Traditional foods and sustainable rural development: exploiting the case of the Comoros tea as a potential source of bioactive compounds

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Solvents and chemicals

Sodium carbonate, Folin-Ciocalteu phenol reagent, sodium acetate, citric acid, potassium chloride, hydrochloric acid, iron(III) chloride hexahydrate, 2,4,6-tripyridyl-S-triazine (TPTZ), 1,2-phenylenediamine dihydrochloride (OPDA), all polyphenolic (caffeic acid, chlorogenic acid, coumaric acid, ferulic acid, hyperoside, isoquercitrin, quercetin, quercitrin, rutin, ellagic acid, gallic acid, (+)-catechin, (-)-epicatechin, castalagin, and vescalagin) and terpenic standards (limonene, phellandrene, sabinene, γ-terpinene, and terpinolene), carotenoids (α-carotene, β-carotene, β-cryptoxanthin, lutein, lycopene, zeaxanthin), potassium dihydrogen phosphate, phosphoric acid and HPLC-grade methanol, tert-Butyl methyl ether, dichloromethane, and acetonitrile were purchased from Sigma-Aldrich (St. Louis, MO, USA). Ethylenediaminetetraacetic acid disodium salt was purchased from AMRESCO (Solon, OH, USA). Sodium fluoride was purchased from Riedel-de Haen (Seelze, Germany). Milli-Q ultrapure water was produced by Sartorius Stedim Biotech mod. Arium (Sartorius, Göettingen, Germany). Cetyltrimethylammonium bromide (cetrimide), ascorbic acid (AA) and dehydroascorbic acid (DHAA) were purchased from Extrasynthése (Genay, France). Acetic acid, ethanol, organic acids (citric acid, malic acid, oxalic acid, quinic acid, succinic acid, and tartaric acid) and HPLC-grade formic acid were purchased from Fluka BioChemika, Buchs, Switzerland.

Extraction protocols

Table S1. Protocols for the extraction of bioactive compounds and nutritional substances.

Chemical class	Sample Weight	Extraction Solvents	Time of Extraction	Homogenisation	Centrifugation	Number of Successive Extraction	Other Actions
Polyphenolic compounds	10 g	methanol : bi-distilled water, 95:5 v/v, pH adjusted with 1.5 mL of 37% HCl (25 mL)	60 min in the dark	1 min	15 min at 3,000 rpm	3	/
Organic acids and monoterpenes	5 g	95% ethanol (25 mL)	30 min in the dark	1 min	10 min at 4,000 rpm	2	/
Vitamin C	5 g	0.1 M citric acid, 2 mM EDTA disodium salt, and 4 mM sodium fluoride in methanol – water, 5:95 v/v (10 mL)	20 min in the dark	1 min	10 min at 4,000 rpm	2	Supernatant acidified with 4 N HCl (pH = 2.2–2.4) and centrifuged for 5 min at 12,000 rpm at 4 °C
Carotenoids	5 g	methanol : tert-butyl methyl ether, 70:30 v/v (35 mL)	30 min in the dark	1 min	15 min at 4,000 rpm	2	/

Chromatographic analysis

Table S2. Chromatographic conditions of the used HPLC methods.

Method	**Classes of Interest**	**Stationary Phase**	**Mobile Phase**	**Wavelength** *(nm)*
A	cinnamic acids, flavonols	KINETEX – C18 column (4.6 × 150 mm, 5 µm)	A: 10 mM KH_2PO_4/H_3PO_4, pH = 2.8 B: CH_3CN	330
B	benzoic acids, catechins	KINETEX – C18 column (4.6 × 150 mm, 5 µm)	A: H_2O/CH_3OH/HCOOH (5:95:0.1 v/v/v), pH = 2.5 B: CH_3OH/HCOOH (100:0.1 v/v)	280
C	monoterpenes	KINETEX – C18 column (4.6 × 150 mm, 5 µm)	A: H_2O B: CH_3CN	250
D	organic acids	KINETEX – C18 column (4.6 × 150 mm, 5 µm)	A: 10 mM KH_2PO_4/H_3PO_4, pH = 2.8 B: CH_3CN	214
E	vitamins	KINETEX – C18 column (4.6 × 150 mm, 5 µm)	A: 5 mM $C_{16}H_{33}N(CH_3)_3Br$/50 mM KH_2PO_4, pH = 2.5 B: CH_3OH	261, 348
F	carotenoids	KINETEX – C18 column (4.6 × 150 mm, 5 µm)	A: ACN B: CH_3OH C: CH_2Cl_2	450

Elution conditions

Method A, gradient analysis: 5%B to 21%B in 17 min + 21%B in 3 min (2 min conditioning time); flow: 1.5 mL min^{-1}

Method B, gradient analysis: 3%B to 85%B in 22 min + 85%B in 1 min (2 min conditioning time); flow: 0.6 mL min^{-1}

Method C, gradient analysis: 30%B to 56%B in 15 min + 56%B in 2 min (3 min conditioning); flow: 1.0 mL min^{-1}

Method D, gradient analysis: 5%B to 14%B in 10 min + 14%B in 3 min (2 min conditioning time); flow: 0.6 mL min^{-1}

Method E, isocratic analysis: ratio of phase A and B: 95:5 in 10 min (5 min conditioning time); flow: 0.9 mL min^{-1}

Method F, isocratic analysis: ratio of phase A, B and C: 75:20:5 in 20 min (5 min conditioning time); flow: 1.0 mL min^{-1}