Food Research International 43 (2010) 2006–2013

Contents lists available at ScienceDirect

Food Research International

journal homepage: www.elsevier.com/locate/foodres

Nutritional evaluation and bioactive microconstituents (carotenoids, tocopherols, sterols and squalene) of raw and roasted chicken fed on DHA-rich microalgae

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ARTICLE INFO

Article history:
Received 19 March 2010
Accepted 30 May 2010

Keywords:
Broilers
Carotenoids
Cholesterol
DHA
Fatty acids
Squalene
Tocopherols

ABSTRACT

Fresh breast fillets, leg fillets, and whole carcass from broilers fed on a docosahexaenoic acid (DHA)-rich microalgae, were analysed for crude composition, energy content, sterols, fatty acids, carotenoids, squalene and tocopherols, and were compared to broilers fed on a conventional diet. DHA supplementation did not cause significant changes of crude composition and sterols – including cholesterol – content. On the contrary, DHA-enriched broiler's whole carcass, breast and legs contained 3.1–5.6 times more DHA, presented lower ω6/ω3 ratios, lower tocopherols content, and contained significantly more carotenoids and squalene compared to conventional samples. The roasting of DHA-enriched breast and leg fillets caused partial loss of DHA, total fat, carotenes, sterols, squalene and tocopherols. The loss of these macro- and microconstituents was more extended in leg fillets compared to breast fillets. The consumption of roasted DHA-rich broiler's breast and legs is expected to cover a significant fraction of daily protein and DHA intake, without overloading the consumer with cholesterol, while providing certain amounts of health promoting microconstituents.

1. Introduction

Chicken meat is consumed worldwide with increasing popularity, as it is an excellent source of protein, has a favourable ratio of unsaturated to saturated fatty acids, and delivers essential vitamins and minerals, while it is among the most affordable meat sources (Hargis & Van Elswyk, 1993). By the end of 90s the daily per capita intake of chicken in European Union countries ranged from 18 g in Finland to 62 g in Cyprus (DAFNE), while in USA chicken annual per capita consumption was more than doubled between 1974 and 2004, from 12.4 to 26.8 kg (Buzby & Farah, 2006).

Given the prevalence of diet related diseases like obesity, diabetes, hypertension, cardiovascular diseases and certain types of cancer, the link between diet and health has become important, and consumers' demand for food products of superior health quality has increased. This need resulted in efforts to modify the lipid composition of poultry meat and eggs and studies were centred on the manipulation of specific fatty acids, like the omega 3 fatty acids.

Omega-3 fatty acids are polyunsaturated fatty acids (PUFA), which are present in our diet as α-linolenic acid (ALA, C18:3ω3) provided mainly by vegetable oils and nuts, and as long-chain ω3 PUFA (LC ω3 PUFA) consisting mainly of eicosapentaenoic acid (EPA, C20:5ω3) and docosahexaenoic acid (DHA, C22:6ω3) provided mainly by fish and seafood. The ω3 PUFA exhibit a wide range of beneficial effects on human health, while they may influence the course of several diseases and disorders (Lagarde, 2008; Shahidi & MIraliakbari, 2004, 2005; Simopoulos, 1991). Among LC ω3 PUFA, docosahexaenoic acid (DHA) has a unique role: it is important for biological membranes, the cerebral cortex, nervous tissues, blood platelets due to its effect on arteries and it is responsible for the development and proper function of the brain and the retina (Lagarde, 2008; Mueller & Talbert, 1988).

The ω3 PUFA are "essential" nutrients, as they cannot be synthesized by humans. ALA can be converted to EPA and DHA in the human body, but the extent of this conversion is, at best, very limited and inefficient (Lagarde, 2008).

Therefore LC ω3 PUFA must be provided through the diet, and several governmental agencies and nutritional organizations have recommended daily intake levels of DHA and EPA ranging from 0.2–0.3 g/day for the general population (Krauss et al., 2000; Ruxton, Reed, Simpson, & Millington, 2004) up to 1.0–4.0 g/day for patients with documented coronary heart disease (CHD) and patients who need to lower triacylglycerols (AHA, 2009). As fish is currently the primary dietary source of DHA and EPA, consumers are encouraged by health authorities to eat more fish. However, seasonal availability, affordability, taste preferences and concern for environmental toxins

Abbreviations: DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; LC ω3 PUFA, long chain ω3 polyunsaturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids.

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doi:10.1016/j.foodres.2010.05.018

often limit fish consumption, thereby excluding the primary source of LC ω3 PUFA (Gebauer, Psota, Harris, & Kris-Etherton, 2006).

The alternative to fish and fish oil supplements is to produce other food sources containing significant levels of EPA and DHA. This led to the formulation of a variety of common processed foods such as bread and milk which were enriched with LC ω3 PUFA from fish oil. However, unless flavour masking techniques like microencapsualtion are involved, this can impair the sensory quality due to susceptibility of the LC ω3 PUFA to oxidation (Lyberg, Fasoli, & Adlercreutz, 2005). An alternative approach was to enrich meat and eggs by the supplementation of rations with dried marine microalgae which are very rich in LC ω3PUFA, especially DHA and do not cause off-flavours (Abrilm & Barclay, 1998; Fredriksson, Elwinger, & Pickova, 2006).

In the present study we compared the crude composition, energy, sterols, tocopherols, carotenoids, squalene, fatty acids and LC ω3 PUFA content of broilers meat enriched by dietary treatment with a DHA-rich microalgae supplement to broilers fed on a conventional diet. As the lipids of marine microalgae have been reported to contain squalene, several sterols and carotenoids (Lewis, Nichols, & McKeekin, 2001; Shahidi, Metusalach, & Brown, 1998), the levels of these microconsituents in the broiler meat samples were also studied. In addition, the effect of roasting of DHA-enriched chicken breast and leg fillets on the macro and microconstituents studied was examined and a nutritional evaluation of the roasted fillets was undertaken.

2. Materials and methods

2.1. Reagents and chemicals

Butylated hydroxytoluene (BHT), boron trifluoride in methanol (14% BF_3/MeOH), cholesterol, 5-α-cholestane, methyl eicosapentaenoate, methyl docosahexaenoate, methyl nonanoate, β-carotene, β-sitosterol, campesterol, stigmasterol and squalene were purchased from Sigma Chemicals. A standard mixture of 37 fatty acid methyl esters (FAME) was purchased from Supelco. α-Tocopherol and δ-tocopherol were obtained from Aldrich (Steinheim, Germany), and γ-tocopherol was purchased from Fluka (Steinheim, Germany). Hexane, chloroform, propanol-2, and acetonitrile of HPLC grade were provided from Merck (Darmstadt, Germany). Bis-(trimethylsilyl)-trifluoroacetamide (BSTFA) was obtained from Aldrich. All other solvents used were of analytical grade.

2.2. Samples and sample preparation

Both the DHA-enriched and the conventional broiler meat samples are commercial products, marketed fresh in Greek supermarkets. All chicken were grown in the same farm, fed with similar diets and were slaughtered at the age of 45–55 days. The DHA enriched chicken received additionally a dried marine microalgae (*Schizochytrium* sp) supplement (DHA Gold®, Martek Biosciences Corporation, Columbia, MD) which, according to the manufacturer, contains around 40% fat, 10% protein and more than 18% DHA. The supplement was provided in such a way that by the slaughter day each bird had received at least 23 g of dried microalgae. Both conventional and DHA-enriched broiler meat samples were brought in the laboratory on the slaughtering day packaged in the way they are marketed, i.e. in plastic trays covered by plastic films, and kept at refrigerator temperature (4 °C). The samples were conventional and DHA-enriched breast fillets, leg fillets and whole chicken. At their arrival in the laboratory, the samples were unwrapped, skin and bones were removed from the whole chicken samples, while the DHA-enriched breast and leg fillets were divided in identical subsamples and half of them were roasted at a domestic electric kitchen oven until well done, at 150 °C for 50 min on a grill that allowed juices to drain. Fillets were weighed before and after roasting; raw breast and leg fillets weighed 440.0 and 306.1 g, while the respective roasted samples weighed 285.7 and 165.2 g. All samples were subsequently homogenized by a tissue grinder. A portion of the homogenized samples was sealed in plastic cups and kept at −40 °C to serve for the determination of total lipids, sterols, fatty acids, carotenoids, squalene, and tocopherols. Another portion was frozen and then lyophilized for 48 h, homogenized by a mixer, sealed in plastic cups, and kept at −20 °C.

2.3. Crude composition and energy determination

Freeze-dried samples were used for the determination of ash, protein, and energy content. Freeze-drying served also for moisture determination, as the water content of freeze-dried samples was found to be less than 1%. The ash content of freeze-dried samples was determined by programmed temperature incineration in a muffle furnace. Total protein was calculated from Kjeldahl nitrogen using a 6.25 conversion factor. Kjeldahl nitrogen was determined in the freeze-dried samples by employing a Buchi (Zurich, Switzerland) B426 digesting apparatus and B316 steam-distillation apparatus. The gross energy content was determined in the freeze-dried samples, by means of an IKA C4000 (IKA Analysentechnik, Heitersheim, Germany) adiabatic calorimeter. Fat content was determined gravimetrically in 10 g portions of fresh meat homogenates after extraction and purification of total lipids according to the method of Folch, Lees and Sloane Stanley (1957). The extracted lipids were weighed, diluted in hexane which contained 20 mg/L BHT and were stored under nitrogen at −40 °C. The lipid samples served also for the determination of sterols, fatty acids, carotenoids, squalene and tocopherols. Lipid content, sterols, fatty acids, carotenoids and squalene were also determined in the DHA-rich microalgae supplement.

2.4. Fatty acids determination

Fatty acids were determined in aliquots of the Folch extracts (containing 10–20 mg of lipids) by GC/MS, in the form of fatty acid methyl esters (FAME), after hot saponification with 2 mL of 0.5 M KOH in MeOH, for 15 min at 90 °C, followed by methylation of fatty acids with 1.5 mL of BF_3/MeOH for 2 min at 90 °C in Teflon lined screw-capped vials. FAME were separated on a 50 m × 0.22 mm inner diameter BPX 70 capillary column (SGE, Melbourne, Australia) coated with a 0.25 μm film of cyanopropyl silicone, using an Agilent Technologies HP 6890 (Avondale, PA) gas chromatograph equipped with an autosampler and with a MSD-5972 mass selective detector, as previously reported (Andrikopoulos, Kalogeropoulos, Falirea, & Barbagianni, 2002). Identification of peaks corresponding to FAME was accomplished by means of a standard mixture of 37 FAMEs and by reference to the NIST 98 (Hewlett-Packard Version, NIST98 Mass Spectral Library Version 1.6) mass spectra library. The mixed FAME standard furthermore served for the calculation of fatty acid response factors, which were applied to the areas derived from the chromatographic traces. Additionally, internal standard quantification was carried out for the LC ω3 PUFA, namely 20:5ω3, 22:5ω3, and 22:6ω3, by constructing reference curves of methyl eicosapentaenoate and methyl docosahexaenoate by employing methyl nonanoate as internal standard. For the quantification of 22:5ω3 the reference curve of 22:6ω3 was used.

2.5. Sterols and squalene determination

Sterols and squalene were determined in the non-saponifiable fraction of the Folch extract (aliquots containing 20–30 mg of lipids) after direct saponification with 0.5 M KOH/MeOH followed by methylation with 10% (v/v) BF_3/MeOH as described by Hwang, Wang, & Choong, 2003. The unsaponifiable fraction was then extracted with 2 mL hexane and added 100 μL 5-α-cholestane 0.4% (w/v) as internal standard. Aliquots of 100 μL of the above extracts were