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Full Length Research Paper

Constituents of *Kawal*, fermented *Cassia obtusifolia* leaves, a traditional food from Chad

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Femented leaves of *Cassia obtusifolia* are used as a substitute of meat or an appetizing agent by people of eastern of Chad and south of Sudan. Chemical composition of the methylene chloride extract from the fermented leaves of this legume was analyzed for the first time by GC and GC/MC. Thirty three constituents were identified. The major components are found to be aliphatic acids and identified as hexanoic acid (27%), butyric acid (10.4%) and valeric acid (6.3%) with lesser amounts of p-ethylphenol (17.2%) and p-methylphenol (13%). Examination of the protein fraction from leaves indicated 20.2% in the crude leaves and 12.9% in the fermented leaves. The participation of 10 g of fermented *C. obtusifolia* leaves to the daily requirements in essential amino acids of an adult is from 13 to 25% of needed amount. Moreover this traditional food had a high content of potassium and calcium.

Keys words: *Cassia obtusifolia*, leguminous, fermented leaves, food, volatiles, amino acids, trace elements.

INTRODUCTION

Cassia obtusifolia, syn. *Cassia tora* (family Leguminous) is a wild African plant found in wastelands in the rainy season. Its leaves can be fermented (named Kawal) and is used by people from the eastern part of Chad and the southern part of Sudan as a meat replacer or a meat extender.

Although the genus Cassia has been subjected to extensive phytochemical investigations because of their medicinal properties (Sui-Ming et al., 1989; Ching-Kuo et al., 2001; Yun Choi et al., 1990; Yen and Chen, 1998), no important study concerning the volatiles from the crude and the fermented leaves has been done. The last reported study on the fermented leaves of *C. obtusifolia*, which described the phytochemical properties, was published by Dirar et al. (1984, 1985, 1993). Because of the importance of these fermented leaves in the diets of the population, we investigated the volatiles from the methylene chloride extract of the fermented leaves of *C. obtusifolia*, and analysed the composition of amino acids and trace elements in the samples.

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EXPERIMENTAL

Raw materials

The leaves of *C. obtusifolia* were collected from northern N'Djaména (Chad), late in the rainy season when the plant is fully grown (September, 2001). Its taxonomic status was confirmed by comparison the voucher specimens of the plant with those of the herbarium of the "Laboratoire Vétérinaire et Zootechnique de Farcha" (AG n° 3152 and NK n° 63). The fermented leaves samples were obtained according to the processing described below. The leaves are pounded into paste without releasing the juice. The paste is placed in an earthenware and covered with sorghum leaves. The whole jar is buried in the ground up to the neck. Every 3 days the contents are mixed by hand. The fermentation takes about fourteen days. After fourteen days, the strongly smelling black fermented paste is removed and sun-dried.

Solvent extraction of the volatiles

Air-dried fermented leave samples (500 g) were soaked in dichloromethane for 48 h. The solvent was removed by filtration, and the fresh CH_2Cl_2 was then added to the sample for another 48 h. The CH_2Cl_2 extracts were combined and evaporated under reduce pressure to give 2.8g of a greenish viscous residue (yield: 1.7%).

Mineral solution from the fermented leaves samples

Fermented leaves (10 g) oven dried at 110°C and ground to fine powder were incinerated at 550°C according to the processing previously described (AFNOR,1981). The ash (1 g) was then dissolved in 1 L of water acidified with H_2SO_4 15%.

Analytical techniques

The volatiles were analysed by gas chromatography (GC) and gas chromatography-mass spectrometry (CG-MS). Apparatus types and analytical conditions were as previously reported (Mahmout et al., 2001). The percentage composition of the extract was computed from the CG peak areas without using correction factors. The volatile constituents were identified by their retention indices and by comparison of the mass spectra with those of authentic compounds or with those from literature and databases (Adams, 1989; Jenning and Shibamoto, 1980; Ardrey at al, 1983; HP59943ANSB Database, 1988).

Amino acid analyses: Air-dried and powdered leaves of *C. obtusifolia* were hydrolysed, under nitrogen, in HCl vapour at 120°C for 24 h using a Pico-Tag work station (Waters). Along with 2-β-mercaptoethanol (4%), to preserve sulphur-containing amino acids, 200 µL of 6 N HCl were placed in the hydrolysis tank. After hydrolysis, 10 nmol of glocosamic acid per mg of sample were added as an internal standard. The samples were dried under vacuum in a Speedvac apparatus (Savant Instrument Inc., Farmingdale) and taken up with 0.05 M lithium citrate buffer, pH 2.2. The samples were submitted to ion exchange chromatography on an automatic amino acid analyser (Beckman 3600). Amino acids were detected by the ninhydrin reaction, identified by their retention time and wavelength ratio, and quantified by their absorption at 570 n (440 nm for proline).

Mineral contents: Sodium and potassium were determined with a 410 flame spectrophotometer using butane under the pressure of 2.1 kg/cm^2; the debit was 0.4L/min. Calcium and magnesium were determined with an atomic absorption spectrophotometer (Spectro Varian 20BQ). S-2501 colorimeter method was used for the determination of iron and phosphorus (Buoso et al., 2002).

Table 1. Composition of fermented leaves of *C. obtusifolia.*

RI	Constituent	Percentage
725	propionic acid	0.5
770	isobutyric acid	3.2
849	(Z)-3-hexenol	0.1
855	butyric acid	10.4
860	3-methylbutyric acid	5.5
865	2-methylbutyric acid	2.5
952	valeric acid	6.3
956	Benzaldehyde	0.4
970	Phenol	1.8
975	Trimethylpyrazin	0.3
1030	Benzyl alcool	0.1
1048	Hexanoic acid	27.1
1049	5-Hexenoic acid	0.3
1066	P-Methylphenol	13
1069	Cis-Linalyl oxide	0.5
1071	Tetramethylpyrazine	0.7
1075	Butyl isovalerate	1.9
1078	Isobutyl valerate	2.7
1105	Phenylethanol	1.3
1158	P-Ethylphenol	17.2
1171	(Z)-3 Hexenylisobutyrate	0.1
1238	2,3dihydrobenzofurane	0.2
1246	Phenylethyl acetate	0.4
1280	Isopentyl hexanoate	0.5
1283	2-Methylbutyl hexanoate	0.2
1338	Benzyl Butanoate	0.1
1341	Phenylethyl propionate	0.1
1352	Eugenol	0.2
1437	Phenethyl butyrate	0.9
1460	(E)-B-Ionon-5,6-epoxide	0.7
1480	Phenylethyl 2-methylbutanoate	0.1
1490	Dihydroactinidiolide	0.5
1634	Phenethyl hexanoate	0.8
Total		99.98

RESULTS AND DISCUSSION

Examination of our results given by the GC and the GC-MS which are summarised in Table 1 shows that the extract of fermented leaves of *C. obtusifolia* was essentially made up of aliphatic acids (45.43%). The corresponding esters are 6%, while phenolics account for 32%. The large content of hexanoic acid (27%) and butyric acid (10%) could be identified as responsible for the goat-like and the cheese-like odour of the fermented *C. obtusifolia* leaves. The amount of other aliphatic acids such as 2-methylbutyric (5.48%) and 2-methylbutyric (2.48%) seems to be noteworthy. In fact, it is well known

Table 2. Amino acids composition of *C. obtusifolia*.

Aminoacid	Crude leaves (mg/100g weight)	Fermented leaves (mg/100g weight)	Requirement (mg/50 kg weight Adult /Day)*	Percentage of the daily requirement**
Threonine	980	522	350	14.91
Valine	1606	1282	500	25.64
Cystine	13	4	650 (Cyst+Met)	0.33 (Cyst+Met)
Methionine	11	18		
Isoleucine	1181	987	500	19.74
Leucine	2234	1803	700	25.75
Tyrosine	435	239	700 (Tyr+Phe)	18.01 (Tyr+Phe)
Phenylalanine	1562	1022		
Lysine	1477	790	600	13.16
Histidine	664	356	500	7.12
Arginine	1314	601	-	-
Aspartic acid	3786	1345	-	-
Serine	863	505	-	-
Glutamic acid			-	-
Proline	1769	858	-	-
Glycine	1428	1074	-	-
Alanine	1595	1638	-	-
Total amount of amino acids	**20.2%**	**12.9%**		

*Recommended dietary allowances NRC-National Academy of Sciences, USA, 10th ed. p.17 (estimation for a 50 kg weight adult).
**For a quantity of 10 g of fermented leaves consumed by an adult.

Table 3. Elemental composition of the fermented *C. obtusifolia* from Chad.

Element	Amount (mg/kg)
Na	1310
K	31500
Ca	27250
Mg	1490
P	2800
Fe	0.4
Total ash	**18%**

that these compounds exhale a fruity odour. The occurrence of p-methylphenol (13%) and p-ethylphenol (17.21%) suggests the attack of the bulk phenolic constituents of the raw materials by the yeast during semi-anaerobic fermentation.

The total protein concentration in the dried leaves of *C. obtusifolia* is 20.2% but falls to 12.9% after fermentation. The loss of proteins may result because of their use for the growth of the micro-organisms during fermentation. It could be note in the Table 2 that the fermentation modifies also the relative amounts of amino acids. Only the amounts of Met and Ala increase. The participation of 10 g. of fermented *C. obtusifolia* leaves to the daily requirements in essential amino acids of an adult is from 13 to 25% of the needed amounts. However, the amount of His is only 7% and the sulfur amino acid are negligible, but these constituents are available (usually in excess) in the cereals which are consumed with fermented leaves of *C. obtusifolia*. Although fermentation lowers the protein value, this process seems to be necessary to the palatability and the non-toxicity of these leaves.

The fermented leaves of *C. obtusifolia* contain the major elements needed by human body (see Table 3). Other reports showed that the samples obtained from the Sudan generally had lower quantity of elements compared to those from Chad (Dirar et al., 1985). It appears that locations of Cassia species and their environments have effects on the chemical constituents (Buosco et al., 2002)

In conclusion, these results show that elemental and amino acid compositions of the fermented leaves of *C. obtusifolia* corroborated the nutritional value of this traditional food which is the meat substitute for many poor people in the eastern part of Chad and south of Sudan. Their nutritional value is enhanced when eaten with cereals. This strongly smelling food is also used as flavour whose volatiles are here reported for the first time.

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