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Production, physicochemical, microbiological and nutritional analysis of the liquor obtained from the peelings of *annas Comusus* reinforced by the root extract of *Mondia whitei* harvested in Benin

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Abstract In Benin, *Ananas Comosus* co-products are often left in wild dumpsites for flies and mosquitoes and are a source of foul odour. The main objective of this study is to valorize the co-products of the *Ananas Comosus* processing industry through the formulation of the liquor reinforced with bioactive substances. To do this, the secondary metabolites of the *Mondia whitei* root were identified by staining and precipitation reactions specific to each metabolite. After the elaboration of the liquor, the organoleptic and physicochemical properties of this product were determined. The contents of phenolic compounds in the liquor were evaluated by spectrophotometric assay. The quantification of its mineral salt content was carried out by atomic absorption spectrophotometry. Finally, the microbiological quality of the formulated product was determined according to ISO standards. From the results obtained, it appears that the root of *Mondia whitei* is rich in secondary metabolites. The pH of the formulated liquor is 4.75 and its soluble solids content is 15°Brix. Total phenol concentrations; in flavonoids and in condensed tannins are respectively 11.31 mg GAE/mL; 15.07 µg QE/mL and 34.43 mg LeucE/mL. The resulting liquor is rich in mineral salts (P, K, Ca, Mg, Na, Fe and Mn); its alcohol content is 45%. From a microbiological point of view, it is free of any pathogenic germ and meets the normative requirements. From the sensory analysis, it is well appreciated by all the tasters (>73%).

Keywords: *Ananas Comosus*; secondary metabolites; liquor, analyses; *Mondia whitei*

1. Introduction

Ananas Comosus is the second tropical fruit in world trade and contributes more than 20% to the world production of tropical fruits with 17 million tons [1]. In West Africa, Benin is the second largest producer of *Ananas Comosus* with 160,000 tons [1]. In 2015, Benin's share in world *Ananas Comosus* production was estimated at 315,795 tons, or 1.27% of world production. It contributes about 1.2% of GDP, and 4.3% of agricultural GDP [2]. Releases from the fruit processing industry are obtained every year in significant quantities all over the world [3]. *Ananas Comosus* canning industry generates 25 to 35% of waste per day [4]. One ton of fresh *Ananas Comosus* produces about 400 kg of waste, the pressing of which gives 260 to 300 liters of press juice [5]. Unfortunately in Benin, these rejects are not recycled and are often piled up in uncontrolled dumps located near processing centers [6]. Recent studies have been oriented towards the recovery of these fruit processing rejects. It appears that fruit wastes containing

fermentable sugars can no longer be abandoned in our environment, but they should be converted into useful food products such as wine, vinegar, ethanol, liquor etc.[7]. In Benin, the liquor is not only used during major festivals, ritual, religious and family ceremonies, but also on a daily basis as a sign of hospitality and respect. A good mastery of the transformation of *Ananas Comosus* by-products into a food drink would constitute an important issue, not only in the protection of the environment, but also at the socio-economic level. Despite this socioeconomic importance, little research has been conducted on the processing of *Ananas Comosus* suckers. It is in this logic that the present work aims at the valorization of the co-products of *Ananas Comosus* through the elaboration of the liquor reinforced by the active principles of *Mondia whitei*.

2. Materials and Methods

2.1 Materials It is made up of *Mondia whitéi* root harvested in pobè (southern Benin) and co-products of *Ananas Comosus* collected in Akassato (southern Benin). The yeast strain used is "*Saccharomyces cerevisiae*" purchased on the local market, made in China, under the Angel brand.

2.2 Methods

2.2.1 Preliminary phytochemical screening

Secondary metabolites were carried out by coloration and precipitation reactions specific to each family of metabolites [8], [9], [10]

Table 1: Methods for identification secondary metabolites of *Mondia whitéi* root

Secondary metabolites	Chemical test
Alkaloids	Mayer's test and Dragendroff's test
Anthocyanes	test with hydrochloric acid and ammonia
Anthraquinones	Borntranger's test
Coumarins	Fluorescence at 365 nm
Flavanoids	shinoda test and magnesium powder
Tannins	stiasny test, Ferric chloride and sodium acetate test
Saponins	Frothing test
Leuco anthocyanins	Bate-Smith and metcalf
Mucilages	flaky test
Cyanogenic derivatives	picric acid test
Reducing compound	Fehling's test
Sterols and terpenes	Liebermann-Burchard's test
proteins	Biuret test

2.2.2 Technological diagram for making *Ananas Comosus* liquor

The liquor was obtained according to the method written by CEBENOR [11] and Hêdiblè et al. [12]. Figure 1 below shows the production technology diagram of *Ananas Comosus* liquor.

2.2.3 Determination of phenolic compounds

- **Total phenol content**: Total phenolic content was determined using the Folin-Ciocalteu colorimetric method[13] with some modifications. This method consisted on using a mixture of phosphotungstic and phosphomolybdic acids, which were reduced during the oxidation of phenols into a mixture of tungsten blue oxide and molybdenum. Finally, the absorbance was measured at 760 nm using a spectrophotometer and the total phenol content are expressed in micrograms of gallic acid equivalence per mL (µgGAE/mL)[13], [14], [15].
- **Total flavonoids content**: The method of aluminum trichloride ($AlCl_3$) was used to quantify total flavonoids. This technique was based on the formation of aluminum complex flavonoids. The absorbance was read at 415 nm using a spectrophotometer and total flavonoid content are expressed in micrograms quercetin equivalence per mL (µgQE/mL)[16] .
- **Condensed tannin content**: Vanillin and hydrochloric acid method was used to determine total condensed tannins content. The absorbance was measured at 500 nm using spectrophotometer and tannin content was expressed in micrograms catechin equivalence per mL (µgEC/mL) [17].

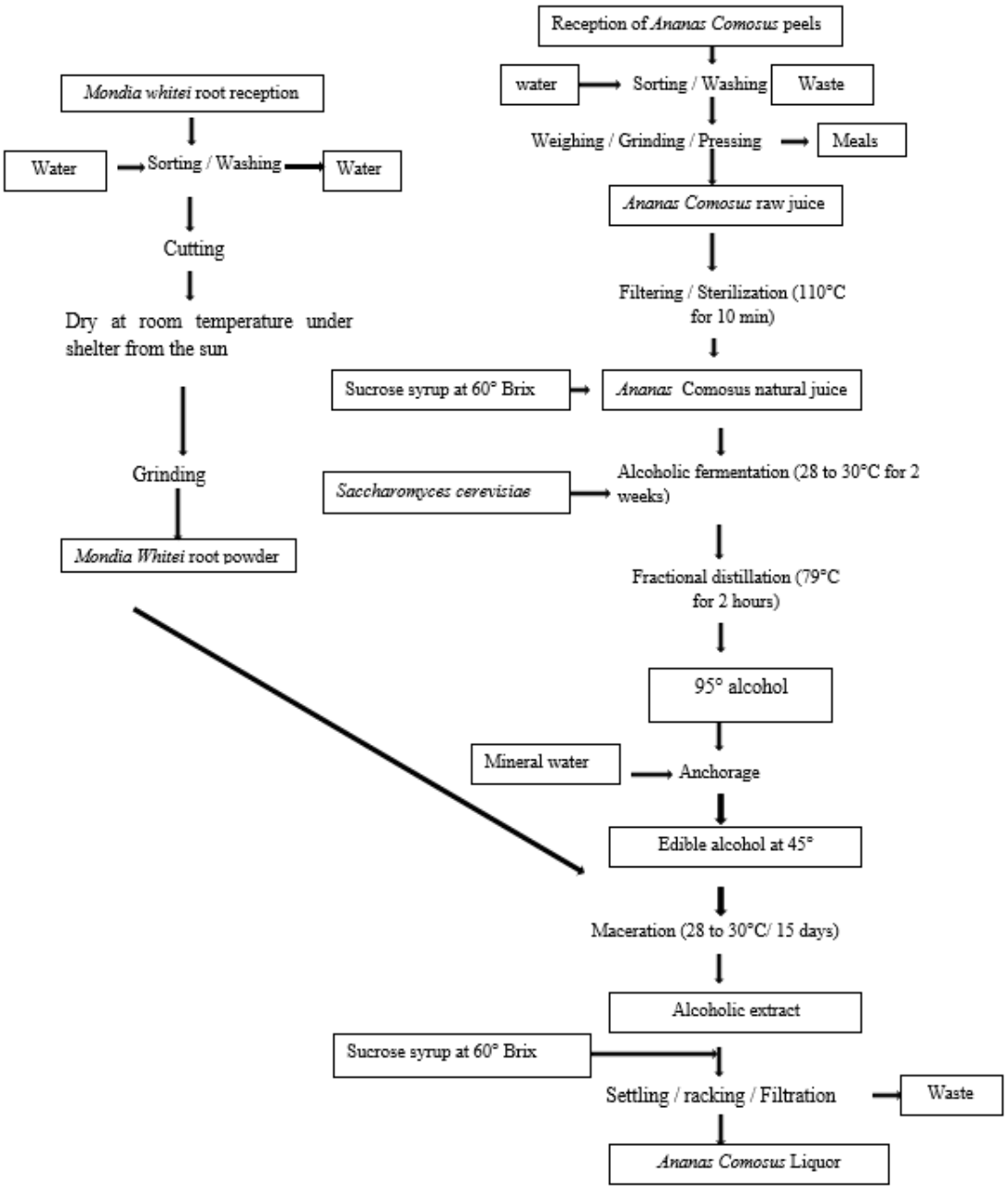

Figure 1: Technological diagram for making Ananas Comosus liquor

2.2.4 Minerals and Trace Elements Content: 5 grams of the sample were cremated following the procedure indicated above. The ash obtained was dissolved in 5 ml of hydrochloric acid (6N) and then evaporated on a hot plate at 125°C. The resulting residue was dissolved and recovered with HNO_3 (0.1M) in a 100 mL flask. This solution was used to obtain the content of the minerals by spectrophotometer Atomic Absorption. The results are expressed in relation to the dry matter [18].

2.2.5. Physicochemical analysis of formulated liquor: The pH was determined using a portable digital pH meter. The titratable acidity, expressed as percentage of citric acid, was determined by titration with NaOH (0.1N) in the presence of phenolphthalein. The soluble dry extract (°Brix) was determined using a portable refractometer of type (HI 96801 of 0-80° Brix. The relative density at 20°C was determined using a pycnometer according to the method

described by Massengo et al[19].The dry matter was determined by drying in an oven at 105°C according to the recommended method [20].The minerals were assayed by Atomic Absorption Spectrophotometry [18].

2.2.6. Microbiological analyzes: The microbiological quality of *Ananas Comosus* liquor was assessed through the determination of mesophilic aerobic flora, coliforms and yeasts and molds. 1 mL of the sample to be analyzed was taken aseptically to which 9 mL of sterile saline peptone water was added. The mixture was vortexed for 2 minutes, serving as the stock solution. Successive tenfold dilutions were prepared for seeding Petri dishes. The total aerobic mesophilic flora was counted using PCA agar (Plate Count Agar) at 30°C for 72 h. 1 mL of each of the dilutions as well as the stock suspension was introduced into different sterile Petri dishes. Then, 20 mL of the agar was poured into the contents of the Petri dishes. The whole was gently homogenized so as to incorporate the inoculum into the agar. After solidification, the agar is poured again to make a second layer. The count of germs was done according to the ISO 6222 1999 standard. The count of coliforms was carried out using VRBA (Violet Red Bile Agar) medium. 1 mL of each dilution was inoculated into a double layer of 10 to 15 mL of VRBA agar then incubated after solidification of the medium at 37°C for 72 h according to the ISO 21528-2: 2008 standard. Yeasts and molds were were counted following incubation of 1 mL of each dilution on Malt Extract Agar (MEA, oxoid, CM 325, Hampshire, England) at 25°C for 72 hours. The count of germs in CFU/mL of samples analyzed was done according to the ISO 21527-1: 2008 standard.

2.2.6. Sensory analysis: Sensory tests were carried out at the end of production. From a panel of tasters, it was determined and evaluated the evolution of the sensory characteristics of the liquor produced according to the hedonic method [21]. The panel of tasters is made up of eighteen (18) people of different sexes and from all social categories, chosen from among the usual consumers of this product. The descriptors or attributes to consider are: color, flavor, aroma, smell, texture, bitterness, viscosity, taste and general impression.

3. Results & Discussion

3.1. Secondary metabolites identified from the root of *Mondia whitéi*

The secondary metabolites of *Mondia whitei* are listed in Table 2. The root of *Mondia whitei* contains tannins, alkaloids, anthraquinones, saponosides, coumarins, flavonoids and reducing compounds. In the bark sample of *Mondia whitéi* collected in Cameroon, reducing compounds, sterols and terpenes have been identified but note the absence of flavonoids [22]. At the level of *Mondia whitéi* harvested in Zagnanando (Benin), tannins, flavonoids, alkaloids, coumarins and the absence of mucilage were identified[23]. In the sample of *Mondia whitéi* collected in Congo, we note the presence of flavonoids, reducing compounds, saponins and tannins[24]. The variation in secondary metabolites observed in our samples compared to previous work could be related to the harvest period, the nature of the soil or climatic factors[25][26]. The presence of metabolites in the root of *Mondia Whitéi* could give the formulated liquor antioxidant, vasculoprotective, antiallergic, anti-inflammatory, antidiarrheal, antimalarial properties[27], .[28], [29], [30], [31], [32], [33], [34].

Table 2: Methods for the identification of secondary metabolites of root of *Mondia whitéi*

Secondary metabolites	Present/Absent
Alcaloids	+
Tannins	-
Flavonoids	+
Anthocyanes	-
Leuco anthocyanins	+
Reducing compound	+
Mucilages	-
Coumarins	+
Cyanogenic derivatives	-
Saponosids	+
Anthraquinones	+
Sterols and terpenes	-

Legends: + : Present ; - : Absent

3.2. Physicochemical characteristics of *Ananas Comosus* liquor

Table 3 presents the values of the physicochemical parameters of *Ananas Comosus* liquor. According to this table, the pH, specific gravity and dry matter of pineapple liquor are respectively 4.75; 0, 97 and 12.22%. The titratable

acidity content of the liquor is 6.40 mg Eac/mL, with an alcohol content of 45% and a soluble solids content of 15° Brix. The pH of the formulated liquor is higher than that obtained on the red grape liquor in Portugal, which varies from 3.68 to 3.81[35]. The titratable acidity content of the liquor produced is well above that reported with the liquor obtained from cashew apple, the contents of which vary respectively from 0.6 to 1.0 mg Eac/mL [12]. The dry matter and soluble solids contents of the liquor produced are lower than those obtained by[12], whose values vary respectively from 6.65 to 24.69% and from 9.2 to 31.6° Brix. However, these values are lower than those obtained by Ferreira et al [35] ranging from 34.44 to 34.85% for dry matter and from 36.5 to 36.7 °Brix for soluble solids. The differences observed in these physicochemical parameters could be due to the degree of maturity of the raw material used.

Table 3: Physicochemical characteristics of *Ananas Comosus* liquor

Parametres	**Ananas Comosus liquor**
pH	4.75
Density	0.97
Dry matter content (%)	12.22
Titratable acidity (mgEac/ mL)	6.40
° Brix (%)	15.00
Alcoholic degree (%)	45.00

3.3. Mineral salt content

Table 3 below shows the mineral content of *Annas Comosus* liquor. From the analysis in Table 3, the *Annas Comosus* liquor produced has a high concentration of mineral salts. The contents of P, K, Na and Ca are respectively 60.00; 128.70; 7.96 and 17.44 mg/L. Its Mg concentrations; Fe and Mn of the liquor are of the order of 14.20; 1.57 and 0.14 mg/L. The contents of mineral salts and trace elements in the liquor from the Ciqual table (2020) [36] have a high content of sodium (72.50 mg/kg), magnesium (30.00 mg/kg) and potassium (300.00 mg/ kg) compared to those found in our work. On the other hand, the iron (0.80 mg/kg) and calcium (10.00 mg/kg) contents are lower than those obtained in the *Annas Comosus of* liquor produced. These differences could be due to the raw material used.

Table 4: Mineral salt content

MSC	**Ca**	**Fe**	**Na**	**Mg**	**K**	**P**	**Mn**
(mg/Kg)	17.44	1.57	7.96	14.20	128.70	60.00	0.14

MSC: mineral salt content ; K: Potassium ; Ca: Calcium ; Na: sodium; P: phosphorus; Mg: Magnesium ; Fe: Iron ; N : nitrogen

3.4. Content of phenolic compounds in the formulated liquor

Table 5 presents the content of phenolic compounds in the liquor produced. This table shows that the liquor contains a significant amount of tannins, flavonoids and total phenols. The total phenol concentration is 9.49 mgEAG/mL. The contents of total flavonoids and condensed tannins are respectively 6.05 µg EQ/mL and 119.5 mgELeuc/mL. The total phenol content obtained from our liquor is lower than the values reported by Ferreira et al[35] between 24.70 and 25.70 mgEAG/mL. On the other hand, it is higher than those obtained by Hêdiblè[12] ranging from 0.26 to 0.55 mgEAG/mL. Indeed, flavonoids from food plants have been reported to provide biological benefits, such as reducing the risk of cancer and cardiovascular disease [37].

Table 5: Content of phenolic compounds in the liquor produced

Phenolic compound	**Total phenol content (µgGAE/mL)**	**Total flavonoids content (µgQE/mL)**	**Condensed tannin content (µgCE/mL)**
	9.49	6.05	119.5

3.5. Microbiological quality

Table 6 presents the results of the microbiological analysis of the liquor produced

From the microbiological point of view (table5), the liquor produced does not contain coliforms. As for yeasts and moulds, they are below the microbiological criteria. These values are in accordance with the beverage standard which indicates that the number of yeasts and molds must not exceed 10^3CFU/mL [38]. The count of the total aerobic mesophilic flora is less than 10^3 CFU/mL. These values meet the normative requirements. Thus, the *Annas Comosus* liquor produced is of acceptable microbiological quality.

Table 6: Microbiological characteristics of *Annas Comosus* liquor

Microorganisms	Liquor	Normative values
TAMF	10^2 UFC/ mL	< 10^3 UFC/mL (*FSD*, 2018)
Total coliforms	Absent	< 10^2 UFC/ mL (*FSD*, 2018)
Yeasts	4.10^1 UFC/ mL	< 10^3 UFC/ mL [38]
Molds	10^1 UFC/mL	< 10^3 UFC/ mL [38]

FSD: *Food Security Division;* **TAMF**: Total Aerobic Mesophilic Flora; **CFU**: Colony Format Unit

3.6. Sensory analysis

Table 7 presents the averages obtained at the end of the sensory tests carried out on the *Annas Comosus* liquor. As for the general impression, this figure shows that the tasters really liked the *Annas Comosus* liquor with an average appreciation rate of 73.38%. Parameters such as color, bitterness and viscosity of *Annas Comosus* liquor with a respective appreciation rate of 68.80%, 62.77% and 67.22% are moderately liked by tasters. In addition, the alcoholic flavor and the sweet aroma of the elaborate liquor having received an average appreciation of 70.55% and 70% are much appreciated by tasters.

Table 7: Sensory analysis of *Annas Comosus* liquor

Parameters	Appreciation rate
Color	68.8
Alcoholic flavor	70.55
Sweet aroma	70
Bitterness	62.77
Viscosity	62.22
General impression	73.38

Legends: 10: I extremely hated; 20: I hated it a lot; 30: I slightly hate; 40: I'm indifferent; 50: I liked it a little; 60: I moderately liked; 70: I loved; 80: I really liked

4. Conclusion

This study made it possible to produce and characterize the liquor from the co-products of *Ananas Comosus* on the physicochemical, microbiological and sensory levels. The phytochemical screening showed that the root of *Mondia whitéi* contains secondary metabolites such as tannins, flavonoids, alkaloids, reducing compounds, anthraquinones, saponins and coumarins. The product produced has an acid pH and high levels of dry matter, titratable acidity and soluble dry matter. The evaluation of phenolic compounds shows that they are rich in total phenols, flavonoids and condensed tannins. Mineral analysis indicates that the liquor contains a significant amount of mineral salts; essential to man. From a microbiological point of view, it is free of any pathogenic germ and meets the normative requirements. From the sensory analysis, it appears that it is well appreciated by all the tasters (>73%). The transformation of *Ananas Comosus* co-products into liquor solves problems related to pollution and is of undeniable economic importance.

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