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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.

A palmeira *tua-tali* (*Corypha utan* Lam.) é uma espécie de planta produtora de seiva, onde a seiva é utilizada como matéria-prima para a produção de açúcar, vinagre e *tua-sabu*, entre outros. O estudo visa determinar o teor de álcool etílico do *tua-sabu*, uma bebida tradicional timorense. As amostras foram coletadas em diferentes municípios de Timor-Leste, incluindo coleta direta no local de produção, bem como disponibilidade em pequenas mercearias e feiras com um total de 39 amostras, compreendendo dezessete amostras no total para cada fração de *tua-ulun* e *tua-klaran* e cinco amostras da fração *tua-ikun*. A cromatografia líquida com detector UV foi utilizada para analisar as amostras, o resultado mostrou que as frações de *tua-ulun* coletadas diretamente no local de produção em cada município apresentaram maiores concentrações de álcool etílico entre 38,1 e 53,6% v/v. Em contraste, as frações coletadas em pequenos supermercados e feiras tinham concentrações de álcoois etílicos de 36,1 a 50,3% v/v e 30,0 a 41,5% v/v, respectivamente. Além disso, a maioria das frações de *tua-ulun* e *tua-klaran* apresentam diferentes e semelhantes concentrações entre si, e também algumas das frações de *tua-klaran* dos pequenos merceários e feiras têm concentrações mais altas que algumas frações de *tua-ulun* e/ou mais baixas de algumas frações de *tua-ikun*. Vale ressaltar que variações e similaridades no teor alcoólico são atribuídas principalmente a fatores na cadeia de produção, materiais de embalagem e suas condições higiênicas, processo armazenamento ao longo da cadeia de suprimentos que levam à disponibilidade do produto ao mercado.
Palavras-chave: *Tua-mutin*, aguardente, bebida tradicional.

1. INTRODUCTION

Corypha utan palm (*Corypha utan* Lam.), also known as *tua-tali* (a name given in *Tétum*, one of the official languages of Timor-Leste), is one of the palm species cultivated in tropical regions and widely distributed in Asian countries from southwest India to northern Australia. This palm tree grows and adapts well in open and low-lying areas, especially in coastal areas with medium and high temperatures [1, 2].

The palm is widely exploited in several countries due to its potential for multiple uses [3], it is well known among Asians for its efficient use in everyday life as a source of food, and some parts of the plant are used as construction material (used to make shelters or shade), fences, ropes, handicrafts (rugs, mats, drums, baskets), among others [4-6]. The inner rib fiber can be used as a raw material for polymer composites, with economic value and can be easily obtained in large quantities. In addition, it is commonly used for food production through its starches for producing bread, biscuits, and *akarbilan* (in *Tétum*), a food product derived from starch inside the palm tree trunk, among others [2, 7, 8].

The *tua-tali* palm species is considered the main source of sap production known as *tua-mutin* (in *Tétum*), alongside other palms such as *lontar palms/akadiru (Borassus flabellifer,* L.) [9]*,* sugar palm/*tua-metan* (*Arenga pinnata* Merr.) [10], and coconut/*nuu (Cocos nucifera* L.) [11]. Fresh sap may be for direct consumption or typically used as raw material for producing various products such as brandy known as *tua-sabu* (in *Tétum*), sugar, and vinegar, among others [4, 12]. The *tua-tali* sap collection process is carried out through the apical meristem cavity (Figure 1), using a technique known as the destructive extraction method [4].

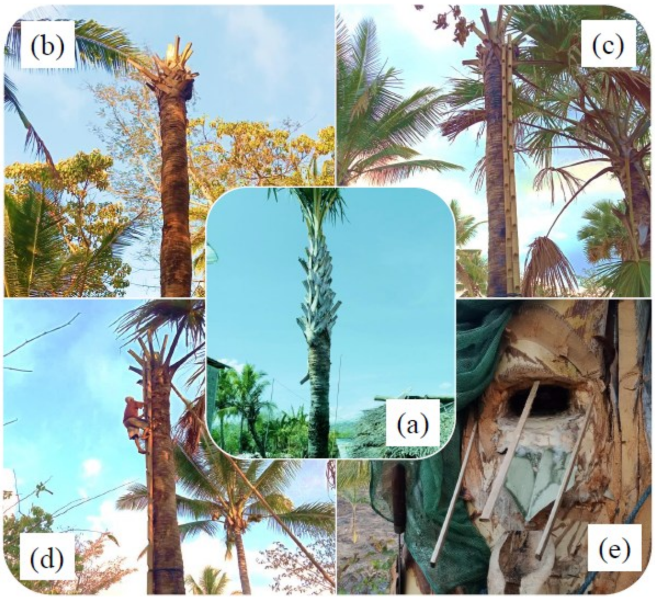

Figure 1: The process of preparing and extracting sap from the tua-tali palm species (Corypha utan Lam) in Timor-Leste. (a)Tua-tali palm selected (b)Remove or cut the stems (c)Preparation of the first cavity of the apical meristem (d) Install or route sap collection tube into cavity and cut daily to maximize sap flow (e) Sap extraction and collection.

This method involves tapping the tree at the apical meristem, which allows for collecting significant quantities of sap where producers stated that a tree could produce between 30 and 40 liters of sap per day, although Nguyen et al. (2016) [13], reported that some trees may produce up to 45 liters per day. The amount of sap collected is suitable for the production of *tua-sabu* brandy.

Fresh palm sap contains sucrose as the main ingredient at about 12 to 15% by weight, with a little reducing sugar, including proteins, fats, and minerals [14]. The composition and quality of palm sap may vary with the location, time, and duration of extraction [15, 16]. A higher amount of sugar in a food matrix is essential for the efficient production of ethyl alcohol [17, 18].

Lalel and Rubak (2024) [19], 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 for the production of brandies. There are very few references available on the study of chemical compositions, mainly the *tua-tali* palm sap, and particularly in Timor-Leste, there are still no previous studies on the subject.

In the context of Timor-Leste, the production of *tua-sabu* normally consists of steps such as sap extraction, fermentation, distillation, separation of the fraction for classification as head *'tua-ulun'*, heart *'tua-klaran'*, and tail *'tua-ikun'* (in *Tétum*), and its final packaging [4]. Each of these stages has its control measures, wherein in the sap extraction process, the droplets flow throughout the day, allowing for continuous collection.

The ethanol formation in the beverage results from a sugar metabolism process caused by microorganisms [18, 20, 21]. In the production of brandy-based palm sap, the fermentation process generally occurs immediately and naturally due to the presence of wild microorganisms present in the sap itself since its collection [13].

The study aims to reveal the concentrations of ethyl alcohol in each fraction consisting of *tua-ulun*, *tua-klaran,* and *tua-ikun* fractions collected from different regions, with samples being collected from three main points: directly at the production sites, small grocery stores, and fairs to monitor the quality throughout its production and supply chain. Since *tua-sabu* lacks a clear identity in terms of quality due to the absence of standards and regulations for its control, this is a step towards contributing to the establishment of standards and legislation to regulate the product in the market and domestic consumption in the future.

In this study, Liquid Chromatography with a UV detector was used to quantify ethanol, with precise amounts of an external standard solution introduced directly into the analyzer without requiring any pretreatment. Standard curves were established, defining detection and quantification limits, which facilitated the evaluation of standard deviation, the accuracy, and precision of the results.

2. MATERIALS AND METHODS

The methodology is based on all processes carried out in the production field and even laboratory analyses (Figure 2).

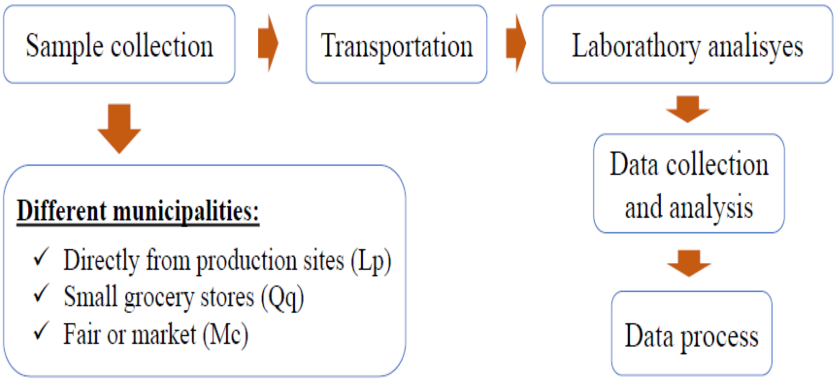

Figure 2: Sample collection and analysis flowchart.

2.1 Samples

The samples (*tua-sabu*) were recently produced locally, sold in small grocery stores and at fairs, and collected from various locations across Timor-Leste. The samples consist of three distilled fractions of *tua-ulun*, *tua-klaran*, and *tua-ikun*. These samples were sourced from different producers, regardless of production techniques or locations, with the selection based on higher production levels, market availability, and local consumption. Details can be found in Table 1.

Table 1: The samples (fractions of tua-sabu) were collected directly from production sites, small grocery stores, and fairs across various regions of Timor-Leste.

The origin of the sample of the *Tua-tali* palm species (*Corypha utan* Lam)							
No.	**Sample collection sites**				**Distillate fractions**		
	Municipality	**Collection site**					
		Lp	Qq	Mc	*Tua-ulun (Cu1)*	*Tua-klaran (Cu2)*	*Tua-ikun (Cu3)*
1	Baucau	3	3	3	3	3	3
2	Viqueque	2	-	2	2	2	-
3	Díli	-	2	2	2	2	-
4	Bobonaro	2	-	2	2	2	-
5	Oe-cusse	-	-	2	1	1	-
6	Covalima	3	2	3	3	3	2
7	Manufahi	-	-	2	1	1	-
8	Ainaro	-	-	2	1	1	-
9	Aileu	-	-	2	1	1	-
10	Ermera	-	-	2	1	1	-
Total		**10**	**7**	**22**	**17**	**17**	**5**
		39			**39**		

Legend: Palm species: **Cu** (*Corypha utan* Lam/*tua-tali* palm); Distilled fractions: **1** (*Tua-ulun*), **2** (*Tua-klaran*), and **3** (*Tua-ikun*), and Samples collection sites: **Lp** (production site), **Qq** (Small grocery store/Kiosk) & **Mc** (Fair/market).

2.1.1 Distillate fractions

The samples (*tua-sabu*) totaled 39 distilled fractions, whereas the *tua-ulun* and *tua-klaran* fractions totaled seventeen samples and *tua-ikun* totaled five samples. Among the fractions, 10 samples were collected directly from production sites, seven from small grocery stores, and 22 from fairs. Each sample location represents a set of three fractions: *tua-ulun*, *tua-klaran*, and *tua-ikun*.

2.1.2 Samples collected at different locations

The samples were collected directly from the production sites, small grocery stores, and fairs across different municipalities of Timor-Leste.

Among the 10 samples collected at production sites, four samples were acquired from municipalities that represent the production of *tua-sabu* from the origin of this palm. The municipalities of Baucau (Cu-Lp1) and Covalima (Cu-Lp6) represented three samples consisting of a fraction of the first (Cu1), second (Cu2), and third (Cu3) distillate for each municipality. In contrast, the municipalities of Viqueque (Cu-Lp2) and Bobonaro (Cu-Lp4) represented two samples composed of the first and second fractions of each municipality.

Additionally, the samples collected in small grocery stores were obtained from four municipalities, representing the viability of *tua-sabu* production from this palm species. The municipality of Baucau provided three samples, one from each fraction of *tua-ulun*, *tua-klaran*, and *tua-ikun*. Meanwhile, the municipalities of Díli and Covalima each provided two samples, consisting of the first and second distillate fractions, respectively.

A total of 22 samples, consisting of fractions of *tua-ulun*, *tua-klaran*, and *tua-ikun*, were collected at fairs across several municipalities. The municipalities of Baucau and Covalima each provided three fractions, consisting of the first, second, and third distillate fractions. Other municipalities, such as Viqueque, Díli, Bobonaro, Oe-cusse, Manufahi, Ainaro, Aileu, and Ermera, each provided two samples, consisting of the first and second fractions.

These samples were selected to account for variations in production, sales, and consumption frequency, allowing for the assessment of potential influences on product quality throughout the supply chain.

2.2 Calibration curve

Calibration of ethanol solutions at concentrations of 1.0 to 6.0 gL^{-1} were prepared using > 99% of ethyl alcohol. In this process, a quantity of ethyl alcohol was weighed for subsequent mixing with ultrapure water, to adjust the concentration between 0.1 and 6.0 gL^{-1}.

2.3 Ethyl alcohol determination

2.3.1 Sample preparation

The samples were previously centrifuged at 1075g for 2 minutes, the supernatant was collected and diluted 1:51, and then filtered using a Merck Millipore Express™ nylon filter with 0.22µm pore size, 20µL of the filtrate was collected, and then injected into liquid chromatography.

Ethyl alcohol concentrations of all analyzed samples were obtained as the average of triplicate analyses of each sample.

2.3.2 Liquid Chromatography Analysis

The analysis was performed at the Biochemical Engineering Laboratory of the School of Agronomy, Federal University of Goiás - UFG. High-Performance Liquid Chromatography (HPLC) was employed to quantify ethyl alcohol in *tua-sabu*. After sample preparation as described in item 2.3, a chromatograph Shimadzu, model Prominence, 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 a mobile phase flow of 5mM perchloric acid at 0.600 $mL.min^{-1}$. All standards used had a purity greater than 99%. Compounds were based on calibration curves constructed with five standard concentration points. The analysis considered retention time, detection limit, quantification limit, concentration range, and correlation coefficients of the calibration curves. To ensure accuracy, all samples were analyzed in triplicate.

2.4 Data analysis

Statistical analyses were performed using Microsoft Office Excel Professional Plus 2016. The results were expressed as the mean percentage of ethyl alcohol content (v/v), including the standard deviation, with the significance level set at <0.05.

3. RESULTS

A total of 39 samples analyzed for the present study were composed of 17 fractions of *tua-ulun*, 17 of *tua-klaran,* and 5 of *tua-ikun*.

3.1. 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 (Cu1-Lp1) contains the highest concentration of ethyl alcohol at 53.6% v/v, followed by those gathered in the municipalities of Covalima (Cu1-Lp6), Viqueque (Cu1-Lp2) and Bobonaro (Cu1-Lp4) with a percentage of ethyl alcohol of 45.6, 41.5 and, 38.1% v/v, respectively (Table 2). The fractions of *tua-klaran,* such as Cu2-Lp1, Cu2-Lp2, Cu2-Lp4, and Cu2-Lp6, had respective ethyl alcohol contents of 40.4, 34.3, 20.7, and 25.3% v/v. Furthermore, two fractions of *tua-ikun* (Cu3-Lp1) and (Cu3-Lp6) showed concentrations of 21.5 and 17.2% v/v, respectively (Table 2).

Table 2: Ethyl alcohol content of the tua-sabu fractions obtained directly from production sites (Lp). The value in the table was the mean of three replicates ± Standard Deviation (SD).

Sample collection sites (Municipality)	Sample code	Distilled Fraction	% (v/v)
Baucau	Cu1-Lp1	*Tua-ulun*	53.6 ±0.01
	Cu2-Lp1	*Tua-klaran*	40.4 ±0.00
	Cu3-Lp1	*Tua-ikun*	21.5 ±0.01
Viqueque	Cu1-Lp2	*Tua-ulun*	41.5 ±0.03
	Cu2-Lp2	*Tua-klaran*	34.3 ±0.10
Bobonaro	Cu1-Lp4	*Tua-ulun*	38.1 ±0.00
	Cu2-Lp4	*Tua-klaran*	20.7 ±0.00
Covalima	Cu1-Lp6	*Tua-ulun*	45.6 ±0.01
	Cu2-Lp6	*Tua-klaran*	25.3 ±0.05
	Cu3-Lp6	*Tua-ikun*	17.2 ±0.00

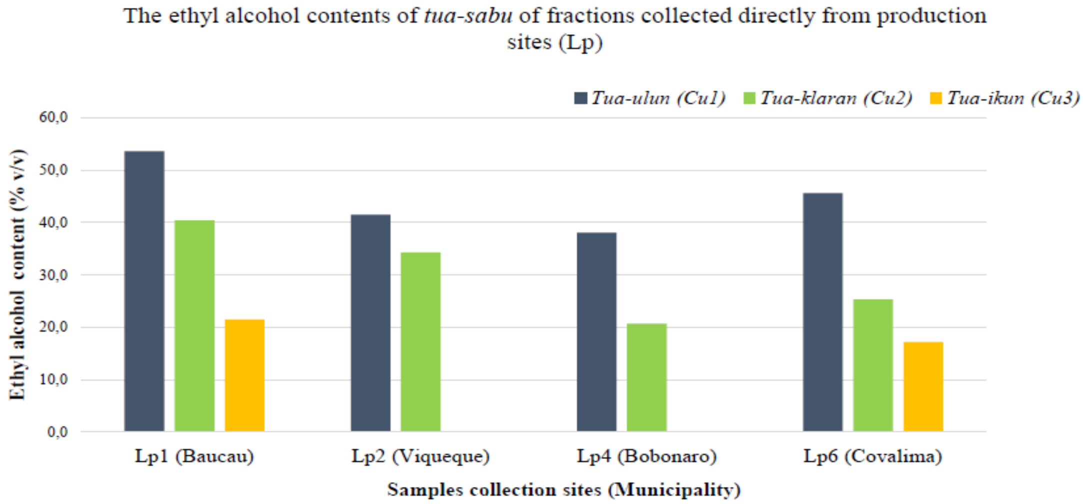

Figure 3: Ethyl alcohol content of the tua-sabu fractions obtained from production sites (Lp).

Among the fractions of each municipality of *tua-ulun*, *tua-klaran*, and *tua-ikun*, there was a significant difference in ethyl alcohol levels (Figure 3). Furthermore, when comparing the fractions from one region to another, differences in alcoholic content were also observed.

For example, the *tua-klaran* fraction (Cu2-Lp4) presented a lower concentration than the other fractions of the same type and was even lower when compared to a *tua-ikun* fraction (Cu3-Lp1). It is worth considering that the variation in alcohol content is likely influenced by factors related to the manufacturing process. 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.

3.2 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 (Cu1-Qq1, Cu2-Qq1, and Cu3-Qq1) 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 (Cu1-Qq3, and Cu2-Qq3) and Covalima (Cu1-Qq6, and Cu2-Qq6) presented ethyl alcohol concentrations of

41.3 and 23.0% v/v, and 36.1 and 29.5% v/v respectively. Additionally, a single *tua-ikun* fraction was collected in a small grocery store in Baucau, containing 16.4% v/v of ethyl alcohol (Table 3).

Table 3: Ethyl alcohol content of the tua-sabu fractions obtained from small grocery stores (Qq). The value in the table was the mean of three replicates ± Standard Deviation (SD).

Sample collection sites (Municipality)	Sample code	Distilled Fraction	% (v/v)
Baucau	Cu1-Qq1	*Tua-ulun*	50.3 ±0.00
	Cu2-Qq1	*Tua-klaran*	31.9 ±0.01
	Cu3-Qq1	*Tua-ikun*	16.4 ±0.00
Dili	Cu1-Qq3	*Tua-klaran*	41.3 ±0.01
	Cu2-Qq3	*Tua-klaran*	23.0 ±0.21
Covalima	Cu1-Qq6	*Tua-ulun*	36.1 ±0.01
	Cu2-Qq6	*Tua-klaran*	29.5 ±0.00

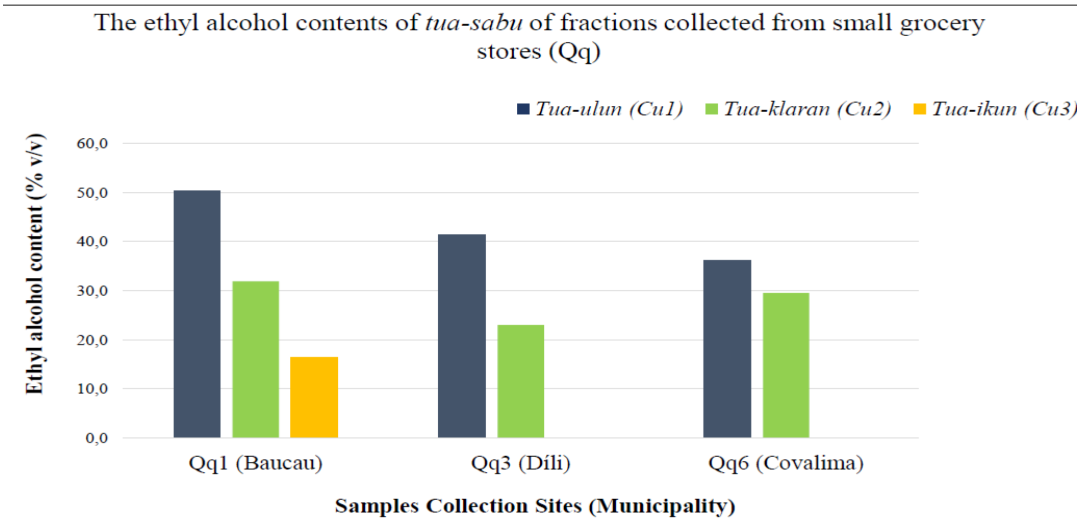

Figure 4: Ethyl alcohol content of the tua-sabu fractions obtained from small grocery stores.

The highest concentration of ethyl alcohol in the first fraction (*tua-ulun*) was found in a sample from the municipality of Baucau (Cu1-Qq1), which contained 50.3% v/v of ethyl alcohol, while the lowest concentration was found in a fraction from the municipality of Covalima (Cu1-Qq6), containing 36.1% v/v (Figure 4). The highest levels of ethyl alcohol in the second fraction (*tua-klaran*) were also found in a sample from Baucau (Cu2-Qq1), with an ethyl alcohol percentage of 31.9% v/v, while the lowest was found in a fraction collected in the municipality of Díli (Cu2-Qq3), containing 23.0% v/v of ethyl alcohol (Figure 4).

It should be noted that the fractions collected in the same place presented significantly different alcoholic concentrations. When observing the same fractions collected in several locations, there is also a large difference in their alcohol content.

3.3 Fractions collected in the fairs

The *tua-ulun* fractions with high concentrations of ethyl alcohol, collected from the fairs, were observed in the municipalities of Baucau (Cu1-Mc1) and Dili (Cu1-Mc3), with percentages of 41.5 and 41.6% v/v, respectively (Table 4). This was followed by samples from the municipalities of Manufahi (Cu1-Mc7), Aileu (Cu1-Mc9), Ainaro (Cu1-Mc8), Covalima (Cu1-Mc6), Ermera (Cu1-Mc10), Oe-cusse (Cu1-Mc5), Viqueque (Cu1-Mc2), and Bobonaro (Cu1-Mc4). 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. The lowest concentration, 18.2% v/v, was found in the sample (Cu2-Mc5) from the municipality of Oe-cusse, while the highest concentration was found in the samples collected in the municipalities of Baucau (Cu2-Mc1) and Díli (Cu2-Mc3), which contained 41.5 and 41.6% v/v of ethyl alcohol, respectively (Table 4).

Table 4: Ethyl alcohol content of tua-sabu fractions obtained from the fairs (Mc). The value in the table was the mean of three replicates ± Standard Deviation (SD).

Sample collection sites (Municipality)	**Sample code**	**Distilled Fraction**	**% (v/v)**
Baucau	Cu1-Mc1	*Tua-ulun*	41.5 ±0.00
	Cu2-Mc1	*Tua-klaran*	21.4 ±0.01
	Cu3-Mc1	*Tua-ikun*	19.5 ±0.00
Viqueque	Cu1-Mc2	*Tua-ulun*	30.8 ±0.00
	Cu2-Mc2	*Tua-klaran*	24.0 ±0.00
Dili	Cu1-Mc3	*Tua-ulun*	41.6 ±0.03
	Cu2-Mc3	*Tua-ulun*	21.4 ±0.08
Bobonaro	Cu1-Mc4	*Tua-ulun*	30.4 ±0.00
	Cu2-Mc4	*Tua-klaran*	23.6 ±0.00
Oe-cusse	Cu1-Mc5	*Tua-ulun*	33.0 ±0.00
	Cu2-Mc5	*Tua-klaran*	18.2 ±0.76
Covalima	Cu1-Mc6	*Tua-ulun*	35.7 ±0.00
	Cu2-Mc6	*Tua-klaran*	23.1 ±0.00
	Cu3-Mc6	*Tua-ikun*	19.7 ±0.00
Manufahi	Cu1-Mc7	*Tua-ulun*	38.1 ±0.11
	Cu2-Mc7	*Tua-klaran*	22.7 ±0.00
Ainaro	Cu1-Mc8	*Tua-ulun*	37.4 ±0.61
	Cu2-Mc8	*Tua-klaran*	19.5 ±0.01
Aileu	Cu1-Mc9	*Tua-ulun*	37.6 ±0.01
	Cu2-Mc9	*Tua-klaran*	23.9 ±0.00
Ermera	Cu1-Mc10	*Tua-ulun*	35.3 ±0.01
	Cu2-Mc10	*Tua-klaran*	20.6 ±0.00

Overall, the fractions collected from various fairs and municipalities have different ethyl alcohol contents, following a descending order from the *tua-ulun* to the *tua-klaran* and *tua-ikun* fractions. Among all *tua-ulun* fractions, Cu1-Mc1 and Cu1-Mc3 have higher ethyl alcohol concentrations, while Cu1-Mc5 contains lower levels. It can be observed that, among the *tua-ulun* fractions, there are differences and similarities in alcohol concentrations (Figure 5).

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 (Figure 5).

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 (Cu2-Lp1), 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.

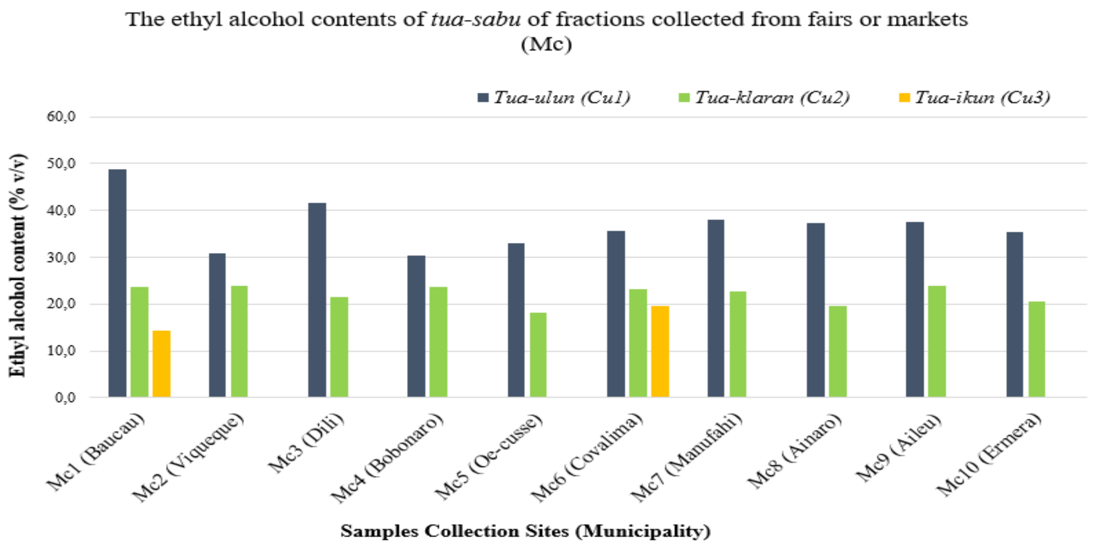

Figure 5: Ethyl alcohol content of the tua-sabu fractions obtained from the fairs.

The other *tua-klaran* fractions, such as Cu2-Lp2 and Cu2-Qq2, 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 Cu2-Lp6 < Cu2-Qq6 and Cu2-Lp4 < Cu2-Mc4, 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 (Figure 6). 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, Cu3-Lp1 (Baucau) and Cu3-Mc6 (Covalima), contained ethyl alcohol concentrations similar to the levels found in the *tua-klaran* fractions collected directly from the production site, such as Cu2-Lp4 from Bobonaro municipality, and in the fractions collected at fairs, including Cu2-Mc3 (Díli), Cu2-Mc5 (Oe-cusse), Cu2-Mc8 (Ainaro), and Cu2-Mc10 (Ermera) (Figure 6).

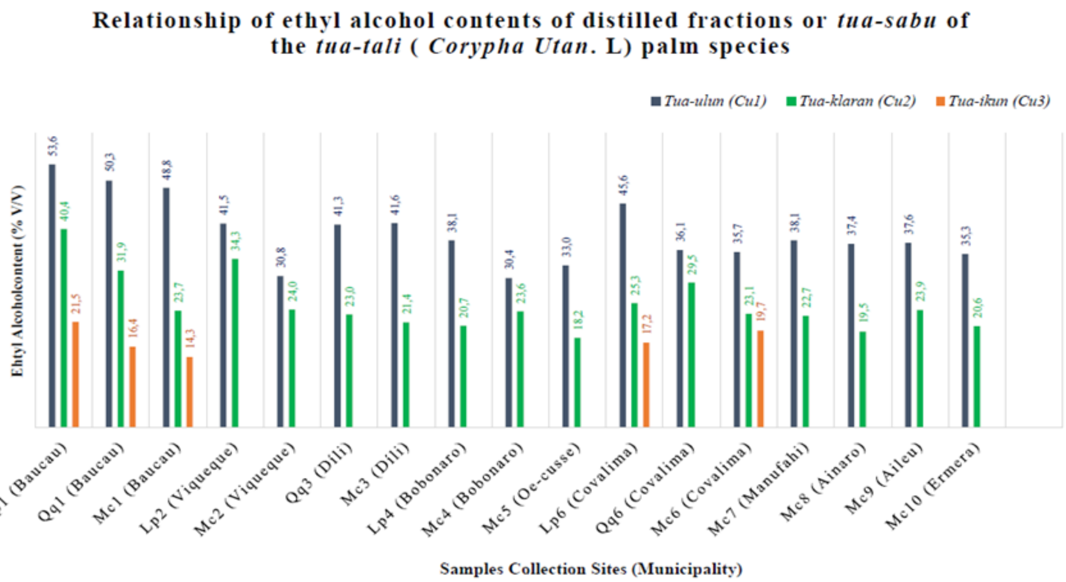

Figure 6: Relationship of the ethanol content of the tua-sabu fractions of the tua-tali (<u>Corypha utan</u> Lam.) palm species.

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.

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 7). 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.

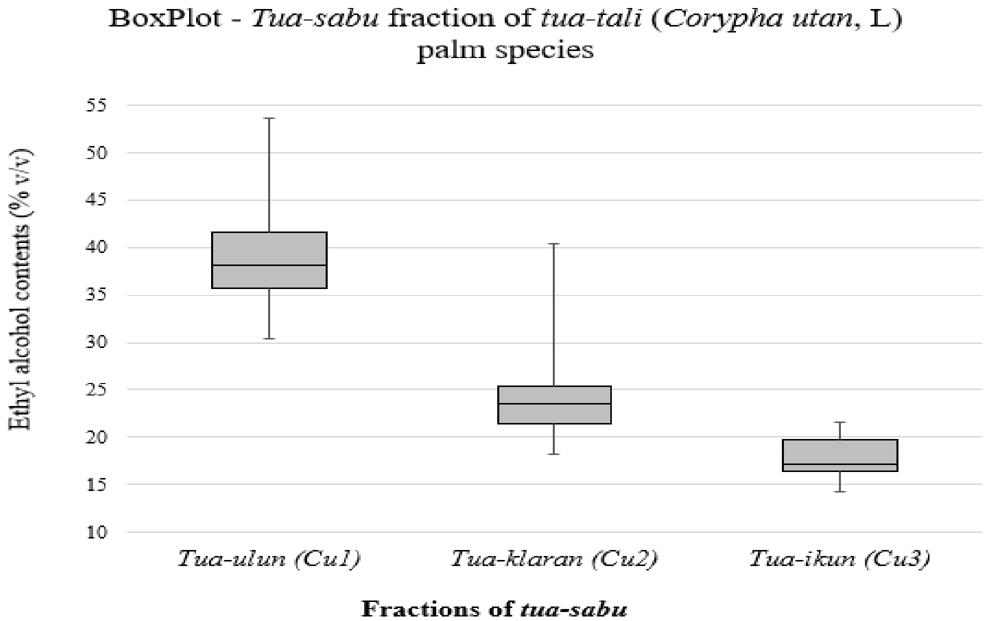

Figure 7: The BoxPlot of tua-sabu fractions of tua-tali (Corypha utan Lam.) palm species.

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.

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 according to Brazilian brandy law, only fractions with percentages ranging from 38.1, and 53.6% v/v, which 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 the establishment of Brazilian legislation allows cachaça obtained exclusively from sugarcane juice may contain 38 to 48% v/v, liquor 15 to 54% v/v, and brandy may contain 38 to 54% v/v of ethyl alcohol at 20 Celsius [22].

4. DISCUSSION

The production process of brandy or *tua-sabu* essentially comprises the following stages: preparation and sap extraction, fermentation (which generally occurs naturally or spontaneously), distillation, separation and collection of fractions, bottling, and distribution for sales. The results showed significant variations in the alcohol content of the sample fractions, whether they were the same or different, including those from the same or different collection regions. Thus, the following factors can be considered the main influences on the variations in the quality of *tua-sabu* produced in the country.

4.1 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 experience of each producer and the geographical location where it is produced [16, 23]. 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 [4]. This variation in techniques and materials consequently affects the quantity and quality of sap produced.

In brandy production, 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. 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 [4]. 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 the fermentation time, preventing microorganisms from acting uniformly and efficiently in the process of converting sugar into ethanol.

4.2 Influence of the fermentation process

In the fermentation process, microorganisms assume their roles by converting sugars into ethyl alcohol, water, and CO_2 [24]. Naknean et al. (2010) [16] stated that 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 [25].

Palm sap is an excellent substrate for the growth of microorganisms for fermentation within an hour or two of collection [26]. After some time, the sap begins to form foam as a result of fermentation, and its sweet taste becomes bitter with a noticeable alcohol content. The sap can be consumed fresh or used as raw material for brandy production.

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 [18]. 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 [27], consequently reducing the yield of ethyl alcohol produced.

In a study carried out by Alzeer and Abou Hadeed (2016) [28], on the temperature and time conditions for the fermentation process of fresh grape juice, the result showed that the formation of ethyl content increased significantly when the juice was kept for a day, and the ethyl alcohol content can still increase drastically when kept for more days, up to about 10 days at the same temperature condition. 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 [28].

The fermentation process used by the Timorese to produce *tua-sabu* is conducted entirely naturally or spontaneously by native microorganisms present in the sap without adding any ingredients such as strains or yeast [4]. One of the factors affecting the quality of *tua-sabu* is the fermentation process, which is carried out without proper control, especially regarding time standards.

A process is conducted entirely in open spaces without environmental control, which can cause imbalances in the performance of the fermentation process [29]. Furthermore, some parameters, such as hygiene conditions or cleanliness of the sap collecting materials or collectors used for the sap store, are essential for the control process at this stage [16, 30]. Thus, similar to the variations in sap collection mentioned above, which consequently affect the time of the fermentation process, this can also be considered one of the factors affecting the quality of the brandies produced.

One of the issues may be related to the use of the plant's peels as a natural ingredient added during the fermentation period. According to the producers, the purpose of adding the peel is to confer color, flavor, and aroma. However, the presence of an ingredient without any study on its safety can have a negative effect due to its potential toxicity, as it can act as an antimicrobial, interfering with and delaying the fermentation process, reducing the yield of the final product, or forming other unwanted compounds [4]. In some regions of Africa, wood bark, leaves, or lime are used as natural antimicrobial agents, generally added to the sap collection container to prevent the immediate action of microorganisms after sap collection [31].

4.3 Distillation and separation of fractions processes

In the production of *tua-sabu*, Timorese producers use different methods, particularly in the materials for distillation structures. These include distillers of various types, usually made of iron and aluminum, as well as earth-based pots in different shapes and sizes, including distillation procedures that also vary slightly between producers and regions. Other components of the distilleries, such as the size and length of the tubes used, also differ significantly [4]. The differences are often related to producers' experiences, procedures, and availability of materials in their distillation system [32].

The cooking techniques used by Timorese producers are generally carried out in open spaces and use firewood as an energy source to provide 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 [4].

According to Liebminger et al. (2021) [33], 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 [34].

In the process of collecting the final distillate, cutting the fractions is essential to define the difference in the desired alcoholic strength, generally classified into fractions such as head, heart, and tail [35, 36], which is the case of the production of Brazilian cachaça from sugarcane juice, representing 5 to 10% for the first distillate, 80 to 85% for the second fraction and 10 to 15% for the last fraction of the distillate [37, 38].

In the production of *tua-sabu*, fraction cuts are generally made into three fractions: *tua-ulun*, *tua-klaran,* and *tua-ikun*. The fraction cut depends on each producer's experience. With total distillate collected is around 10 to 15% of the boiler volume. The total distillate is further separated into three parts: the first collection or *tua-ulun* represents 20 to 25%, the second fraction about 50 to 60%, and the last collection fraction about 20 to 25% of the final volume of the distillate, respectively [4].

Based on the results obtained for each distilled fraction of all analyzed samples, the alcohol content varies significantly from one region to another, even for the same fraction, as shown in Table 2. Furthermore, some *tua-ulun* fractions had concentrations similar to certain *tua-klaran* fractions, as observed in Figure 3. 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 [39]. Therefore, this step requires a standard as a reference point and a minimum measurement of concentrations [40]. This would help avoid large differences in alcohol levels between the same fractions from a region or producer, and also between different regions across the country. It would also prevent variations in alcohol levels between the *tua-ulun*, *tua-klaran*, and *tua-ikun* fractions.

4.4 Bottling process and fractions combination

Product bottling is a fundamental process that initiates the placing of a product for sale and consumption [41]. Furthermore, one of the most important unit operations in the formulation of commercial products is to ensure quality during storage, transportation, sale, and consumption [42, 43].

Brandies produced in Timor-Leste are mostly stored in plastic containers from the beginning of the collection of distillate fractions until its intended for sale. These containers are plastic bottles, as well as polyethylene terephthalate (PET) bottles, mineral water packaging, and high-density polyethylene (HDPE) gallons for cooking oil packaging. Furthermore, to prevent leakage, the lids are always sealed with low-density polyethylene (LDPE) plastic [4]. They 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 [44, 45].

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 [46-48].

On the other hand, considering the increasing interest of consumers and the demand for goods, most products entering the fair have their fractions often mixed. For example, a fraction of *tua-ulun* may be combined with *tua-klaran* and/or *tua-ikun* [4]. This can cause changes in the products, bringing the concentration of ethyl alcohol closer from one fraction to another, as can be seen in the results of the fractions listed in Table 2, and Table 3.

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. On the other hand, the first and last fractions are discarded or can be used for another purpose [49].

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 [50].

The *tua-sabu* fractions presented ethyl alcohol concentrations that varied depending on the fractions and collection sites. Most *tua-ulun* fractions presented concentrations between 30.4 and 53.6% v/v, *tua-klaran* fractions presented concentrations between 18.2 and 40.4% v/v, while *tua-Ikun* fractions presented concentrations between 14.3 and 21.5% v/v. It is noted that some fractions that present concentrations within the 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 6). 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 lack of a national quality standard to regulate and control the national production of products, including *tua-sabu* and their circulation in the markets, thus continuing to allow the majority of consumers continue to seek stronger and more concentrated fractions, mainly the *tua-ulun* fraction or the combination of the *tua-ulun* and *tua-klaran* fractions. In addition, weak fractions, such as the combination of the *tua-ulun* and *tua-ikun* fraction or the *tua-klaran* and *tua-ikun* fraction, are also intended for normal consumption without realizing the health consequences.

Alcarde et al. (2010) [51], reported that the head fraction of brandy contains alcohol-soluble compounds that have a low boiling point, such as methanol, including 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 water or water-soluble, 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.

4.5 Product supply chain and its storage conditions

After processing or manufacturing a product, steps such as distribution, transportation, and sale follow, and each step has its own storage conditions. Distribution, transportation, and sales are among the stages that require maximum attention and care in storage related to environmental aspects [44, 48]. 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 [52].

The distribution of *tua-sabu* involves various forms of transportation and sales, which are carried out freely without any control, particularly regarding environmental conditions. This can lead to chemical and physical changes, causing oxidation and contamination from packaging materials due to exposure to sunlight. These factors may contribute to a reduction in alcohol levels and the formation of undesirable compounds in the product. According to Fontoura et al. (2016) [53], physical and microbiological contamination can occur during distribution and transportation, altering the quality of the products.

5. CONCLUSION

The present study determined the ethyl alcohol content of different fractions of *tua-sabu*, consisting of *tua-ulun*, *tua-klaran*, and *tua-ikun*, exclusively from the *tua-tali* palm species, originating from various regions of Timor-Leste. The recently produced fractions, mainly *tua-ulun*, demonstrate higher levels of alcohol content in sequence, for the fractions sold in small grocery stores and fairs. The qualities of the products demonstrate a representative identity of each region, regardless of technical differences in production, and how the products are transported and reach the market. Overall, the results demonstrate a noticeable change in concentration reductions throughout the sale, therefore, this could be an opportunity to establish a national standard exclusively to control and regulate the availability of products, contributing to the improvement of the production and supply chain, thus ensuring the product quality and public health. Furthermore, it is worth highlighting that it is necessary to improve the processes involved in the production and supply chain, avoiding mixing fractions to guarantee the quality and safety of the products, making them more competitive, and enabling their entry into international trade. Finally, it is suggested that future work investigate other quality parameters that ensure that products meet the minimum quality standard required and contribute to enriching information on product identity to establish legislation in the future.

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