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Enhancing colour and oxidative stabilities of reduced-nitrite turkey meat sausages during refrigerated storage using fucoxanthin purified from the Tunisian seaweed *Cystoseira barbata*

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The present study investigated the angiotensin-I converting enzyme (ACE) inhibitory activity and the antioxidant properties, *in vitro* and in cured meat sausages containing reduced levels of sodium nitrite, of fucoxanthin extracted from the Tunisian brown seaweed *Cystoseira barbata* (CBFX). Results revealed that CBFX exhibited great scavenging activities against DPPH free radicals ($EC_{50} = 136$ μg/ml), peroxyl radicals in the linoleate-β-carotene system ($EC_{50} = 43$ μg/ml) and hydroxyl radicals generated by Fenton reaction (DNA nicking assay). A considerable ferric reducing potential was also recorded for CBFX ($EC_{50} = 34$ μg/ml). It is interesting to note that CBFX was found to modulate the ACE activity, which is the key enzyme involved in the blood pressure regulation, with an EC_{50} of 5 μg/ml. When fucoxanthin was supplemented, the concentration of sodium nitrite added to cured turkey meat sausages was reduced from 150 to 80 ppm, coupled with the enhancement of colour and oxidative stabilities. Thus, CBFX, with noticeable antioxidant and antihyertensive effects, could be used as a natural additive in functional foods to alleviate potential human health hazards caused by carcinogenic nitrosamines formation.

1. Introduction

Recently, the attention towards pigments from natural sources has increased (Kirti et al., 2014). The natural pigments are essentially represented by carotenoids, flavonoids (anthocyanins) and some tetrapyrroles (chlorophylls and phycobiliproteins) (Kirti et al., 2014). Carotenoids, a vital class of lipidic soluble functional compounds with colours ranging from yellow to red, are widely synthetized in plants, algae, bacteria and fungi. However, they can not be synthesized *in vivo* by humans and only consumed through diet (Ruiz-Sola and Rodríguez-Concepción, 2012). Carotenoids include two main subclasses of non-polar hydrocarbon carotenes and polar compounds named xanthophylls comprising oxygenated derivatives such as hydroxyl, keto, epoxy, methoxy, or carboxylic acid groups (Kirti et al., 2014). Seaweeds (macroalgae) serve as an important source of carotenoids. Fucoxanthin is a well known xanthophyll (3′-acetoxy-5,6-epoxy-3,5′-dihydroxy-6′,7′-didehydro-5,6,7,8,5′,6′-hexahydro-β- β-caroten-8-on) with a unique carotenoid structure including an allenic bond, a 5,6-monoepoxide, 9 conjugated double bonds and a conjugated carbonyl group in the polyene chain of the molecule (Fig. 1) (Piovan et al., 2013). Fucoxanthin (FX) is the main carotenoid produced in brown algae as a component of the light-harvesting complex for photosynthesis and photoprotection. FX has received considerable attention because of its diverse health-promoting properties. The antioxidant properties of FX have been suggested as being the main mechanism that afford beneficial health effects (D'Orazio et al., 2012). Fucoxanthin has been reported to effectively scavenge chemically-generated free radicals, to quench singlet oxygen (Sachindra et al., 2007) and to act as a preventing agent against cardiovascular risk factors such as obesity, diabetes, high blood pressure, chronic inflammation and high triglycerides and cholesterol concentrations (Jeon et al., 2010).

There is an increasing interest in meat products processed with less or no additives. Sodium nitrite in combination with salt is widely used in the production of cured meat products. Nitrite or nitric oxide (derived from nitrite) are effective against lipid

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oxidation by chelating iron or by reacting with lipid peroxyl radicals to form non-radical addition products, thereby breaking the radical chain processes characteristic of lipid oxidation (Zanardi et al., 2004). However, some epidemiological and toxicological studies have demonstrated a potential relationship between nitrites, N-nitroso compounds and the risk of cancer. Nitroso compounds belong to the family of potent human carcinogens known as N-nitrosodimethylamine, which are easily formed by the interaction of a secondary amino compound with nitrites under specific conditions (Bryan et al., 2012; Herrmann et al., 2015). In order to reduce the risk of nitrosamine formation, EUDirective 2006/52/EC (EU, 2006) refers to the necessity of reducing the use of nitrites in meat products rather than their total replacement. The amount of nitrite permitted for use as a food additive in cured meat is currently 150 mg/kg (150 ppm) (EU, 2006). Several approaches, including addition of ascorbic acid or other antioxidants, have been conducted to reduce nitrosamines formation (Alahakoon et al., 2015). Food industries generally used synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) to control oxidation process. However, the use of these synthetic compounds has been linked to carcinogenic effects in long-term consumption (Botterweck et al., 2000; Williams et al., 1999). Thereby, the search for naturally occurring compounds with antioxidant activity has increased dramatically. Ascorbic acid is commonly added in cured meat products to reduce the formation of carcinogenic nitrosamines as well as to retard lipid oxidation in turkey meat sausages containing nitrite as curing agent. Natural pigments acting as colorants and antioxidants could be also used to partially replace nitrites (Alahakoon et al., 2015).

Several studies demonstrated that fucoxanthin was a safe antioxidant compound and had no genotoxic/mutagenic effect on the bone marrow cells of mice. No mortality, no abnormalities in gross appearance and no abnormal changes in liver, kidney, spleen, and gonadal tissues were induced by fucoxanthin (Beppu et al., 2009; Iio et al., 2011a, 2011b; Peng et al., 2011). The chemical synthesis of fucoxanthin is very expensive and as brown seaweeds are potential sources of fucoxanthin, the possiblity of obtaining directly this carotenoid from the brown seaweed *Cystoseira barbata* should not be overlooked. Moreover, the reduction of nitrite amounts in meat products is highly requested, and until now, there are no studies that have been conducted on the effects of fucoxanthin as a partial substitute of nitrites in meat sausages. Therefore, the aim of the present study was to isolate fucoxanthin from a Tunisian brown seaweed (*C. barbata*) (CBFX), to assess its antioxidant and antihypertensive (ACE inhibition) properties and to evaluate the effect of different CBFX levels coupled to a reduced dose of sodium nitrite on enhancing the redness and delaying lipid oxidation and colour loss of turkey meat sausages.

2. Materials and methods

2.1. Materials

1,1-Diphenyl-2-picrylhydrazyl (DPPH), β-carotene, linoleic acid, 3-[2-pyridyl]-5,6-diphenyl-1,2,4-triazine-4,4'-disulfonic acid monosodium salt hydrate (ferrozine), butylated hydroxyanisole (BHA), Butylated hydroxytoluene (BHT), ferrous sulfate heptahydrate ($FeSO_4$, 7 H_2O), angiotensin I-converting enzyme (ACE) from rabbit lung, hippuril histidine leucine (HHL), methanol HPLC grade were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals, namely ascorbic acid, potassium ferricyanide, trichloroacetic acid (TCA), ferrous chloride ($FeCl_2$), ferric chloride ($FeCl_3$), D-deoxyribose, thiobarbituric acid (TBA), H_2O_2, Tween 40, ethanol, chloroform and other solvents, were of analytical grade.

2.2. Seaweed material and extraction procedure of fucoxanthin

The marine brown seaweed *C. barbata* was collected from Kerkennah Island (Sfax, Tunisia), in November 2012. Fresh seaweeds were thoroughly washed with seawater and placed in plastic bags. On their arrival at the laboratory, the seaweed thalli were washed again with tap water then with distilled water and dried in shadow (25 ± 2 °C) away from sunlight and heat for 20 days. Dried seaweed thalli were milled in a mechanical grinder to obtain a fine and homogeneous powder (0.2 mm mesh size), then were stored in sealed amber glass bottles at 4 °C.

FX was extracted from the dried powder of *C. barbata* (CBFX) as described by Haugan et al. (1992) with slight modifications. The algal powder (100 g) was extracted twice with acetone: methanol (7:3) for 24 h at 30 °C under stirring (250 rpm). The pooled extract was evaporated to dryness under reduced pressure in a rotary evaporator (Stuart RE 300B, UK) and re-dissolved in 100 ml methanol. In a separatory funnel, the dried extract dissolved in methanol was mixed with 10 ml water and 100 ml *n*-hexane and swirled 4 times. The lower methanol-water phase was recuperated in a new funnel, mixed with 300 ml water and 400 ml diethyl ether. Finally, the upper ether phase containing CBFX was collected and vacuum dried using rotary evaporator. The CBFX fraction obtained was dissolved in 5 ml of *N*-hexane.

2.3. Purification of CBFX by silica gel column chromatography

CBFX was purified from the crude extract by open silica gel column chromatography (SGCC) equilibrated with hexane, as described by Sangeetha et al. (2009). Firstly, the column was washed by successive *N*-hexane: acetone solvant (9:1, v/v) and (8:2, v/v) to remove chlorophylls and carotenoids other than

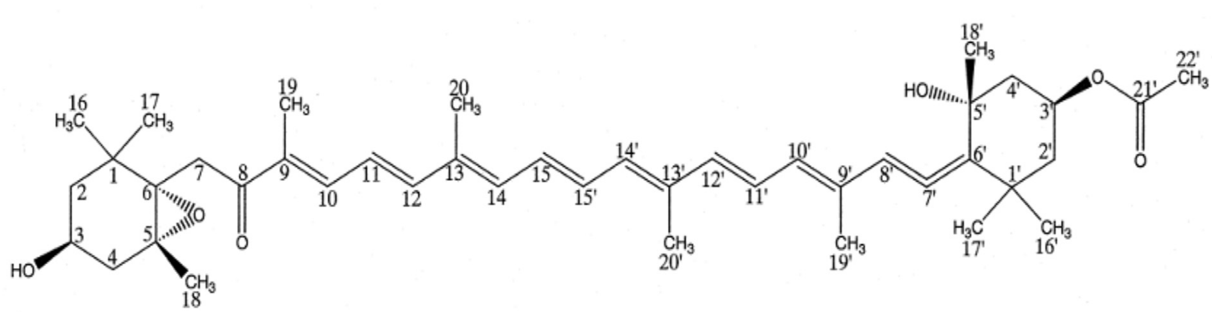

Fig. 1. Chemical structure of all-*trans*-fucoxanthin.