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Antioxidant properties of extracts from *Ginkgo biloba* leaves in meatballs

Joanna Kobus-Cisowska *, Ewa Flaczyk, Magdalena Rudzińska, Dominik Kmiecik

Faculty of Food Science and Nutrition, Poznan University of Life Sciences, Wojska Polskiego 31, 60-634 Poznan, Poland

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ABSTRACT

The aim was to determine the effect of Ginkgo leaf extracts on the stability of lipids and cholesterol in pork meatballs over 21 days of refrigerated storage. The antioxidants used were characterized by their antioxidant activity towards lipids and cholesterol. Extracts were prepared from green and yellow leaves from *Ginkgo biloba* L. trees. Water, acetone and ethanol were used as extractants. The extracts showed stabilizing effects on both lipid and cholesterol oxidation processes. The lipid oxidation process of pork meatballs was mostly inhibited by the aqueous and ethanolic extracts of the yellow leaves. Their antioxidant activity was higher than that of BHT. All the extracts had a stabilizing effect on cholesterol and most of them inhibited the formation of oxidized derivatives. The acetone and ethanol extracts of green leaves and the ethanol extract of yellow leaves inhibited the formation of cholesterol oxidation products formation most effectively.

1. Introduction

Extracts of *Ginkgo biloba* L. leaves are interesting because of their multifaceted action. The chemical composition of the anatomical parts of *Ginkgo biloba* L. was identified by Kawamura, as well as Furkawa. They showed that Gingko leaves are rich in such substances as polyphenols, terpenoids and vitamins (Del Tredici, 1991). These substances belong to a group of natural antioxidants. Therefore, it was decided to investigate this material as a natural antioxidant in stored meat products. A previous study indicated that extracts from yellow and green Ginkgo (*Ginkgo biloba*) leaves have *in vitro* model systems with strong antioxidant activity (Kobus et al., 2009, Flaczyk et al., 2009). Positive correlations were found between the flavonol content, chelating activity, reducing power, and DPPH• scavenging activity. In addition, among the analyzed Ginkgo preparations, the yellow ones were richer in flavonol aglycones. Moreover, the extracts with the highest flavonol content demonstrated excellent activity against linoleic oxidation in a β-carotene–linoleate model system. The results from this study suggest that extracts prepared from both yellow and green Ginkgo leaves may constitute a good source of natural antioxidants and could prolong shelf-life of, for example meat. Leaves (green) from Gingko trees are harvested during the growing period. As shown in previous model studies, yellow leaves are also a rich source of antioxidants.

Meat and meat products exhibit varied nutritive qualities because of their different protein and lipid contents, which are dependent on the species, part of the carcass and type of product (Biesalski, 2005). Meat and meat products, due to the presence of fat, are unstable: they deteriorate easily and during storage undergo changes, mainly oxidation. These changes, apart from microbial growth, are the main cause of the limited shelf-life of meat foodstuffs. Moreover, compounds formed as a result of auto-oxidation may have an adverse effect on the quality and flavor of the final product (Ahn, Nam, Du, & Jo, 2001; Ahn & Nam, 2008; Frankel, 2001).

At the beginning of the lipid oxidation process, free radicals of lipid hydroperoxides and peroxides are formed (Boselli et al., 2005). Such products often react with each other, forming new compounds, of which the most important are aldehydes, ketones, hydroxy compounds, di- and trihydroxy esters, cyclic compounds and polymers Frankel, 2001; Hur, Park, & Joo, 2007; Kmiecik, Korczak, Rudzinska, Gramza-Michałowska, & Hęś, 2009; Kmiecik et al., 2011).

Another important component of the meat lipid fraction is cholesterol (3β-cholest-5-en-3-ol), with four conjugated rings, one hydroxyl group and one double bond in the molecule. Oxidation of cholesterol, as with unsaturated fatty acids, occurs in the presence of light and in an environment of singlet oxygen molecules. However, products of this oxidation are scarce (Baggio & Bragagnolo, 2006a,b; Smith, 1996).

However, to date no studies have been conducted on the influence of extracts from green and yellow Ginkgo leaves on lipid and cholesterol stability in meat products even though the natural origin of an antioxidant is generally accepted as an important consideration for food use. Therefore, the antioxidant effect of green and yellow Gingko leaf extracts on the oxidation of lipid and cholesterol constituents in a meat model system was investigated.

* Corresponding author. Tel.: +48 61 848 73 07; fax: +48 61 848 73 26.
E-mail address: joanna.kobus@up.poznan.pl (J. Kobus-Cisowska).

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2. Materials and methods

2.1. Chemicals

All solvents and chemicals used were analytical or GC grade. Cholesterol and oxysterol standards, including 5α-cholestan (5α-C), 5-cholesten-3β,19-diol (19-OH-C), 5-cholesten-3β,7α-diol (7α-OH-C), 5-cholesten-3β,7β-diol (7β-OH-C), 5-cholesten-3β-ol-7-on (7-keto-C), 5α,6α-epoxy-cholestan-3β-ol (α-epoxy-C), 5β,6β-epoxy-cholestan-3β-ol (β-epoxy-C), cholestan-3β,5α,6β-triol (triol-C), 5-cholesten-3β,25-diol (25-OH-C), and 5-cholesten-3β,20-diol (20-OH-C), were purchased either from Sigma-Aldrich (St. Louise, MO, USA) or from Steraloids (New Port, RP, USA).

2.2. Preparation of extracts

2.2.1. Ginkgo leaves

Green *Ginkgo biloba* L. var. *Hippocrates* leaves were harvested in August (marked as G), and yellow leaves were harvested in October (marked as Y) in 2009. Leaves came from a plantation in Baranowo belonging to the Poznan University of Life Sciences. Leaves, including leaf stalks, were dried at 40 °C until a moisture content of 8% was achieved, and then were ground in a laboratory mill (Retsch, type GM 200, Haan, Germany). The degree of comminution was by use a 0.03–0.8 mm-sized mesh.

2.2.2. Preparation of extracts

Twenty grams of ground plant leaves were extracted with 1 L solvent at atmospheric pressure under the following conditions: water at 95 °C during 15 min infusion (GW, YW), acetone–water (3:2 v/v) at 40 °C during 90 min extraction (GA, YA), or ethanol (96%) at 18 °C during 16 h maceration (GE, YE) (Kmiecik, Korczak, Rudzinska, Gramza-Michałowska, & Hęś, 2009). The solution was cooled and filtered using a Whatmann 1 filter. All samples were vacuum evaporated and lyophilized. The extracts were stored in a dark, dry and cool place. The bioactive compound composition of the extracts is presented in Table 1. The characterisation of these compounds and the antioxidant activity of the extracts have been analyzed previously (Kobus et al., 2009).

2.3. Meatball preparation and storage

The meatballs were prepared as described by Flaczyk, Rudzinska, Wąsowicz, Korczak, and Amarowicz (2006) and Hes, Waszkowiak, and Szymandera-Buszka (2012) with modifications. Samples of raw meat — (pork belly 64.2%, shoulder 35%; all without nitrate and nitrite) were purchased from local stores in Poznan (Poland) and were twice ground in a grinder with mesh sizes of 10 and 5 mm with sodium chloride (0.8%). The meat batter was divided into 8 parts. To each part of the batter the extracts were added. Extracts were added to meat at 500 ppm in relation to the meat batter (previous own studies), while BHT was added at 200 ppm (the maximum limit in meat) (Kobus et al., 2009). A control sample without extract was also prepared. Thus the batters contained

1) GW — water infusion of green Ginkgo leaves, 500 ppm,
2) GA — acetone extract of green Ginkgo leaves, 500 ppm,
3) GE — ethanol extract of green Ginkgo leaves, 500 ppm,
4) YW — water infusion of yellow Ginkgo leaves, 500 ppm,
5) YA — acetone extract of yellow Ginkgo leaves, 500 ppm,
6) YE — ethanol extract of yellow Ginkgo leaves, 500 ppm,
7) Control
8) BHT 200 ppm.

The meatballs, approx. 50 g were subjected to thermal processing in a Rational Combi-Dämpfer CCC-6102 convection oven (with hot air circulation, without water vapor, time: 20 min, until 72 °C was recorded inside the meat balls). Samples were then cooled to room temperature and packaged (Multivac, type A 300/16) in vacuum bags (PE/PA, 75 μm thick) and stored for 21 days at 4 ± 1 °C. Analyses were conducted after 1, 7, 14 and 21 days of storage. For each kind of meatball three productions were done and the results averaged. Analysis were performed in three replications. The chemical composition of the meatballs after cooking is presented in Table 2.

2.4. Methods

2.4.1. Lipid extraction

The process was with a chloroform:methanol 2:1 v/v mixture. After the separation phase, chloroform was evaporated in a vacuum evaporator and the lipid fraction retained (Flaczyk et al., 2006). The moisture, protein, fat and ash content were determined according to AOAC (1990).

2.4.2. Fatty acid composition

The fatty acid composition of the methyl ester derivatives was determined by GC (Wąsowicz, Zawirska-Wojtasiak, & Rudzińska, 2001).

Fatty acids were separated using a Hewlett Packard 5890 GC gas chromatograph (Agilent, Wilmington, DE, USA) equipped with Supelcowax-10 (Supelco, USA) capillary columns (30 m × 25 mm × 25 μm) and FID. Oven temperature was initially set at 60 °C and increased to 210 °C at 12 °C/min. The injector temperature was set at 240 °C, split ratio at 1:25 and detector temperature at 260 °C. The carrier gas was ultra-high-purity helium at a flow rate of 1 mL/min. Fatty acids were identified by comparing their retention times with commercially available standards.

2.4.3. Peroxide value (PV)

The peroxide value was determined by titration with 0.02 M sodium thiosulfate and was expressed in meq O_2/kg (ISO3960, 2005).

2.4.4. Anisidine value (AV)

The determination was based on ISO 6885 (2008). The method is based on the reaction of aldehydes present in the sample with a p-anisidine solution in ice-cold acetic acid and measurement of the absorbance of the yellow complex formed at 350 nm (Metertek SP-830, Taiwan).

2.4.5. TBARS determination (TBARS)

TBARS was determined according to Pikul, Leszczyński, and Kummerow (1989). Distillates were reacted with thiobarbituric acid (TBA) in boiling water and the absorbance measured at 532 nm

Table 1
The bioactive components of Ginkgo leaf extracts.

Components [mg/g s.m.]	Green leaves			Yellow leaves		
	Water infusion	Acetone extract	Ethanol extract	Water infusion	Acetone extract	Ethanol extract
Polyphenols	56.86 ± 2.35	203.5 ± 4.47	204.44 ± 0.64	179.96 ± 3.48	247.54 ± 1.04	137.28 ± 1.46
Phenolic acids	8.77 ± 0.12	14.16 ± 0.17	8.17 ± 0.08	13.80 ± 0.07	11.45 ± 0.12	11.12 ± 0.05
Flavonols	0.88 ± 0.02	1.96 ± 0.03	0.51 ± 0.02	3.15 ± 0.05	4.50 ± 0.03	1.81 ± 0.03

Results are the mean values of three determinations ± standard deviation.