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Acceptability, nutritional and antioxidant properties of spice formulations based on *Coelocaryon oxycarpum* (Cox), ginger and pepper.

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ABSTRACT

Objectives: This study aims to carry out formulations of spices based on *Coelocaryon oxycarpum* (Cox), ginger and pepper then to determine the best formulations from the hedonic test, and to analyse the biochemical characteristics and the antioxidant properties.

Methodology and results: Cox, ginger and pepper spice powders were obtained by drying and grinding each spice. Powders obtained were used to make the formulations, and then hedonic test made it possible to select appreciated formulations. The proximate and antioxidant properties using standard analytical methods were then determined. The blend 1, 4, 6 and 10 were selected, the incorporation of 30% of ginger and pepper in the powder of the pulp of the fruit of Cox, has improved the nutritional characteristics and antioxidant properties. The blend 4 and 10 expressed the best free radical scavenging capacities DPPH.

Conclusion and application of results: Cox is aromatic plant with interesting nutritional characteristics. Four blend based on these spices, having the most interesting sensory characteristics, were selected by the panel of tasters. These are the blend 1: 100% Cox, Blend 4: 70% Cox and 30% ginger, Blend 6: 80% Cox and 20% pepper, and Blend 10: 70% Cox, 20% Ginger and 10% of pepper. The incorporation of ginger and pepper in proportions of 30% in the powder of the pulp of the fruit of Cox, has improved the nutritional characteristics and antioxidant properties of spice blends. These spice blends could be potential sources of protein, minerals, vitamins and natural sources of antioxidants easily accessible and beneficial to consumers. These spices could be recommended for seasoning dishes and as good sources of natural antioxidants. These formulations would be a good alternative for the nutritional management of people suffering from metabolic diseases like hypertension and diabetes.

Keywords: *Coelocaryon oxycarpum*, blend, pepper, ginger, antioxidants activities

INTRODUCTION

Aromatic plants are part of every culture in the world. They are composed of herbs and spices that are generally used as aromatic agents in cooking and in medicine to treat various conditions (Lai and Roy, 2004). The spices could be derived from a dried bark, root or plant substance of a plant while the grass is called as part of green plant leaves, or any herbaceous plant (Iris *et al.,* 2006). These plants also have many properties, including antioxidants, antimicrobial, pharmaceutical and nutritional (Peter *et al.,* 2012). Due to their phytochemicals commonly known as antioxidants such as polyphenols, phenolic acids, tannins, flavonols, isoflavones and curcuminoids (Nobuji, 2000), spices and herbs help improving the flavour and organoleptic properties of foods (Kivilompolo *et al.,* 2007). The high proportion of antioxidants confers various health benefits to herbs and spices (Balentine *et al.,* 1999). In Côte d'Ivoire, *Coelocaryon oxycarpum* (Cox) is an edible aromatic plant found in the eastern part of Cote d'Ivoire (Sinh *et al.,* 2017). Cox fruit is consumed as spice well appreciated by local population with a high socio-cultural value. The sun-dried form is preferentially used for sauces (soups) for the care of lactating women for its health benefits. More and more the dried form is mixed with other spices to enhance food taste and exploring for new flavours.

Studies revealed that consumption of mixed spices could be a source of protein and essential amino-acid supplements (Bouba, 2009). Ginger (*Zingiber officinale*) and pepper (*Capiscum nigrum*) have been reported to provide nutritional benefit such anti-flatulent, anti-bacterial, anti-fungal, anti-diabetic (Srinivasan, 2005) when they are mixed with other spices. The objectives of This study aims to carry out formulations of spices based on *Coelocaryon oxycarpum* (Cox), ginger and pepper then to determine the best formulations from the hedonic test, and to analyse the biochemical characteristics and the antioxidant properties.

MATERIAL AND METHODS

Samples: The plant materials used in this study are fruits of Cox, and the black peppers (*Piper guineense*) which were harvested respectively in Bondoukou (North-Eastern Côte d'Ivoire) and Azaguié (South Côte d'Ivoire). Then the fresh rhizomes of yellow ginger (*Zingiber officinale*) purchased at urban market (Abidjan, Côte d'Ivoire). These spices were stored in the laboratory in a freezer (-10 ° C) and then reduced to powder (Sinh *et al.,* 2018). These different spices were pitted, washed with distilled water, drained, grated and dried in an oven at 45°C for 72 h. The dried samples were ground into fine powder using a Moulinex-type mixer. All mixed products were conditioned in dry labelled boxes and tightly closed and stored in a desiccator at 25° C (AOAC, 1995). For this study, ten blended proportions of spices using Cox, ginger and pepper were tested as described in Table 1 according to Wakil and Kazeem (2012). Cox with no blending was used as control.

Figure 1: Photographs of the pulp of the fresh fruit of *Coelocaryon oxycarpum*

Table 1: Spice blending proportions

Blends	Cox (%)	Ginger (%)	Pepper (%)
Blend 1	100	0	0
Blend 2	90	10	0
Blend 3	80	20	0
Blend 4	70	30	0
Blend 5	90	0	10
Blend 6	80	0	20
Blend 7	70	0	30
Blend 8	70	15	15
Blend 9	70	10	20
Blend 10	70	20	10

Sensory analyses: For this study two kind of sensory evaluation were carried out on blending formulation. The first analyses were dealing with on A 9- point hedonic test scale to evaluate the acceptability score of blended spices according to the method (Hu *et al.*, 2018). The samples were evaluated for flavour, taste, appearance of spiciness, duration of spiciness, colour, odour characteristics and overall acceptability of each sample.

Proximate analysis: Proximate parameters (moisture, fats, protein, crude fibre and ash) of the four spices formulations were determined using the Association of Official Analytical Chemists (**AOAC, 1990**) method.

The carbohydrate content was determined by calculation using the difference method (FAO, 2004): Carbohydrates: 100 – (% moisture + % proteins + % lipids + % ash).

Reducing sugar content was determined by extraction with 80% neutral aqueous ethanol followed by evaporation of the ethanol and subsequent measurement using the dinitrosalicylic acid method according to (Bernfeld, 1955).

Total soluble sugar content in ethanolic extract was assessed using the phenol-sulfuric acid method according to (Dubois *et al.*, 1956).

Calorific value (dry matter basis): (% proteins × 2.44) + (% carbohydrates × 3.57) + (% lipids × 8.37).

Quantitative phytochemical analysis: Phenolics determination was carried out according to the Folin-Ciocalteu procedure

(Singleton *et al*, 1999). Flavonoids, and tannins, were determined by the methods described by (Meda *et al.*, 2005), and Baibridge *et al.* (1996**)** respectively.

Phenolic Compounds by HPLC Analysis: The individual phenolic compounds were analysed by the method described by (Ho *et al.* 1999) using HPLC for the determination of molecules. The methanolic extract previously prepared (50 mL) was diluted into 100 mL of distilled water and 20 µL were analysed using an Analytical HPLC unit (HPLC (Shimadzu Corporation, Japan) equipped with the binary pump (LC-6A) coupled to UV-VIS Detector (SPD-6A). Phenolic compounds were separated on a column ICSep ICE ORH-801(Length 25cm) at 30°C. The mobile phase consisted of 50 mM NaH4H2PO4 to pH 2.6 (Eluent A), a solution of acetonitrile/ NaH4H2PO4 (80:20, v/v) (Eluent B) and 200 mM acid o-phosphoric pH 1.5 (Eluent C). The operating time was 70 min with a flow rate of 1 mL/min. Phenolic compounds in methanolic extract of each spices formulation were identified through comparison of their retention times and UV-visible spectra with those obtained by injection of the standard solution under the same conditions. Peak area was used for quantification purposes, using external calibration with standards.

Antioxidant Assay by DPPH: The DPPH free-radical scavenging activity was determined by the methods described by (Choi *et al.*, 2002) with some modifications. Various concentrations (2 mL) of each spices formulation (aqueous extract) as well as the ascorbic acid used in this study as a reference were added to 1 mL of a DPPH radical solution in methanol for testing the antioxidant activity of these spice formulations. The absorbance was measured at 517 nm against blank by using a Spectrophotometer (Spectrophotometer invisible model MS-A 5100). DPPH radical scavenging activity was calculated by using the following equation:

% inhibition = (Absorbance of control – Absorbance of sample) x 100 / Absorbance of control.

The 50 % Inhibition Concentration (IC50: Concentration of compound decreasing the absorbance of a DPPH· solution by 50 %) was determined graphically.

Statistical analysis: All experiments were conducted in triplicate and submitted a one-way analysis of variance (ANOVA I) followed by Duncan's test to compare averages of the spices formulations.

RESULTS

Sensory analysis: In general, the descriptors of different spice formulations were rated as pleasing by the panellists (Table 2). Indeed, the descriptors of colour (6.13 ± 1.64 - 7.73 ± 1.02), smell (6.23 ± 1.43 -7.52 ± 1.26), aroma (5.60 ± 1.55 - 6.70 ± 1.02) and taste (5.44 ± 1.77 - 6.81 ± 1.71) of the spices had scores above 5. In addition, the average score (score > 6) of the different descriptors of each spice formulation shows a very high appreciation of these formulations. However, the spice formulations most highly rated by the panellists considering the rank assigned to them were blend 1, 4, 6 and 10.

Table 2: Sensory attributes of blends spices

Spices (% of blending)	*Cox*	*Cox* and ginger formulations			*Cox* and pepper formulations			*Cox*, ginger, and pepper formulations		
	Blend 1	Blend 2	Blend 3	Blend 4	Blend 5	Blend 6	Blend 7	Blend 8	Blend 9	Blend 10
Colour	7.19 ± 0.93	6.93 ± 1.56	6.13 ± 1.64	7.56 ± 1.5	7.18 ± 0.93	7.73 ± 1.02	7.06 ± 0.77	6.82 ± 1.32	6.84 ± 1.98	7.45 ± 1.66
Smell	6.89 ± 1.46	6.46 ± 1.85	6.38 ± 1.62	6.94 ± 1.53	6.70 ± 1.40	7.52 ± 1.26	6.66 ± 1.34	6.44 ± 1.64	6.23 ± 1.43	6.80 ± 1.63
Aroma	6.70 ± 1.02	5.81 ± 1.88	5.63 ± 1.70	5.88 ± 1.7	6.19 ± 1.72	6.28 ± 1.67	5.89 ± 1.69	5.60 ± 1.55	5.91 ± 1.47	6.35 ± 1.82
Taste	6.22 ± 0.98	5.98 ± 1.87	6.25 ± 1.88	5.44 ± 1.77	5.56 ± 1.65	6.44 ± 1.69	5.88 ± 1.25	5.95 ± 1.29	5.61 ± 1.77	6.81 ± 1.71
Score	6.75 ± 0.68	6.30 ± 1.79	6.10 ± 1.71	6.46 ± 1.63	6.41 ± 1.43	6.99 ± 1.41	6.37 ± 1.26	6.20 ± 1.45	6.15 ± 1.66	6.85± 1.71
Ranking	4±0.46	2.8±0.91^{a}	3±0.71^{a}	4.25±0.74^{b}	3.45±1.71^{b}	4.31±1.46^{c}	3.15±1.5^{a}	3.12±0.51^{b}	2.53±0.28^{a}	3.95±0.9^{c}

Data are represented as mean and standard deviation values. Liking scores were based on a 9-point hedonic scale (1 = dislike extremely, 9 = like extremely). Mean values followed by different letters on the same line for the same formulation group are statistically different *(α = 0,05).*

Blend 1 :100 Cox, Blend 2 (90% Cox+10% gin), Blend 3 (90% Cox+20% gin), Blend 4 (70% Cox+30% gin), Blend 5 (90% Cox+10% pep), Blend 6 (80% Cox+20% pep), Blend 7 (70% Cox+30% pep), Blend 8 (70% Cox+15% gin+15% pep), Blend 9 (70% Cox+10% gin+20% pep), Blend 10 (70% Cox+20% gin+10% pep).

Proximate analysis: The nutrient composition of the different spice formulations (blend 1, 4, 6 and 10) is reported in Table 3. These results show that the different spice formulations are excellent sources of ash, protein and fibre. Indeed, the ash content of these spice formulations varies from 5.09 ± 0.11 to 5.67 ± 0.2 % with the highest significant value ($p \leq 0.05$) recorded at the level of blend 4 (70% Cox+30% gin). As for the protein content of the different spice formulations, it is present a significant difference at the threshold of 5% and is between 13.54 ± 1.01% (Blend 4) and 17.12 ± 1.04% (Blend 6). Regarding fibre, which is the highest nutrient, it ranges from 37.26 ± 0.24 % (Blend 4) to 46.98 ± 1.20 % (Blend 1). On the other hand, these spices contain a significant proportion of lipids (7.57 ± 0.20 to 8.08 ± 0.19 %), although static analysis did not show any significant difference ($p>0.05$) between the different contents.

Table 3: Proximate composition of the four spice formulations.

Composition	Formulations			
	Blend 1	**Blend 4**	**Blend 6**	**Blend 10**
Ash (%DM)	5.09 ± 0.11[a]	5.67 ± 0.2 [b]	5.24 ± 0.08[a]	5.55 ± 0.14 [b]
Moisture (%)	11.55 ± 0.37 [b]	9. 93 ± 0.28 [a]	9.59 ± 0.20 [a]	10.19 ± 0.04 [a]
Fat (%DM)	7.57 ± 0.20 [a]	7.84 ± 0.21 [a]	8.08 ± 0.19 [a]	8.01 ± 0.20 [a]
Protein (%DM)	16.16 ± 1.14 [b]	13.54 ± 1.01 [a]	17.12 ± 1.04 [b]	14.01 ± 1.01 [a]
Carbohydrates (%DM)	24.20 ± 0.64 [a]	35.67 ± 0.81 [c]	26.49 ± 0.54 [b]	33.0 ± 0.72 [c]
Crude Fibre (%DM)	46.98 ± 1.20 [c]	37.26 ± 0.24 [a]	43.05 ± 0.62 [b]	38.54 ± 0.33 [a]
Reducing Sugars (mg/100gDM)	3.89 ± 0.04 [c]	3.63 ± 0.04 [b]	3.18 ± 0.03 [a]	3.36 ± 0.02 [b]
Total Sugars (mg/100gDM)	8.48 ± 0.21 [b]	8.61 ± 0.13 [b]	7.37 ± 0.25 [a]	8.0 ± 0.23 [b]
Caloric Energy (Kcal/100gDM)	189.19 ± 1.73[a]	226.05 ± 1.69 [c]	203.96 ± 1.27 [b]	221.15 ±1.16[c]

The means ± standard deviations, affected by different letters on the same line are significantly different at the 5% threshold according to Duncan's test.

Phytochemical composition: Table 4 shows the phenolic compound contents of the different spice formulations. These results show a significant difference at the 5% threshold. From the results, it can be noted that the highest polyphenol content is recorded with Blend 1 formulation (2287.42 ±106.89 mg GAE/100 g DM) and the lowest with Blend 6 (2033.59 ±83.46 mg GAE/100 g DM). In addition, tannin contents varied from 281.59 ± 0.62 mg tannic acid/100 g DM (Blend 6) to 347.77 ± 4.11 mg tannic acid/100 g DM (Blend 4). Flavonoid contents were between Blend 10 (179.09 ± 2.75 mg quercetin/100 g DM) and Blend 1 (211.57 ± 3.88 mg quercetin/100 g DM).

Individual phenolic compound: Data presented in Table 5 show the individual phenolic compound of the different formulations spices. It was noticed that all spices formulations contain higher low of quercetin (ranged from 5.05 ± 1.23 to 19.54 ±2.56 µg/100g), epicatechin (ranged from 3.21 ± 0.94 to 9.09 ± 0.75 µg/100g) and arbutin (ranged from 0.78 ± 0.10 to 8.08 ± 0.67 µg/100g). However, catechin were found at higher levels in the blend 6 (563.78 ± 18.06 µg/100g).

Table 4: Composition of the polyphenols in the four formulations spices

Compounds	Formulations			
	Blend 1	**Blend 4**	**Blend 6**	**Blend 10**
Polyphenols (mg GAE/100 g DM)	2287.42 ±106.89[b]	2124.83 ± 62.38[a]	2033.59 ±83.46[a]	2052.12 ±65.70[a]
Tannins (mg tannic acid/100 g DM)	314.95 ± 1.28[b]	347.77 ± 4.11[c]	281.59 ± 0.62[a]	320.16 ± 2.51[c]
Flavonoids (mg quercetin/100 g DM)	211.57 ± 3.88[b]	183.68 ± 2.98[a]	183.78 ± 2.79[a]	179.09 ± 2.75[a]
Catechin (µg/100g DM)	494.95 ± 8.03[b]	415.05 ± 4.29[a]	563.78 ± 18.06[c]	476.11 ± 9.51[b]
Quercetin (µg/100g DM)	6.22 ± 1.53[a]	19.54 ±2.56[c]	5.05 ± 1.23[a]	14.52 ± 2.06[b]
Arbutin (µg/100g DM)	0.78 ± 0.10[a]	8.08 ± 0.67[c]	1.84 ± 0.10[b]	6.18 ± 0.47[c]
Epicatechin (µg/100g DM)	3.21 ± 0.94[a]	9.09 ± 0.75[c]	3.8 ± 0.76[a]	6.81 ± 0.71[b]

The means ± standard deviations, affected by different letters on the same line are significantly different at the 5% threshold according to Duncan's test.

DPPH Free-Radical Scavenging Activity: The results on the DPPH scavenging activity showed that the inhibition rates increase with the aqueous extract concentrations of the different spice formulations as well as the ascorbic acid used as a reference in this study. The blend 10 has the strongest DPPH free-radical scavenging activity. This activity was followed by blend 4, blend 6 and blend 1. All data were represented in figure 2.

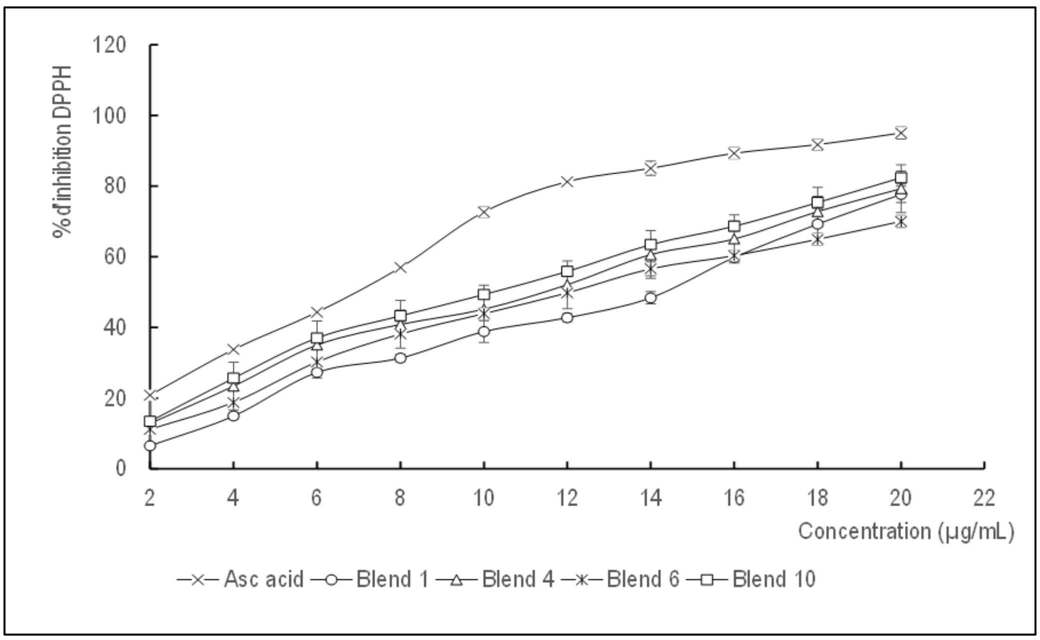

Figure 2: Percentage inhibition of free radical DPPH as a function of the different concentrations of aqueous extracts of the four spices formulations and ascorbic acid.

DISCUSSION

The hedonic test showed that blends 4, 6 and 10, were well appreciated by the panellists. These formulations, which approximates the classification of the control sample composed of 100% cox (blend 1). Sensory analysis of the spice blends showed sensory attributes influenced by aroma and colour with respect to blend 4. Blend 6 was motivated by its taste and odour and blend 10 dominated by flavour, taste, aroma and colour. Proximate analysis for this spice formulations showed that a moisture content significantly less than 12% and a low content in fat. These low contents are due to adequate drying. According to (Aprotosoaire *et al.,* 2016), drying food would help improve the compounds responsible for the flavour of food. The ash contents of the spice blends were significantly higher for blend 4 and blend 10. This increase in ash content is mainly due to the addition of ginger in these formulations. Indeed, an analysis done by (Nwinuka *et al.*, 2005) showed a relatively high ash contents in ginger (6.40 ± 0.15%). Vunchi *et al*. (2011) showed in their study that the consumption of foods with high ash content brings a significant amount of mineral salts to the body. Protein levels for formulations varied between 13.54 ± 1.01% (Blend4) and 17.12 ± 1.04% (Blend 6) and are higher than those of spices such as *Tetrapleura tetraptera* (5.0 ± 0.3 g / 100g MS) and *Xylopia aethiopica* (7.9 ± 0.3 g / 100g MS) (*Bouba et al.,* 2012). Regarding the carbohydrate content, blend 4 and 10 obtained the highest values (35.67 ± 0.81 and 33.0 ± 0.72 (%DM)). Proteins, fats and carbohydrates (Ijarotimi and Kershinro, 2013) provide energy value in foods. The phenolic compounds, phenol and other phytochemicals found in foods are bioactive compounds which can neutralize free radicals and that play a role in several diseases prevention (Doss *et al.,* 2010). This study showed significantly different ($p < 0.05$) polyphenol contents from one formulation to another. Polyphenol contents between 2287.42 ± 106.89 (blend 1) and 2033.59 ± 83.46 (mg GAE/100 g DM) (blend 6) are higher than the total polyphenol content of 35 different herbs and medicinal plants for which contents were between 0.8 to 42.1 mg of Gallic acid equivalent /g dry weight (DM) (Kahkonen *et al.,* 1999). The extracts of these formulations were used in different concentrations to show their antioxidant activities. The DPPH radical scavenging power of these spice formulations could be due to its fullness in individual polyphenols, tannins, flavonoids and phenolic compounds. These works directed by (Sharif *et al.,* 2008) showed that antioxidants extracted from plants are mainly phenolic compounds including flavonoids, tannins and phenolic acids. The IC50 value of the spice extract calculated from the logarithmic regression were obtained the best DPPH radical scavenging activities observed in blend 4 and 10.

CONCLUSION AND APPLICATION OF RESULTS

The study showed that the organoleptic properties of spices, the proximate composition and the presence of antioxidant compounds (phenolic acids, flavonoids and tannins) demonstrated some level of antioxidant activities of Cox. These parameters were enhanced through the addition of ginger and pepper. The hedonic analyses, which served as a reference for selected well-appreciated blends, made it possible to select blends 4, 6 and 10. Immediate analyses of the species revealed a sufficient amount of flavonoids, phenolics and tannins in the extracts of spice formulations. However, a strong scavenging activity for DPPH was observed in blend 4 and 10. These spice blends can be preserved as potential sources of certain biochemical compounds and natural sources of accessible and beneficial antioxidants for consumers. Blend pepper and ginger in the Cox formulation provides nutritional benefit by improving the hot spicy taste and the content of phenolic compounds.

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