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Nutritional evaluation and functional properties of traditional composite salad dishes

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ABSTRACT

Crude composition and bioactive microconstituents were determined in three traditional vegetable salad dishes, namely cabbage, Greek and lettuce salad, and a dietary evaluation from their consumption was undertaken. In addition, the salads' *in vitro* anti-inflammatory and antiatherogenic potential were evaluated. The salads under concern were good sources of dietary fiber, carotenoids, vitamin C, squalene, phytosterols, polyphenols, and terpenic acids. Phytosterols and squalene in lettuce and cabbage salads originated mainly from olive oil, while the bulk of simple polyphenols and terpenic acids in Greek salad originated from the table olives and oregano used for its preparation. In addition, salads' extracts inhibited lipid oxidation in total serum and exerted cytoprotective activity in stimulated peripheral blood cells and anti-inflammatory activity by downregulating the proinflammatory mediators TNF-α, IL-6 and MCP-1, their activity correlating with their phenolic and terpenics content.

1. Introduction

The Mediterranean Diet (MD) is grounded on the principles of variety, nutrient rich and natural, minimally or not processed food consumption. One of the major constituents of the MD is the high consumption of fruits and vegetables, which, in addition to vitamins and minerals, contain phytochemicals, such as flavonoids and other phenolics (Naczk & Shahidi, 2006). Also, olive oil is a very important element in MD, composed mainly of the mixed triacylglycerols of oleic acid and palmitic acid, along with traces of squalene and sterols, and with several antioxidant phenolic compounds (Boskou, 2009).

Traditional Greek diet has been considered a typical example of the healthy MD, the adherence to which has been associated with reduction in mortality related to coronary heart disease and cancer (Trichopoulou, Costacou, Bamia, & Trichopoulos, 2003), and with increased survival among the elderly (Trichopoulou et al., 2005). In the traditional Greek diet, cold salads are constituted of fruits and vegetables including cultivated leafy vegetables and also root vegetables, usually prepared with virgin olive oil and, in the case of the so-called "Greek salad", also with feta cheese. Traditional Greek salads are therefore appetizing combinations of the above items that contain various nutrients and phytochemicals interacting with each other and with olive oil compounds. Nutritional evaluation and functional properties of individual items have been established in several studies. However, the amount and orchestrating role of these materials in salad dishes prepared following the traditional recipes of Greek cuisine have not been examined yet. The aim of this study was to evaluate the nutritional characteristics of three salad dishes – namely cabbage salad, Greek salad and lettuce salad – most commonly consumed in Greece. Furthermore, the antiatherogenic potential of salad extracts on total serum lipids and their cytoprotective and anti-inflammatory effects in mononuclear cells were tested.

2. Materials and methods

2.1. Materials, reagents and chemicals

RPMI-1640 culture media was purchased from Gibco (Grand Island, NY, USA). Ficoll-Paque Plus was from GE Healthcare Biosciences (Piscataway, NJ, USA). Fetal bovine serum (FBS), penicillin/streptomycin and trypsin/EDTA were supplied by PAA Company (Somerset, UK). Quantikine sandwich ELISA kits for the measurement of interleukin-6 (IL-6), monocyte chemoattractant protein-1 (MCP-1) and tumor necrosis factor–alpha (TNF-α) were from R&D Systems (Oxford, UK). Lactate dehydrogenase (LDH) cytotoxicity assay kit was from ABCAM (Cambridge, MA, USA). Phosphate buffer saline (PBS) tablets, lipopolysaccharide (LPS) and copper sulphate were purchased from Sigma-Aldrich (St Louis, MO, USA). A

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standard mixture of 37 fatty acid methyl esters was purchased from Supelco (Bellefonte, CA, USA). Butylated hydroxytoluene (BHT), boron trifluoride in methanol (14% BF_3/MeOH), 2,4,6-tris (2-pyridyl)-s-triazine (TPTZ), 5-α-cholestane, cholesterol, β-sitosterol, stigmasterol, campesterol, squalene, ascorbic acid, β-carotene, *p*-hydroxybenzoic acid, ursolic acid, vanillin, *p*-coumaric acid, syringic acid, gallic acid, *p*-hydroxyphenylacetic acid, resveratrol, luteolin, chlorogenic acid, ferulic acid, catechin and tyrosol were obtained from Sigma (Steinheim, Germany). Folin-Ciocalteu reagent, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox®), bis-(trimethylsilyl)-trifluoroacetamide (BSTFA), 1,1-diphenyl-2-picrylhydrazyl radical (DPPH•), homovanillic alcohol, phloretic acid, cinnamic acid, oleanolic acid, quercetin and 3-(4-hydroxyphenyl)-1-propanol were obtained from Aldrich (Steinheim, Germany). Caffeic acid, protocatechuic acid, and sinapic acid were from Fluka (Steinheim, Germany). 3,4-dihydroxyphenylacetic acid, chrysin and genistein were from Alfa Aesar (Karlsruhe, Germany); hydroxytyrosol, kaempferol, and naringenin from Extrasynthèse (Genay-Cedex, France); vanillic acid from Serva (Heidelberg, Germany); epicatechin from Biochemika (Steinheim, Germany); thymol from Riedel-de Haen (Seelze, Germany). All the solvents used were of HPLC grade and were purchased from Merck (Darmstadt, Germany) or Aldrich (Steinheim, Germany).

2.2. Salad dish preparation

The ingredients used for salads' preparation, shown in Table 1, were purchased from local supermarkets in Athens. Care was taken in choosing fresh fruits and vegetables of superior quality, by checking their appearance, smell and texture and selecting items free of bruises, spots or cracks. In the laboratory, fruits and vegetables were thoroughly washed with cold water, excess water was drained, and then they were peeled and/or chopped. Commercial extra virgin olive oil (EVOO) of the Koroneiki cultivar was used in all salads. Salads were prepared according to specific recipes provided by a chef (Palisidis) Individual ingredients were weighed prior composing the different salad dishes(Table 1). The prepared salad dishes were subsequently homogenized with a domestic mixer (Kenwood KMC010, New Lane, Havant, Hampshire, USA) and were freeze-dried (Holten, Allerod, Denmark). Freeze dried samples were kept in air-tight glass containers at −40 °C and were used for all analyses, except ascorbic acid, which was determined in fresh salads on the day of preparation.

Table 1
Ingredients used for salad preparation.

Type of salad	Ingredients	Weight (g)
Cabbage salad	Cabbage	393.2
	Carrots	97.5
	Fresh lemon juice	23.3
	Extra virgin olive oil (EVOO)	13.4
	Garlic	4.3
	Canned capers	20.9
	Total weight	552.6
Greek salad	Tomatoes	606.1
	Cucumber peeled	175.2
	Onions	85.9
	Sweet peppers (green peppers)	109.0
	Feta cheese	110.0
	Kalamon table olives (flesh only)	66.3
	Extra virgin olive oil	19.1
	Canned capers	11.4
	Dried oregano	1.0
	Total weight	1184.1
Lettuce salad	Lettuce	250.4
	Spring onions (scallions)	48.6
	Extra virgin olive oil	12.8
	Vinegar	10.1
	Total weight	321.9

2.3. Chemical analyses

2.3.1. Crude composition and energy content

Moisture was calculated from the weight loss during freeze-drying, as the water content of lyophilized samples was found to be less than 3 g/100 g. The ash content of freeze dried samples was determined by a programmable muffle furnace. Total lipids were determined gravimetrically after extraction of lipids according to Bligh and Dyer (1959). Crude fiber was determined according to Weende's method using a Dosi Fiber apparatus (Selecta, S.A., Barcelona); Weende crude fiber is the ash-free residue of the acid-alkali treatment. The gross energy content was determined in the freeze-dried samples, by means of an IKA C4000 (IKA Analysentechnik, Germany) bomb calorimeter.

2.3.2. Fatty acids

Fatty acids were determined as methyl esters by GC/MS in aliquots of the Bligh Dyer extracts, as previously described (Kalogeropoulos et al., 2010).

2.3.3. Sterols and squalene

Sterols and squalene were determined in aliquots of the Bligh Dyer extracts by GC/MS after hot saponification with 0.5 mol/L KOH/MeOH and derivatisation to trimethylsilyl ethers with BSTFA, as described by Kalogeropoulos et al. (2010), by employing 5-a-cholestane as internal standard.

2.3.4. Carotenoids

Carotenoids were determined in the Bligh-Dyer extracts with the spectrophotometric method of Fredriksson, Elwinger, and Pickova (2006), by measuring the absorbance at 450 nm using a Specord 20 (Analytik Jena, Jena, Germany) spectrophotometer and performing external standard quantification with β-carotene standard solutions. The carotenoids content was expressed as mg β-carotene/100 g fresh weight.

2.3.5. Ascorbic acid

Ascorbic acid was determined in fresh homogenized salad samples on the day of their preparation by the 2,4-dinitrophenylhydrazine method of Roe (1961). Briefly, 0.1 g of sample was homogenized with ice-cold 0.4 g/100 g oxalic acid (5 mL) with an UltraTurrax T25 (IKA-Werke, Staufen, Germany) homogeniser and centrifuged for 10 min at 800 *g*. The ascorbic acid concentration in the supernatant was determined after reaction with 2,4-dinitrophenylhydrazine by recording absorbance at 520 nm using a Specord 20 spectrophotometer.

2.3.6. Polyphenols extraction

Polyphenols were isolated by the procedure described by Chouchouli et al. (2013), slightly modified. For this purpose, freeze-dried salad samples and EVOO (1 g) were extracted with methanol (5 × 10 mL) using a Vortex and a sonicator bath. After the first extraction, samples were soaked overnight in the extracting solution in refrigerator (4 °C). The extracts were separated by centrifugation, were combined and, after solvent removal by vacuum evaporation (Centrivap, Labconco, MO, USA), the residues were dissolved in methanol (2 mL), sonicated for 10 min and syringe-filtered through 0.45 μm membrane filters. Extractions were carried out in triplicate. The extracts were sealed in GC vials and kept at −40 °C. Aliquots of salad methanolic extracts, at concentrations corresponding to 0.2 mg fresh salad/mL MeOH, were used for the