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The effect of natural antioxidants extracted from plant and animal resources on the oxidative stability of soybean oil

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ABSTRACT

The objective of this study was to evaluate and compare the antioxidant activity of protein hydrolyzates isolate (PHI) from Crucian carp (*Carassius carassius*) fish and cow's intestine along with microwave-assisted olive leaf extract (OLE) encapsulated by Arabic gum and maltodextrin, in soybean oil. The antioxidant activity of PHIs at three concentrations of 200, 500 and 1000 mg/kg and OLE samples containing 70 mg/kg total phenolics during 20 days storage was evaluated by peroxide value, TBA value, p-anisidine value and Rancimat stability test. The fish PHI at concentration of 1000 mg/kg, cow's intestine PHI at 500 and 1000 mg/kg and OLE encapsulated with Arabic gum showed best oxidative protection activity (more than BHT at 100 and 200 mg/kg). OLE had a suitable antioxidant activity in soybean oil and encapsulation improved the thermal stability of phenolic compounds, but on the other hand, it decreased the antioxidant efficiency of OLE.

1. Introduction

The oxidation reactions are the main reason of deterioration in edible oils and fats during storage or heat treatments such as frying and cooking. Autoxidation which is the most common oxidation phenomenon happens through a reaction between oxygen and unsaturated fatty acids via an auto-catalytic process consisting of a free radical chain mechanism. This chain includes initiation, propagation, and termination stages which could be cyclical once started (Shahidi, 2005). Primary antioxidants (natural or synthetic) prevent autoxidation by giving their hydrogen to free radicals formed during initial stages of autoxidation. There is still doubt about safety and the approval, usage level, type and application of synthetic antioxidants and they have been regulated in most countries (Akoh & Min, 2008; Gunstone, 2011; Shahidi, 2005). In recent years, a global trend has been observed toward the substitution of synthetic antioxidants with natural ones such as olive leaf extract (Bouaziz, Fki, Jemai, Ayadi, & Sayadi, 2008; Rafiee, Jafari, Alami & Khomeiri, 2011), myricetin, catechin, genistein and caffeic acid (Michotte et al., 2011) and many others. The goal of these studies was to reduce the application of synthetic antioxidants because of their potential negative health effects and as a result of consumer demand. Yanishlieva and Marinova (2001) have reviewed the studies applying natural antioxidants for stabilization of edible oils until the year 2000 and more recent studies in this area have been reviewed by Taghvaei and Jafari (in press). It has been concluded that most of natural additives have more antioxidant activity and thermal stability than synthetic ones in different edible oils.

Protein hydrolyzates isolate (PHI) from animal resources have been reported to own suitable oxidative prevention activities, such as chicken intestinal mucosa (Damle, Harikumar, & Jamdar, 2010), smooth hound (Bougatef et al., 2009), sheep visceral mass (Bhaskar, Modi, Govindavaju, & Radh, 2007) and sardinelle by-products (Bougatef et al., 2010). Sarmadi and Ismail (2010) and Bernardini et al. (2011) also reviewed the antioxidant properties of bioactive peptides from animal sources.

The exact mechanism of oxidation prevention for these peptides has not fully been understood yet. Some studies have revealed that free radicals scavenging, metal ions chelating and oxygen quenching can be the main mechanisms of oxidation prevention from bio-active peptides (Sarmadi & Ismail, 2010). Antioxidant activity of these peptides is more related to their composition, structure, and hydrophobicity.

The Crucian carp (*Carassius carassius*) fish from Cyprinidae family is a non-commercial fish which is unwelcome in fish

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growing pools. Its undesirability is not only because of its bad taste and flavor, but also because of low cooking quality since it is considered as a waste fish whereas *C. carassius* fish muscle is a suitable source of protein and bioactive peptides (FAO, 2012; IFIS, 2009).

At animal slaughterhouses, by-products such as visceral mass constitute nearly 60%–70% of the slaughtered carcasses (Bhaskar et al., 2007). Cow's intestine is also a waste by-product from slaughterhouses which is made of a considerable portion of muscle tissues and can be considered as a suitable source for producing bio-active peptides.

It has been reported that olive is a good source of phenolic compounds with antioxidant activity (Rafiee, Jafari, Alami, & Khomeiri, 2011c). It has been proved that olive leaf extract has a suitable oxidation prevention effect in various edible oils (Bouaziz et al., 2008; Rafiee, Jafari, Alami, & Khomeiri, 2011a, 2011b).

Microwave-assisted extraction (MAE) is a novel technology which has been studied extensively in recent years mainly in order to reduce the extraction time and solvent consumption (Amarni & Kadi, 2010; Camel, 2000; Taghvaei, Jafari, Nowrouzieh, & Alishah, in press). It has been reported that natural antioxidants due to their phenolic structure are more exposed to microwave and many researches have been done in order to extract phenolic compounds such as isoflavones (Terigar et al., 2010), Gallic acid, Vannilic acid, Catechin, p-Coumaric acid, Ferulic acid and may others phenolic compounds (Proestos & Komaitis, 2008), tocopherols and tocotrienol (Zigoneanu, Williams, Xu, & Sabliov, 2008) and many others from various plant resources. It has been concluded that MAE decreased the extraction time and solvent usage and increased the amount of extracted phenolic compounds. This is due to the polarity of phenolic compounds. Higher the polarity of a compound, higher the movement during microwave radiation. This leads to a better extraction of such compounds (Camel, 2000; Proestos & Komaitis, 2008).

Encapsulation which has gained increased interest to the food industry is defined as a technique in which tiny particles or droplets are surrounded by a coating wall to give small capsules. This technique increases stability of bio-active compounds against oxidation, evaporation, reaction or migration during processing, heat treatments and storage and also controls the release of the incorporated compounds (Calvo, Castano, Lozano, & Gomez, 2012; Mourtzinosa, Papadakisb, Igoumenidisc, & Karathanos, 2011). The encapsulation of natural antioxidants such as vitamin E (Yang & McClements, 2013), α-tocopherol (Somchue, Sermsri, Shiowatana, & Siripinyanond, 2009), grape marc extract (Spigno et al., 2013), raspberry leaf, hawthorn, ground ivy, yarrow, nettle and olive leaf extracts (Cvitanovic et al., 2011), have been studied widely in recent years. Fanga and Bhandari (2010) also reviewed the encapsulation of phenolic compounds by different methods. The effectiveness of oxidation prevention properties of plant extracts depends, among others, on preserving the stability, bioactivity and bioavailability of the polyphenols during storage and heat treatments. Encapsulation not only can preserve the antioxidant compounds from destruction, but also can decrease the bitter taste of these phenolics in food formulations. Our hypothesis for encapsulation of OLE was to use lower amounts of antioxidants in a more effective way. Regarding the facts that the oxidation rate of edible oils is low at first days of storage and the effective mechanism of antioxidants is cyclic, keeping the lower amounts of antioxidants in a protected environment and releasing them slowly into the oxidation cycle can not only decrease the usage amount, but also increase the efficiency of antioxidants.

Although in recent years some studies have been performed to evaluate the antioxidant activity of natural compounds extracted from plant or animal resources, but there is still a limited amount of published results comparing these two natural antioxidants together and comparable with synthetic antioxidants in edible oils. The objectives of this study were to evaluate the antioxidant activity of phenolic compounds from a plant resource (OLE) and compare the antioxidant activity with that of bio-active peptides from animal resources (Crucian carp fish PHI and cow's intestine PHI) in soybean oil in order to avoid the application of synthetic antioxidants and evaluating the effect of encapsulation on stability and release of phenolic compounds during storage.

2. Materials and methods

2.1. Natural antioxidant preparations

The Crucian carp fish samples were purchased from a local market in Gorgan (Iran) and after cutting off the head, tail and viscera, the fish was peeled off and the remaining was minced. The cow's intestine also obtained from a local slaughterhouse in Gorgan (Iran) and disinfected and minced completely by a lab-scale blender (Moulinex, Germany). The preparations of both PHIs were performed according to Guerard, Guimas, and Binet (2002) and Ovissipour et al. (2009). Briefly, the minced samples were heated at 85 °C for 20 min in order to denaturize proteins and inactivate endogenous enzymes. The heated mixtures were cooled immediately and mixed with sodium phosphate buffer 1:2 (w/v). They were homogenized in a blender (Moulinex, Germany) for about 2 min at ambient temperature. The pH of mixtures was adjusted to the optimum activity of alcalase (pH 8.5) by adding sodium hydroxide. At the next stage, alcalase (with a declared activity of 2.4 Anson U/g and a density of 1.18 g/ml), which is a bacterial endoproteinase from a strain of *Bacillus licheniformis*, was added to the mixtures. All reactions were performed in 250 ml glass vessels, in a shaking incubator (Jaltajhiz, Iran) with constant agitation at 0.2 g. The enzymatic reactions were terminated by heating the solutions at 85 °C for 20 min, assuring the enzyme inactivation. The final hydrolyzates were cooled to room temperature and centrifuged at 6700 g at 10 °C for 20 min by a Hettich D-7200 (Tuttlingen, Germany) centrifuge; then supernatants were dried by a freeze-drier model Operon FDB-5503 (korea) at −20 °C.

Olive leaves of Koroneiki varieties were collected from Gorgan (North of Iran) and were dried at oven (Memmert, ULM 400, Germany) at 40 °C for two days; then powdered by a Sunny model SFP-820 laboratory mill (40 mesh).

Refined soybean oil without any antioxidant additive was purchased from a local oil refining factory (Alia Golestan co.).

2.2. Microwave assisted extraction of phenolic compounds from olive leaf

A microwave oven (Samsung, model: CF3110N-5, Korea) was modified for oil extraction. The modified MAE system consisted of a volumetric flask (500 ml) coupled with a condenser at the top and a magnetic stirrer beneath as illustrated in Fig. 1. The microwave output was 900 W with 2450 MHz frequency and its inner cavity dimensions were 400 mm × 300 mm × 250 mm. For each extraction, 4 g of dried leaves was blended with 40 ml of methanol/water (8:1) solvent in a 500 ml volumetric flask and were placed in microwave oven and while magnetic stirring, 6 min of irradiation were performed (8 s power on and 15 s power off in order to prevention of super-boiling of solvent). After that, the methanol extract was filtered and solvent removed under reduced pressure at 50 °C by means of rotary evaporator (IKA RV 10 basic, Japan) and remaining dried by means of freeze-drier model Operon FDB-5503 at −20 °C.