In addition to industrial alcoholic beverages, imported or produced in Timor-Leste, *tua-sabu* stands out as a traditional alcoholic beverage of considerable economic value and viability in the market, with its production capacity intended solely for local sales and consumption (BELO *et al.*, 2025). The beverage is currently sold at various points of sale, directly from the production sites, roadsides, homes, market stalls, kiosks, and traditional markets, and is becoming an important source of income for the producing communities.

BELO *et al.* (2023) reported that the selling price of *tua-sabu* directly at the production sites was relatively high, with the *tua-ulun* fraction generally hovering around US\$5 per liter, and the *tua-klaran* fraction selling for an average of US\$3.50 per liter. And products sold in markets, whether for the *tua-ulun* and *tua-klaran* fractions alone, or for combinations of *tua-ulun* and *tua-klaran* fractions considered strong or moderate,

generally sell for prices ranging from US\$13 to US\$15 and US\$10 to US\$12 per 5 liters, respectively.

The authors also reported that current selling prices, both in small supermarkets and in fairs or markets, range from US\$3 to US\$5 per liter for the *tua-ulun* fraction, between US\$2 and US\$3 per liter for the *tua-klaran* fraction, and around US\$1.5 to US\$3 for the mixed or combined fractions. The aspects of quality, category of fractions, origin of beverages, demand, and accessibility have a positive impact on the variation in product prices on the market (MC WILLIAM, 2022; BELO *et al.*, 2023). Due to the lack of a national quality standard and regulation, the product currently circulates and is sold without restrictions in the country.

Regarding alcohol consumption, in Timor-Leste, consumption begins in early adolescence. The most commonly consumed alcoholic beverages are industrial and imported, such as red wine, beer, brandy, and whiskey, among others. Locally produced beverages, such as *tua-mutin* and *tua-sabu*, are also consumed on numerous occasions throughout the country.

In the absence of a national quality standard, most local consumers of *tua-sabu* continue to prefer stronger fractions, especially the *tua-ulun* fraction or the combination of *tua-ulun* and *tua-klaran* fractions. Furthermore, moderate or weaker fractions, such as the combination of *tua-ulun* and *tua-ikun* or *tua-klaran* and *tua-ikun* fractions, are also intended for regular consumption, without understanding the health consequences (BELO *et al.*, 2023). This preference, without adequate standards and measures, can generate risks and pose significant health problems for humans.

ALCARDE, SOUZA, & BELLUCO (2010) reported that the head fraction of brandy contains alcohol-soluble compounds with low boiling points, such as methanol, acetaldehyde, and ethyl acetate, which are produced during the distillation stage. In addition, the tail fraction contains a high concentration of compounds with a boiling point higher than that of water or water-soluble compounds, such as acetic acid and furfural. Conversely, the alcohol-soluble and water-soluble compounds present in high concentrations in beverages can cause several health problems, which is why the way Timorese consume *tua-sabu* represents a public health concern, putting consumers at risk of serious illness and even death.

According to the WORLD HEALTH ORGANIZATION (2018), the results of the investigation showed that Timor-Leste was one of the countries with the highest youth

alcohol consumption, at 15.7%, involving adolescents aged between 13 and 17 years. This shows greater social concern for the country.

Worldwide, harmful alcohol use causes 3% of all deaths, representing 1.8 million people each year (WHO, 2018). It is estimated that alcohol use has caused about 20-30% of diseases worldwide, such as esophageal cancer, liver disease, homicide and other intentional injuries, epilepsy, and traffic accidents. Excessive alcohol consumption also puts a person at greater risk of cardiovascular disease. In most countries, alcohol-related deaths are often highest between the ages of 45 and 54. Still, the association between early age of alcohol use and patterns of use in adults makes the status of alcohol consumption among adolescents important (WHO, 2018).

In a study carried out by JANI *et al* (2021) in the United Kingdom on the risks of consuming various alcoholic beverages, the results showed that the consumption of brandies was associated with a 25% higher risk of mortality, 31% of MACE (Mortality, Major Adverse Cardiovascular Event), a side effect of heart disease, 48% higher risk of liver cirrhosis and 10% higher risk of accidents/self-harm compared to red wine. Similarly, beer/cider consumption was associated with approximately 18% increased risk of mortality, 16% with MACE, 36% with increased risk of liver cirrhosis, and 11% with increased risk of accidents/self-harm.

CARVALHO *et al* (2021) carried out a study analyzing the relationship between alcohol consumption and the prevalence of hypertension in patients admitted to the Gleno Inpatient Health Center, of Ermera municipality. The results showed that patients who consumed alcohol had positive and significant results, eventually suffering from hypertension with increased blood pressure compared to patients without alcohol consumption. Furthermore, patients admitted to Hospital Nacional de Guido Valadares (HNGV) were also positively affected by hypertension caused by alcohol consumption (PINTO *et al.*, 2022a), and the study reported that the population of the Baucau municipality's alcohol consumption also influenced the incidence of hypertension (PINTO *et al.*, 2022b).

In addition to causing illness and death, excessive consumption can also interfere with human psychological development, negatively impacting social aspects such as the workplace, school, social activities, and leisure. Consumption can disrupt some of the paths of young Timorese from an early age, leading them to drop out of school early and adopt different lifestyles (BELO *et al.*, 2023).

In Timor-Leste, incidents associated with alcohol consumption are frequent, such as arguments, fights, homicides, and traffic accidents, in addition to the direct risk to health, leading to various diseases (WHO, 2018). The core of the problems of alcohol consumption in the country is the lack of social control and the implementation of prohibition rules that exist in the political system of government to control the existence of trade and free consumption of alcoholic beverages throughout the country, as in the market and within society.

Given this, the government of Timor-Leste relaunched its consumer protection policy and created a decree law to regulate the trade and consumption of alcoholic beverages, thereby protecting and guaranteeing the health of consumers. The government of Timor-Leste, through Decree-Law ^{no.} 26/2016, established an entity to execute the powers to inspect, supervise, and control the exercise of economic activities, sanitary conditions, and quality control in the food sector under the law in force, which aims to eliminate, reduce, or prevent risks to the health of consumers (TIMOR-LESTE, 2016a).

Therefore, in Article 20 of Decree-Law ^{no.} 8/2016 on “Consumer Protection”, deviant commercial practices are not permitted, and it is prohibited to place any capital or services on the market without complying with applicable rules, laws, and regulations. Each product must provide, at a minimum, accurate, correct, clear, and objective information about the characteristic, quality, quantity, composition, price, guarantee, expiration date, and origin, among others, as well as the risks associated with consumer health and safety, which is provided for in article 21 (TIMOR-LESTE, 2016b).

The law on the protection of consumer rights and interests in Timor-Leste provides for sanctions for irregularities in marketed products; however, the absence of a functional quality control system may increase the risk of consuming unsuitable products. To prevent abuses and protect consumer rights, it is recommended that a quality seal or legal mark be implemented to regulate the trade of products, in addition to informing citizens of their rights in the event of violations (TIMOR-LESTE, 2016b).

This would guarantee greater security for consumers, ensuring that the products they purchase are manufactured and marketed in accordance with safety and quality standards.

2. 7. Prospects

Tua-sabu production in the country is growing rapidly, keeping pace with the growing number of local connoisseurs and consumers. Currently, it is one of the most

popular beverages, available throughout the country, and is sold at various points of sale, including production sites, roadsides, homes, market stalls, kiosks, and traditional markets, becoming an important source of income for the producing communities.

The current development of the beverage has positively impacted on the quality of life of the rural communities involved, generating additional jobs and increasing the income of local families, thus contributing to the improvement of their socioeconomic conditions.

In the production of *tua-sabu*, the diversity of raw materials used, and the experience and technical knowledge of the producers result in products differentiated in terms of quality, factors that are reflected in the prices charged. The beverage has finally established itself in the market, with current prices that provide satisfactory expectations, in addition to maintaining its high recognition as a heritage and cultural identity of the country.

Its attractiveness, due to growing consumer demand and the viability of sap production plants, opens up opportunities for expanding medium-scale production, with promising prospects for the future. Therefore, medium-scale industrialization is considered a viable alternative, in addition to its expansion to meet local demand, which could bring *tua-sabu* to the global market.

Conversely, despite the significant increase in demand for *tua-sabu*, production capacity remains limited and insufficient to meet the country's entire domestic demand. The lack of a national quality and safety standard is a concern, resulting in *tua-sabu* being circulated and sold without restrictions within the country and without adequate regulations regarding its quality and hygiene.

This poses a significant problem, especially regarding the type of container used for bottling, in addition to restricting its access to the international market.

2. 8. Research gaps

A major problem for the development of *tua-sabu* is the absence of a national quality standard and regulations.

In 2016, the government of Timor-Leste established the Agência de Investigação e Fiscalização da Atividade Econômica, Sanitária e Alimentar, Instituição Pública (AIFAESA.IP), through Decree-Law No. 26/2016. This is an agency responsible for ensuring quality control of food and beverage products, supervising their transportation

conditions, and the sanitary conditions of production and marketing sites (TIMOR-LESTE, 2016a).

And, two years later, through Decree-Law No. 10/2018, the Instituto de Qualidade e Metrologia de Timor-Leste (IQTL.IP) was created to implement and manage the national quality system and other qualification systems, promoting and coordinating activities that contribute to the credibility of economic agents, acting as a national metrology laboratory (TIMOR-LESTE, 2018).

However, the full functioning of both institutions has not yet reached full operation, facing infrastructure challenges, e.g., the absence of laboratories and adequate equipment to analyze the quality of beverages and other food products. Investing in improving the agency's infrastructure is essential for it to perform its functions more effectively. In addition to the lack of national regulations and quality standards, *tua-sabu* has not yet had any quality parameters studied and, to date, remains an unregistered product in the country.

Given these issues, it is necessary to implement Good Manufacturing Practices (GMPs) that cover all aspects of the production process, from sourcing and receipt of raw materials to final product distribution, ensuring product safety and quality for consumers. GMPs are legal requirements in various sectors, and their implementation not only ensures product safety and quality but also increases the credibility of producers and the market.

It would also be important for the government, academic, and non-governmental organizations (NGOs) to promote awareness campaigns based on GMP guidelines, encouraging producers and retailers to adopt more sustainable practices by using returnable bottles and recyclable packaging, thereby ensuring product quality and safety for consumers, as well as environmental protection.

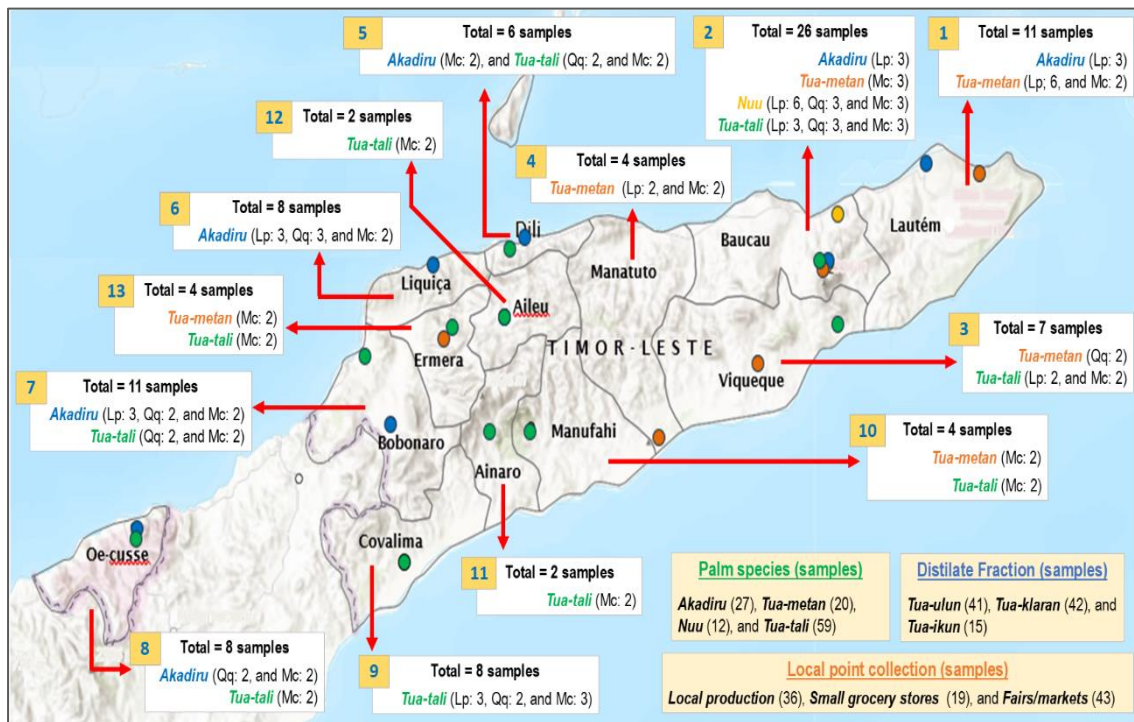
CHAPTER III: MATERIALS AND METHODS

3. 1. Study area and sampling design

The samples used in this study are fractions of *tua-sabu* (alcoholic beverage) derived from the sap of the palm species *akadiru*, *tua-metan*, *nuu*, and *tua-tali*, coded as respective codes Pl, Ap, Cn, and Cu. Additionally, the distilled fractions consist of three distinct fractions: *tua-ulun*, *tua-klaran*, and *tua-ikun*, represented by the respective codes 1, 2, and 3. These samples originate from Timor-Leste at coordinates -8°47'41.08" S 126°08'12.90" E.

Samples were collected over five months, between August and December 2023, at three main points, based on the production, distribution, and sale of fresh produce, produced directly at the production sites (Lp), sold in small grocery stores (Qq), and fairs/markets (Mc) in thirteen municipalities of Timor-Leste (Figure 9).

Figure 9. The samples (fractions of *tua-sabu*) were collected directly from production sites, small grocery stores, and fairs or markets in several regions of Timor-Leste.



The samples were sampled in multiple municipalities and points of sale, palm species, distillate fractions, and producers to represent settings with the highest observed levels of production, sales, and consumption, while documenting production techniques rather than excluding them.

3. 2. Sample distribution details

A total of 98 samples of *tua-sabu* distillate fractions were analyzed: ***tua-ulun*** (n = 41), ***tua-klaran*** (n = 42), and ***tua-ikun*** (n = 15). Samples originated from four palm species-*akadiru* (*Borassus flabellifer*, n = 27), *tua-metan* (*Arenga pinnata*, n = 20), *tua-tali* (*Corypha utan*, n = 39), and *nuu* (*Cocos nucifera*, n = 12), and were obtained at three points of acquisition: production sites (n = 36), kiosks/grocery stores (n = 19), and fairs/markets (n = 43), across multiple municipalities of Timor-Leste.

Sampling was stratified to reflect sap-extraction contexts with similar techniques and comparable sap volumes. The resulting distribution by fraction, species, and point of acquisition is summarized below:

3. 2. 1. Distribution of samples from palm trees by extraction of inflorescence sap.

Were analyzed of 59 samples from *akadiru*, *tua-metan*, and *nuu*: ***akadiru*** (n = 27: *tua-ulun* 11; *tua-klaran* 11; *tua-ikun* 5); ***tua-metan*** (n = 20: *tua-ulun* 9; *tua-klaran* 9; *tua-ikun* 2); ***nuu*** (n = 12: *tua-ulun* 4; *tua-klaran* 5; *tua-ikun* 3). In summary, the samples consist of: *tua-ulun* (n = 24), *tua-klaran* (n = 25), and *tua-ikun* (n = 10) fractions.

Samples collected directly from the production sites (Lp) totalled (n = 26), of which the *akadiru* palm (PILp) represented (n = 12) samples, across municipalities of Lautém (n = 3), Baucau (n = 3), Liquiça (n = 3), and Bobonaro (n = 3). The *tua-metan* species (ApLp) represented (n = 8), Lautém (n = 6), and Manatuto (n = 2) samples; Additionally, *nuu* species (CnLp) totaled (n = 6) samples, were collected in the municipality of Baucau.

Samples collected from small grocery stores (Qq) totaled (n = 12) samples, with *akadiru* species (PlQq) represented (n = 7); municipalities of Liquiça (n = 3), Bobonaro (n = 2), and Oe-cusse (n = 2) samples. Additionally, the *tua-metan* species (ApQq) have (n = 2), municipality of Viqueque, and *nuu* species (CnQq) represented (n = 2) samples from the municipality of Baucau.

Finally, a total of 21 came from fairs or markets (Mc) in various municipalities: the *akadiru* palm (PlMc) (n = 8); from municipalities of Dili (n = 2), Liquiça (n = 2), Bobonaro (n = 2), and Oe-cusse (n = 2) samples. *Tua-metan* palm (ApMc), representing (n = 10) samples; two samples from each of the municipalities of Lautém (n = 2), Baucau (n = 2), Viqueque (n = 2), Manatuto (n = 2), Manufahi (n = 2), and Ermera (n = 2), and *nuu* species (CnMc) represented (n = 3), were collected in the municipality of Baucau.

3. 2. 2. *Distribution of palm samples by sap extraction through the cavity of the soft apical meristem.*

The samples totaled 39 fractions, with *tua-ulun* (n = 17), *tua-klaran* (n = 17), and *tua-ikun* (n = 5) fractions, with an allocation of: production sites (n = 10: *tua-ulun* 4, *tua-klaran* 4, and *tua-ikun* 2); kiosks/grocery stores (n = 7: *tua-ulun* 3, *tua-klaran* 3, and *tua-ikun* 1), and fairs/markets (n = 22: *tua-ulun* 10, *tua-klaran* 10, and *tua-ikun* 2) fractions, across the several municipalities as well as municipality of Baucau, Viqueque, Dili, Bobonaro, Oe-cusse, Covalima, Manufahi, Ainaro, Aileu, and Ermera

3. 3. **Sample handling and storage**

Each sample was collected in 10 mL clear borosilicate glass with threads glass vials. After collection, the samples were carefully stored in a Styrofoam box at room temperature and transported by air to the LabFood - food analysis laboratory of the Graduate Program in Food Science and Technology (PPGCTA) of the School of Agronomy (EA) of the Federal University of Goiás (UFG) for analysis. Upon arrival at the laboratory, the samples were stored in a safe place, at room temperature, away from direct sunlight.

3. 4. **Sample preparation for analysis**

To determine the alcoholic strength (ABV), the samples were pre-centrifuged at 1075 g for 2 minutes. The supernatant was collected, diluted 51-fold, and then filtered through a Merck Millipore Express nylon filter with a porosity of 0.22 μm . A 20 μL aliquot of the filtrate was collected and injected into the liquid chromatography system.

For volatile profile analysis, 1 mL of each sample was added to a 2 mL clear borosilicate glass vial. Without any pretreatment, the samples were analyzed directly by liquid injection.

3. 5. **Determination of alcoholic strength (ABV)**

3.5.1. Calibration curve

Calibration of ethyl alcohol solutions at concentrations of 1,0 to 6,0 g/L was prepared using > 99% of ethyl alcohol. Where a quantity of ethyl alcohol was weighed

for subsequent mixing with ultrapure water, to adjust the concentration between 0.1 and 6.0 g/L.

3.5.2. Liquid chromatography analysis

The analysis was carried out in the Biochemical Engineering Laboratory of the School of Agronomy at the Federal University of Goiás - UFG. High-Performance Liquid Chromatography (HPLC) was employed to determine the concentrations of ethyl alcohol in *tua-sabu*. After sample preparation as described in section 3.4, a Shimadzu Prominence Chromatography was used with a Shim-pack SCR 102HG column protected by an SCR 102HG pre-column.

The substances were visualized using the refractive index (RID-20A) and UV-VIS (SPD-20A) detectors, maintaining the oven temperature at 50°C and flow of the mobile phase of 5 mM perchloric acid at 0.600 mL.min⁻¹. The standards used all had purities greater than 99%.

The determination of the compounds was carried out based on calibration curves constructed with five standard concentration points, considering retention time, detection limit, quantification limit, concentration range, and correlation coefficients of the calibration curves. All samples were analyzed in triplicate.

3. 6. Analysis of volatile compounds

The analysis was carried out in the LabFood of the Food Engineering laboratory of the School of Agronomy at the Federal University of Goiás (UFG). The analyses were carried out using a gas chromatograph coupled to a mass spectrometer (Shimadzu Nexis GC 2030/GC-MS-QP2020 NX, AOC-6000), equipped with a capillary column, SH-Stabilwax-ms (30m, 250µm id, 0.25µm), using electron impact (70 eV) as an ionization source.

The programmed oven temperature was 40 to 250 °C, and the total time for analysis was 55 min. Helium 5.0 was used as the carrier gas, with a pressure of 4.7 psi and a flow of 1.56 mL. min⁻¹, and linear speed of 45.0 cm/s⁻¹. The Injector, interface, and ion source temperature were maintained at 250°C. The temperature of the injector and detector interface was 250°C.

Direct sample injection was used with a sampling volume of 1 μ L. Chromatographic data were collected using LabSolutions/GC-MS Solution Version 4.50 SPI software, copyright©1999-2018 Shimadzu Corporation.

3. 7. Statistical analysis

Statistical analysis of the data was performed independently of the analyzed parameters, as described below:

Microsoft Office Excel Professional Plus 2016 was used to determine the alcoholic strength (ABV), and the results were expressed as the mean percentage of ethyl alcohol (% v/v), including the standard deviation. All analyses were performed in triplicate.

Analysis of volatile compounds was performed using the MetaboAnalyst 6.0 web platform. Data were logarithmically transformed (base 2), and the data matrix tables were normalized by summation and autoscaling. Analysis of variance (ANOVA) and the t-test were performed as univariate statistical evaluations, and variables with $p < 0.05$ were considered significant.

Principal component analysis (PCA) was performed as a multivariate statistical evaluation for pattern recognition and exploratory data analysis. The distributions of the PCA data were visualized using the first two principal components of the score plot, and the compounds considered important for the separation of the groups were related to their corresponding groups by comparing the coordinates on the loading and score plot axes.

CHAPTER IV: RESULTS AND DISCUSSION

4.1. RESULTS

The results and discussion obtained are divided into two parts: consisting of determinations of the alcoholic strength, and the identifications of the volatile organic compounds.

4.1.1. PART I. Physicochemical characterization of *tua-sabu*

In this first part of the results, the *tua-sabu* fractions derived from four palm species are presented, which are divided into two subparts based on production processes in sap obtaining by non-destructive and destructive methods, including the times required in the fermentation process, distillation, and the quantities of sap produced.

4.1.1.1. Alcoholic strength (ABV) of *tua-sabu* fractions from the *akadiru*, *tua-metan*, and *nuu* palm species.

A total of 59 samples analyzed for the present study were composed of twenty-four (24) fractions of *tua-ulun*, twenty-five (25) fractions of *tua-klaran*, and ten (10) fractions of *tua-ikun*. Ethyl alcohol concentrations or alcoholic strengths of all analyzed samples were obtained through the average triplicate analysis of each sample. The values of ethyl alcohol concentrations in each fraction of *tua-sabu* from each palm species are presented in Tables 6, 7, and 8.

4.1.1.1.1. *Akadiru* (*Borassus flabellifer* L.)

All distilled fractions of the *akadiru* palm species were obtained from the municipalities of Lautém, Baucau, Liquiça, Bobonaro, and Oe-cusse. These municipalities are considered the region that represents the largest production of *tua-sabu* derived from this palm species. The distilled fractions collected directly at the production sites (Lp) included fractions of *tua-ulun*, *tua-klaran*, and *tua-ikun*, which presented different concentrations of ethyl alcohol. The sequence of municipalities and their corresponding ethyl alcohol concentrations for each fraction is as follows: 35.0, 30.0, and 17.3; 37.4, 19.6, and 13.3; 31.2, 25.3, and 16.8; 49.4, 17.3, and 17.3 % (v/v), respectively.

Table 6. The alcoholic strength (ABV) of the *tua-sabu* fractions obtained from the *akadiru* palm species.

Type of palm species	Sample collection sites (municipality)	Sample code	Distilled Fraction	Ethyl alcohol (mg/L)	% (v/v)	
<i>Lontar/akadiru</i>	Lautém	P11Lp1	<i>Tua-ulun</i>	275,9 ±0,00	35,0 ±0,00	
		P12Lp1	<i>Tua-klaran</i>	240,3 ±0,02	30,5 ±0,00	
		P13Lp1	<i>Tua-ikun</i>	136,5 ±0,01	17,3 ±0,00	
	Baucau	P11Lp2	<i>Tua-ulun</i>	294,9 ±0,32	37,4 ±0,04	
		P12Lp2	<i>Tua-klaran</i>	154,4 ±0,90	19,6 ±0,11	
		P13Lp2	<i>Tua-ikun</i>	104,6 ±0,19	13,3 ±0,02	
	Dili	P11Mc5	<i>Tua-ulun</i>	175,8 ±1,31	22,3 ±0,17	
		P12Mc5	<i>Tua-klaran</i>	109,8 ±0,04	13,9 ±0,00	
	Liquiça	Liquiça	P11Lp6	<i>Tua-ulun</i>	245,8 ±0,00	31,2 ±0,00
			P12Lp6	<i>Tua-klaran</i>	199,8 ±0,01	25,3 ±0,00
			P13Lp6	<i>Tua-ikun</i>	132,3 ±0,02	16,8 ±0,00
		Liquiça	P11Qq6	<i>Tua-ulun</i>	245,8 ±0,00	31,2 ±0,00
			P12Qq6	<i>Tua-klaran</i>	175,0 ±0,04	22,2 ±0,01
			P13Qq6	<i>Tua-ikun</i>	131,0 ±0,00	16,6 ±0,00
		Liquiça	P11Mc6	<i>Tua-ulun</i>	273,3 ±0,01	34,6 ±0,00
			P12Mc6	<i>Tua-klaran</i>	82,9 ±0,00	10,5 ±0,00
		Bobonaro	Bobonaro	P11Lp7	<i>Tua-ulun</i>	389,7 ±0,04
	P12Lp7			<i>Tua-klaran</i>	136,2 ±1,92	17,3 ±0,24
	P13Lp7			<i>Tua-ikun</i>	136,3 ±1,18	17,3 ±0,15
	Bobonaro		P11Qq7	<i>Tua-ulun</i>	310,8 ±0,01	39,4 ±0,00
			P12Qq7	<i>Tua-klaran</i>	105,3 ±0,02	13,3 ±0,00
			P11Mc7	<i>Tua-ulun</i>	207,6 ±0,03	26,3 ±0,00
	Oe-cusse	Oe-cusse	P12Mc7	<i>Tua-klaran</i>	128,7 ±0,30	16,3 ±0,04
			P11Qq8	<i>Tua-ulun</i>	247,5 ±0,10	31,4 ±0,01
Oe-cusse		P12Qq8	<i>Tua-klaran</i>	117,7 ±0,03	14,9 ±0,00	
		P11Mc8	<i>Tua-ulun</i>	291,7 ±0,02	37,0 ±0,00	
		P12Mc8	<i>Tua-klaran</i>	114,0 ±0,03	14,5 ±0,00	

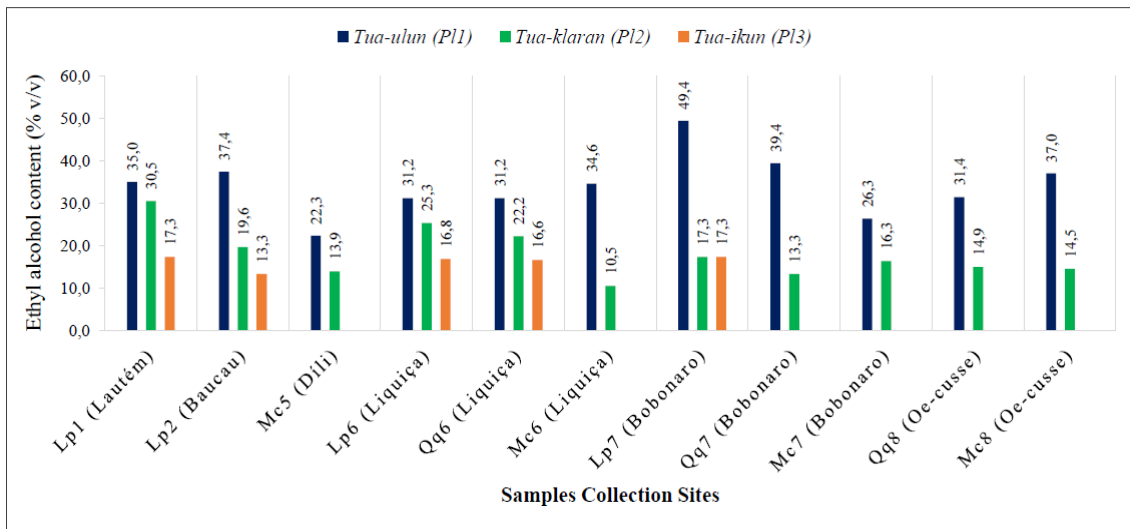
Observation: Type of palm species: **PI** (*Akadiru* palm), Distillate fraction: **PI1** (*tua-ulun*), **PI2** (*tua-klaran*), and **PI3** (*tua-ikun*), and samples collection sites: **Lp** (Production site), **Qq** (Small grocery store/Kiosk), **Mc** (Fair/Market). Source: authors, 2025.

The results indicate that the *tua-ulun* fraction consistently presents higher concentrations of ethyl alcohol in all locations studied. This suggests that the initial fraction of the distillation process retains more alcohol, probably due to the lower boiling points of ethanol compared to other compounds present in palm sap distillate. The variation in alcohol content between different fractions also highlights the importance of controlling the distillation process to achieve the desired quality and alcohol content of the beverage.

Additional analyses and comparisons between different municipalities reveal that production techniques and environmental factors in each region can influence the alcohol strength of *tua-sabu* (Figure 10). For example, the fraction of *tua-ulun* collected directly at the production site (P11Lp7) in Bobonaro's contains an alcoholic strength (ABV) of $49.4 \pm 0.0\%$ (v/v), being considered superior to all other fractions of *tua-ulun* collected

at production sites, grocery stores, and fairs in other municipalities suggesting more efficient distillation practices or variations in the composition of the palm sap. Already, the fraction of *tua-ulun* collected at the fair in the municipality of Dili (P11Mc5) is considered to have the lowest concentration of ethyl alcohol among the remaining fractions of *tua-ulun*.

Figure 10. Alcoholic strength (ABV) of (% v/v) *tua-sabu* of the *akadiru* palm species.



Source: authors, 2025.

It is observed that the *tua-ulun* fractions, such as P11Mc5 and P11Mc7, had ethyl alcohol concentrations lower than or close to those of the other fractions of *tua-klaran*. Furthermore, the *tua-ulun* fractions (P11Lp6, P11Qq6, and P11Qq8) had an ethyl alcohol concentration close to the *tua-klaran* fraction (P12Lp1) collected in the municipality of Lautém. Regardless of the *tua-klaran* fractions, they have ethyl alcohol concentrations ranging from 10.5 and 30.5% (v/v). Some of these concentrations are considered low or close to the concentrations of the *tua-ikun* fractions, which have an ethyl alcohol concentration range of 13.3 to 17.3% (v/v).

The difference and proximity of the alcoholic strength (ABV) of one fraction to others are often related to the experience of each producer in the production process, mainly involving the distillation and separation of the distilled fractions and the bottling process. Furthermore, considering the supply chain issues that involve inadequate storage, transportation, distribution, and sales process, along with factors such as the mixing of a small portion of the first fraction with the second fraction or the third fraction, this can result in reduced alcohol levels (BELO *et al.*, 2023).

The majority of the fractions contain ethyl alcohol concentrations considered low by Brazilian legislation, which sets standards for alcoholic beverages classified as cachaças and/or distillates for human consumption, allowing an alcohol content between 38 and 48% or 38 and 54% (v/v) (MAPA, 2005). Exceptions include fractions such as P11Lp7 and P11Qq7, collected directly at the production site and a small grocery store in the municipality of Bobonaro, which can be included within this legislation.

4.1.1.1.2. *Tua-metan* palm (*Arenga pinnata* (Wurmb) Merr.)

The distilled fractions of the *tua-metan* species were acquired in the municipalities of Lautém, Baucau, Viqueque, Manatuto, Manufahi, and Ermera.

Table 7. The alcoholic strength (ABV) of the *tua-sabu* fractions obtained from the *tua-metan* palm species.

Type of palm species	Sample collection sites (Municipality)	Sample code	Distilled Fraction	Ethyl alcohol (mg/L)	% (v/v)
<i>Arenga pinnata</i> <i>/tua-metan</i>	Lautém	Ap1Lp1T	<i>Tua-ulun</i>	481,5 ±0,07	61,0 ±0,01
		Ap2Lp1T	<i>Tua-klaran</i>	288,9 ±0,40	36,6 ±0,05
		Ap3Lp1T	<i>Tua-ikun</i>	53,0 ±0,35	6,7 ±0,04
		Ap1Lp1I	<i>Tua-ulun</i>	410,4 ±0,04	52,0 ±0,01
		Ap2Lp1I	<i>Tua-klaran</i>	279,5 ±0,85	35,4 ±0,11
		Ap3Lp1I	<i>Tua-ikun</i>	155,5 ±1,01	19,7 ±0,13
	Baucau	Ap1Mc1	<i>Tua-ulun</i>	245,3 ±1,66	31,1 ±0,21
		Ap2Mc1	<i>Tua-klaran</i>	222,6 ±1,42	28,2 ±0,18
	Viqueque	Ap1Qq3	<i>Tua-ulun</i>	308,4 ±0,99	39,1 ±0,13
		Ap2Qq3	<i>Tua-klaran</i>	155,5 ±1,27	19,7 ±0,16
	Manatuto	Ap1Lp4	<i>Tua-ulun</i>	279,0 ±0,09	35,4 ±0,01
		Ap2Lp4	<i>Tua-klaran</i>	237,4 ±0,07	30,1 ±0,01
		Ap1Mc4	<i>Tua-ulun</i>	374,4 ±0,05	47,4 ±0,01
		Ap2Mc4	<i>Tua-klaran</i>	125,6 ±0,14	15,9 ±0,02
	Manufahi	Ap1Mc10	<i>Tua-ulun</i>	208,2 ±0,06	26,4 ±0,01
		Ap2Mc10	<i>Tua-klaran</i>	80,3 ±0,02	10,2 ±0,00
	Ermera	Ap1Mc13	<i>Tua-ulun</i>	242,1 ±0,00	30,7 ±0,00
		Ap2Mc13	<i>Tua-klaran</i>	127,7 ±0,08	16,2 ±0,01

Observation: Type of palm species: **Ap** (*Tua-metan* palm), Distillate fraction: **Ap1** (*tua-ulun*), **Ap2** (*tua-klaran*), and **Ap3** (*tua-ikun*), and sample collection sites: **Lp** (Production site), **Qq** (Small grocery store/Kiosk), **Mc** (Fair/Market). Source: authors, 2025.

The results showed that in the fractions collected directly from the production sites in the municipality of Lautém, each fraction contains concentrations of ethyl alcohol as follows: the first fraction Ap1Lp1T (61.0%), Ap2Lp1T (36.6%), Ap3Lp1T (6.7%) and Ap1Lp1I (52.0%), Ap2Lp1I (35.4%) and Ap3Lp1I (19.7%) of v/v.

Additionally, the fraction of *tua-ulun* (Ap1Lp4) and *tua-klaran* (Ap2Lp4) collected at the production site in the municipality of Manatuto had an ethyl alcohol percentage of 47.4 and 15.9% v/v, respectively. This demonstrated a variation in ethyl alcohol levels from one location or producer to another, even though they are collected directly from production sites.

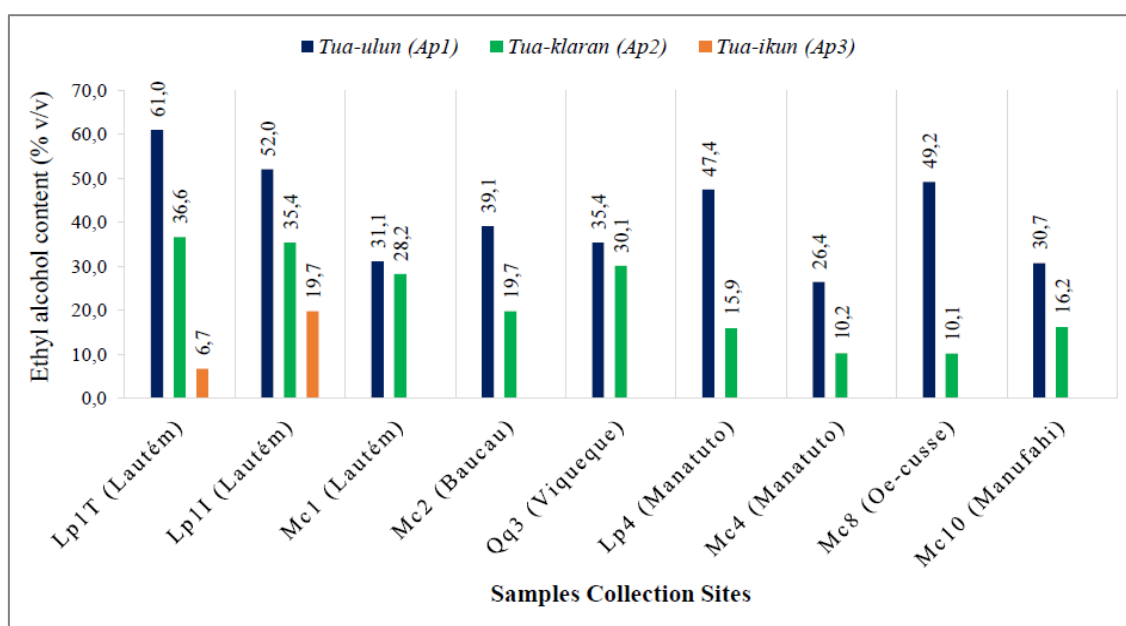
Furthermore, observing the difference between the fractions collected directly at the production site from the municipality of Lautém and those distributed to the market for sale, it is evident that there was a significant reduction in the alcohol content of the *tua-ulun* collected at the fair, which was almost half of that found in the fraction directly at the production site, as this may be related to factors such as separation or cutting of distillate fractions and also other factors related to the sanitation of materials for bottling, environmental conditions during storage and transportation process and products stored for long periods during the sales process.

Two fractions collected in the small grocery store in the municipality of Viqueque, composed of the first fraction containing a concentration of ethyl alcohol of 35.4% and 30.1% v/v for the second fraction of the distillate, appear to have concentrations somewhat close to each other. This may have occurred due to factors such as inadequate separation or cutting of fractions during the distillation process. This can lead to variations since the cutting process is generally determined based on experience and lacks a reference standard. Additionally, the combination of fractions during sale can also contribute to these differences.

The fractions collected at fairs in different municipalities consisted of *tua-ulun* and *tua-klaran* fractions from each municipality, with Lautém having concentrations of 31.3% and 28.2% v/v, Baucau 39.1% and 19.7% v/v, Manatuto 26.4% and 10.2% v/v, Manufahi 30.7% and 16.2% v/v, and Ermera 49.2% and 10.1% v/v of ethyl alcohol. It can be observed that the fractions present variations in the concentrations of ethyl alcohol from one region to another, demonstrating differences in the quality of the product depending on where it was produced or sold.

One of the fractions of *tua-ulun* collected at a fair in the municipality of Manatuto had a very low concentration compared to the other fractions of *tua-ulun* and was also considered low when compared to some fractions of *tua-klaran*, such as Ap2Lp1T, Ap2Lp1I, Ap2Mc1, and Ap2Qq3 (Figure 11). These variations can be attributed to the location of production, supply chain conditions, and combinations of fractions, among other factors.

Figure 11. Alcoholic strength (ABV) of (% v/v) *tua-sabu* of *tua-metan* palm species



Source: authors, 2025.

The fraction of *tua-ulun* collected at a fair in the municipality of Manatuto had a very low concentration compared to the other *tua-ulun* fractions and was also lower than some *tua-klaran* fractions, such as Ap2Lp1T, Ap2Lp1I, Ap2Mc1, and Ap2Qq3, including a fraction of *tua-ikun* of Ap3Lp1I. It is worth noting that these variations in concentrations demonstrate that product quality varies by production and sales region. This can be influenced by factors such as production processes, supply chain issues, and environmental conditions, which can affect the product and reduce the concentration of ethyl alcohol.

In some cases, the *tua-ulun* fractions have ethyl alcohol concentrations close to each other. Producers in the eastern region of the country draw on family experience in the production process; it is important to highlight the techniques and instruments used to produce the beverages. Conversely, the concentrations were similar in the *tua-ulun* (Ap1Qq3) and *tua-klaran* (Ap2Qq3) fractions collected in small grocery stores. This could be due to mixing the *tua-ulun* and *tua-klaran* fractions or inadequate separation during distillation.

The ethyl alcohol concentrations obtained for most of the analyzed fractions are too low to be classified as brandies under Brazilian legislation, which requires an ethyl alcohol concentration between 38 and 54% (v/v) under specific storage conditions at 20 °C (MAPA, 2005). Among the samples, only the *tua-ulun* fractions such as Ap1Lp1T,

Ap1Lp1I, Ap1Mc2, Ap1Lp4, and Ap1Mc13 meet the ethyl alcohol concentration requirements set forth in this legislation.

4.1.1.1.3. *Nuu* (*Cocos nucifera* L.)

Table 8 presents alcoholic strength (ABV) data for *tua-sabu* fractions obtained from the *nuu* species across different sample collection sites in Baucau. The table details ethyl alcohol concentrations for three different distilled fractions (*tua-ulun*, *Tua-klaran*, and *Tua-ikun*) from three types of locations (production sites, small grocery stores, and fairs).

Table 8. The alcoholic strength (ABV) of the *tua-sabu* fractions was obtained from the *nuu* species.

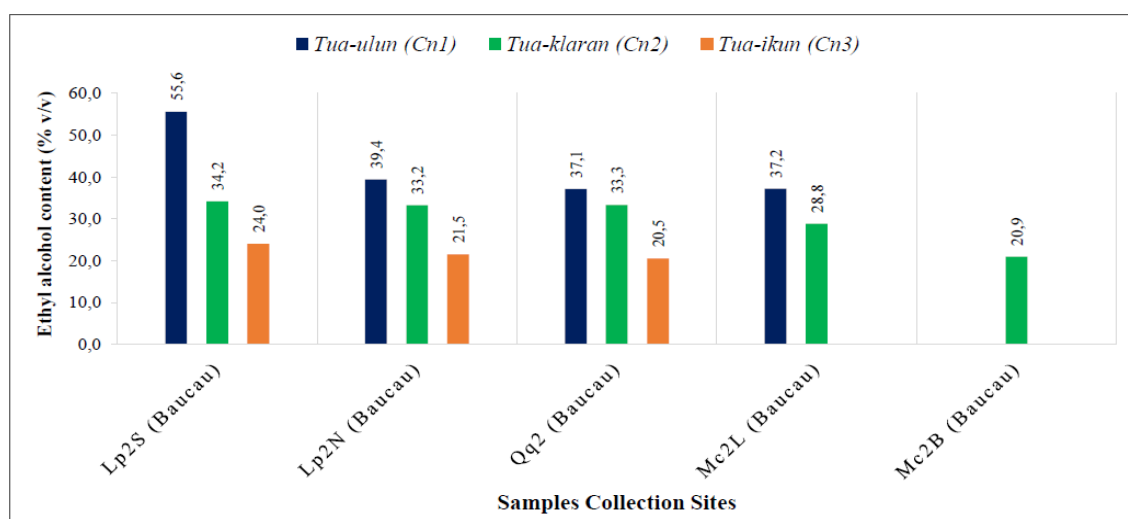
Type of palm species	Sample collection sites (Municipality)	Sample code	Distilled Fraction	Ethyl alcohol (mg/L)	% (v/v)
<i>Cocos nucifera</i> / <i>Nuu</i>	Baucau	Cn1Lp2S	<i>Tua-ulun</i>	439,1 ±0,10	55,6 ±0,01
		Cn2Lp2S	<i>Tua-klaran</i>	270,0 ±0,03	34,2 ±0,00
		Cn3Lp2S	<i>Tua-ikun</i>	189,2 ±0,04	24,0 ±0,00
		Cn1Lp2N	<i>Tua-ulun</i>	310,7 ±0,03	39,4 ±0,00
		Cn2Lp2N	<i>Tua-klaran</i>	262,1 ±0,14	33,2 ±0,02
		Cn3Lp2N	<i>Tua-ikun</i>	169,5 ±0,02	21,5 ±0,00
		Cn1Qq2	<i>Tua-ulun</i>	292,5 ±0,06	37,1 ±0,01
		Cn2Qq2	<i>Tua-klaran</i>	262,5 ±0,01	33,3 ±0,00
		Cn3Qq2	<i>Tua-ikun</i>	161,4 ±0,02	20,5 ±0,00
		Cn1Mc2L	<i>Tua-ulun</i>	293,1 ±0,11	37,2 ±0,01
		Cn2Mc2L	<i>Tua-ulun</i>	227,4 ±1,55	28,8 ±0,20
		Cn2Mc2B	<i>Tua-klaran</i>	164,92 ±1,54	20,9 ±0,20

Observation: Type of palm species: **Cn** (*Nuu*), Distillate fraction: **Cn1** (*tua-ulun*), **Cn2** (*tua-klaran*), and **Cn3** (*tua-ikun*), and sample collection sites: **Lp** (Production site), **Qq** (Small grocery store/Kiosk), **Mc** (Fair/Market). Source: authors, 2025.

Six samples were collected directly at the production site, two samples each from the first (Cn1Lp2S), second (Cn2Lp2S), and third (Cn3Lp2S); (Cn1Lp2N), second (Cn2Lp2N), and third (Cn3Lp2N) fractions, with ethyl alcohol concentrations of 55.6%, 34.2%, and 24.0%; 39.4%, 33.2%, and 21.5% v/v, respectively. Furthermore, the fractions collected at the small grocery stores (Cn1Qq2, Cn2Qq2, and Cn3Qq2) and the fair (Cn1Mc2L, Cn2Mc2L, and Cn2Mc2B) presented ethyl alcohol concentrations of 37.1%, 33.3%, 20.5%, and 37.2%, 28.8%, 20.9% (v/v), respectively.

In general, all fractions present very well-perceived concentrations, arranged in descending order from *tua-ulun* to *tua-ikun*. However, there was a change or reduction in the concentrations of ethyl alcohol in the *tua-ulun* fractions collected at the small grocery stores (Cn1Qq2) and in the fair (Cn1Mc2B), compared to those collected directly at the production site.

Figure 12. Alcoholic strength (ABV) of (% v/v) *tua-sabu* of *nuu* species



Source: authors, 2025.

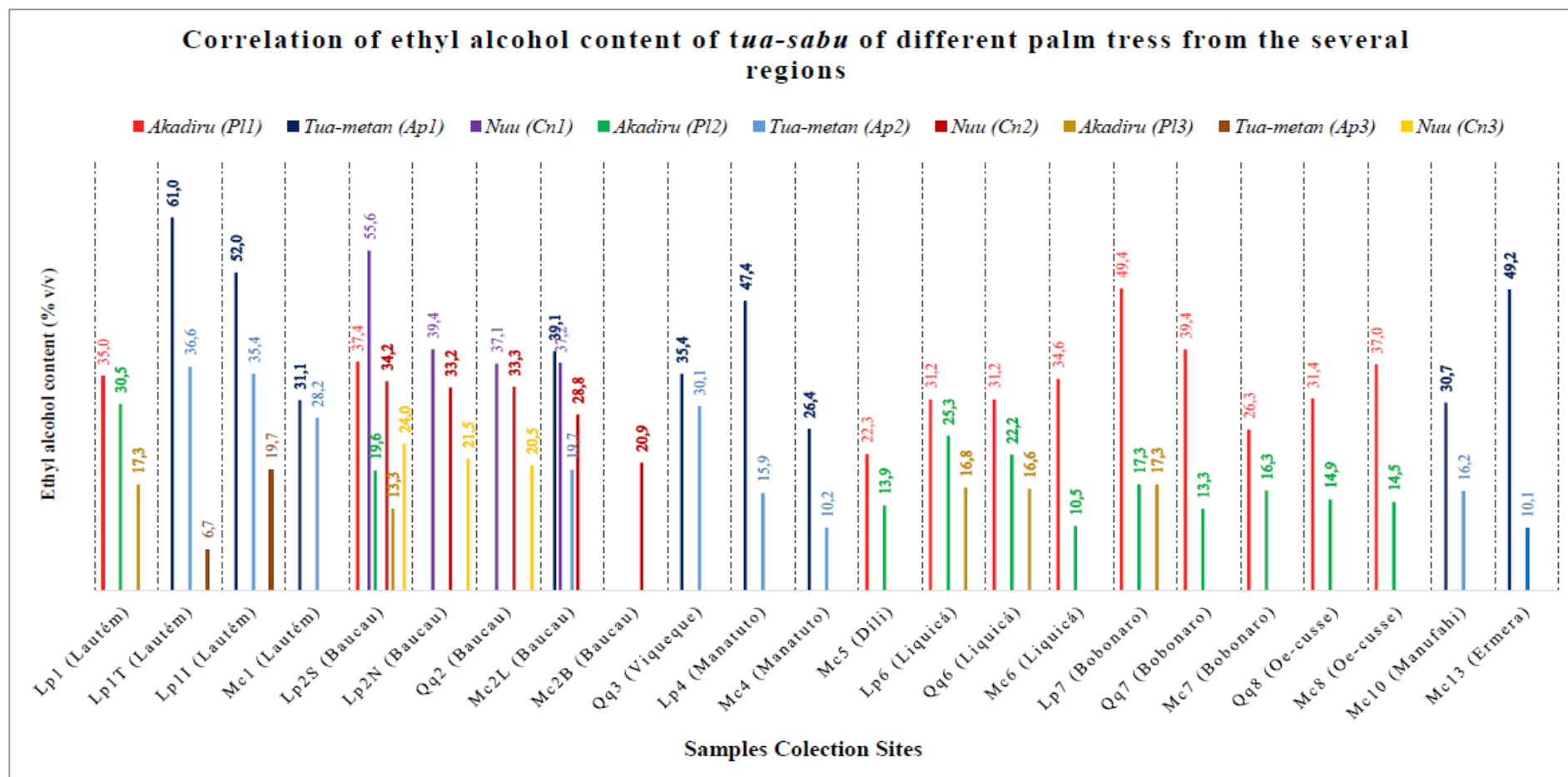
Additionally, the two fractions of *tua-klaran* (Cn2Mc2L and Cn2Mc2B) obtained at the fair have ethyl alcohol concentrations comparable to or lower than those of the *tua-ikun* fractions (Cn3Lp2S/Cn3Qq2) collected at the production site and in the small grocery store.

According to Brazilian legislation for distilled alcoholic beverages classified as brandy, only two fractions of *tua-ulun*, Cn1Lp2 (S and N), collected directly at the production site, meet the criteria with ethyl alcohol concentrations of 55.6% and 39.4% v/v. The remaining fractions, which have lower concentrations, do not qualify under this legislation for brandy but may be classified as liqueurs, defined as having an alcohol content between 15% and 54% vol at 20 °C (BRASIL, 2005).

All fractions exhibit concentrations that decrease sequentially from *tua-ulun* to *tua-ikun*, and there was a slight variation in concentrations during commercialization, both in small markets and at the fair, when compared to fractions collected directly from production sites. These changes reflect the management of product distribution and transportation, including inadequate storage conditions such as exposure to open air and sunlight, use of unsuitable packaging materials, poor hygiene practices, and mixing of fractions.

The production of *tua-sabu* from *Cocos nucifera* sap remains limited compared to other palm tree species, largely due to the scarcity of experienced producers, particularly in extracting sap from this plant. This has resulted in a reduced number of samples collected.

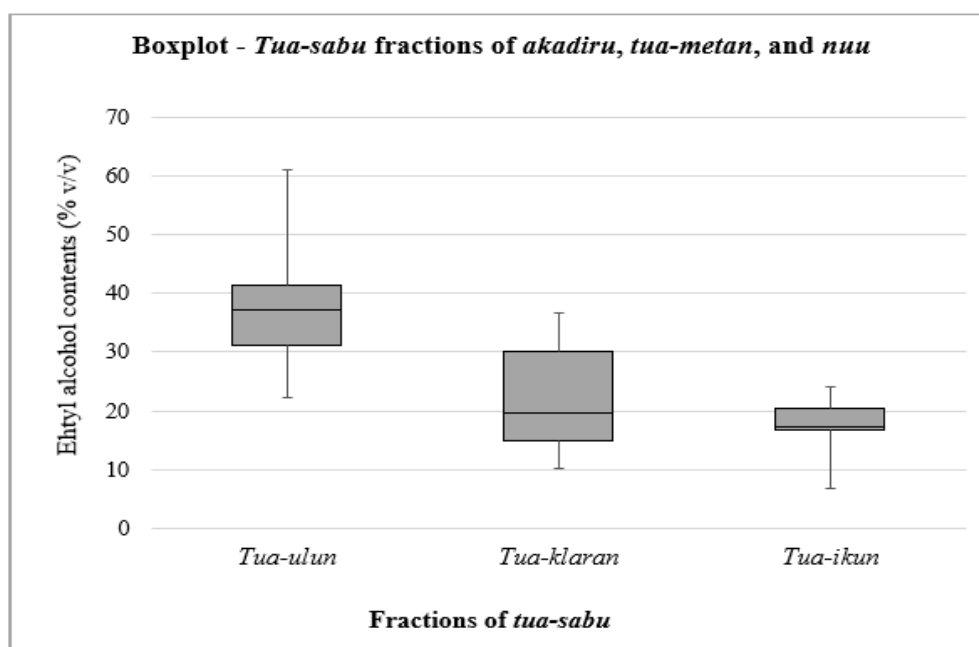
Figure 13. Relationship of the alcoholic strength (ABV) of (% v/v) of *tua-sabu* fractions from different palm species, collection points, and various regions of Timor-Leste.



Source: authors, 2025.

The overall graph (Figure 13) showed that the fractions exhibited large variations in concentration, even when originating from the same palm species or different species, and from the same or different collection points. This demonstrates the influence of the types and qualities of the raw materials, the conditions involved in the production processes (fermentation, distillation, and separation of fractions), the use of inadequate packaging materials with poor hygiene, the handling, storage, and transport of the product, and management throughout the commercialization process, including inadequate hygiene practices and some instances of fraction mixing.

Figure 14. The BoxPlot of *tua-sabu* fractions of palms of *akadiru*, *tua-metan*, and *nuu* species.



Source: authors, 2025.

As can be observed in the box plot (Figure 14), all fractions showed well-defined concentrations, decreasing sequentially from *tua-ulun* to *tua-ikun*. Furthermore, none of the *tua-klaran* fractions meet the Brazilian legal requirements for aguardente or cachaça for human consumption.

4.1.1.2. Alcoholic strength (ABV) of *tua-sabu* fractions of the *tua-tali* palm species.

Alcoholics' strengths (ABV) of all analyzed samples were obtained as the average of triplicate analyses of each sample. The values of ethyl alcohol concentrations in each fraction of *tua-sabu* from each palm species are presented in Table 9, Table 10, and Table 11.

4.1.1.2.1. Fractions collected directly from production sites

The results showed that the samples collected at the production sites presented different concentrations. The *tua-ulun* fraction collected in the municipality of Baucau (Cu1Lp2) contains the highest concentration of ethyl alcohol at 53.6% v/v, followed by those gathered in the municipalities of Covalima (Cu1Lp9), Viqueque (Cu1Lp3), and Bobonaro (Cu1Lp7) with a percentage of ethyl alcohol of 45.6, 41.5, and 38.1% v/v, respectively.

The fractions of *tua-klaran*, such as Cu2Lp2, Cu2Lp3, Cu2Lp7, and Cu2Lp9, had respective ethyl alcohol contents of 40.4, 34.3, 20.7, and 25.3% v/v. Furthermore, two fractions of *tua-ikun* (Cu3Lp2) and (Cu3Lp9) showed concentrations of 21.5 and 17.2% v/v, respectively.

Table 9. Alcoholic strength (ABV) of the *tua-sabu* fractions was obtained directly from production sites (Lp).

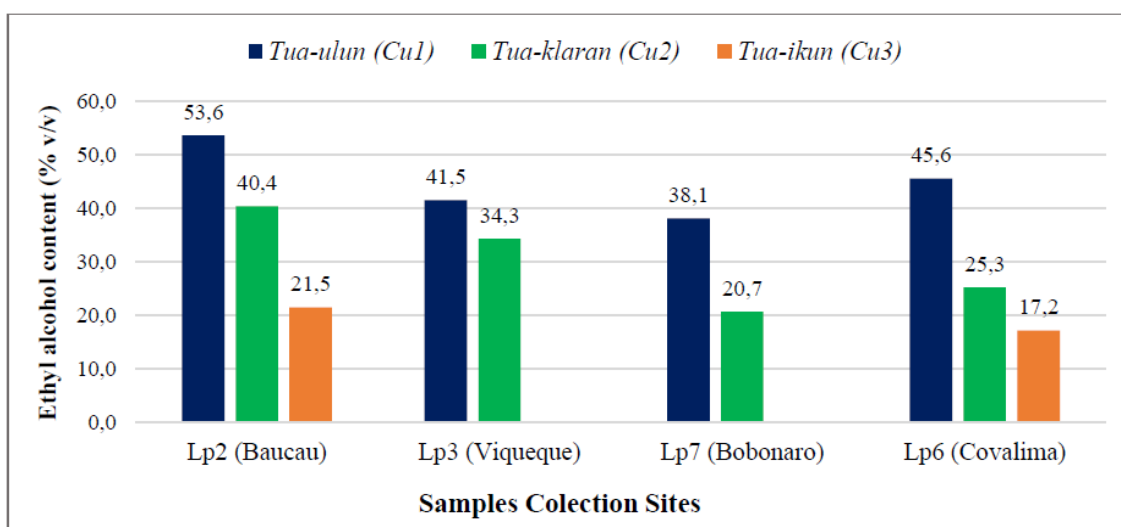
Sample collection sites (Municipality)	Sample code	Distilled Fraction	% (v/v)
Baucau	Cu1Lp2	<i>Tua-ulun</i>	53.6 ±0.01
	Cu2Lp2	<i>Tua-klaran</i>	40.4 ±0.00
	Cu3Lp2	<i>Tua-ikun</i>	21.5 ±0.01
Viqueque	Cu1Lp3	<i>Tua-ulun</i>	41.5 ±0.03
	Cu2Lp3	<i>Tua-klaran</i>	34.3 ±0.10
Bobonaro	Cu1Lp7	<i>Tua-ulun</i>	38.1 ±0.00
	Cu2Lp7	<i>Tua-klaran</i>	20.7 ±0.00
Covalima	Cu1Lp9	<i>Tua-ulun</i>	45.6 ±0.01
	Cu2Lp9	<i>Tua-klaran</i>	25.3 ±0.05
	Cu3Lp9	<i>Tua-ikun</i>	17.2 ±0.00

Legend: The value in the table was the mean of three replicates ± Standard Deviation (SD)

Among the fractions of each municipality of *tua-ulun*, *tua-klaran*, and *tua-ikun*, there was a significant difference in ethyl alcohol levels. Furthermore, when comparing the fractions from one region to another, differences in alcoholic content were also observed. For example, the *tua-klaran* fraction (Cu2Lp7) presented a lower concentration than the other fractions of the same type and was even lower when compared to a *tua-ikun* fraction (Cu3Lp2).

It is worth considering that the variation in alcohol content is likely influenced by factors related to the manufacturing process.

Figure 15. Alcoholic strength (ABV) of the *tua-sabu* fractions obtained from production sites (Lp).



Source: authors, 2025.

Each region has different experiences and methods regarding the use of stills, the quality and quantity of saps, time control during fermentation and distillation, and the estimated quantity of distillate for separating the fractions, among others.

4.1.1.2.2. Fractions collected in the small grocery stores

The fractions collected in small grocery stores in different regions showed varying concentrations of alcohol content. The samples collected in the municipality of Baucau (Cu1Qq2, Cu2Qq2, and Cu3Qq2) showed respective ethyl alcohol percentages of 50.3, 31.9, and 16.4% v/v. However, each of the samples collected in municipalities such as Dili (CuQq5 and Cu2Qq5) and Covalima (Cu1Qq9 and Cu2Qq9) presented ethyl alcohol concentrations of 41.3 and 23.0% v/v, and 36.1, and 29.5% v/v, respectively.

Table 10. Alcoholic strength (ABV) of the *tua-sabu* fractions obtained from small grocery stores (Qq).

Sample collection sites (Municipality)	Sample code	Distilled Fraction	% (v/v)
Baucau	Cu1Qq2	<i>Tua-ulun</i>	50.3 ±0.00
	Cu2Qq2	<i>Tua-klaran</i>	31.9 ±0.01
	Cu3Qq2	<i>Tua-ikum</i>	16.4 ±0.00
Dili	Cu1Qq5	<i>Tua-klaran</i>	41.3 ±0.01
	Cu2Qq5	<i>Tua-klaran</i>	23.0 ±0.21
Covalima	Cu1Qq9	<i>Tua-ulun</i>	36.1 ±0.01
	Cu2Qq9	<i>Tua-klaran</i>	29.5 ±0.00

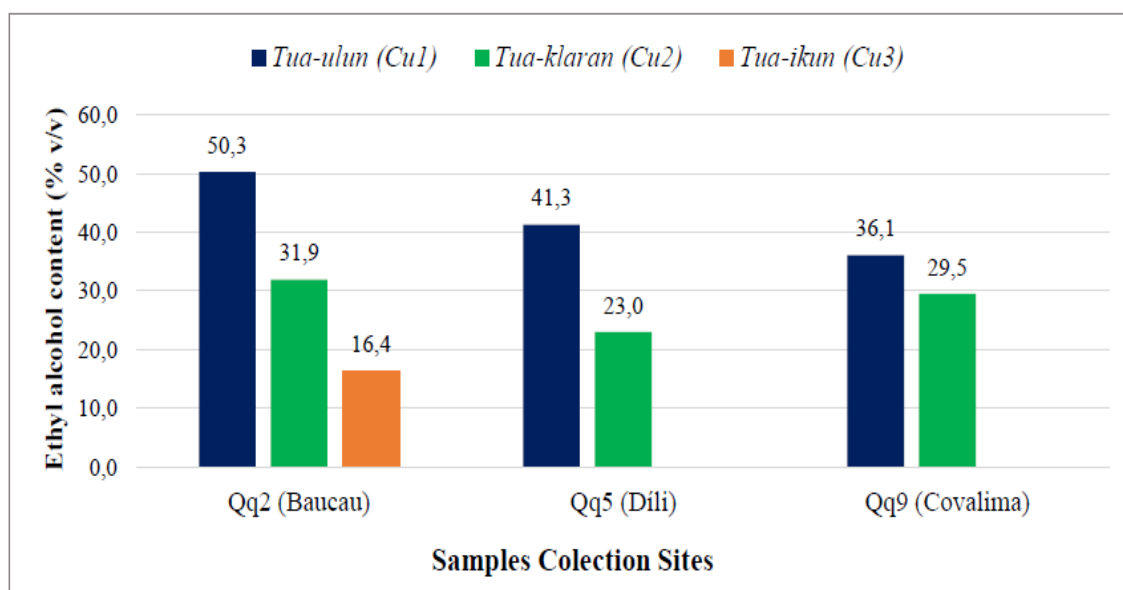
Legend: The value in the table was the mean of three replicates ± Standard Deviation (SD)

Additionally, a single *tua-ikun* fraction was collected in a small grocery store in Baucau, containing 16.4% v/v of ethyl alcohol.

The highest concentration of ethyl alcohol in the first fraction (*tua-ulun*) was found in a sample from the municipality of Baucau (Cu1Qq2), which contained 50.3% v/v of ethyl alcohol, while the lowest concentration was found in a fraction from the municipality of Covalima (Cu1Qq9), containing 36.1% v/v.

On the other hand, the highest levels of ethyl alcohol in the second fraction (*tua-klaran*) were also found in a sample from Baucau (Cu2Qq2), with an ethyl alcohol percentage of 31.9% v/v, while the lowest was found in a fraction collected in the municipality of Dili (Cu2Qq5), containing 23.0% v/v of ethyl alcohol. It should be noted that the fractions collected in the same place presented significantly different alcoholic concentrations.

Figure 16. Alcoholic strength (ABV) of the *tua-sabu* fractions obtained from small grocery stores (Qq).



Source: authors, 2025.

When observing the same fractions collected in several locations, there is also a large difference in their alcohol content.

4.1.1.2.3. Fractions collected at the fairs

The *tua-ulun* fractions with high concentrations of ethyl alcohol, collected from the fairs, were observed in the municipalities of Baucau (Cu1Mc2) and Dili (Cu1Mc5), with percentages of 48.8 and 41.6% v/v, respectively. This was followed by samples from the

municipalities of Manufahi (Cu1Mc10), Aileu (Cu1Mc12), Ainaro (Cu1Mc11), Covalima (Cu1Mc9), Ermera (Cu1Mc13), Oe-cusse (Cu1Mc8), Viqueque (Cu1Mc3), and Bobonaro (Cu1Mc7). These samples presented decreasing concentrations of ethyl alcohol of 38.1, 37.6, 37.4, 35.7, 35.3, 33.0, 30.8, and 30.4 (% v/v), respectively.

Additionally, in *tua-klaran*, the lowest concentration, 18.2% v/v, was found in the sample (Cu2Mc8) from the municipality of Oe-cusse, while the highest concentration was found in the samples collected in the municipalities of Viqueque (Cu2Mc3) and Aileu (Cu2Mc12), which contained 24.0 and 23.9% v/v of ethyl alcohol, respectively.

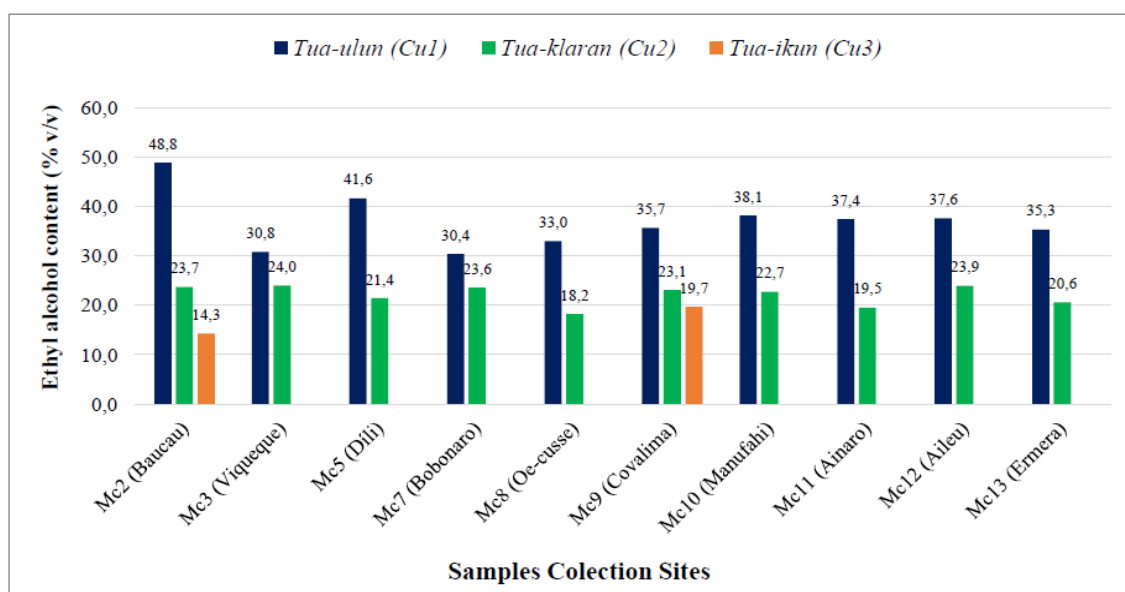
Table 11. Alcoholic strength (ABV) of *tua-sabu* fractions obtained from the fairs (Mc).

Sample collection sites (Municipality)	Sample code	Distilled Fraction	% (v/v)
Baucau	Cu1Mc2	<i>Tua-ulun</i>	48.8 ±0.00
	Cu2Mc2	<i>Tua-klaran</i>	21.4 ±0.01
	Cu3Mc2	<i>Tua-ikun</i>	19.5 ±0.00
Viqueque	Cu1Mc3	<i>Tua-ulun</i>	30.8 ±0.00
	Cu2Mc3	<i>Tua-klaran</i>	24.0 ±0.00
Dili	Cu1Mc5	<i>Tua-ulun</i>	41.6 ±0.03
	Cu2Mc5	<i>Tua-ulun</i>	21.4 ±0.08
Bobonaro	Cu1Mc7	<i>Tua-ulun</i>	30.4 ±0.00
	Cu2Mc7	<i>Tua-klaran</i>	23.6 ±0.00
Oe-cusse	Cu1Mc8	<i>Tua-ulun</i>	33.0 ±0.00
	Cu2Mc8	<i>Tua-klaran</i>	18.2 ±0.76
Covalima	Cu1Mc9	<i>Tua-ulun</i>	35.7 ±0.00
	Cu2Mc9	<i>Tua-klaran</i>	23.1 ±0.00
	Cu3Mc9	<i>Tua-ikun</i>	19.7 ±0.00
Manufahi	Cu1Mc10	<i>Tua-ulun</i>	38.1 ±0.11
	Cu2Mc10	<i>Tua-klaran</i>	22.7 ±0.00
Ainaro	Cu1Mc11	<i>Tua-ulun</i>	37.4 ±0.61
	Cu2Mc11	<i>Tua-klaran</i>	19.5 ±0.01
Aileu	Cu1Mc12	<i>Tua-ulun</i>	37.6 ±0.01
	Cu2Mc12	<i>Tua-klaran</i>	23.9 ±0.00
Ermera	Cu1Mc13	<i>Tua-ulun</i>	35.3 ±0.01
	Cu2Mc13	<i>Tua-klaran</i>	20.6 ±0.00

Legend: The value in the table was the mean of three replicates ± Standard Deviation (SD)

Overall, the fractions collected from various fairs and municipalities have different ethyl alcohol contents, following a descending order from the *tua-ulun* to *tua-klaran* and *tua-ikun* fractions. Among all *tua-ulun* fractions, Cu1Mc2 and Cu1Mc5 have higher ethyl alcohol concentrations, while Cu1Mc8 contains lower levels. It can be observed that, among the *tua-ulun* fractions, there are differences and similarities in alcohol concentrations.

Figure 17. Alcoholic strength (ABV) of the *tua-sabu* fractions obtained from the fairs (Mc).



Source: authors, 2025.

The fraction collected in the municipality of Baucau presented the highest concentration of ethyl alcohol, while the lowest concentration was found in the sample from Bobonaro. These samples indicate a significant difference in alcohol levels; however, the majority of fractions show similar or very close concentrations. Additionally, similar concentrations are observed in the *tua-klaran* fractions collected from all municipalities.

Most of the *tua-klaran* fractions collected from all locations showed variations in ethyl alcohol concentration, even though they were collected from the same places of origin. Fractions produced directly from production sites generally had higher alcohol contents compared to some *tua-ulun* fractions collected from small grocery stores and fairs. This can be observed in a sample collected directly from the production site in the municipality of Baucau (Cu2Lp2), which had a higher alcohol content than all of the *tua-klaran* fractions sold in small grocery stores and fairs. In addition, it was considered superior compared to most of the other *tua-ulun* fractions collected from small grocery stores and fairs in several other regions.

The other *tua-klaran* fractions, such as Cu2Lp3 and Cu2Qq3, also showed a similar pattern in their distribution to the fairs. Furthermore, some *tua-klaran* fractions from production sites presented lower concentrations compared to similar fractions collected from small grocery stores and fairs. This is observed in the fractions $Cu2Lp9 < Cu2Qq9$

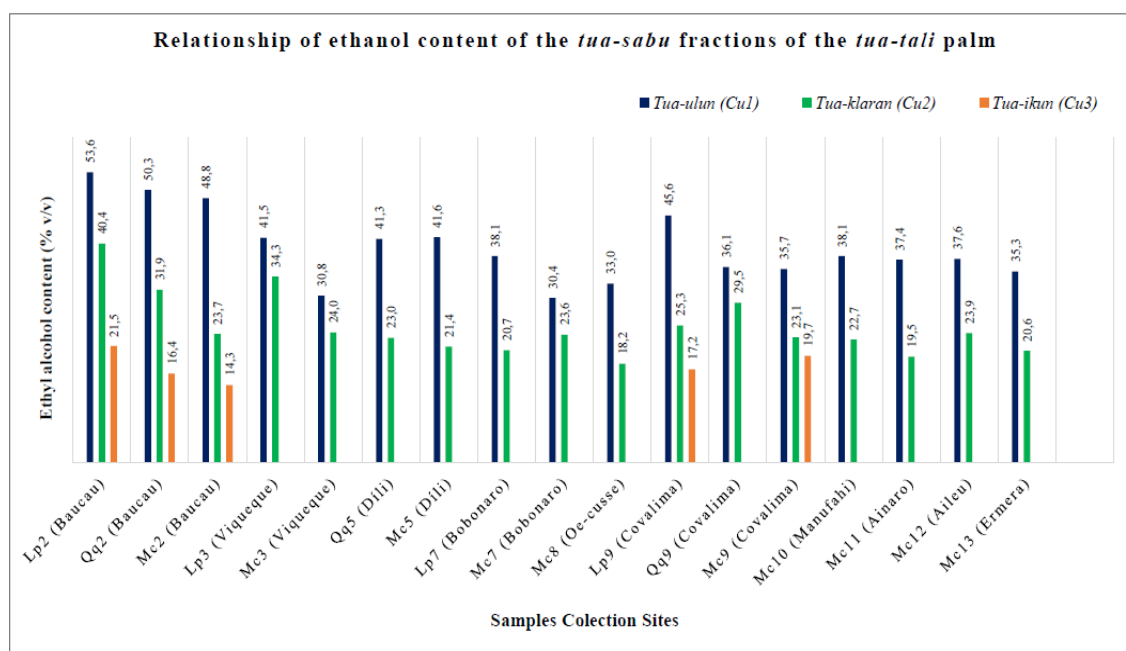
and Cu2Lp7 < Cu2Mc7, with respective ethyl alcohol concentrations of 23.0% < 24.0% and 25.3% < 29.5% v/v.

Although the variation in alcohol content is not very significant, it still indicates a difference in quality between the fractions. This variation is likely influenced by the environmental conditions during the production and sale process, as well as the storage conditions of the packaged product. Additionally, the types of containers or bottles used for packaging, their hygienic conditions, and the combination of fractions may also have contributed to the differences.

Meanwhile, the *tua-ikun* fractions, Cu3Lp2 (Baucau) and Cu3Mc9 (Covalima), contained ethyl alcohol concentrations similar to the levels found in the *tua-klaran* fractions collected directly from the production site, such as Cu2Lp7 from Bobonaro municipality, and in the fractions collected at fairs, including Cu2Mc5 (Díli), Cu2Mc8 (Oe-cusse), Cu2Mc11 (Ainaro), and Cu2Mc13 (Ermera).

It can be observed that samples collected directly from production sites and their distribution for sale in small grocery stores and fairs within a single region or municipality, even if from the same fraction, show a decrease in concentrations throughout their distribution. This is evident in samples collected from the municipalities of Baucau, Viqueque, Bobonaro, and Covalima.

Figure 18. Relationship of the alcoholic strength (ABV) of the *tua-sabu* fractions of the *tua-tali* (*Corypha utan* Lam) palm species.



Source: authors, 2025.

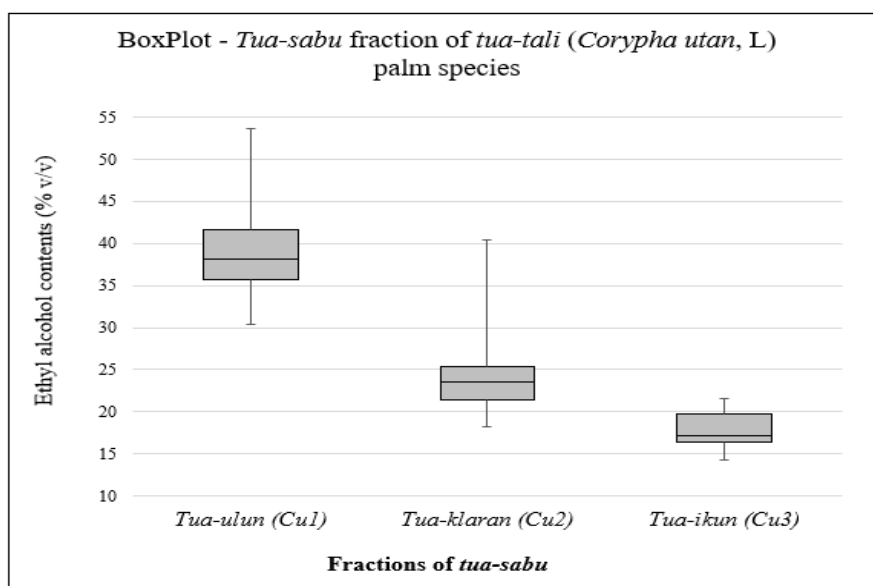
Overall, it can be observed that among the fractions collected at the three different collection sites, the highest concentrations were found in the fractions collected directly at the production site. These concentrations decreased throughout their distribution to small grocery stores and fairs, even if originating from the same region, as observed in samples from the municipalities of Baucau, Viqueque, Bobonaro, and Covalima.

The fractions collected directly from the production site contained higher concentrations, followed by those sold in grocery stores and fairs, mainly for the *tua-ulun* fractions. Meanwhile, the *tua-klaran* and *tua-ikun* fractions showed variations in ethyl alcohol levels, with some fractions sold at fairs containing higher alcohol levels than those sold in small grocery stores, and vice versa (Figure 18).

These variations are likely related to factors such as the transport and storage conditions of the products throughout their distribution and sale. It is worth considering that the variations and significant changes in concentration from one sample to another are related to factors throughout their production, distribution, and sales.

The main factors that should be highlighted include the hygienic conditions of the materials used in product packaging, inadequate storage and transport management, direct contact of the product with sun exposure or open environments with air contact, the combination of fractions, and potential irregularities during the fairing process, among others. These influences can consequently alter the alcohol content of the products and lead to the formation of other undesirable secondary compounds.

Figure 19. The BoxPlot of *tua-sabu* fractions of *tua-tali* (*Corypha utan* Lam) palm species



Source: authors, 2025.

Note that all of the *tua-ulun* and *tua-klaran* fractions collected at different locations had a concentration of ethyl alcohol ranging from 30.4 to 53.6% v/v and 18.2 to 40.4% v/v, respectively (Figure 19). According to Brazilian brandy law, only fractions with percentages ranging from 38.1% to 53.6% v/v should be included in this regulation.

Fractions with ethyl alcohol concentrations of 18.2 to 37.6% v/v are considered too low to be classified as brandy under current legislation. However, they can be classified as liquor. According to Brazilian legislation, cachaça obtained exclusively from sugarcane juice may contain 38 to 48% v/v, while liquor may contain 15 to 54% v/v, and brandy may contain 38 to 54% v/v of ethyl alcohol at 20°C (BRASIL, 2005).

4.1.2. PART II. Volatile compound profile

A total of 98 samples of *tua-sabu* fractions composed of *tua-ulun*, *tua-klaran*, and *tua-ikun* fractions originating from *akadiru*, *tua-metan*, *tua-tali*, and *nuu* species were collected from different locations and sites in the Timor-Leste region, and a total of 50 volatile compounds were identified.

The compounds are grouped based on the chemical classes of the compounds, such as aldehydes, alcohols, organic acids, esters, and ketones, including other compounds (furans, alkaloids, plastics, and sugar derivatives), as can be seen in Table 12. Each fraction of the analyzed samples presented a different number of compounds, which may represent their unique characteristics.

The difference in the number of compounds formed is related to the type of raw materials and the processes involved in the production steps, such as fermentation, distillation, storage, and maturation, which are important factors that determine the quality and nature of the formation of these compounds (CAPOBIANGO *et al.*, 2013; LIU *et al.*, 2023).

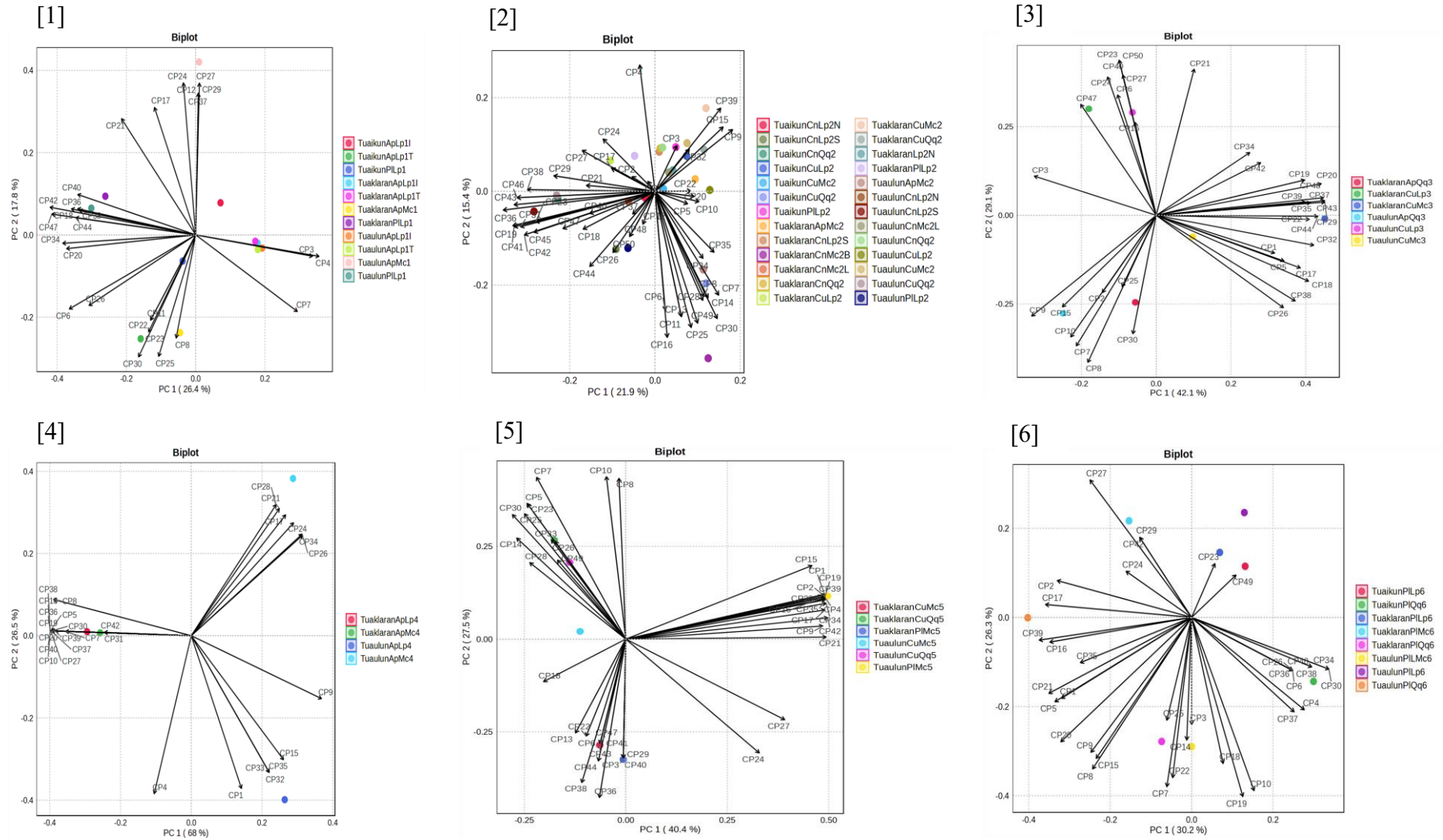
Table 12. Total of compounds found in 98 *tua-sabu* fractions analyzed.

Chemical class of compounds	Name	Code
Alcohol	Methyl Alcohol	CP1
	2-Butanol	CP2
	1-Propanol	CP3
	1-Propanol, 2-methyl-	CP4
	1-Butanol	CP5
	1-Propanol, 3-ethoxy-	CP6
	Propylene Glycol	CP7
	1-Butanol, 2-methyl-	CP8
	1-Butanol, 3-methyl-	CP9

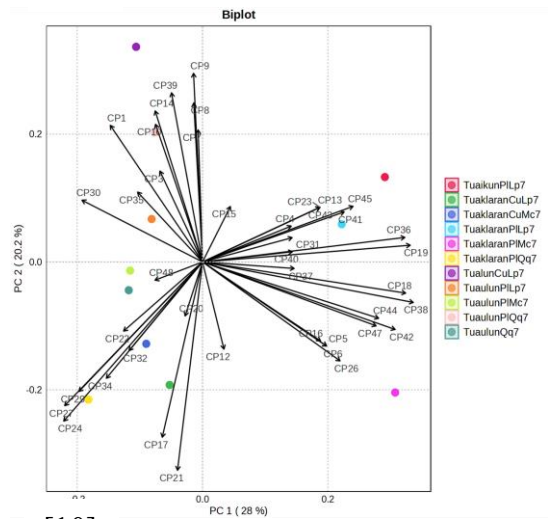
	2,3-Butanediol, [R- (R*, R*)]-	CP10
	2,3-Butanediol, [S- (R*, R*)]-	CP11
	3,3-Diethoxy-1-propanol	CP12
	Tetrahydro-4H-pyran-4-ol	CP13
	2-Furanmethanol	CP14
	Phenylethyl Alcohol	CP15
	Glycerin	CP16
	Catechol	CP17
Acids	Acetic acid	CP18
	Propanoic acid	CP19
	Propanoic acid, 2-methyl-	CP20
	Butanoic acid	CP21
	Butanoic acid, 3-methyl-	CP22
	Pentanoic acid	CP23
	Octanoic acid	CP24
	Lactic acid	CP25
	n-Decanoic acid	CP26
Aldehydes	Acetaldehyde	CP27
	Acetaldehyde, hydroxy-	CP28
	5-(Hydroxymethyl) dihydrofural	CP29
	5-Hydroxymethylfurfural	CP30
	Furfural	CP31
	2-Hydroxy-gamma-butyrolactone	CP32
	Benzaldehyde, 4-hydroxy-	CP33
Esther	Ethyl formate	CP34
	Ethyl Acetate	CP35
	Ethyl Acetate (Ethane, 1,1-diethoxy-)	CP36
	Formic acid, 1-methyl ethyl ester	CP37
	Propanoic acid, 2-hydroxy-, ethyl ester	CP38
Ether	Oxirane, (ethoxymethyl)-	CP39
Ketones	Acetone	CP40
	Acetoin	CP41
	2-Propanone, 1-hydroxy-	CP42
	Butyrolactone	CP43
	Dihydroxyacetone	CP44
	2(3H)-Furanone, dihydro-4-hy	CP45
	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-	CP46
Others (Furan, Alkaloids, plastics, and sugar derivatives)	Benzofuran, 2,3-dihydro-	CP47
	Bis(2-ethylhexyl) phthalate	CP48
	Caffeine	CP49
	.beta.-D-Glucopyranoside, met	CP50

PCA analysis was performed on volatile organic compounds of the 98 samples representing 41 fractions of *tua-ulun*, 42 fractions of *tua-klaran*, and 15 fractions of *tua-ikun*, with 27 fractions of *akadiru* palm species, 20 fractions of *tua-metan* palm, 39 fractions of *tua-tali* palm, and *nuu* represented by 12 fractions. Of the data variations observed in Figure 20, and Figure 21. The two principal components (PC1 and PC2) of each PCA are displayed in the data information.

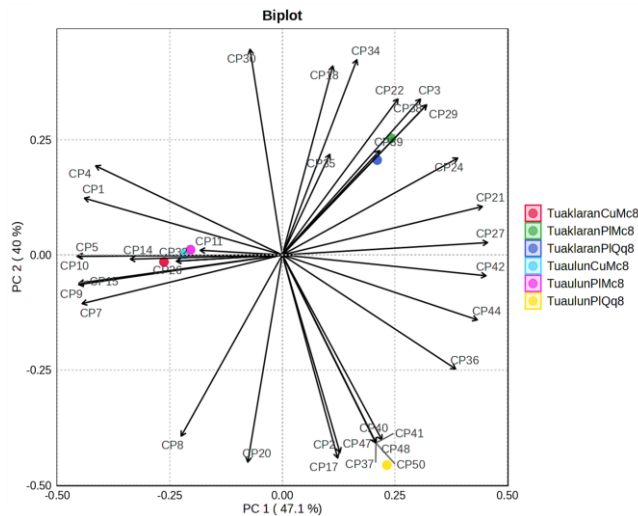
Figure 20. PCA of all fractions from different locations (municipalities)



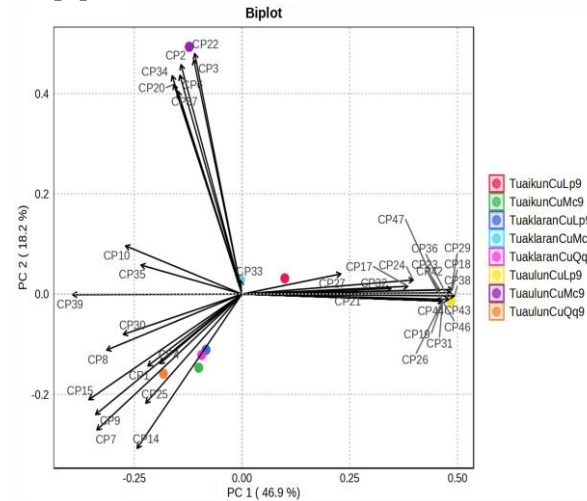
[7]



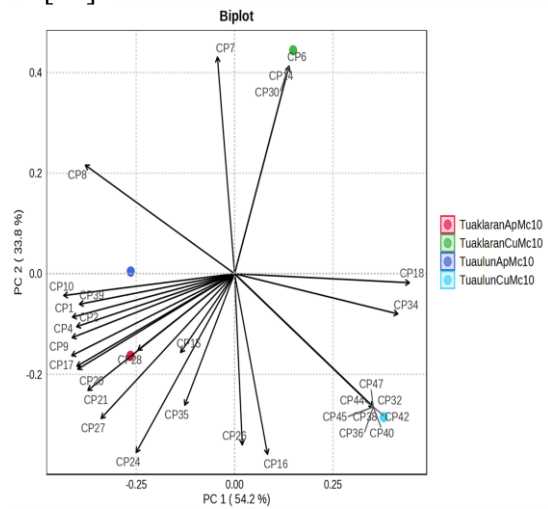
[8]



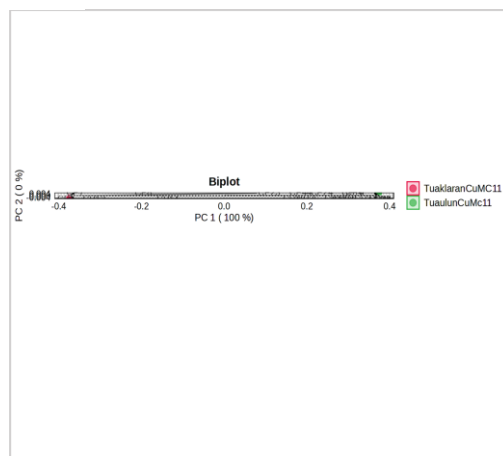
[9]



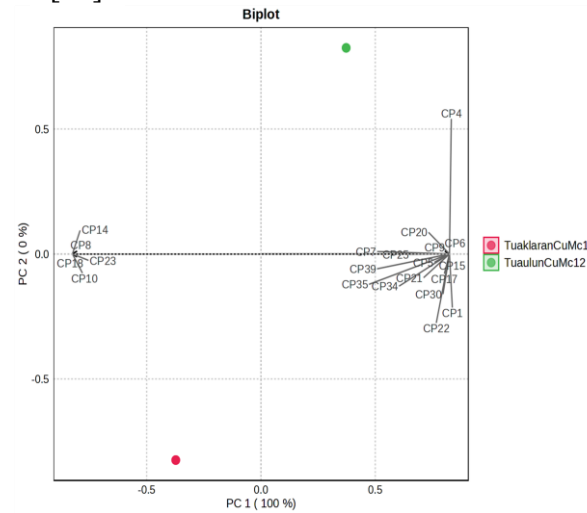
[10]

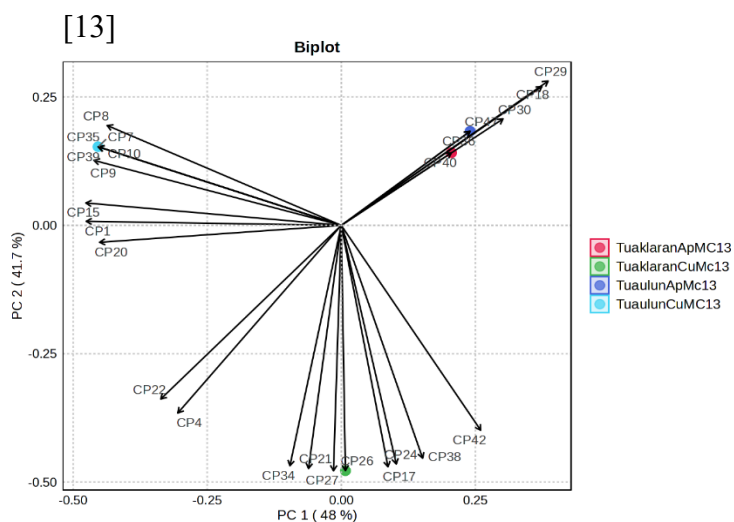


[11]



[12]





The proximity between the points on the graph indicates similar compositions of volatile compounds, suggesting that they share identical aromatic profiles. The closer the points (samples) are, the more similar the composition of the compounds that characterize them.

The length of the vectors or arrows representing the compounds indicates the importance of each compound in differentiating the samples. The longer the vector, the greater the importance and the more characteristic the compound is as a marker for the sample, while shorter vectors represent less influential compounds. Regarding the direction of the vectors, a vector pointing directly towards a group of samples indicates that the compound is more closely associated with that group, while a vector pointing in the direction of a single sample suggests that this compound is contributing to the composition of that sample.

4.1.2.1. Municipal Source Sample

Lautém municipality (Figure 20.1)

The graph shows that PC1 explains 26.4% of the total variation, and PC2 explains 17.8%. Together, they account for a significant portion of the data's variability, 44.2%. The sample groups are mainly differentiated by compounds based on the concentrations of compounds in the right side of PC1, such as 1-Propanol (CP3), 1-Propanol, 2-methyl- (CP4), and Propylene Glycol (CP7), which are abundantly associated with samples such as Tuaulun(ApLp1I and ApLp1T) and Tuaklaran(ApLp1I and ApLp1I) fractions.

The compounds as 1-Propanol, 3-ethoxy- (CP6), Acetic acid (CP18), Propanoic acid, 2-methyl- (CP20), n-Decanoic acid (CP26), Ethyl formate (CP34), Ethyl Acetate

(Ethane, 1,1-diethoxy-) (CP36), Propanoic acid, 2-hydroxy-, ethyl ester (CP38), Acetone (CP40), 2-Propanone, 1-hydroxy- (CP42), Dihydroxyacetone (CP44), and Benzofuran, 2,3-dihydro- (CP47) at left side of PC1 are associated with samples such as Tuaulun(PIIp1) and Tuaklaran(PIIp1).

A fraction from Tuaulun(ApMc1) isolated in the upper left corner of PC2 is associated with Compounds as Catechol (CP17), Butanoic acid (CP21), Octanoic acid (CP24), 5-(Hydroxymethyl) dihydrofural (CP29), and Formic acid, 1-methyl ethyl ester (CP37). However, a group of samples from Tuaklaran(ApMc1) and Tuaikun(ApLp1T) are differentiated by Compounds of 1-Butanol, 2-methyl- (CP8), Butanoic acid, 3-methyl- (CP22), Pentanoic acid (CP23), Lactic acid (CP25), and 5-Hydroxymethylfurfural (CP30) at the negative side of PC2. The clustering of these samples suggests that they have more similar chemical compositions and/or share chemical profiles.

The fractions Tuaikun(ApLp1I, PIIp1, and ApLp1T, in shades of red, blue, and green) are more dispersed, particularly on the left and upper sides of the graph. This indicates that the *tua-ikun* fraction has a distinct chemical composition compared to the other two fractions.

PCA analysis suggests that the main difference in *tua-sabu* composition lies in the fractions (*Tua-ulun*, *Tua-ikun*, and *Tua-klaran*) rather than in the suppliers (Lp, Qq, and MC) or plant species (Pl and Ap). Each fraction has a specific chemical profile defined by a distinct set of compounds.

Baucau municipality (Figure 20.2)

The PCA, explained through PC1 and PC2, accounts for 21.9% and 15.4% of the total variance of the data, respectively; together, they account for 37.3% of the variance.

The visualization of the sample distributions for each fraction shows that they are scattered near the center of the graph, demonstrating that the differentiation is driven by the fractions (*tua-ulun*, *tua-klaran*, and *tua-ikun*), as there does not appear to be a clear separation of the samples based on plant species (Pl, Ap, Cn, and Cu) and their suppliers (Lp, Qq, and Mc).

The main compounds that best characterize the overall variability in the two-dimensional plane (PC1 vs PC2) are compounds such as Methyl Alcohol (CP1), 2-Butanol (CP2), and 1-Propanol, 2-methyl- (CP4) from PC1 and compounds such as Propanoic acid (CP19), Ethyl Acetate (Ethane, 1,1-diethoxy-) (CP36), and 4H-Pyran-4-

one, 2,3-dihydro-3,5-dihydroxy-6- (CP46) from PC2, further indicating that the samples have a more diverse and less homogeneous chemical composition. Little variation can be observed within this separation, mainly based on plant species (Cn/Lp and Cn/Lp). Considering that each fraction of these samples has a specific chemical profile defined by a distinct set of compounds.

One of the fractions, TuaklaranCnMc2B (purple dot), located in isolation in the lower right corner of the graph, presents a more distinct chemical profile than all the other fractions, being mainly influenced by compounds such as 2,3-Butanediol, [S-(R*, R*)]- (CP11), Tetrahydro-4H-pyran-4-ol (CP13), 2-Furanmethanol (CP14), Glycerin (CP16), Lactic acid (CP25), Acetaldehyde, hydroxy- (CP28), 5-Hydroxymethylfurfural (CP30) and Caffeine (CP49).

Viqueque municipality (Figure 20.3)

The distribution of the samples is clearly separated by the clustering of sample groups based on plant species (Ap vs Cu) in both principal component directions, and a clear clustering is also observed according to their suppliers (Lp, Qq, and Mc). This suggests that both plant species and their suppliers are considered important factors influencing the separation of these samples.

Distribution of the sample on the positive side of PC1 as a fraction of Tuaklaran(CuMc3), blue dot, is influenced by the compounds Catechol (CP17), Acetic acid (CP18), Propanoic acid (CP19), Propanoic acid, 2-methyl- (CP20), Butanoic acid, 3-methyl- (CP22), 5-(Hydroxymethyl) dihydrofural (CP29), 2-Hydroxy-gamma-butyrolactone (CP32), Ethyl Acetate (CP35), Formic acid, 1-methyl ethyl ester (CP37), Oxirane, (ethoxymethyl)- (CP39), Butyrolactone (CP43), Dihydroxyacetone (CP44), and Bis(2-ethylhexyl) phthalate (CP48). And the fraction of Tuaulun(CuMc3), yellow dot, is more influenced by Methyl Alcohol (CP1), 1-Butanol (CP5), Catechol (CP17), Acetic acid (CP18), n-Decanoic acid (CP26), and Propanoic acid, 2-hydroxy-, ethyl ester (CP38).

The samples located on the negative side of PC1/PC2 originate from the Ap species and are influenced by compounds such as 2-Butanol (CP2), Propylene Glycol (CP7), 1-Butanol, 2-methyl- (CP8), 1-Butanol, 3-methyl- (CP9), 2,3-Butanediol, [R-(R*, R*)]- (CP10), Phenylethyl Alcohol (CP15), and 5-Hydroxymethylfurfural (CP30).

Additionally, the samples like Tuaklaran and Tuaulun(CuLp3), located in the upper quadrant of PC2/PC1-, are influenced by compounds 1-Propanol (CP3), 1-Propanol, 3-

ethoxy- (CP6), Glycerin (CP16), Butanoic acid (CP21), Pentanoic acid (CP23), Octanoic acid (CP24), Acetaldehyde (CP27), Acetone (CP40), Benzofuran, 2,3-dihydro- (CP47), and. beta. -D-Glucopyranoside, met (CP50). A different group of compositions defines these samples than the others, which makes them chemically distinct.

Manatuto municipality (Figure 20.4)

Principal component analysis (PCA), through components PC1 and PC2, explains 68% and 26.5% of the total variance of the data, respectively, with a total of 94.5% of the variance. The distribution of the samples in the graph shows a significant separation based on the supplier (Lp vs Mc), demonstrating that this was an important factor influencing the separation of the samples.

Through PC1, it is possible to separate the samples based on the different fractions (*tua-ulun* vs *tua-klaran*). Within these groups, on the positive side of PC1, there was still a separation between the *tua-ulun* fractions, favoring the distinction based on their suppliers, where each one presents a distinct chemical profile, mainly influenced by a specific set of compounds.

For example, the *tua-ulun* fraction from supplier Lp (TuaulunApLp4, blue dot) in the lower right corner of the graph is characterized by compounds from the chemical classes alcohol, aldehyde, and esters, consisting of Methyl Alcohol (CP1), 1-Propanol, 2-methyl- (CP4), 1-Butanol, 3-methyl- (CP9), Phenylethyl Alcohol (CP15), 2-Hydroxy-gamma-butyrolactone (CP32), Benzaldehyde, 4-hydroxy- (CP33), and Ethyl Acetate (CP35). And, the TuaulunApMc4 fraction from the Mc supplier, on the other hand, is characterized by compounds including Catechol (CP17), Butanoic acid (CP21), Octanoic acid (CP24), n-Decanoic acid (CP26), Acetaldehyde, hydroxy- (CP28), and Ethyl formate (CP34). Considering that the fractions of *tua-ulun* are chemically distinct is driven by the high concentration of specific compounds of 1-Propanol, 2-methyl- (CP4), and Acetaldehyde, hydroxy- (CP28).

The *tua-klaran* fractions Tuaklaran(ApMc4 and ApLp4, red and pink dots) are clustered on the left side of the graph, associated with groups of compounds from the chemical classes of alcohols, followed by groups of compounds such as esters, acids, aldehydes, ketones, and ethers, consisting of 1-Butanol (CP5), Propylene glycol (CP7), 1-Butanol, 2-methyl- (CP8), 2,3-Butanediol, [R-(R*, R*)]- (CP10), Glycerin (CP16), Ethyl acetate (Ethane, 1,1-diethoxy-) (CP36), Formic acid, 1-methylethyl ester (CP37), Propanoic acid, 2-hydroxy-, ethyl ester (CP38), Acetic acid (CP18), Propanoic acid, 2-

methyl- (CP20), 5-Hydroxymethylfurfural (CP30), Furfural (CP31), Acetone (CP40), 2-Propanone, 1-hydroxy- (CP42) and Oxirane, (ethoxymethyl)- (CP39).

Dili municipality (Figure 20.5)

Sample TuaulunPIMc5 (yellow dots) originates from the PI species and is located in isolation on the positive side of PC1, influenced by a set of compounds that differentiate it from the other samples. These groups of compounds are attributed to the chemical classes of alcohols, aldehydes, ketones, esters, and ethers, consisting of Methyl Alcohol (CP1), 2-Butanol (CP2), Propanoic acid (CP19), Propanoic acid, 2-methyl- (CP20), 2-Hydroxy-gamma-butyrolactone (CP32), Benzaldehyde, 4-hydroxy- (CP33), Oxirane, (ethoxymethyl)- (CP39), and others grouped.

Samples TuaklaranCuQq5 and TuaulunCuQq5 (green and purple dots) originates from the Cu species and Qq supplier, located in the left quadrant of PC1-/PC2+, are characterized by the presence mainly of compounds as 1-Butanol (CP5), Propylene Glycol (CP7), 1-Butanol, 2-methyl- (CP8), 2,3-Butanediol, [R-(R*, R*)]- (CP10), Pentanoic acid (CP23), and n-Decanoic acid (CP26), and others in the upper left quadrant, indicating that these fractions share similar chemical profiles.

On the other hand, the fractions of Tuaklaran(CuMc5 and PIMc5, red and blue dots), in the lower part of the negative PC2/PC1 axis, are correlated with the compounds Propanoic acid, 2-hydroxy-, ethyl ester (CP38), Ethyl Acetate (Ethane, 1,1-diethoxy-) (CP36), Tetrahydro-4H-pyran-4-ol (CP13), Dihydroxyacetone (CP44), and others that are in the quadrant.

The graph showed that the distribution of the samples is based primarily on the plant species and that their suppliers also have little influence on the separation of these samples.

Liquiça municipality (Figure 20.6)

The biplot shows the main variation (30.2% in PC1 and 26.3% in PC2). The graph shows that the samples exhibit a heterogeneous distribution in the two-dimensional plane and do not present clear groupings based on the fraction classifications (*tua-ulun*, *tua-klaran*, and *tua-ikun*). This can be observed in the samples as TuaklaranPIMc6 (cyan dots) and TuaulunPILp6 (purple dots) on the positive side of PC2, in the TuaulunPIQq6 (orange dots) on the negative side of PC1, and in the TuaikunPIQq6 (green dots) on the

PC1+/PC2- side. These distributions demonstrate that each fraction has a distinct chemical composition, characterized by a specific set of compounds, with the fraction serving as the primary differentiating factor among these samples.

For example, TuaulunPIQq6, the orange dots, is grouped in isolation on the left side of PC1, being strongly influenced by the compounds Oxirane, (ethoxymethyl)- (CP39), Glycerin (CP16), and Butanoic acid (CP21), and, to a lesser extent, by Catechol (CP17) and 2-Butanol (CP2). The TuaklaranPIMc6 fraction, the cyan dot, in the upper left part of the graph, is more influenced by compounds such as Acetaldehyde (CP27), 5-(Hydroxymethyl) dihydrofural (CP29), 2-Propanone, 1-hydroxy- (CP42), and Octanoic acid (CP24). Meanwhile, the TuaikunPIQq6 fraction (green dots), isolated on the positive side of PC1, is more associated with compounds 1-Propanol, 3-ethoxy- (CP6), n-Decanoic acid (CP26), 5-Hydroxymethylfurfural (CP30), Ethyl formate (CP34), and the other compounds in that quadrant.

Regarding their suppliers (Lp, Qq, and Mc), there was only one grouping of samples originating directly from the production site (Lp) in relation to the others on the positive side of PC1/PC2, indicating their proximity and similar chemical compositions. This suggests that the collection dots should also be considered as a secondary factor contributing to the variation of these samples.

Bobonaro municipality (Figure 20.7)

PC1 explains 28% of the total variation in the data, and PC2 explains 20.2% of the variation. Both explain about 48.2% of the total variation in the data, which is a reasonable representation. The graph shows that the samples did not exhibit clear groupings based on their suppliers (Lp, Qq, and Mc), and in some cases, there was a cohesive grouping in relation to the plant species, mainly the Cu species on the negative side of PC1. Even so, each of these samples possesses distinct chemical characteristics, defined by different sets of compounds.

Most of the samples are scattered across the graph, with the TuaklaranPIMc7 samples (pink dots) on the PC1+/PC2- side and the TuaulunPILp7 fraction (purple dot) in the upper left corner (PC1-/PC2+), as an isolated sample dot, being the most distinct from each other and from the rest of the group. Each of these fractions has a distinct and specific chemical composition. One of the samples from Qq supplier, such as TuaulunPIQq7, with salmon-colored dots in the upper left corner, is influenced by compounds such as Methyl Alcohol (CP1), 1-Propanol (CP3), 2,3-Butanediol, [R-(R*,

R*)]- (CP10), 2-Furanmethanol (CP14), Ethyl Acetate (CP35), and Oxirane, (ethoxymethyl)- (CP39).

TuaklaranPIQq7 (the yellow dot in the lower right corner of the graph, close to 0.2 in PC1) is mainly influenced by the compounds whose vectors point in its direction, such as Octanoic acid (CP24), Acetaldehyde (CP27), 5-(Hydroxymethyl) dihydrofural (CP29), and Ethyl formate (CP34), including Butanoic acid (CP21) and Catechol (CP17) also contribute significantly in this sense.

In contrast, the TuaklaranCuMc7 (blue dot), TuaklaranPILp7 (light green dot), TuaulunCuLp7 (yellow dot), TuaulunPIMc7 (light green dot), and TuaulunQq7 (light blue dot) samples form a cohesive group around the origin of the graph, in the center-left, suggesting that these samples share similar compound profiles. Furthermore, the TuaikunPILp7 sample (red dots) also stands out as a separate group. Overall, the graph reinforces that the separation of the sample groups is influenced by factors such as the origin of the collection point/suppliers and the plant species.

Oe-cusse municipality (Figure 20.8)

The PCA results indicate that the first two components, PC1 and PC2, explain a total of 87.1% of the data variability (47.1% + 40%). Samples are identified by two different characteristics, such as Plant Species (Cu and Pl) and Supplier (Mc and' Qq). The proximity between the points on the graph indicates similarity in compound composition.

The graph, through PC1, allows us to observe the formation of sample groups based on the palm species. On the positive side of PC1, there is a group of samples from the *akadiru* plant species (Pl), and on the opposite side, a group formed by samples of the *tua-tali* palm species (Cu). The distribution based on their suppliers (Mc and Qq) shows that the samples are slightly more separated from each other. This suggests that the supplier is the main factor influencing the separation of these samples, outweighing the effect of the plant species.

One of the samples from supplier Qq, TuaulunPIQq8 (yellow dots), is isolated and located in the lower quadrant of the graph, being strongly influenced by compounds 2-Butanol (CP2), Catechol (CP17), Formic acid, 1-methyl ethyl ester (CP37), Acetone (CP40), Acetoin (CP41), Dihydroxyacetone (CP44), and .beta.-D-Glucopyranoside, met (CP50), and is considered chemically very different from the other samples.

Covalima municipality (Figure 20.9)

The graph shows that PC1 and PC2 explain, respectively, 46.9% and 18.2% of the total variance in the data. The analysis suggests that the *tua-sabu* fractions (*tua-ulun*, *tua-ikun*, and *tua-klaran*) are the main differentiating factor between the samples, and not the supplier. This can be observed in the fact that most samples from different suppliers clustered quite closely together. And, in fact, in some cases, the position of some fractions was influenced by distinct groups of compounds that characterize them.

One of the fractions of TuaulunCuMc9, represented by purple dots, is plotted separately in the upper left corner of the graph and is further characterized by distinct sets of compounds, such as 2-Butanol (CP2), 1-Propanol (CP3), 1-Propanol, 3-ethoxy- (CP6), Propanoic acid, 2-methyl- (CP20), Butanoic acid, 3-methyl- (CP22), Ethyl formate (CP34), and Formic acid, 1-methyl ethyl ester (CP37).

Therefore, the TuaulunCuLp9 fraction, yellow dot on the right side of the graph, is strongly associated with compounds of Catechol (CP17), Acetic acid (CP18), Pentanoic acid (CP23), Octanoic acid (CP24), 5-(Hydroxymethyl) dihydrofural (CP29), Furfural (CP31), Ethyl Acetate (Ethane, 1,1-diethoxy-) (CP36), Propanoic acid, 2-hydroxy-, ethyl ester (CP38), 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6- (CP46), and 2-Propanone, 1-hydroxy- (CP42), and Benzofuran, 2,3-dihydro- (CP47).

Manufahi municipality (Figure 20.10)

PC1, which explains 54.2% of the data variation, and PC2, which explains 33.8% of the variation. Both explain most of the total variability (88%), which is an excellent value for interpretation.

The distribution of the plant species samples (Ap and Cu) shows cohesive groupings in terms of PC1, with the samples of the Cu palm species allocated on the positive side of PC1 and those of the Ap palm species on the opposite side. Within the group of the two fractions originating from the Cu palm species, as TuaulunCuMc10 and TuaklaranCuMc10 present the greatest difference in their composite composition, confirming that they consequently present distinct chemical profiles.

Where, TuaklaranCuMc10 (green dot), in the upper right corner, is associated with compounds of 1-Propanol, 3-ethoxy- (CP6), Propylene Glycol (CP7), 5-Hydroxymethylfurfural (CP30), and 2-Furanmethanol (CP14).

Whereas the TuaulunCuMc10 (cyan dot) is located in the lower right part of the graph, strongly associated with compounds of Benzofuran, 2,3-dihydro- (CP47), 2-Hydroxy-gamma-butyrolactone (CP32), Dihydroxyacetone (CP44), Propanoic acid, 2-hydroxy-, ethyl ester (CP38), 2-Propanone, 1-hydroxy- (CP42), Ethyl Acetate (Ethane, 1,1-diethoxy-) (CP36), Acetone (CP40), and 2(3H)-Furanone, dihydro-4-hy (CP45) (which are clustered together) and also with compounds of Acetic acid (CP18) and Ethyl formate (CP34).

The sample distribution on PCA suggested that the greatest effect on the differentiation of these samples seems to be more related to the fractions (*tua-ulun* and *tua-klaran*) than to the plant species (Ap and Cu).

Ainaro municipality (Figure 20.11)

In this particular graph, the horizontal axis (PC1) explains 100% of the variance, which is an unusual and extreme result. This means that all the difference in your data is concentrated on a single dimension, so PC2 explains no variance (0%).

The data are represented by two groups of samples, TuaklaranCuMc11 (red dot) and TuaulunCuMc11 (green dot). Both groups of samples appear at approximately the same point on the graph, around -0.4 on the PC1 axis. This indicates that the *tua-klaran* and *tua-ulun* samples, derived from the Cu palm species collected from the market (Mc), are extremely similar in terms of the measured compounds. The complete overlap suggests that there are no significant differences between the *tua-ulun* and *tua-klaran* fractions, which the principal component analysis (PCA) reveals as a high similarity between the two groups.

Aileu municipality (Figure 20.12)

The primary variation in the data is along the horizontal axis (PC1), which accounts for 100% of the variability. This means that the differences between the *tua-ulun* and *tua-klaran* fractions are almost entirely explained by their distinct chemical compositions.

The graph shows that the *tua-ulun* and *tua-klaran* fractions, although originating from the same plant species (Cu) and the same supplier (Mc), present completely different compound profiles.

The TuaulunCuMc12 fraction is characterized by several compounds, mainly 1-Propanol, 2-methyl- (CP4), which is positioned in the same direction, including a positive

association with other compounds located in the same quadrant. While the TuaklaranCuMc12 fraction is defined by a group of compounds that includes 2-Furanmethanol (CP14), Acetic acid (CP18), and is associated with other compounds in the same quadrant.

Ermera municipality (Figure 20.13)

The horizontal axis (PC1) explains 48% of the total variability of the data, and the vertical axis (PC2) explains 41.7% of the variability. The graph shows that samples TuaulunApMc13 (blue dot) and TuaklaranApMc13 (red dot), originating from the same plant species, are clustered in the upper corner of the graph, on the positive side of PC1/PC2, indicating that they share similar chemical profiles.

The TuaulunApMc13 fraction is further characterized by Acetic acid (CP18), 5-(Hydroxymethyl) dihydrofural (CP29), 5-Hydroxymethylfurfural (CP30), Ethyl Acetate (Ethane, 1,1-diethoxy-) (CP36), Acetone (CP40), and Benzofuran, 2,3-dihydro- (CP47). The TuaklaranApMc13 sample, on the other hand, is more characterized by compound Acetone (CP40) and shows positive influences with the other compounds in its direction.

In contrast, the samples based on the Cu plant species present completely different fractions, defined by a distinct set of compounds. The TuaulunCuMc13 fraction is characterized by main compounds such as Propylene Glycol (CP7), 1-Butanol, 2-methyl- (CP8), 1-Butanol, 3-methyl- (CP9), 2,3-Butanediol, [R-(R*, R*)]- (CP10), Ethyl Acetate (CP35), and Oxirane, (ethoxymethyl)- (CP39), including contributions from other compounds in the same quadrant.

The distribution of these samples is primarily attributed to the fractions that exhibit different chemical compositions that characterize them, with the plant species being a secondary factor in the distribution of these samples.

4.1.2.2. Plant Species Source

Akadiru palm (Tua-ulun; Figure 21a)

The graph shows that this sample stands out from the others, suggesting a very distinct compound profile compared to all the others. One of the samples of Lp supplier from the municipality of Baucau TuaulunPILp2, the green dot, is isolated at the extreme of PC1+/PC2-, and is strongly associated with Ethyl acetate (ethane, 1,1-diethoxy-) (CP36) and ethyl 2-hydroxypropanoate (CP38), which point almost directly towards it,

and also to other compounds such as 2(3H)-furanone, dihydro-4-hydroxy (CP45), lactic acid (CP25) and Furfural (CP31), which are located in the same quadrant at small angles relative to the point, indicating a strong positive correlation with this sample.

TuaulunPIMc5 (light pink dot) and TuaulunPIQq6 (light green dot) are most influenced by Butanoic acid (CP21), followed by Glycerin (CP16) and Catechol (CP17), which point in a similar direction, indicating a strong positive correlation. Additionally, they are also significantly influenced by Propanoic acid (CP19), 2-Propanone, 1-hydroxy- (CP42), and 1-Butanol, 2-methyl- (CP8), which are aligned in the same general direction as these samples.

TuaulunPILp6 (blue dot) and TuaulunPIMc7 (purple dot), and most of the samples located at the top of the graph, are most influenced by the CPs that have the largest positive projections on the PC2 axis, such as 5-(Hydroxymethyl) dihydrofural (CP29), Formic acid, 1-methyl ethyl ester (CP37), 1-Propanol (CP3), Butanoic acid, 3-methyl- (CP22), 2-Furanmethanol (CP14), and Bis(2-ethylhexyl) phthalate (CP48). However, TuaulunPILp1 (red dot) and TuaulunPIQq8 (dark blue dot) are clustered in the upper right part of the graph, near the center, suggesting that they are chemically similar, despite coming from different municipalities.

The samples that cluster at the top of the graph originate from different locations and suppliers, show a strong correlation with PC2, and are associated with 5-(Hydroxymethyl) dihydrofural (CP29), Formic acid, 1-methyl ethyl ester (CP37), 1-Propanol (CP3), 2-Furanmethanol (CP14), Dihydroxyacetone (CP44), and Butanoic acid, 3-methyl- (CP22), presenting similar characteristics.

In summary, the samples form distinct clusters in the center and upper part of the graph. The samples from the Lp supplier (Lp1, Lp2, and Lp6) are distributed between the upper and lower right quadrants, where the samples from the Mc suppliers, as Mc6, Mc7, and Mc8, are concentrated in the upper part of the graph, and Mc5 is located in the lower left quadrant. Additionally, the samples from the Qq supplier, as well as Qq6, Qq7, and Qq8, do not display a cohesive cluster, indicating that the samples have quite varied compound profiles.

The proximity between points on the graph indicates similarity. For example, samples from Mc supplier are closer to each other, suggesting that, despite the differences, they have closer chemical profiles than samples from supplier. The graph suggests that both the geographical origin (municipality) and its suppliers (Lp, Qq, and Mc) are the main factors influencing the variability of these samples.

Akadiru palm (Tua-klaran; Figure 21b)

Through the graphic it is possible to observe the formation of some groups, such as the set of dots in purple, light blue and red (TuaklaranMc7, TuaklaranPILP7, and TuaklaranPILp1), upper left of the graph near the center, indicating that these fractions are chemically similar to each other, and are strongly associated with the Benzofuran, 2,3-dihydro- (CP47), n-Decanoic acid (CP26), Ethyl Acetate (Ethane, 1,1-diethoxy-) (CP36), Formic acid, 1-methyl ethyl ester (CP37), 3,3-Diethoxy-1-propanol (CP12), and Glycerin (CP16).

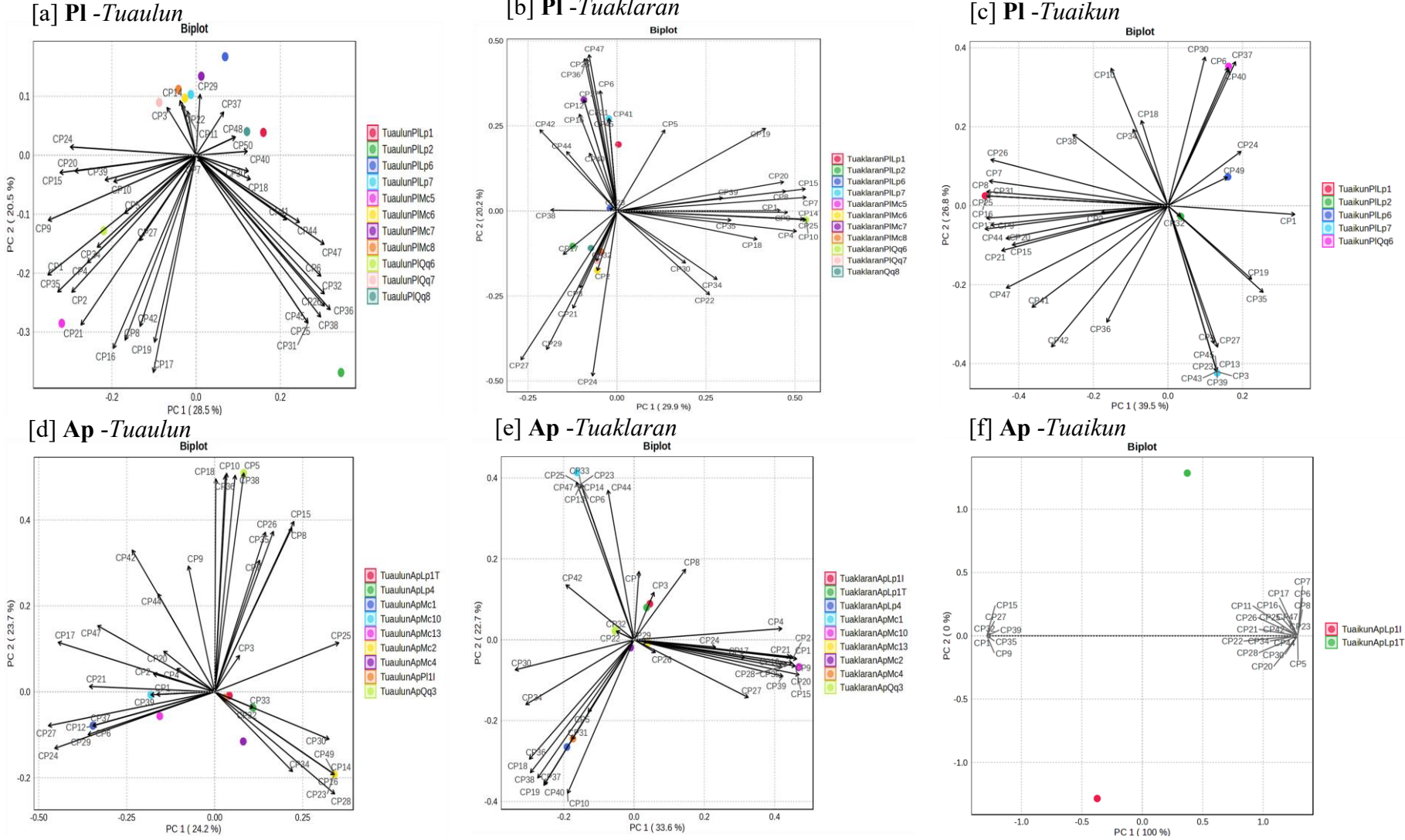
On the opposite side, samples such as Tuaklaran(PILp2, green dot; PIQq8, dark blue dot; PIMc8, orange dot; PIMc5, pink dot; PIQq7, light pink dot, and PIMc6, yellow dot) are very close, indicating that, despite coming from different municipalities and collection points or supplier, their compound profiles are quite similar. These samples are strongly correlated with the 2-Butanol (CP2), 1-Propanol (CP3), Butanoic acid (CP21), Octanoic acid (CP24), Acetaldehyde (CP27), and 5-(Hydroxymethyl) dihydrofural (CP29).

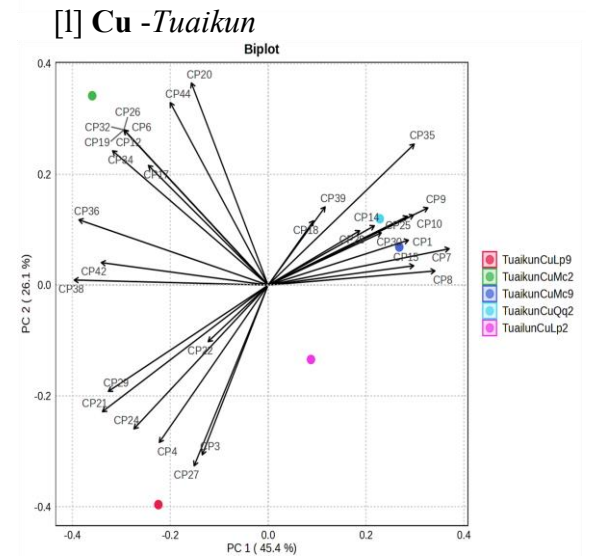
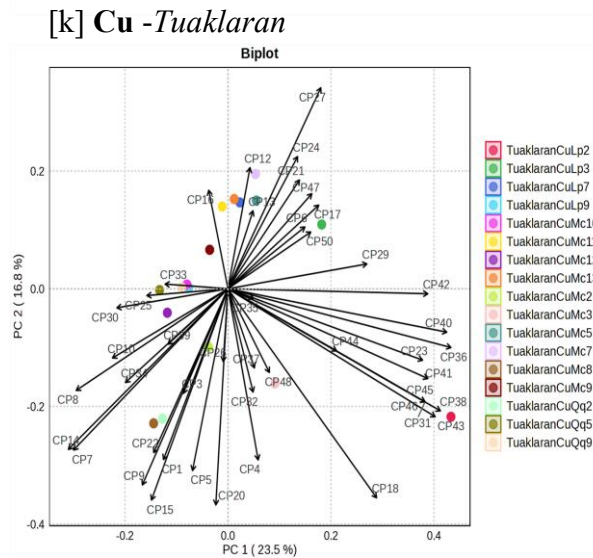
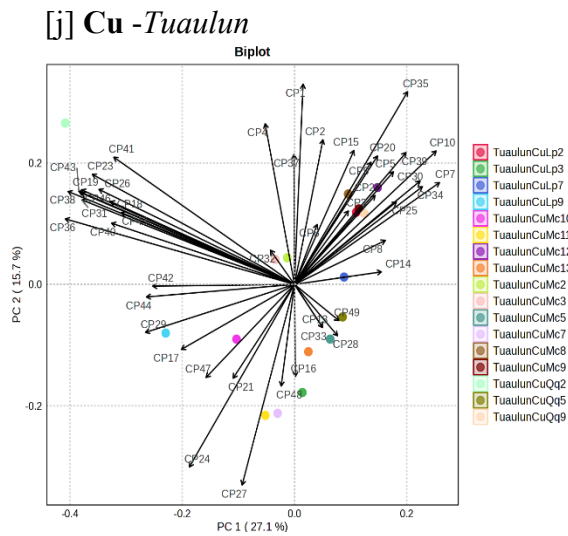
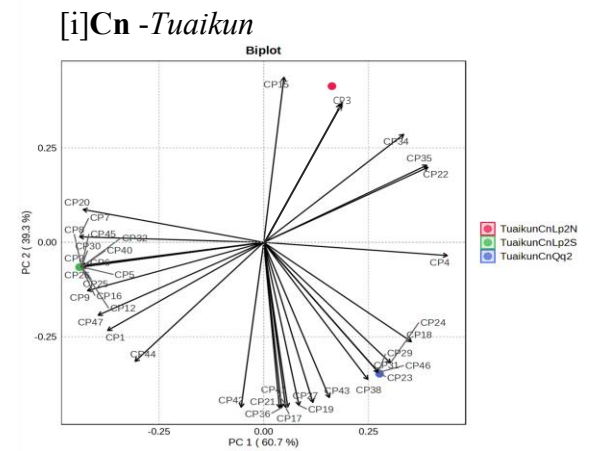
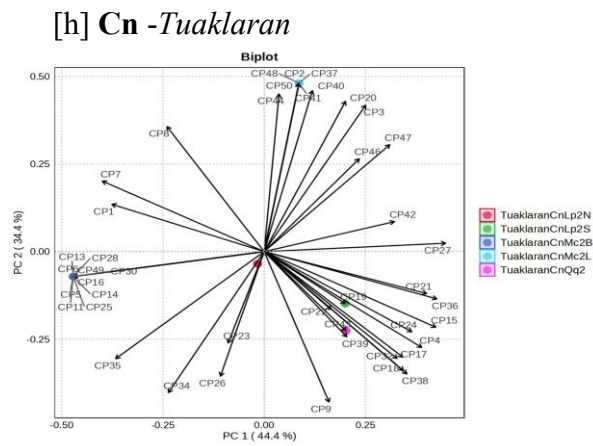
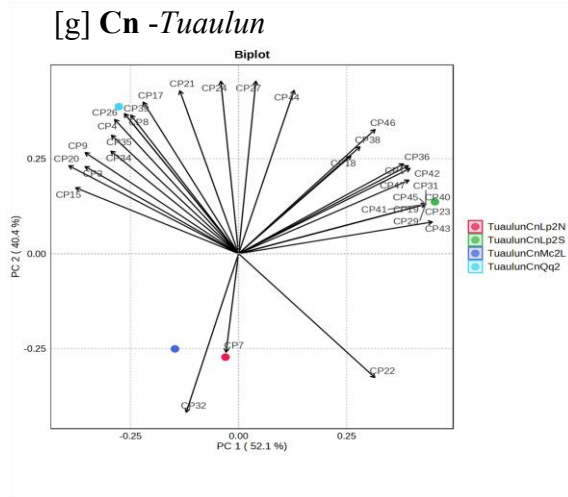
Additionally, the fraction of TuaklaranPIQq6 (lime green dot) is isolated is strongly associated with compounds, as Phenylethyl Alcohol (CP15), 1-Butanol, 3-methyl- (CP9), Propylene Glycol (CP7), Lactic acid (CP25), and 2-Furanmethanol (CP14), including other CPs, in the direction and proximity of the point, also exerting significant influences that contribute to its chemical profile.

Regarding the geographical origin of the samples, in the cluster of samples in the upper left corner of the graph, a distinct subgroup composed of samples from the municipality of Bobonaro (Lp7 and Mc7) was observed, and similarly, this can be observed in the group of samples at the bottom of the graph for the samples from the municipality of Oe-cusse (Qq8 and Mc8).

Furthermore, the samples from the municipality of Liquiça (Lp6 and Qq6) are also clustered near the central line of the graph, sharing similar chemical profiles in some specific compounds. However, in relation to their suppliers (Lp, Qq, and Mc), they do not show clear groupings like those based on municipal origin. In summary, the supplier is the main factor in the separation of these samples, while the groupings based on geographical origin (municipality) contribute little to the variability of these samples, being considered a secondary factor.

Figure 21. PCA of all fractions of different palm species





Akadiru palm (Tua-ikun; Figure 21c)

The graph showed that the samples exhibited greater dispersion, with each sample being influenced by a distinct set of compounds. In some cases, certain samples, such as TuaikunPILp2 (green dots) and TuaikunPILp6 (blue dots) on the right side of PC1, near the center of the graph, exhibit a similar compound profile in specific compounds.

However, samples such as TuaikunPIQq6 (pink dots) isolated in the upper right corner, TuaikunPILp7 (light blue dots) isolated in the lower right quadrant, and TuaikunPILp1 (red dots) isolated on the left side of PC1, each present a distinct chemical profile, defined by a different set of compounds.

The biplot analysis suggests that both the supplier (Lp vs. Qq) and the geographical origin (municipality) directly impact the composition of the compounds, contributing to the variability of these samples, since the samples do not show a clear grouping.

Tua-metan palm (Tua-ulun; Figure 21d)

PC1 accounts for 24.2% of the total variation in the data, and PC2, 23.7%. Together, they explain about 47.9% of the variability. The graph shows that, despite the samples being from the same fraction, they exhibit some distinct groupings, mainly based on their suppliers (Lp, Qq, and Mc).

For example, in the upper right corner, there is an isolated sample from the supplier (Qq) from the municipality of Viqueque, while on the positive side of PC1, there is a clear grouping of samples from the Lp supplier from the municipalities of Lautém (subregion I & T), and Manatuto; however, on the negative side of PC1, there is an evident group of most samples from the Mc supplier of Lautém, Manufahi, and Ermera municipalities.

Furthermore, on the negative side of PC2, especially for the TuaulunApMc2 sample (yellow dot) from the municipality of Baucau and the TuaulunApQq3 sample (light green dot) from the municipality of Viqueque, both from the Mc supplier, they also form a clear grouping, exhibiting similar chemical compositions.

Regarding the geographical origin of the samples, the distribution of the samples did not show clear groupings, as observed in relation to the suppliers, suggesting that geographical location plays a significant role in the variability of these samples, with suppliers acting as a secondary factor.

Tua-metan palm (Tua-klaran; Figure 21e)

PC1 explains 33.6% of the data variability, and PC2 explains 22.7%. Together, they account for 56.3% of the total variability.

A sample from the Mc supplier from the municipality of Lautém (TuaklaranApMc1, cyan dots) is located in isolation in the upper left corner of the graph, strongly associated with Tetrahydro-4H-pyran-4-ol (CP13), 2-Furanmethanol (CP14), Pentanoic acid (CP23), Lactic acid (CP25), Benzaldehyde, 4-hydroxy- (CP33), Benzofuran, 2,3-dihydro- (CP47), 1-Propanol, 3-ethoxy- (CP6), and Dihydroxyacetone (CP44). This composition of compounds makes it unique, with different chemical characteristics from others.

Furthermore, the TuaklaranApMc10 sample (pink dots) from the municipality of Manufahi, located in the lower right corner of the graph, is strongly associated with Methyl Alcohol (CP1), 2-Butanol (CP2), Phenylethyl Alcohol (CP15), Propanoic acid, 2-methyl- (CP20), Oxirane, (ethoxymethyl)- (CP39), Propanoic acid, 2-methyl- (CP20), Butanoic acid (CP21), and 1-Propanol, 2-methyl- (CP4), including other compounds in this quadrant that contribute positively.

Meanwhile, both samples Tuaklaran(ApLp4, blue dot, and ApMc4, orange dot) from Lp and Mc suppliers from Manatuto municipality, are very close to each other at the bottom of the graphs, exhibiting similar chemical profiles and being specific in Acetic acid (CP18), Propanoic acid (CP19), Propanoic acid, 2-hydroxy-, ethyl ester (CP38), Acetone (CP40), Formic acid, 1-methyl ethyl ester (CP37), and in the other quadrants. This suggests that there was no chemical alteration during their distribution to the market.

The graph shows that the samples were grouped both by their geographical origin, TuaklaranAp (Lp4 and Mc4) from the municipality of Manatuto, and by their supplier, TuaklaranAp (Lp2I and Lp2T; Mc12 and Mc13), suggesting that both origins contributed to the variability of these samples, defined by a set of distinct compounds that they share or that define a sample as having a chemically unique profile.

Tua-metan palm (Tua-ikun; Figure 21f)

The graph shows that it is possible to differentiate the TuaikunApLp1I, red dot, and TuaikunApLp1T, green dot, where (1I and 1T represent different geographical origins, sub-regions within the municipality of Latém), which are separated by PC1. This suggests

that they are distinctly different in terms of their chemical composition. Considering this, geographical origin is a determining factor that influences the separation of these samples.

TuaikunApLp1I is strongly associated with compounds of Methyl Alcohol (CP1), 1-Butanol, 3-methyl- (CP9), 3,3-Diethoxy-1-propanol (CP12), Phenylethyl Alcohol (CP15), Acetaldehyde (CP27), 2-Hydroxy-gamma-butyrolactone (CP32), Ethyl Acetate (CP35), and Oxirane, (ethoxymethyl)- (CP39). TuaikunApLp1T, on the other hand, is strongly associated with compounds of 1-Butanol (CP5), 1-Propanol, 3-ethoxy- (CP6), Propylene Glycol (CP7), 1-Butanol, 2-methyl- (CP8), Catechol (CP17), Propanoic acid, 2-methyl- (CP20), Butanoic acid (CP21), Butanoic acid, 3-methyl- (CP22), Pentanoic acid (CP23), Lactic acid (CP25), n-Decanoic acid (CP26), 1-Butanol, 2-methyl- (CP28), 5-Hydroxymethylfurfural (CP30), Ethyl formate (CP34), 2-Propanone, 1-hydroxy- (CP42), and Benzofuran, 2,3-dihydro- (CP47).

Nuu species (Tua-ulun; Figure 21g)

PC1 explains 52.1% of the total variance, and PC2 explains 40.4% of the total variance. Explain 92.5% of the total variance, meaning the graph is a highly accurate representation of the original data.

The distribution of the samples in the graph did not show a clear grouping based on their geographical origins (L, N, and S, representing sub-regions within the municipality of Baucau) and their suppliers (LP, Qq, and Mc). Biplot analysis shows that both are crucial factors determining the chemical profiles that differentiate these samples.

It is observed that the samples TuaulunCnLp2N (purple dots) and TuaulunCnMc2L (red dots), located in the lower quadrant of the graph, although originating from different suppliers (Lp and Mc) and distinct geographical origins (L and N), show a positive correlation with compounds such as Propylene Glycol (CP7), Butanoic acid, 3-methyl- (CP22), and Pentanoic acid (CP32).

The other two samples, such as the one from supplier Qq, located in the upper left corner of the graph, and the TuaulunCnLp2S fraction, originating directly from the production site, located on the right side of the graph, showed distinct chemical characteristics, defined by a different set of compounds.

The differentiation of these samples is driven by compounds that show the longest arrows on the PC1 axis in the upper right quadrant, in the positive direction, such as 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6- (CP46), Propanoic acid, 2-hydroxy-, ethyl

ester (CP38), and compound Butanoic acid, 3-methyl- (CP22) in the lower quadrant, in the negative direction of PC2.

Sample TuaulunCnLp2S is strongly associated with Pentanoic acid (CP23), 5-(Hydroxymethyl) dihydrofural (CP29), Furfural (CP31), Ethyl Acetate (Ethane, 1,1-diethoxy-) (CP36), Acetone (CP40), Butyrolactone (CP43), 2(3H)-Furanone, dihydro-4-hy (CP45), and shows a positive correlation with the other samples in the same quadrant. However, TuaulunCnQq2 is strongly associated with compounds such as 1-Butanol, 2-methyl- (CP8), Catechol (CP17), Butanoic acid (CP21), n-Decanoic acid (CP26), 1-Propanol, 2-methyl- (CP4), Oxirane, (ethoxymethyl)- (CP39), and also shows a positive correlation with the other compounds in the same quadrant or in nearby quadrants that are in its direction.

Nuu species (Tua-klaran; Figure 21h)

PC1 explains 44.4% and PC2 explains 34.4%, totaling 78.8% of the total variation. The samples are dispersed, and there was no clear clustering based on geographical origin (L, N, S, and B) or their suppliers (Lp, Qq, and Mc). Each sample presents a different composition of compounds (CPs), which may indicate that both geographical origin and supplier are the main factors distinguishing the samples, defined by a distinct set of compounds.

Among these samples, a cohesive cluster was observed in the lower right corner, comprising the supplier's samples (Qq, pink dot, and Lp, green dot). This suggests that the two share similar chemical profiles in some specific compounds.

A fraction of TuaklaranCnMc2L (light blue dot), isolated in the upper right corner of the graph, is strongly associated with 2-Butanol (CP2), Formic acid, 1-methyl ethyl ester (CP37), Bis(2-ethylhexyl) phthalate (CP48), and .beta.-D-Glucopyranoside, met (CP50), including positive contributions from 1-Propanol (CP3), Propanoic acid, 2-methyl- (CP20), Acetone (CP40), Acetoin (CP41), 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6- (CP46), and Benzofuran, 2,3-dihydro- (CP47).

On the other hand, a sample of TuaklaranCnMc2B (blue dot), to the left of PC1 or PC2-, at the bottom of the graph, being isolated, presents different chemical characteristics from the others, being more associated with 1-Butanol (CP5), 2,3-Butanediol, [S-(R*, R*)]- (CP11), Tetrahydro-4H-pyran-4-ol (CP13), 2-Furanmethanol (CP14), Glycerin (CP16), Lactic acid (CP25), Acetaldehyde, hydroxy- (CP28), 5-

Hydroxymethylfurfural (CP30), Caffeine (CP49) and positively with other CPs in the same quadrant.

Nuu species (Tua-ikun; Figure 21i)

PC1 explains 60.7% of the data variation, and PC2 explains 39.3% of the data variation. Together, these two components explain 100% of the variation, which is an excellent result for data visualization.

The graph illustrates a high dispersion of the samples, each possessing a unique chemical characteristic defined by a distinct set of compounds. This suggests that the geographical origin (N and S) of the samples and their suppliers (Lp and Qq) are important factors contributing to the separation or variability of these samples.

The graph clearly shows that the samples are separated into three distinct groups, each with its own unique composition defined by a distinct set of compounds. This demonstrates that geographical origin is the main factor of variation within the Lp supplier, resulting in these separations variability of these samples. The Qq supplier results in a profile (TuaikunCnQq2) that is negatively correlated with most of the characteristics of TuaikunCnLp2S and distinct from TuaikunCnLp2N, showing that product distribution is crucial in this case.

Where the TuaikunCnLp2N fraction (red dot), located in the upper right quadrant, is the most influenced by compounds such as 1-Propanol (CP3), Phenylethyl Alcohol (CP15), Ethyl formate (CP34), Ethyl Acetate (CP35), and Butanoic acid, 3-methyl- (CP22). Each of the TuaikunCnLp2S (green dot) and TuaikunCnQq2 (blue dot) fractions is influenced by the main compounds that point directly to them and also shows a positive correlation with the other compounds that point to them or that are in the same quadrant or nearby.

Tua-tali palm (Tua-ulun; Figure 21j)

The graph through PC1 explains 27.1% of the variance, while PC2 explains 15.7%. The samples are broadly and heterogeneously distributed in relation to their geographical origin (municipality represented by sequential numbers 1, 2, 3, ...), suggesting that the geographical origin factor plays a crucial role in the variability of these samples.

On the other hand, within each supplier, a cohesive grouping was observed in Mc supplier, as can be seen in the group of samples presented at the bottom of the graph,

which shows the origin of the municipalities in subgroups e.g., Ainaro (yellow dot), Bobonaro (silver dot) plotted to the left of PC1; Dili (orange dot) and Ermera (light blue dot) plotted on the positive side of PC1, while the samples from the municipalities of Baucau (lime green dot) and Viqueque (salmon dot) are on the PC1-/PC2+ side near the origin of the graph, the samples from the municipalities of Oe-cusse, Covalima, and Aileu (gray, red, and purple dots) are clustered in the right corner of PC1 or to the left of PC2.

The distribution of the samples within their respective suppliers, forming cohesive groups, suggests that these suppliers act as a secondary factor contributing to the variability of these samples. A sample from supplier Qq in the municipality of Baucau (TuaulunCuQq2) is isolated in the upper left corner of the graph, strongly associated with compounds Acetoin (CP41), Butyrolactone (CP43), Pentanoic acid (CP23), and Ethyl Acetate (Ethane, 1,1-diethoxy-) (CP36), which point in the same direction, being the main compounds responsible for distinguishing this group. The close clustering of the samples suggests that they share similar chemical compositions.

A sample from the municipality of Covalima (TuaulunCuLp9, cyan dot) also stands out from the others, especially from the samples of the same supplier (Lp), being strongly influenced by compounds Catechol (CP17), 5-(Hydroxymethyl) dihydrofural (CP29), 2-Propanone, 1-hydroxy- (CP42), and Dihydroxyacetone (CP44), Octanoic acid (CP24), and also by the other compounds that are in its direction.

Tua-tali palm (Tua-klaran; Figure 21k)

The PCA explains a total variability of 40.3% of the data, with PC1 explaining 23.5% and PC2 explaining 16.8% of the data variability.

The graph shows a heterogeneous distribution of the samples, mainly in relation to their geographical origins (municipalities). On the other hand, most of the samples concentrated near the center of the graph showed some cohesive groupings of samples from the Mc supplier, which can be observed in the samples clustered in the upper left quadrant of the graph in relation to PC2 or directly on the PC1 line. These samples originate from various municipalities, such as Dili, Bobonaro, Ainaro, and Ermera.

Conversely, in the lower quadrant of the graph, towards the negative side of PC2, are the samples from the municipalities of Baucau, Viqueque, Oe-cusse, and Aileu. The proximity of these samples is mainly influenced by a specific set of compounds that they share.

The samples cluster quite distinctly, suggesting significant differences in their chemical composition, as is the case with one of the samples from Lp supplier in the municipality of Baucau, sample TuaklaranCuLp2, represented by the pink dot, located isolated in the lower corner of the graph (PC1+/PC2-), highly associated with the main compounds such as Furfural (CP31), Propanoic acid, 2-hydroxy-, ethyl ester (CP38), Acetoin (CP41), Butyrolactone (CP43), 2(3H)-Furanone, dihydro-4-hy (CP45) and 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6- (CP46), including those located in the same quadrant or nearby. This sample presents a distinct chemical profile that makes it unique and different from the other samples.

Furthermore, a group of samples, composed of samples from the municipalities of Baucau from Qq supplier (TuaklaranCuQq2, cyan dots) and from Oe-cusse from supplier Mc (TuaklaranCuMc8, brown dots), forms a cluster influenced by the main compounds Methyl Alcohol (CP1), 1-Butanol (CP5), Propylene Glycol (CP7), 1-Butanol, 3-methyl- (CP9), 2-Furanmethanol (CP14), Phenylethyl Alcohol (CP15) and Butanoic acid, 3-methyl- (CP22), including the other compounds located in the same quadrant or nearby, which are responsible for conferring the chemical profile.

In summary, significant variability is observed in the samples, mainly originating from different municipal sources, including their suppliers (Lp, Qq, and Mc), which are considered a secondary factor contributing to this variability.

Tua-tali palm (Tua-ikun; Figure 211)

The PCA axes (PC1 and PC2) explain 45.4% and 26.1% of the total data variation, respectively. The graph shows a clear clustering of samples based on source (supplier). Samples TuaikunCuMc2 (green dot) and TuaikunCuLp9 (red dot) are well separated from the others.

The green dot (TuaikunCuMc2), located in the upper left corner of the graph, is clearly distinct from the other groups and presents a high concentration of compounds strongly associated with compounds of 2-Hydroxy-gamma-butyrolactone (CP32), n-Decanoic acid (CP26), 1-Propanol, 3-ethoxy- (CP6), Propanoic acid (CP19), Dihydroxyacetone (CP44), Propanoic acid, 2-methyl- (CP20), and Ethyl formate (CP34), which point in its direction. This suggests that these compounds are the main contributors to the variation in this sample. However, the red dot (TuaikunCuLp9), located at the bottom of the graph, presents a high concentration of 1-Propanol, 2-methyl- (CP4),

Acetaldehyde (CP27), and 5-(Hydroxymethyl) dihydrofural (CP29), pointing toward this group, indicating a higher concentration of these compounds in this sample.

Samples TuaikunCuQq2 (light blue dot) and TuaikunCuMc9 (dark blue dot) are close to each other in the upper right corner of the graph. This may indicate a greater similarity in chemical composition between them, despite coming from different suppliers. Both fractions are strongly associated with compounds of Ethyl Acetate (CP35), 1-Butanol, 3-methyl- (CP9), 2,3-Butanediol, [R-(R*, R*)]- (CP10), Propylene Glycol (CP7), 1-Butanol, 2-methyl- (CP8), and Methyl Alcohol (CP1), and are positively associated with other compounds in the quadrant.

The isolated pink dot (TuaikunCuLp2) in the middle of the graph does not appear to cluster with any of the other samples. The absence of compound arrows pointing directly to it suggests that its composition is not dominated by any of the CPs shown in the graph, or that it is an outlier. In summary, the PCA analysis suggests that the main reason why the samples separate differently in their chemical profiles may be related to their local origin and suppliers.

4.1.2.3. Association between samples (fractions) and compounds (CPs)

A large number of samples were analyzed, where the fractions showed similar or different chemical profiles, defined by sets of distinct compounds.

Table 13. Association between samples (fractions) and main compounds (CPs)

Code	Compounds	Samples
CP1	Methyl alcohol	Tuaulun (ApLp4, PIMc5, PIQq7, CuMc8 & CuMc3), Tuaklaran (CuQq2, PIMc8 & ApMc10) and Tuaikun (ApLp1I, CuQq2 & CuMc9)
CP14	2-Furanmethanol	Tuaulun (PILp6, CuMc6, PIQq7, PIMc7, CuQq9 & CuMc8) Tuaklaran (ApMc1, CuQq2, CnMc2B, PIQq6, CuMc8, CuMc10 & CuMc12).
CP18	Acetic acid	Tuaulun (CuMc3, CuLp9, CuMc10, CuMc12 & ApMc13), Tuaklaran (CuMc3, ApMc4 & ApLp4) Tuaikun (CnQq2, CuQq2 & CuLp9).
CP27	Acetaldehyde	Tuaulun (CuLp3 & ApLp4) Tuaklaran (CnMc2B, PILp2, CuLp3, PIMc5, PIMc6, PIQq7, PIQq8 & PIMc8) Tuaikun (ApLp1I & CuMc9).
CP29	5-(Hydroxymethyl) dihydrofural	Tuaulun (CnLp2S, PILp6, CuLp9 & ApMc13) Tuaklaran (PILp2, CuMc3, PIMc5, PIMc6, PIQq7, PIQq8 & PIMc8) Tuaikun (CuLp9).
CP30	5-Hydroxymethylfurfural	Tuaulun (ApMc2, ApQq3, CuQq5, PILp7 & ApMc13) Tuaklaran (CnMc2B, ApQq3, ApMc4, ApLp4, CuQq5, CuMc10 & ApMc13)

CP31	Furfural	Tuaikun (ApLp1T & PIQq6) Tuaulun (PILp2, CnLp2S & CuLp9) Tuaklaran (CuLp2, ApMc4, ApLp4 & PILp7).
CP34	Ethyl formate	Tuaulun (ApLp4, CuMc9 & CuMc10) Tuaklaran (PIQq7) Tuaikun (ApLp1T, CnLp2N, CuMc2 & PIQq6).
CP35	Ethyl Acetate	Tuaulun (CnQq2, CuLp2, ApLp4, PIMc5, P11Lp7, CuMc8, CuQq9, CuMc9 & CuMc13) Tuaklaran (CuMc3) Tuaikun (ApLp1I, CuQq2, CnLp2N & CuMc9).
CP40	Acetone	Tuaulun (PILp1, PIMc7, CuMc7, PIQq8, CuMc10 & ApMc13) Tuaklaran (PILp1, ApLp4, ApMc4, PIMc5, CuMc5 & ApMc13)
CP41	Acetoin	Tuaulun (CnLp2S & PIQq8) Tuaklaran (CuLp2, PIMc5 & CuMc5).
CP43	Butyrolacton	Tuaulun (CnLp2S, CuQq2 & CuLp9) Tuaklaran (CuLp2, CuMc3 & PILp7) Tuaikun (CnQq2 & PILp7).
CP48	Bis(2-ethylhexyl) phthalate	Tuaulun (PILp1, PILp2, CuLp3, PIMc7, CuMc7, PIQq8, CuMc11 & CuMc13) Tuaklaran (CnMc2L & CuMc3).

4.2. DISCUSSION

The production of alcoholic beverages, particularly ethyl alcohol, involves a complex and multi-step process. Numerous factors are crucial in the production process, including the selection of raw materials, the various steps involved, and the operating systems, which must adhere to good practice requirements (BLACK; WALKER, 2023).

These encompass control actions throughout the production chain, such as sap preparation, fermentation conditions, distillation techniques, fraction separation, the bottling process, and supply chain management up to the point of sale. In addition, geographic factors and technical variations involved in each producer's production are also considered (SARMA *et al.*, 2022).

4.2.1. PART I. Physicochemical characterization of *tua-sabu*

The results showed significant variations in the alcohol content of the sample fractions, whether they were from the same or different collection regions. Thus, the factors that may influence the variation in product quality and the change in concentrations among the fractions of *tua-sabu* are the following:

4.2.1.1. Influence of Raw Materials

The raw material is one of the main components that determines the quality of the product. Therefore, it requires attention to specific aspects regarding the state, quantity,

and quality, including its preparation mechanisms. Furthermore, the quality and quantity of the sap also vary depending on the type of palm tree, the experience of each producer, and the geographical location where it is produced (NAKNEAN *et al.*, 2010; SUDHA *et al.*, 2019; BELO *et al.*, 2023).

The way Timorese producers prepare and collect sap varies greatly from one producer or region to another in terms of extraction processing techniques and collector materials, among others (see BELO; BRICENO; PEREIRA, in press). This variation in methods and materials consequently affects the quantity and quality of sap produced.

The sap extracted from *akadiru*, *tua-metan*, and *nuu* palms is extracted through the inflorescences using a non-destructive method. The extraction process and the amount of sap produced show similarities between *akadiru* and *nuu* palm, with each flower producing between 3 and 5 liters of sap per day. In contrast, *tua-metan* palms can produce an average of 8 to 10 liters daily (BELO *et al.*, 2023).

According to MC WILLIAM (2022), independently, a well-managed *akadiru* palm tree can produce an amount of sap of up to 10 liters per day, while a *tua-metan* palm tree can produce 20 to 24 liters per tree per day. Still, the amount and flow of sap produced in *akadiru* and *tua-metan* palms vary greatly depending on factors such as plant physiology, soil fertility, seasonal factors, climatic conditions, and site location, among others. Furthermore, it can also be considered that the experience and technique of sap extraction are important at this stage.

Due to variations in sap amounts, the collection times for the fermentation process also vary greatly as the *akadiru*, *tua-metan*, and *nuu* must join the sap every day to reach the desired volumetric quantity until the penultimate day scheduled for the fermentation process (BELO *et al.*, 2023). Therefore, the process probably would not have been adequate in converting all the sugars contained in the sap itself and may not have been efficient in obtaining a good yield of the alcohol produced.

Where the fermentation stage is a process that requires many controls to obtain the highest alcoholic yield, the efficiency of the process in this stage will be a great contribution to the increase in ethyl alcohol formation (SANTIAGO-URBINA; VERDUGO-VALDEZ; RUIZ-TERÁN, 2013). In this sense, it is worth considering that in one of the steps before moving on to the fermentation process, it is necessary to add a quantity of sap depending on the desired volume to facilitate control of the time of the fermentation process, so that the conversion can occur more efficiently.

The sap of the *tua-tali* palm tree is mostly extracted by the destructive method through the apical cavity of the meristem. The amount of sap that this palm tree requires depends on each producer or region, whereas producers in the eastern region of the country, mainly in the municipality of Baucau, stated that they often need to collect sap over two to three days to reach the desired amount according to the distiller's capacity, with this palm tree being capable of producing 30 to 40 liters of sap (see BELO; BRICENO; PEREIRA, in press).

Thus, the sap collected on the first day rested longer compared to the second day, as in the following two days, the volume is sufficient and can be subjected to the subsequent process (distillation). Therefore, it is worth noting that the time required for the fermentation process can vary by at least 24 hours for collection on the first day, while sap collected the next day may require up to 24 hours less (BELO *et al.*, 2023).

A region in the municipality of Viqueque reported that the extraction and distillation process can be carried out in a single day, as extraction is generally done from two or more trees at the same time. This means that sap production can reach an average of 40 to 50 liters per day. Meanwhile, the producers in the western region of the country, mainly in the municipalities of Covalima and Bobonaro, follow the same process as in Viqueque, since in these regions it is possible to extract sap from up to three trees per day.

The producers themselves stated that the quantities of sap produced can reach an average of 80 to 100 liters per day. Therefore, the distillation process is carried out immediately, a few hours after the extraction. Thus, the resting time of the fermentation process varies from the beginning of the sap flow to the last collection, considering time zero. It is generally less than 24 hours, depending on the collection, as the sap flow is continuous. This fact revealed an inconsistency in controlling time applied in the fermentation process, which prevents microorganisms from acting uniformly and efficiently in the process of converting sugar into ethanol.

Based on the results obtained, it can be observed that all samples of the different types of palm trees analyzed present varying alcohol concentrations. It should be noted that the chemical composition of the sap may be one of the factors that influence the variation in ethyl alcohol concentrations of these beverages. Since the composition and quality of palm sap vary depending on the location, time, and duration of extraction (NAKNEAN *et al.*, 2010). A large amount of sugar in a food matrix is essential, as it is

responsible for the production of ethyl alcohol (ABDEL-BANAT, 2010; VARELA, 2016).

In a study carried out by NAKNEAN *et al* (2010), investigating the total sugar and total soluble solids content in the sap of the palm tree (*Borassus flabellifer* Linn), or *akadiru*, reported that a total sugar content between 10.36 and 16.94% and total soluble solids between 10.67 and 17.33° Brix.

SARMA *et al.* (2022) reported that saps obtained from palm species such as *Cocos nucifera*, *Borassus flabellifer*, and *Arenga pinnata* contain total sugar contents of 10.08 - 16.50 g, 9.88 - 17.32 g, and 10.00 - 15.00 g, respectively, and LALEL & RUBAK (2024) reported that saps obtained from *tua-tali* palm contain a total soluble solids content ranging from 8.5 to 14.1 °Brix. However, in the context of sap production in Timor-Leste for direct, fresh consumption or as a raw material in alcoholic beverage production, there are no prior studies that detail the sugar content of the sap.

Therefore, in certain cases, differences in the amount of sugar present in the raw materials can lead to differences in the concentration of ethyl alcohol produced. Furthermore, SANTIAGO-URBINA; VERDUGO-VALDEZ; RUIZ-TERÁN (2013) reported that the increase in the population of yeasts responsible for ethyl alcohol fermentation can vary the concentrations of ethyl alcohol in distilled alcoholic beverages produced mainly from palm tree sap.

4.2.1.2. Influence of the fermentation process

Fermentation is a process of converting sugar into ethyl alcohol, water, and CO₂ through metabolic processes in the presence of microorganisms (ALZEER; HADEED, 2016; BLACK; WALKER, 2023; APENTENG *et al.*, 2024). This step is crucial in ethyl alcohol production. These microorganisms, to perform their role as modifiers, need a conditional environment that allows them to carry out activities throughout the entire process (NOSE, 2006; ABDEL-BANAT, 2010; VARELA, 2016).

The production of traditional alcoholic beverages in the context of Timor-Leste is adopted as a spontaneous fermentation process in the presence of microbiological flora, such as native bacteria and microorganisms. The spontaneous fermentation process involves natural inoculation by microorganisms (microbiota) present in the local environment (SERAFIM; FRANCO, 2015).

These microorganisms are generally found on the surfaces of sap collection materials and in parts of the distilleries used (ALCARDE *et al.*, 2012; BLACK;

WALTER, 2023). According to NAKNEAN *et al* (2010), the microorganisms were present from the beginning of the sap collection process, and were generally found on the surfaces of sap collection materials and in parts of the distilleries used.

Palm sap is an excellent substrate for the growth of microorganisms for fermentation within an hour or two of collection (MATHURIN *et al.*, 2020). After some time, the sap begins to form foam because of fermentation, and its sweet taste becomes bitter with a noticeable alcohol content. In the fermentation process, for microorganisms to perform their functions efficiently, there needs to be a conditional environment that allows them to carry out activities throughout the entire process (VARELA, 2016).

Wine can form more alcohol if it continues fermenting for more than a day; however, the longer it remains, it will begin to turn into vinegar or produce more acids (CHANDRASEKHAR *et al.*, 2012; MC WILLIAM, 2022), consequently reducing the yield of ethyl alcohol produced.

According to CHANDRASEKHAR *et al* (2012), they reported that wine or palm sap are excellent substrates for the growth of microorganisms for fermentation within one or two hours after collection. Therefore, the wine can become rich in alcohol if it continues to ferment for more than a day. However, the longer it remains, it will begin to turn into vinegar (CHANDRASEKHAR *et al.*, 2012; MC WILLIAM, 2022), which can consequently reduce the yield of ethyl alcohol produced.

The study carried out by AZEER; HADEED (2016), when investigating the temperature and time conditions for the fermentation process of fresh grape juice, showed that when the juice is kept for a day at room temperature it can significantly increase the formation of ethyl alcohol, therefore, if kept for more days, up to about 10 days, at the same temperature condition, the alcoholic strength (ABV) can increase drastically. The authors further reported that the availability of oxygen can contribute to the reduction of ethyl alcohol through oxidation, thus forming more acetic acid as a product, where the product becomes more acidic (ALZEER; HADEED, 2016).

In addition, the process is conducted entirely in open spaces without environmental control, which can cause imbalances in the performance of the fermentation process (WEISS *et al.*, 2022). Some of the parameters, e.g., hygiene conditions or cleanliness of the sap collecting materials or collectors used for the sap store, are also essential for the controlling process at this stage (NAKNEAN *et al.*, 2010; SANTIAGO-URBINA, VERDUGO-VALDEZ; RUIZ-TERÁN, 2013).

The fermentation process adopted by the Timorese to produce *tua-sabu* is conducted entirely naturally or spontaneously by native microorganisms present in the sap, without the addition of any ingredients, such as strains or yeast. One of the factors affecting the quality of *tua-sabu* is the fermentation process, which is carried out without proper control, especially regarding environmental factors (BELO *et al.*, 2023).

Furthermore, Timorese *tua-sabu* producers don't have a standard timeframe that specifies the fermentation process in days or hours, but rather an exact time based on their experience. The time required for the process varies greatly, depending on each producer's experience, the type of palm sap, and the desired quantity of sap. Therefore, the fermentation process can take up to 94 hours, or sometimes, when the collected sap reaches the desired volume, according to the distiller's measurements, it can be distilled immediately, a few hours after extraction (see BELO; BRICENO; PEREIRA, in press).

Thus, just as the variations in sap collection mentioned earlier affect the fermentation process time, this is considered one of the fundamental factors that can affect the yield and quality of distilled beverages produced. In this sense, independent studies related to the fermentation process are very important to determine a more precise period and the ideal conditions for improving products in the future.

On the other hand, BELO *et al.* (2023) reported that some producers incorporate sun-dried plant bark as a natural ingredient into the sap during fermentation. In this process, according to the producers, the purpose of adding the peel is to confer color, flavor, and aroma to the final product. However, the presence of an ingredient without scientific recognition can have a negative effect due to its potential toxicity, as it may act as an antimicrobial, inhibiting the microorganisms responsible for the fermentative action, reducing the yield of the final product, or forming other undesirable compounds. On the one hand, African sap producers generally use tree bark or leaves of wood or lime as natural antimicrobial agents that are usually added to the sap collection container to prevent rapid fermentation that can occur during sap collection (FRANCISCO-ORTEGA; ZONA, 2013).

4.2.1.3. *Influence of distillation processes*

In Timor-Leste, the production of *tua-sabu* features varied production systems, with distillation apparatuses and procedures that differ slightly from one producer to another, and from region to region. There are several types of distilleries made from different materials, such as iron-based and aluminum stills, including earth-based pots.

Furthermore, the equipment used is presented in different measurements or sizes (BELO *et al.*, 2023). The differences are often related to the producers' experiences, procedures, and the availability of materials in the description of the distillation system (CARVALHO *et al.*, 2016).

It is also possible to find very similar production systems in terms of physical infrastructure made up of materials used to produce *tua-sabu* of *akadiru*, *tua-metan*, and *nuu*, from the producing communities in the municipalities of Baucau and Viqueque. Consequently, this leads to similarities in the qualities of the products produced, with a few variations in ethyl alcohol concentrations observed between them.

The *tua-tali* fractions also present similar alcohol concentrations, with slight differences, as can be observed in the fractions produced in the municipalities of Viqueque, Bobonaro, and Covalima. This difference may be related to the production methods, particularly the use of stills with the same base and distillation procedure (Figure 7a, b, and e).

In the eastern region of Timor-Leste, particularly in the municipality of Lautém, producers, as reported by MC WILLIAM (2022), have independently developed distillation systems for *tua-sabu* from *akadiru* and *tua-metan*. These systems typically consist of an open oven or fireplace, a clay pot with an iron base for the still body, and a long bamboo-based condenser for cooling and collecting the distillate.

The cooking techniques used by Timorese producers are generally carried out in open spaces and use firewood as an energy source to provide for heating throughout the process. As a result, controlling the temperature and pressure is difficult. Furthermore, most of the tubes used as condensers lack a refrigeration system, which can cause issues that affect the alcohol evaporation process, consequently reducing the quality and yield of the final product (BELO *et al.*, 2023).

LIEBMINGER *et al.* (2021) noted that improvements in distillation techniques allow for a better balance between heating parameters, which determine evaporation rates, and reflux conditions, crucial for enhancing the quality of alcoholic beverages. Therefore, in this stage, temperature control is essential as a measure to estimate and control the transport of volatile compounds with the apparent ethyl alcohol concentration (HELLER; EINFALT, 2022).

According to LIEBMINGER *et al.* (2021), improving the quality of alcoholic beverages would have a decisive role. Therefore, it is necessary to improve distillation techniques to achieve a balance between heating parameters, which determine the

evaporation rates, and reflux conditions. Furthermore, temperature control is considered essential at this stage, as a measure to estimate and control the transport of volatile organic compounds up to the apparent concentration of ethyl alcohol (HELLER; EINFALT, 2022).

4.2.1.4. Influence of the separation or cutting of distilled fractions

The cutting or separation of fractions is a fundamental step in determining the concentration that classifies fractions into head, heart, and tail (RODRIGUES *et al.*, 2019; SERAFIM; PEREIRA-FILHO; FRANCO, 2016), which is the case of the production of Brazilian cachaça from sugarcane juice, representing 5 to 10% of the total distillate volume for the first distillate or head fraction, 80 to 85% for the heart or second fraction and 10 to 15% of the total distillate volume for the last fraction of the distillate or tail fraction (SERAFIM *et al.*, 2012; RODRIGUES *et al.*, 2019).

In the production of *tua-sabu*, fraction cuts are generally made into three fractions: *tua-ulun*, *tua-klaran*, and *tua-ikun*. The separation process is often guided by observational experience due to the lack of equipment for measuring alcohol concentration and the absence of defined standards for fraction separation. Producers typically estimate the separation of fractions, with total distillate collected is around 10 to 15% of the boiler volume composed of the first collection or *tua-ulun* represents 20 to 25%, the second fraction or *tua-klaran* about 50 to 60%, and the last collection fraction or *tua-ikun* about 20 to 25% of the final volume of the distillate, respectively (BELO *et al.*, 2023).

Based on the results obtained for each distilled fraction of all analyzed samples, as shown in Figure 13, and Figure 18, the alcohol content varies significantly from one region to another, even for the same fraction. Furthermore, some *tua-ulun* fractions had concentrations similar to certain *tua-klaran* fractions. This indicates that the separation of fractions is the most important measure, as it is capable of determining the concentration of ethyl alcohol in the different fractions (LUNA; LÓPEZ; PÉREZ-CORREA, 2021).

This step requires a standard as a reference point and a minimum measurement of concentrations (PORTUGAL *et al.*, 2017). This would help avoid large differences in alcohol levels between the same fractions from a region or producer, and between different regions across the country. It would also prevent variations in alcohol levels

between the *tua-ulun*, *tua-klaran*, and *tua-ikun* fractions. It would also prevent variations in alcohol levels between the *tua-ulun*, *tua-klaran*, and *tua-ikun* fractions.

According to the results obtained for each distilled fraction of all analyzed samples, the ethyl alcohol concentrations obtained vary greatly between the fractions of *tua-ulun*, the proximity of concentrations between *tua-ulun* and *tua-klaran*, such as the fractions of Pl1Lp1 and Pl2Lp1; Ap1Mc1 and Ap2Mc1; Ap1Qq3 and Ap2Qq3, and Cn1Lp2 and Cn2Lp2 (Figure 13); Cu2Lp2 and Cu1Lp3, Cu1Qq5, Cu1Mc5; Cu2Lp3 and Cu1Mc8, Cu1Qq9, Cu1Mc9, Cu1Mc13 (Figure 18).

Furthermore, some fractions of *tua-ulun* and *tua-klaran* had lower ethyl alcohol concentrations than some fractions of *tua-ikun*. It is considered that the step of separating distillate fractions during distillation is one of the main steps capable of determining the concentration of ethyl alcohol in the distillate, differentiating between fractions. Therefore, this step must be carried out carefully and standardized with adequate separation to avoid the occurrence of any changes, especially in the distillate concentrations.

4.2.1.5. *The influence of product bottling and the combination of distillate fractions*

The packaging or bottling of the product is essential, as it is the initial phase of placing the product for sale and consumption (VERGHESE *et al.*, 2015). Furthermore, one of the most important unit operations in the formulation of commercial products is to ensure quality during storage, transportation, sale, and consumption (ALHENDI; CHOUDHARY, 2013; SIMON; AMOR; FÖLDÉNYI, 2016).

The packaging process involves the function of protecting, preserving, conserving, and delaying the loss of quality of food and beverages products, in this sense, materials generally require ideal conditions depending on the type and characteristics of the products, as packaging materials have different properties concerning contact with products, it's are sensitive to factors such as exposure to light, temperature conditions, contact with air open, among others (COLES; RICHARD; KIRWAN, 2011; AZEREDO; FARIA; BRITO, 2012; FONTOURA; CALIL; CALIL, 2016; JAIME; BOCOLI; FARIA, 2018) (SOHAIL; SUN; ZHU, 2018; JAIME; BOCOLI; FARIA, 2018; VERMA *et al.*, 2021). .

Most *tua-sabu* distillates in Timor-Leste are bottled in various reused, non-sterilized containers made from both permeable and impermeable materials or plastic bottles, e.g., PET, HDPE, and LDPE, to prevent leakage (BELO *et al.*, 2023), chosen

based on material availability and accessibility. The authors also highlighted that bottles are often used without adequate hygiene conditions, cleaning is usually limited to removing minimal amounts of remaining water or oil, and the bottles are immediately used to bottle the products.

Therefore, to maintain the product's quality characteristics, the equipment or materials used must undergo minimal thermal processing or pasteurization to eliminate pathogenic microorganisms (AADIL *et al.*, 2019; SOHAIL; SUN; ZHU, 2018).

The use of these materials can cause some degradation in the products through direct contact with the containers themselves, which probably causes some unforeseen reactions and leads to a decrease in ethyl alcohol concentrations due to the lack of hygiene in the preparation of these materials and their use (NOGUEIRA *et al.*, 2023).

In addition, the use of inappropriate packaging materials can cause changes in the packaged products, making them unsafe, due to the migration of some compounds from the materials themselves to the product, which can negatively influence the quality of the product (BRASIL, 2005; ALMEIDA *et al.*, 2023; NOGUEIRA *et al.*, 2023).

The use of any packaging material depends on the type and characteristics of the product, as the material itself can interact and leach undesired compounds into the product, which causes unforeseen reactions that lead to changes in the quality of the product, making it unsafe for consumer health (BRASIL, 2005; SOHAIL; SUN; ZHU, 2018).

On the other hand, due to growing consumer interest and market demands, accelerating product turnover, most products entering the fair tend to be a blend of fractions. For example, a certain amount of *tua-ulun* fractions is often blended with *tua-klaran*, which has low concentrations of ethyl alcohol as a fortifier (BELO *et al.*, 2023). This can be considered a factor contributing to changes in product quality, particularly in ethyl alcohol levels, as evidenced by the results for the fractions listed in Table 4.

According to local producers (interviewed), reported that the volume of sap extracted per tree/day can reach 30 to 40 liters for a period of two to three months; however, the quantity produced depends on several factors such as the stages of preparation, production season, climatic factors, soil fertility, and productivity, including the age and size of the plant. This form of extraction is similar to the process carried out in African and Western countries, where extraction is done by felling or incising the apical meristem of the palm tree (Table 5).

The collection of sap in this process involves drilling or excavating the cavity in the soft apical meristem of the tree trunk and inserting a tube or opening directed into traditional containers such as a calabash pot or terracotta clay pot, or containers such as plastic bottles (FRANCISO-ORTEGA; ZONA, 2013; DJENI *et al.*, 2020).

In the production of Brazilian cachaças and distillates, the legislation only allows the heart fraction or fraction of the second distillate, which is intended for consumption, in which the ethyl alcohol concentration is generally determined between 38 and 54% v/v for cachaça, 38 to 48% v/v for distillates and 15 to 54% v/v for mixed alcoholic beverages (BRASIL, 2005). On the other hand, the first and last fractions are discarded or can be used for another purpose.

In Indonesia, alcoholic beverages are divided into three categories: Category A consists of beverages such as shandy, light alcoholic beverages, spirits and beer, among others; Category B consists of aromatic beverages such as malt wine, fruit wine, vegetable wine and rice wine, cider, honey wine/mead, toddy, traditional Balinese alcoholic beverage (*Anggur Brem Bali*), among others; while Category C consists of spirits such as brandy, arak, cocktails, fruit brandy, whiskey, rum, gin, among others, where each category can contain ethyl alcohol by volume up to 5%, 5 to 20% and 20 to 55%, respectively (AUDRINE, 2021).

The *tua-sabu* fractions presented ethyl alcohol concentrations that varied depending on the fractions and the collection sites. As observed, the *tua-ulun*, *tua-klaran*, and *tua-ikun* fractions presented minimum and maximum concentrations of ethyl alcohol between 30.4 and 53.6%; 18.2 and 40.4% and 14.3 and 21.5% (v/v), respectively.

It is noted that some fractions that present concentrations within Brazilian legislation for both brandy and cachaça are, for the most part, *tua-ulun* fractions, and only a single *tua-klaran* fraction, direct from local production in the municipality of Baucau, is considered in compliance with these laws (Figure 18).

And, most of the *tua-klaran* fractions can be considered in the Indonesian legislation for the brandy category. Still, it is worth highlighting that it is necessary to improve the main processes involved, from distillation, fraction cutting control, packaging material conditions, avoiding the mixing of fractions, and storage conditions during transportation and marketing.

Due to the absence of national standards and regulations, *tua-sabu* is currently a product that circulates freely in the market with greater prominence, and the consumption

of combined fractions or *tua-ulun* continues to be practiced in the country, without any health consequences being noted.

ALCARDE, SOUZA, & BELLUCO (2010) reported that the head fraction of brandy contains alcohol-soluble compounds with low boiling points, such as methanol, acetaldehyde, and ethyl acetate, which are produced during the distillation stage. In addition, the tail fraction contains a high concentration of compounds with a boiling point higher than that of water or water-soluble compounds, such as acetic acid and furfural.

In contrast, the alcohol-soluble and water-soluble compounds present in high concentrations in beverages can cause several health problems, which is why how Timorese people consume them can generate diseases that can put consumers' lives at risk of serious illnesses and even lead to death (ALCARDE; SOUZA; BELLUCO, 2010).

4.2.1.6. *Product supply chain and its storage conditions*

The distribution, transportation, and sales are among the stages that require maximum attention and care in storage related to environmental aspects (AADIL *et al.*, 2019; NOGUEIRA *et al.*, 2023). In this sense, the temperature and open air are some of the factors that can cause changes in the quality and safety characteristics of the product during the supply chain, considering factors such as exposure to sunlight, presence of open air, and environments with temperatures inappropriate for the product specification, among others (MACIEL; FRANCO; YOSHIDA, 2012; HARDY, *et al.*, 2023).

In Timor-Leste, the distribution and transportation of alcoholic beverages, particularly *tua-sabu*, to fairs are typically uncontrolled concerning transport conditions, beginning from the production site and extending to the point of sale. This can lead to some chemical and physical changes, leading to product oxidation and contamination from packaging materials due to exposure to sunlight, which can contribute to a decrease in the ethyl alcohol concentration and the formation of some undesirable compounds in the product.

According to FONTOURA, CALIL & CALIL (2016), physical and microbiological contamination can occur during distribution and transportation, altering the quality of the products. Therefore, it is necessary to have maximum control over the storage management of products during transportation and sale to avoid any change in product quality.

4.2.2. PART II. Volatile compound profile

The volatile organic compounds identified in the sample fractions were classified and clustered by PCA into chemical classes, including alcohols, acids, aldehydes, ketones, and other organic compounds, e.g., furans, Alkaloids, plastics, and sugar derivatives (Table 12).

4.2.2.1. Analysis based on samples from municipal sources

Sample sizes for the study ranged from a minimum of 2 to a maximum of 26 samples from different municipalities. These samples consisted primarily of *tua-ulun*, *tua-klaran*, and *tua-ikun* fractions from four palm species (Pl, Ap, Cn, and Cu), and were collected at three different collection points (Lp, Qq, and Mc). However, not all municipalities had samples from all fractions, plant species, and collection points as planned. This depended on the availability of samples related to their production base and the movement of products within the target municipalities studied (see BELO; BRICENO; PEREIRA, in press).

For example, the municipality of Baucau produces all plant species but experienced some shortage of fractions and samples originating from collection points during sample collection. Furthermore, in the municipality of Ainaro, there is no information on the production of any of the plant species in the municipality, so the samples were obtained exclusively through market movements, as well as the other municipalities that presented several samples, as previously presented in items 2.5.1 of chapter II and 3.1 of chapter III.

Regarding the results of the PCA analysis, it is possible to observe that samples from the same municipality or from different municipalities present similar and distinct chemical profiles, mainly attributed to the fractions, plant species, and suppliers.

As can be observed, the samples from the municipality of Lautém, although originating from the species *akadiru* (Pl) and *tua-metan* (Ap), were collected at different points, consisting of (Lp, Qq, and MC). The main differences in composition reside primarily in the plant species (Pl and Ap), with the fractions and suppliers considered secondary factors in the variability of these samples, which represent the chemical characteristics of the products, and each fraction has a specific chemical profile defined by a distinct set of compounds. The same behaviors are also observed in samples of the plant species *akadiru* and *tua-tali* from suppliers Qq and Mc in the municipality of Dili.

It is worth noting that these differences in the proportions of compounds that give rise to chemical characteristics in the discrimination of samples may be related to production processes that involve different technical knowledge among producers, such as use of materials, quality of raw materials, operational conditions throughout the fermentation process, distillation, cutting of fractions, and its distribution, are crucial factors that result in differences in the quality of the products produced (SERAFIM *et al.*, 2011; MOREIRA; NETTO; DE MARIA, 2012; BELO *et al.*, 2025).

Meanwhile, samples from the municipalities of Baucau, Liquiça, Covalima, Manufahi, Ainaro, Aileu, and Ermera showed the main differences attributed primarily to the fractions, and the palm species and their suppliers, with secondary factors contributing to the variability of these samples. This phenomenon is generally strongly related, mainly, to the quality of the plant material; even if they come from the same location or producer, their quality may vary, reflecting the original characteristics of the agricultural products (MENDONÇA *et al.*, 2014; SCHOENINGER *et al.*, 2014).

In the municipalities of Viqueque and Bobonaro, the variability in the samples is mainly attributed to both plant species and suppliers, which may be related to the quality of the raw materials and their supply chains. Furthermore, samples from the municipality of Manatuto and Oe-cusse show that the variability of the samples was mainly influenced by their supplier and, secondarily, by the fraction or plant species, demonstrating the importance of the supply chain.

In general, considering also that differences in the composition of product along the supply chain, specifically for distilled beverages in Timor-Leste, can result from a mixture of fractions that compromise quality or, more commonly, from alterations or changes in product quality during its distribution to the market, primarily due to factors such as transportation and storage conditions (BELO *et al.*, 2025; SCHOENINGER *et al.*, 2014)

4.2.2.2. Analysis based on samples from plant species

The samples of the *akadiru*, *tua-metan*, and *tua-tali* palm species are composed of fractions of *tua-ulun*, *tua-klaran*, and *tua-ikun* from different collection points (Lp, Qq, and Mc) in several municipalities.

Based on the results, PCA analyses showed that these samples present variable quality characteristics. The greater internal variability in the composition of these samples is strongly linked to factors such as suppliers, with samples from a given collection point

(Lp, Qq, and Mc) and their location of origin (municipalities). These different characteristics are possibly attributed to the quality of the raw materials used, the cultivation conditions with different management or manufacturing methods, and their storage conditions throughout their distribution to the market.

MENDONÇA *et al.* (2014) state that, during the production process, the quality of the raw material, the lack of control during the cutting of the fractions, among others, are the main factors that influence the quality of the products produced. Considering that the way in which Timorese producers produce *tua-sabu* presents a wide range of complexities, regarding the heterogeneity of methods from one producer to another, consequently leading to product differentiation (BELO *et al.*, 2023).

MIRANDA *et al.* (2007) also found a great variability in the chemical composition of beverages, reflecting the difficulties faced by producers throughout the production process, including the use of inappropriate materials and inadequate hygiene, which compromise the quality standard of the product.

Samples originating from the *nuu* species also consist of the *tua-ulun*, *tua-klaran*, and *tua-ikun* fractions. Due to the scarcity of production and its rare occurrence in other municipalities, the samples were collected exclusively in the municipality of Baucau, as the producer, and the samples originated from the collection points/supplier (Lp, Qq, and Mc).

Although these samples present the same fractions, PCA showed that the fractions have broad chemical compositions, which can be explained by the geographical origin (L, N, S and B), supplier factor (Lp, Qq, and Mc), which influences the determination of the chemical profile and the separation of these samples. These problems may be related to the processes involved in the production chain, inadequate storage and transportation management, the use of unsuitable packaging materials, and a lack of hygiene. Product adulteration can also occur, including the combination of different fractions during the handling or distribution of the product in the market.

These occurrences may be related to inadequate storage and transportation management, the use of inappropriate packaging materials, and inadequate hygiene. Product adulteration may also occur, including mixing of fractions during movement or circulation to the market (BELO *et al.*, 2025)

A product's supply chain is one of the key stages that can compromise product quality, as it involves a variety of conditions that always require maximum control to prevent unwanted occurrences. The use of appropriate packaging materials, storage

management, and transportation is crucial to ensuring and maintaining product quality (BORTOLETTO; SIVELLO; ALCARDE, 2018).

In summarizing the large number of samples and their associated compounds, as presented in Table 13, the main compounds represent each group of chemical classes of compounds considered important for the sensory characteristics of beverages, such as color, aroma, and flavor, including the main contaminant compounds that are harmful to human health. The main compounds, as listed in the table, that are strongly associated with the samples are classified within the chemical class groups of compounds such as alcohols, acids, aldehydes, esters, ketones, and plastic derivatives.

4.2.2.3. Alcohols

Methyl alcohol is an alcohol resulting from the degradation of pectin by the presence of pectic yeasts during the fermentation process, passing into the distilled beverage during the distillation process. Due to its low boiling point, it is typically concentrated in the head fraction of the distilled beverage (PEREIRA; ACEVEDO; ALCARDE, 2024).

As this study demonstrated that the compound is associated with all fractions, constituting fraction as Tuaulun(ApLp4, PlMc5, PlQq7, CuMc8 & CuMc3), Tuaklaran(CuQq2, PlMc8 & ApMc10), and Tuaikun(ApLp1I, CuQq2 & CuMc9) (Table 13), this may be related to improper cutting of the fractions or may have occurred due to some adulteration of the product or improper handling during storage.

Methyl alcohol is considered an undesirable organic contaminant to distilled beverages, as it is a highly toxic compound in excessive consumption (BORTOLETTO; SIVELLO; ALCARDE, 2018), capable of causing poisoning that initially manifests with headache, nausea, blindness, and even death (CARUSO; NAGATO; ALABURDA, 2010). According to Brazilian legislation, the maximum permitted limit is ≤ 20 mg 100 mg/L AA (BRASIL, 2005).

Most higher alcohol compounds are strongly associated with all fractions analyzed in this study, being the main compounds responsible for the sensory characteristics of these beverages. Higher alcohols are those compounds resulting from the reduction of aldehydes by the degradation of sugars and amino acids that can form during fermentation (BORTOLLETO *et al.*, 2015; MAIA *et al.*, 2020; LIMA *et al.*, 2022).

These alcohols constitute one of the largest groups of aromatic compounds in distilled beverages, generally formed throughout the production process, in the main

stages such as fermentation and distillation (FRANCISKIEVICZ *et al.*, 2022), their quantities produced depending on the characteristics and compositions of the raw materials used (OLIVEIRA *et al.*, 2020). The presence of these compounds in distilled beverages plays a vital role in contributing to the sensory profile, especially the characteristic flavors and aromas (NASCIMENTO *et al.*, 2022).

Specific alcohols of 3 to 4 carbon, such as 1-propanol, 1-butanol, and 2-butanol, are compounds formed during the production process through bacterial contamination in various pathways, and are considered organic compounds that contaminate the beverage, especially distilled beverages (SOUZA, 2013; OLIVEIRA *et al.*, 2020). Meanwhile, alcohols consisting of 4 to 5 carbons such as 1-propanol -2-methyl- or isobutanol, and 1-butanol -3-methyl or isoamyl are formed in different ways depending on the yeasts present in the process medium during alcoholic fermentation, whose concentrations vary with the composition of the raw material used for the production of the distilled beverage (CAPOBIANGO *et al.*, 2015; MOURET *et al.*, 2012; LIMA *et al.*, 2022).

The presence of these compounds in the beverage, even in small quantities, makes a great contribution to the characteristic aroma of the beverage (CORTÉS *et al.*, 2011). On the other hand, the excess of higher alcohol present in beverages can alter the quality characteristics of the beverage, form unpleasant odors, and cause negative effects on human health, in addition to leading to product rejection (OLIVEIRA *et al.*, 2020; NASCIMENTO *et al.*, 2022; PEREIRA; ACEVEDO; ALCARDE, 2024).

Given the problems related mainly to the excessive quantities they can cause, the main way to control them is to maintain the appropriate temperature between 28 and 32 °C during the fermentation process, the pH of the must or broth, intense oxygenation in the fermentation tanks and avoid distillations for too long, including inadequate separation of fractions (OLIVEIRA *et al.*, 2020; SILVA *et al.*, 2020; LIMA *et al.*, 2022).

4.2.2.4. Acids

The formation of volatile acids in distilled beverages is generally influenced by factors such as time and temperature conditions applied in the fermentation stage, types of yeasts and microorganisms involved in the process, quality of the raw materials used (must, among others), including hygiene conditions throughout the beverage manufacturing process (CAPOBIANGO *et al.*, 2013).

Volatile acids are measured in terms of acetic acid, a consequence of bacterial contamination during and post-fermentation, where excessive levels of these compounds

are generally related mainly to spoilage by lactic and acetic bacteria. These bacteria ferment the wine and increase the acidity of the medium (PORTUGAL *et al.*, 2017; OLIVEIRA *et al.*, 2023). In addition, their high concentration is also promoted by the oxidation of ethanol (BORTOLETTO *et al.*, 2015; DOS SANTOS *et al.*, 2021). In this sense, it is essential to have maximum control, especially during and post-fermentation, to avoid contamination by acetic bacteria and prevent the formation of acetic acid.

Acetic acid is a compound responsible for the acidity of the beverage, with a burning and pungent sensation after swallowing, generating sensory rejection. This compound is formed during distillation and can accumulate together with the heart fraction during the cutting of the fraction due to its solubility in water and the applied boiling temperature higher than that of water (ODELLO *et al.*, 2009; MIAO *et al.*, 2022).

In this sense, controlling the cutting of the “tails or *tua-ikun*” fractions is important to reduce the formation of these compounds (BORTOLETTO *et al.*, 2018; SANTIAGO *et al.*, 2014). The *tua-ikun* fraction generally contains more acid compared to the other first two fractions, such as *tua-ulun* and *tua-klaran*, due to the presence of undesirable bacteria after the preparation of the beverage (ALMEIDA *et al.*, 2020; OLIVEIRA *et al.*, 2021).

The result of this study showed that acetic acid and other acidic derivatives are not only present in the *tua-ikun* fractions, but are also strongly associated with several *tua-ulun* and *tua-klaran* fractions (Table 13), demonstrating that their presence in these fractions is related to several factors, such as inadequate cuts of the fractions, lack of hygiene of packaging materials, degradation of ethanol due to lack of adequate storage management, combination of fractions, among others.

Excess acids can increase acidity levels and weaken the aromas of beverages (BARBOSA *et al.*, 2022). In addition, they can generate unpleasant odors and flavors that can affect the aromatic balance, leading to the rejection of the product by consumers and, consequently, a decrease in its commercial value (ODELLO *et al.*, 2009; YU *et al.*, 2023; PEREIRA; ACEVEDO; ALCARDE, 2024).

Propionic acid and n-decanoic acid are secondary organic compounds that can be produced during the refining or distillation process (DIÉGUEZ *et al.*, 2002). Furthermore, the formation of propanoic acid is also associated with the presence of a microbial community during fermentation (MAIO *et al.*, 2022; DONGMO *et al.*, 2017; CAETANO *et al.*, 2022). However, n-butanoic acid is one of the important compounds responsible for the flavor and aroma of beverages in adequate amounts; when in excessive levels, it

causes the beverage to have an unpleasant smell and taste (MIAO *et al.*, 2022; YU *et al.*, 2023).

4.2.2.5. Aldehydes

Aldehydes are formed depending on the yeast strains that act in the fermentation process, including the conditions in which the fermentation and distillation processes are carried out (CAETANO *et al.*, 2022; DATO *et al.*, 2005). Furthermore, aldehydes are products formed by the oxidation reaction of alcohols, amino acids, and fatty acids (CORTÉS *et al.*, 2011; BORTOLLETO *et al.*, 2015). These compounds play a more important role and are responsible for the peculiar characteristics of alcoholic beverages, mainly flavor and aroma (OLIVEIRA *et al.*, 2020; LIMA *et al.*, 2022).

Acetaldehyde is the most abundant compound among the group of aldehyde compounds and is formed by acetic bacteria or by the auto-oxidation of ethanol, being considered an important and desirable compound in beverages (LIU; PILONE, 2000; CAMPAGNOL *et al.*, 2012; SILVA *et al.*, 2020).

The *tua-ulun* fraction may present high concentrations of acetaldehyde together with other volatile compounds, such as ethyl acetate and methanol, as these compounds have a lower boiling point than ethanol (Kokoti *et al.*, 2023).

Meanwhile, furfural and 5-hydroxymethylfurfural (5-HMF) can be formed by the pyrogenation of organic matter, the presence of yeast residues, or thermal degradation of sugars, mainly by the dehydration of pentoses and hexoses during the wine cooking process (YAYLAYAN; KEYHANI, 2000; MASSON *et al.*, 2007; BORTOLLETO *et al.*, 2015). Furthermore, these compounds can also be released in the final stages of the Maillard reaction and caramelization (MOREIRA *et al.*, 2012).

This study demonstrates that both furfural and 5-hydroxymethylfurfural are present in the *tua-ulun* and *tua-klaran* fractions, including *tua-ikun* (Table 13), which reflects an imbalance in the distillation process, mainly due to inadequate temperature control during cooking, improper separation of fractions, and mixing of fractions during commercialization.

In addition to the important aromatic functions represented above, excessive levels of these compounds in beverages can cause a negative sensory impact, contributing to a burnt and caramelized flavor, affecting the aromatic balance of the products, and leading to consumer rejection (MOURET *et al.*, 2012), since aldehydes containing up to eight or

more than ten carbon atoms give the products a tasteless appearance and present unpleasant flavors and aromas to the beverages.

Furthermore, the compounds can have negative effects on human health when consumed in excessive amounts (DATO *et al.*, 2005; BORTOLETTO *et al.*, 2017) and can cause effects such as symptoms of intoxication, hangover, nausea, vomiting, agitation, sweating, confusion, and headaches (MOREIRA *et al.*, 2012; OLIVEIRA *et al.*, 2020). Therefore, the temperature control provided during the distillation process and the separation of the fraction are fundamental factors to avoid the excessive formation of these compounds.

4.2.2.6. Esters

Ethyl acetate is the first most abundant compound in the ester class (CAPOBIANGO; OLIVEIRA; CARDEAL, 2013). It is a naturally occurring compound in distilled alcoholic beverages that plays a crucial role in sensory characteristics, particularly aroma and flavor (MOREIRA; NETTO; DE MARIA, 2012). Furthermore, it is a secondary compound formed by alcoholic fermentation during the fermentation process by the reaction between ethyl alcohol and acetic acid because of yeast metabolism (SILVA *et al.*, 2020).

Ethyl acetate is one of the compounds with low boiling points, such as methanol and acetaldehyde, which are typically found in the first fractions of distillates (PEREIRA *et al.*, 2006; PEREIRA; ACEVEDO; ALCARDE, 2024). This study showed that the compound is strongly associated with several *tua-sabu* fractions (*tua-ulun*, *tua-klaran*, and *tua-ikun*) (Table 13), which may have occurred due to fraction mixing practices or some adulteration of the product and inadequate handling during storage.

In excessive concentrations, the compound is characterized by strong and pungent odors that can leave the drink with an unpleasant smell, similar to that of a solvent (MOREIRA; NETTO; DE MARIA, 2012; ZANGHELINI *et al.*, 2024).

4.2.2.7. Ketones

Acetoin is a compound of the ketone group that is produced by the conversion of sugars by strains of the bacterium *Bacillus subtilis* that exist in plants or is a product of the fermentative metabolism of several microorganisms (VALLE-RODRÍGUEZ *et al.*, 2012; WANG *et al.*, 2012; WRIGHT; KLASSON; KIMURA, 2020). The amount produced depends on the processes involved, such as fermentation and maturation, and it

is a compound that gives aroma to beverages even in small portions but can affect the flavor when present in excessive levels (WRIGHT; KLASSON; KIMURA, 2020; JABLONSKI *et al.*, 2021).

Dihydroxyacetone is formed by the conversion of glycerol through microbial synthesis during the fermentation period (BLACK, 2013; POLJUNGREED; BOONYARATTANAKALIN; YUKPHAN, 2017) or can be formed by the degradation of the carbohydrate product in caramelized form or by the Maillard mixture reaction (DAGLIA *et al.*, 2013).

Ketones can have negative effects on human health when present in excessive levels, can cause prolonged inhalation, irritate mucous membranes, cause headaches, confusion, narcotic effects, and can also lead to coma (MOREIRA *et al.*, 2012). For example, excess Gamma Butyrolactone can induce deep sleep, nausea, vomiting, and also increase the risk of coma and death (GALLART-MATEU; GUARDIA; GARRIGUES, 2021).

4.2.2.8. *Plastic-derived compounds*

Di(2-ethylhexyl) phthalate is a chemical derived from phthalates and is a synthetic flexibility-enhancing substance commonly incorporated into plastics (PILLO *et al.*, 2024). GIULIANI *et al.* (2020) stated that the presence of plastic-based compounds, possibly a consequence of their accumulation along the production chain and their accidental release from packaging materials.

Phthalates and their metabolites have become a concern, especially in products for pregnant women and children. Phthalates themselves can cause serious problems for both humans and the environment, depending on their physical and chemical characteristics, and their association with various human diseases therefore represents a global problem (GIULIANI *et al.*, 2020).

As this study showed, some samples, consisting of Tuaulun(PILp2, CuLp3, PIMc7, CuMc7, PIQq8, CuMc11 & CuMc13), Tuaklaran(CnMc2L & CuMc3), and Tuaikun (CnLp2S) (Table 13) from different locations and collection points (Lp, Qq, and Mc), present the presence of this compound. This reflects the inappropriate use of plastic-based packaging and sealing materials used in the bottling process, which may have contributed to their presence in these fractions through direct contact and leaching into the beverages.

Overall, the way Timorese producers manufacture *tua-sabu*, in terms of the characteristics of the raw materials, the types of materials used, and their hygienic

conditions, including the processes involved in the production and distribution chain, is highly likely to contribute to the formation of these compounds.

In this sense, improving the production of high-quality products requires the adoption of Good Manufacturing Practices (GMP) at all stages, including the careful selection of raw materials, strict control of fermentation and distillation conditions and processes, the establishment of standards for the fraction cutting process, adequate hygienic conditions of the materials used, the use of appropriate packaging materials, careful storage of products, avoiding the combination or mixing of fractions and careful control of the market to guarantee the quality of these products, thus facilitating the reduction of health risks and their future exports.

CHAPTER V: FINAL CONSIDERATION

The artisanal brandy of *tua-sabu* is a traditional beverage for most Timorese, demonstrating its sociocultural and economic importance, and widespread recognition by Timorese society from ancient times to the present day. The development of this research consists of a pioneering study on the physical-chemical profile of the fractions of *tua-sabu*, a traditional alcoholic drink from East Timor obtained from the sap of the *akadiru*, *tua-metan*, *nuu*, and *tua-tali* palm trees, since no study has yet been carried out with the same purpose as this work.

Based on the results obtained in the analyses, the quality of each fraction is demonstrated, whether collected in the same or different production areas, collection points (directly at the production site, in supermarkets and markets), plant species or producers, presenting significant variations and similarities alcoholic strength between them, with these behaviors being attributed to the main factors throughout their production and commercialization.

Most of the *tua-ulun* samples collected, mainly from small markets and grocery stores, show low alcohol concentrations compared to the *tua-klaran* fractions and some of the *tua-ikun* fractions collected directly at the production site, indicating low quality and making them unsuitable for consumption. In addition to the low alcohol concentration, some samples also show a strong association with compounds that represent potential risks to human health, such as methanol and 2-furanomethanol, acetaldehyde, 5-(hydroxymethyl) dihydrofuran, furfural, ethyl formate, butyrolactone, bis(2-ethylhexyl) phthalate, among others.

Regarding quality and safety standards, the beverage is still a concern due to the lack of control measures in the country, resulting in *tua-sabu* being circulated and sold without restrictions within the country and without adequate regulations regarding its quality and hygiene. This poses a significant problem, especially regarding the type of container used for bottling, in addition to restricting its access to the international market.

Furthermore, most Timorese consumers of *tua-sabu* often seek out stronger fractions, especially *tua-ulun*, even combining fractions such as *tua-ulun* with *tua-klaran* or *tua-ulun* with *tua-ikun*, unaware that these fractions may contain, in excess, several extremely harmful compounds capable of causing serious health problems to consumers, e.g., the risk of various illnesses and death.

To prevent this, investment in scientific research is crucial to disseminating scientific information related to its most appropriate use or consumption, contributing to improving public health in the country. To this end, it is suggested that establishing a national quality standard, regulated by specific laws, including the implementation of Good Manufacturing Practices (GMPs), will be essential for better development by improving product quality and safety, strengthening the local economy, and protecting public health in Timor-Leste.

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Appendix 1. The artisanal spirit *tua-sabu* and its sociocultural importance in Timor-Leste: a review. Article published in the Journal “PRINCIPIA”.

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A aguardente artesanal *tua-sabu* e sua importância sociocultural em Timor-Leste: uma revisão

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RESUMO: O *tua-sabu* é uma aguardente tradicional de Timor-Leste, produzido a partir da seiva de palmeiras. Essa bebida possui uma longa história, que remonta a tempos antigos, e tem sido reconhecida e valorizada em eventos culturais, como cerimônias rituais e outras celebrações importantes. A produção do *tua-sabu* envolve a colheita da seiva, a fermentação natural, a destilação, a separação de frações e o engarrafamento. A bebida apresenta aspectos de cor, sabor e aroma diferenciados, sendo uma das bebidas tradicionais mais apreciadas pelos consumidores timorenses. Para a maior parte da comunidade agrícola de Timor-Leste, a produção do *tua-sabu* representa uma fonte de renda familiar e de provisões para as necessidades diárias. Todavia, é crucial aprimorar as técnicas e etapas de produção para garantir a qualidade dos produtos artesanais, considerando que ainda falta um controle de qualidade adequado para fins de consumo e para a inserção no mercado. A ausência de infraestrutura laboratorial e de normas técnicas específicas de controle pode comprometer a qualidade e a segurança dos produtos, o que acarreta riscos para a saúde dos consumidores. Esta revisão teórica é necessária pois o processo e as etapas de produção do *tua-sabu* em Timor-Leste ainda são pouco explorados e documentados, sendo um passo importante para a divulgação científica e o desenvolvimento dessa bebida típica, amplamente consumida pelos timorenses, mas ainda pouco conhecida internacionalmente.

Palavras-chave: bebida alcoólica; cadeia produtiva; identidade sociocultural; palmeiras; seiva.



Appendix 2. Exploring *Tua-Sabu* (Brandy) in Timor-Leste: Production, Marketing, and Consumption Patterns. A review. This article is currently in the process of being published in the journal "CIÊNCIA E NATURA" and is awaiting publication.



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Section

Exploring *Tua-Sabu* (Brandy) in Timor-Leste: Production, Marketing, and Consumption Patterns - A review

Explorando *Tua-Sabu* (Aguardente) no Timor-Leste: Padrões de Produção, Comercialização e Consumo - Uma revisão

Appendix 3. Alcoholic strength (ABV) of *tua-sabu* (brandy) from Timor-Leste: derived from the sap of the *tua-tali* palm (*Corypha utan*, L). Article published in the Journal “SCIENTIA PLENA”.



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Ethyl alcohol content of *tua-sabu* (brandy) from Timor-Leste: derived from the sap of the *tua-tali* palm (*Corypha utan* Lam.)

Teor de álcool etílico do *tua-sabu* (aguardente) de Timor-Leste: derivado da seiva de palmeira *tua-tali* (*Corypha utan* Lam.)

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The *tua-tali* palm (*Corypha utan* Lam.) is a species of plant that produces sap, where the sap collected is used as raw material for producing sugar, vinegar, and *tua-sabu*, among others. The study aimed to determine the ethyl alcohol content of *tua-sabu*, the traditional brandy of Timorese. The samples were collected in different municipalities in Timor-Leste, including direct collection at the production site, as well as availability in small grocery stores and fairs, with a total of 39 samples, comprising seventeen samples in total for each fraction of *tua-ulun* and *tua-klaran*, and five samples of the *tua-ikun* fraction. Liquid Chromatography with a UV detector was used to analyze the samples, the result showed that the *tua-ulun* fractions collected directly at the production site in each municipality had higher concentrations of ethyl alcohol between 38.1 and 53.6% v/v. In contrast, fractions collected at the small grocery stores and fairs had concentrations of ethyl alcohol of 36.1 to 50.3% v/v and 30.0 to 41.5% v/v, respectively. Furthermore, most of the *tua-ulun* and *tua-klaran* fractions have different and similar concentrations to each other; even some of the *tua-klaran* fractions sold in small grocery stores and fairs have higher concentrations than some *tua-ulun* fractions and/or less than the *tua-ikun* fractions. It is worth noting that significant variations and similarities in alcohol content are mainly attributed to factors in the production chain, packaging materials and their hygienic conditions, storage mechanisms, and processes throughout the supply chain that lead to product availability at the fair.

Keywords: *Tua-mutin*, brandy, traditional beverage.

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