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Developing New Natural Plant Products from the Spice *Xylopi* aethiopi

Ghana and Liberia

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ABSTRACT

The objective of this study was to assess the quality and chemistry of *Xylopi* aethiopi pods, seeds and capsules from both Liberia and Ghana. When the pods are crushed, the two main products include capsules and seeds, with the seeds making up to 35% of the pods. Seeds are mostly black in color (68-77%) with some brown (32-23%) indicative of varying maturation within seed pods during fruit ripening. Moisture of the pods was higher in the samples from Ghana (10.4%) than in Liberia (8.3%). Pods and seeds were low in ashes, protein, and fats showing low nutritional properties. The aromatic volatiles components of the pods, capsules, and seeds were dominated by α -pinene, β -pinene and 1,8 cineole. While the ripe and dried fruits are often the product of commerce, by separating out the seeds, which contain lower amounts of α - and β - pinene and higher amounts of 1,8 cineole than the whole pods, the seeds by themselves could be commercialized as a seed spice providing a different overall sensory profile characterized by less spice and pungency and more fresh notes.

INTRODUCTION

Xylopi, *Xylopi* aethiopi, one of 150 species in the Annonaceae family, is native to savanna zones of West Africa and now naturalized across North Africa. As a medium-sized tree with a trunk

diameter of 60cm it can reach 30m height with a straight stem and smooth bark and branched crown. Its fruits are curved pods, containing about 15 carpels, which are arranged in bouquets of 12-20 bacciferous capsules (Tairu et al., 1999). The tree flowers twice annually (March-July and October-December) while fruiting occurs in December-March and June-September. The fruits are harvested with the inflorescence and sun-dried for 4-7 days (Orwa et al., 2009). The fruits are highly aromatic, and used to spice and flavor foods as well as in cosmetics and personal hygiene products such as face creams (Ayedoun et al., 1996). The fixed oil from the seeds have been used to create shampoos due to its high saponification value (Ajiwe et al., 1998). *Xylopi* has been used in traditional medicine in many West African countries, including Ghana and Liberia, where according to the World Health Organization (WHO), 80% of the population in those countries still use plant preparations to treat their illnesses (Choumessi et al., 2012).

Grinding the *xylopi* pods release volatile components, responsible for the refreshing aroma of the spice; the pods also contain other non-volatile secondary metabolites including polyphenols, flavonoids, tannins, and saponins; and sterols such as sitosterol, stigmasterol, and campesterol (Ezekwesili et al., 2010).

The essential oil of *xylopi* is known to contain more than 100 different aromatic volatiles. In one study, the aroma volatiles in the oil from the pods

contained principally β -pinene (18%), terpinen-4-ol (8.9%), sabinene (7.2%), α -terpineol (4.1%), 1, 8-cineole (2.5%), mytenol (2.4%), and kaurane derivatives (4.2%) (El-Kamali and Adam, 2009). Samples from Ghana showed a similar composition with high levels of α -pinene (8%), β -pinene (19%), 1,8 cineole (12%), sabinol (5%) and mirtenol (7%). The profile was dominated by monoterpenes (>75%) with lower amounts of sesquiterpenes (<8%) (Juliani et al., 2008a).

Xylopi extracts were reported to have potential applications in food preservation and considered to be generally of low toxicity and environmentally safe (Fetse et al., 2016). Xylopi has been studied for its potential as an antioxidant in foods (Adegoke et al., 2003). Studies on the fruits essential oil of xylopi have shown a variety of mono- and sesquiterpenes, with the main compounds varying depending on the geographical location and time of harvest (Karioti et al., 2004). Studies confirmed that essential oils from the dry fruit collected in Egypt, Benin and Mali contained high levels of the monoterpenes β -pinene and 1,8-cineole (Karioti et al., 2004). Dried fruits collected in Benin contain the monoterpene sabinene, while the fruits from Egypt are rich in the monoterpene 4-terpineol (Karioti et al., 2004). Other studies have also reported contents of diterpenes such as 13-epimanoyl oxide, kaur-16-ene (Elhassan et al., 2010) and xylopic acid (Woode et al., 2012). An ethnobotanical survey showed that xylopi is one of the most used Non-Timber Forest Products in Liberia (Juliani et al., 2012).

The objective of this study was to assess the chemistry and quality of xylopi pods and their separated parts (e.g. seed and capsule), of samples coming from Liberia and Ghana. As volatiles from the seeds has been scarcely studied, this work is part of an endeavor to identify new uses and applications for African plant products such as xylopi, given that the chemical composition relates directly to the aroma, taste, and bioactivity of a spice.

MATERIALS AND METHODS

Two sets of samples from Liberia and Ghana were obtained from local markets in Ghana and

Liberia. Botanical authentication was conducted by local botanical experts. Pods were cracked manually with the help of a mortar, pods were weighed before the analysis, then the parts (seeds and capsules) were weighed to determine percentage mass distribution. Moisture, total ashes, total phenol, crude fats, and proteins were determined (Food Chemical Codex, 1996; Juliani et al., 2009), with a modification of a protocol to determine crude fats by hexane extraction using a soxhlet apparatus. Bulk density was determined by weighing a volume of seeds in a graduated cylinder (10 mL), a procedure based on a protocol developed for black pepper (IPC, 2020).

The composition of volatile oils was determined by static headspace using 20 mL vials with 100 mg of ground xylopi pod parts. One mL of the headspace was injected into a gas chromatograph (GC-2010 Plus) coupled to a mass spectrometer (MS) (Gas Chromatograph Mass Spectrometer GCMS-TQ8040 System). Samples were injected with an autosampler (AOC-6000 Series), at an injection temperature of 250 °C, in an SH-Rxi-5Sil MS Column (30 m \times 0.25 mm i.d., 0.25 μ m film thickness). The heating temperature for the headspace vial was 70 °C for 5 mins with agitation. GC oven temperature program was set at 35°C, rate at 4 °C/min up to 220 °C, held for 5 min. Helium constant flow was set at 1 ml/min. Individual peak identifications were made by their mass spectra and retention indices (Juliani et al., 2008b).

RESULTS AND DISCUSSION

The color of the pods of *Xylopi aethiopica* from Ghana and Liberia were typically dark to black-brown, with both samples having spicy and fresh notes. Traditionally, the whole pods of xylopi are grounded and added to foods as a spice. To find new uses and applications of this spice, the possibility of using only the seeds as a product was a core objective. We observed that the seeds made up to 34.7% in the pods from Ghana, while 24.7% in the pods from Liberia (Table 1), values were not significantly different from each other. We also observed two types of seed color, black seeds (68.61% and 76.78%) being the highest amount of

seeds compared to brown seeds (31.39% and 23.28%) in samples from Liberia and Ghana, respectively (Table 2). Foreign and fine particles were assessed from the seeds (Table 1). Samples from Ghana showed higher amounts of foreign

particles (soil, sand and other unwanted materials) (1.5%) as compared to the Liberian samples (1.27%) (Table 2), though differences were not significant.

Table 1. Mass percentage distribution of *Xylopi*a *aethi*o*p*i*c*a seeds in pods from Liberia and Ghana.

	Ghana	Liberia	P value
Mass percentage	(%, g/100g) ¹	(%, g/100g)	
Total amount of seeds (%)	34.72 ± 1.26 ²	24.78 ± 1.77	0.19
Black seeds to whole fruit	26.63 ± 0.32	16.92 ± 0.09	0.03*
Brown seeds to whole fruit	8.11 ± 0.92	7.86 ± 1.68	0.94
Black seeds to total amount of seeds	76.78 ± 1.86	68.61 ± 4.54	0.42
Brown seeds to total amount of seeds	23.28 ± 1.80	31.39 ± 4.54	0.42
Foreign particle	1.48 ± 0.96	1.27 ± 0.26	0.81
Fine particle	0.03 ± 0.01	0.008 ± 0.003	0.07

¹Percentage, g/100 g dry weight ²Mean ± Standard error. * Significant (T test)

Moisture content levels of the seeds from Liberia showed a slightly lower percentage (8.32 %) than seeds from Ghana (10.41%). This may account for the very slight moldy scent of the seeds from the two countries. High moisture level (higher than 10%) implies seeds were not dry enough and so can easily encourage the presence of off odors. A preferred and acceptable moisture content level for

seed products should often be below 10% to maintain quality, or problems of deterioration may be soon visual (Juliani, 2008a).

Total mineral content as shown by the amount of ashes of the seeds from Liberia (3.11%) and Ghana (3.35%) were significantly different. Also, the ashes of the capsule from Liberia (4.42%) and Ghana (4.82%) were significantly different.

Table 2. Proximate analysis of the seeds, capsules and whole fruits of *Xylopi*a *aethi*o*p*i*c*a from Ghana and Liberia.

	Ghana (%, w/w) ¹	Liberia (%, w/w)	P value
Moisture-whole fruit	10.41 ± 1.12 ²	8.32 ± 0.42	0.14
Ashes- seed	3.35 ± 0.03	3.11 ± 0.07	0.01*
Ashes –capsule	4.82 ± 0.02	4.42 ± 0.00	0.00*
Crude fats-seed	1.22 ± 0.07	1.58 ± 0.14	0.12
Bulk density-seed	0.33 ± 0.00	0.37 ± 0.00	0.06
Total phenols -whole fruit	4.37 ± 0.24	5.54 ± 0.27	0.02*
Total phenols –seed	2.33 ± 0.43	3.63 ± 0.64	0.00*
Total phenols –capsule	4.99 ± 0.26	5.30 ± 0.21	0.45
Total proteins- whole Fruit	0.86 ± 0.17	3.89 ± 0.68	0.01*
Total protein -seed	2.85 ± 0.47	3.35 ± 0.66	0.3

¹Percentage, g/100 g dry weight. ²Mean ± Standard error, * Significant (T test)

Additional studies were conducted to the seeds to determine their chemical and nutritional values. Protein analysis for the pods from Liberia showed a slightly higher percentage (3.35%) compared to seeds from Ghana (2.85%) (Table 2).

However, the whole fruit showed a higher protein percentage for the Liberian seeds (3.89%) compared to the Ghanaian whole fruit (0.86%) (Table 2). Overall, the protein levels in the seeds are low for the seeds from Ghana and Liberia, the

amount of total proteins in the whole fruits are even lower.

Total fats of the seeds were typically low, with seeds from Liberia (1.58%) containing a higher amount of total fats compared to Ghana (1.22%) samples, not significantly different (Table 2). The low amounts of fats in these samples suggest that their use in cosmetics (Ajiwe et al., 1998) may not be economically viable.

The percentage of total phenols in the whole pods from Liberia was significantly higher (5.54%) than the one from Ghana (4.37%). Total phenolic content in the seeds was also significantly higher in Liberia seeds (3.63%) compared to Ghana seeds (2.33%) (Table 2). While the total phenols in the capsules are not significantly different from Liberia (5.3%) and Ghana (4.99%). Even though the seeds contain a high amount of total phenols, the whole pods contain the highest level of total phenols. With the purpose of providing additional tests to help in the characterization of the *Xylopi*a seeds, the bulk density of the seed was 0.37 for Liberia and 0.33 for Ghana, with values not significantly different (Table 2).

Results indicated differences in the chemical aromatic volatile compositions in different parts of the pods of *X. aethi*opica (Table 3). The whole intact fruits (pods) contained volatiles characterized by high levels of α -pinene (25.58% in Ghana and 26.32% in Liberia) and β -pinene (49.59% in Ghana and 41.65% in Liberia) and low levels of 1,8-cineole (6.46% in Ghana and 17.06% in Liberia) (Table 4). The amounts of α -pinene and β -pinene are similar in Liberia and Ghana samples, while 1,8-cineole is higher in Liberia whole fruits. Other studies have reported a similar profile, particularly with samples from Ghana being dominated also by α -pinene (8%), β -pinene (19%) and 1,8-cineole (12%) (Juliani et al., 2008a).

The whole fruits breakdown in capsules and seeds revealed that ground capsules had a similar composition to that of the whole pods, with few variations in whole pods containing slightly higher amounts of α -pinene (24.09%) and β -pinene (45.08%) in Ghana samples. While in Liberia samples is the opposite, the capsules contain a

slightly higher amount of α -pinene (29.26%) and β -pinene (41.04%). Hexanal was not found in the capsules of Liberia or Ghana samples. Some other compounds not present that we had expected in the capsules include elemene, germacrene and guaiane. Phellandrene was detected only in the capsules of Liberia samples (0.17%), and not in the Ghana samples (Table 3). 1,8-Cineole is present in higher amounts in the Liberia capsules (14.68%) compared to the Ghana capsules (9.03%) (Table 3).

The aromatic volatiles in the seeds indicated that only the seeds contain hexanal in the Ghana samples (5.88%), while in the Liberia samples the seeds (3.82%) and the whole fruits (1.0%) contained hexanal (Table 3). Seeds from the two countries contained similar amounts of α -pinene and β -pinene. However, there were higher amounts of 1,8-cineole in the seeds (11.12%, 19.34%, for Ghana and Liberia). The whole fruits contained 6.46% and 17.06% respectively. These results suggest that besides the spicy aroma, associated by the presence of α - and β -pinenes, the higher amounts of 1,8-cineole present in the seeds, would give impart a fresher and mintier note and flavor. The cluster analysis showed the presence of two main clusters segregated by the chemical volatile profile (Fig. 1).

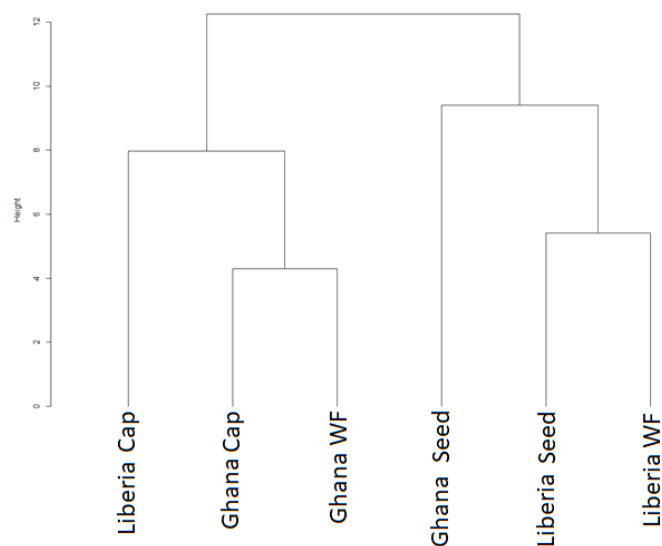


Figure 1. Cluster analysis of the essential oil chemical composition of *Xylopi*a *aethi*opica whole fruits (WF), and their parts (Cap, capsules) from Ghana and Liberia.

Table 3. Essential oil composition of the seeds, capsules and whole fruits of *Xylopi* aethiopi

RT ¹	RI ²	Component	Ghana			Liberia		
			Capsule	Seed	Whole Fruit	Capsule	Seed	Whole Fruit
			(%)	(%)	(%)	(%)	(%)	(%)
5.652	787	Hexanal	-- ³	5.88 ±0.321 ⁴	--	--	3.82 ±0.59	1.00 ±0.14
6.942	876	Hexanol ⁶	0.19 ±0.01	0.15 ±0.01	0.19 ±0.00	0.13 ±0.01	0.13 ±0.03	0.24 ±0.03
7.549	920	α-Thujene	1.17 ±0.01	1.19 ±0.01	2.34 ±0.02	0.91 ±0.02	0.53 ±0.12	0.45 ±0.01
7.647	929	α-pinene	24.09 ±0.26	19.40 ±0.65	25.58 ±0.20	29.26 ±0.55	25.61 ±1.67	26.32 ±0.74
7.855	947	Camphene	0.55 ±0.02	0.38 ±0.05	0.60 ±0.02	0.43 ±0.01	0.30 ±0.02	0.27 ±0.06
7.896	951	Thuja-2,4(10) diene	0.26 ±0.02	0.61 ±0.05	0.30 ±0.01	0.21 ±0.01	0.54 ±0.08	0.83 ±0.04
8.109	969	Sabinene	6.43 ±0.17	2.41 ±0.06	5.42 ±0.13	2.38 ±0.04	1.22 ±0.21	1.03 ±0.03
8.176	974	β-pinene	45.08 ±0.74	41.28 ±1.33	49.59 ±1.00	43.07 ±0.52	41.04 ±1.10	41.65 ±0.76
8.275	983	Mentha diene ⁴	2.88 ±0.02	1.07 ±0.08	1.89 ±0.17	--	0.19 ±0.04	0.08 ±0.02
8.347	989	Mentha diene ⁴	1.20 ±0.03	0.30 ±0.04	0.72 ±0.08	--	--	--
8.452	997	Phellandrene	--	0.14 ±0.03	--	0.17 ±0.01	0.16 ±0.01	0.11 ±0.00
8.652	1018	para Cymene	2.71 ±0.15	3.52 ±0.30	2.36 ±0.14	4.01 ±0.13	3.10 ±0.17	2.62 ±0.16
8.703	1024	Limonene	1.85 ±0.03	1.56 ±0.12	1.46 ±0.04	3.12 ±0.09	--	--
8.743	1028	1,8 Cineole	9.03 ±0.50	11.12 ±0.73	6.46 ±0.47	14.68 ±1.69	19.34 ±1.12	17.06 ±0.99
8.977	1053	Sabinene hydrate trans	0.26 ±0.01	0.33 ±0.02	0.20 ±0.01	0.09 ±0.01	0.09 ±0.01	0.07 ±0.02
9.103	1066	Sabinene hydrate cis -	--	0.18 ±0.04	0.17 ±0.0	--	0.05	0.15 ±0.06
9.213	1078	Unknown	0.32 ±0.19	0.09 ±0.01	0.16	--	--	--
9.328	1089	Terpinolene	--	0.25 ±0.03	--	0.16 ±0.02	0.23 ±0.08	0.30 ±0.03
9.365	1093	Nonenal 2	--	0.58 ±0.12	--	--	0.14 ±0.06	0.14
9.613	1122	Campholenal	0.07	0.11 ±0.03	0.07	0.06 ±0.01	0.11 ±0.03	0.24
9.745	1138	Limonene oxide	--	0.25 ±0.07	--	--	0.16 ±0.06	0.40 ±0.23
9.943	1162	Pinocarvone	0.43 ±0.06	0.57 ±0.07	0.33 ±0.09	0.27 ±0.04	0.53 ±0.13	0.60 ±0.20
10.077	1178	Terpin-4-ol ⁴	0.08 ±0.01	0.20 ±0.03	0.08 ±0.00	--	0.09	0.19 ±0.01
10.217	1194	Myrtenal	0.47 ±0.06	1.01 ±0.15	0.40 ±0.07	0.35 ±0.06	0.76 ±0.25	1.12 ±0.25
10.309	1206	Dyhydro carveol iso-	0.08	0.13 ±0.02	0.08 ±0.00	--	0.12 ±0.02	0.20 ±0.02
11.259	1335	δ-Elemene	0.25 ±0.01	0.77 ±0.01	0.18 ±0.02	0.17 ±0.01	0.15 ±0.03	0.31 ±0.03
11.474	1365	Unknown	--	0.44 ±0.10	--	--	0.39 ±0.14	1.94 ±1.33
11.579	1380	β-Cubebene	0.57 ±0.02	3.12 ±0.34	0.74 ±0.14	0.39 ±0.01	0.82 ±0.09	1.56 ±0.38
11.646	1389	Unknown sesquiterpene	--	0.27 ±0.04	--	--	0.07	0.11 ±0.02
11.909	1429	γ-Elemene	--	1.05 ±0.16	--	--	0.12 ±0.02	--
11.987	1441	β-(z)-Farnesene	--	0.31 ±0.04	--	--	--	--
12.229	1477	Germacrene D	--	0.34 ±0.03	--	--	0.14 ±0.01	0.13 ±0.01
12.303	1488	α-Selinene	0.29 ±0.20	0.33 ±0.03	--	0.23 ±0.02	0.12 ±0.01	--
12.381	1500	Trans β Guaiene	--	0.39	--	--	--	0.03

¹Retention time, component listed in order of elution. ²Retention index on a 5% methyl/95% phenyl silicon. ³not detected.

⁴Mean ± Standard error). ⁵Tentative identification, isomer not identified.

The Ghana and Liberia whole fruits showed a distinctive chemical profile, as they were in separate clusters. However, there was not a clear geographic pattern concerning the pod parts. As the capsules from Ghana and Liberia are grouped with the Ghana whole fruits, while the seeds from Ghana and Liberia are grouped with Liberian whole fruits.

This study following a volatile-chemistry approach has shown that the seeds from the pods of xylopia can be developed as a new spice product as the seeds will have a unique chemical profile. In the last several years, spice medleys, a mixture of several spices (e.g. black, white, green peppercorns, allspice, among others) have become very popular in the marketplace. Thus, xylopia seeds could be a new addition. The challenge will be in the engineering to efficiently and economically separate the seeds from the capsules.

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