

## ANTIOXIDANT ACTIVITIES OF *GINKGO BILOBA* EXTRACTS: APPLICATION IN FREEZE STORED MEAT DUMPLINGS

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**Background.** The following paper is a report from research on the influence of addition of natural antioxidants applied in the form of ethanol extracts from green and yellow leaves of *Ginkgo biloba* (0.05%) on the rate of lipid oxidation in meat dumplings stored under freezing conditions.

**Material and methods.** Experimental material comprised meat dumplings were prepared from materials purchased in a retail store in the city of Poznań. Extracts from ground green and yellow Ginkgo leaves were produced by single extraction with ethanol. Oxidation stability of meat dumplings was analysed using such indexes as anisidine value, peroxide value, as well as test with thiobarbituric acid.

**Results.** On the basis of the obtained results it was observed that the usage of antioxidants combined with vacuum packaging of the product was most effective in slowing down the formation of oxidation products. The added ethanol extracts from *Ginkgo biloba* leaves limited the rate of oxidation of the fats in the product; however, their efficacy was similar as that obtained with the usage of the BHT synthetic antioxidant used for comparative purposes.

**Conclusions.** The added extracts did not cause negative changes in the sensory analysis of the meat, it is claimed that ethanol extracts from *Ginkgo biloba* leaves may be used as antioxidants to prolong stability of meat dumplings.

**Key words:** *Ginkgo biloba* leaves, natural antioxidants, boiled dough pockets filled with meat, lipids, oxidation

### INTRODUCTION

Processes of fat oxidation in foodstuffs, commonly referred to as rancidity, are not irreversible and have a tremendous effect on product quality, since they cause organoleptic changes and the formation of many harmful compounds [Morrissey et al. 1998, Serdaroğlu et al. 2008].

Oxidation of fats in animal origin products occurs most frequently during storage of meat and other products, and the primary pre-condition for its initiation is the presence of atmospheric oxygen or oxygen absorbed by fat during meat storage. The strongest initiators of the oxidation process include the action of light rays, temperature and prooxidative metals. Low temperatures of storage (cold and in freeze storage) slow down the induction process of free radical chain reactions, while it does not mean that changes in fat do not occur [Hęś and Korczak 2007, Jayathilakan et al. 2007, Zaborowska et al. 2001, Ziemiański and Budzyńska-Topolowska 1991]. Taste and aroma characteristic of rancid fat appear mainly as a result of the presence of low molecular volatile substances, such as aldehydes, ketones or free fatty acids. Auto-oxidation of lipids considerably deteriorates organoleptic attributes of meat products [Flaczyk et al. 2009, Kmiecik and Korczak 2006, Korczak et al. 2004, Ziemiański and Budzyńska-Topolowska 1991].

For these reasons fat oxidation processes need to be eliminated or delayed as much as possible, which is connected with an extension of stability of meat products and maintenance of their high quality.

Food of plant origin and to a lesser extent also animal origin foodstuffs comprise the primary source of natural antioxidants. Antioxidant compounds of plant origin are represented first of all by polyphenols (phenolic acids and an extensive group of flavonoids together with anthocyanins and tannins), vitamins (A, C, tocopherols), carotenoids, organic acids, calcium, selenium. Nowadays, in meat products, there are applied natural antioxidants of spice, herbs, tea and grape skin extracts [Duda-Chodak et al. 2008, Nissen et al. 2004, Sikora et al. 2008, Szajdek and Borowska 2004]. In turn, antioxidants of animal origin include minerals such as calcium and selenium, as well as isomers of linolic acid, carnosine, anserine and protein hydrolysates of animal origin [Flaczyk et al. 2006].

Studies on the introduction of natural antioxidants to food as functional additives indicate a positive effect of these compounds on the inhibition of fat oxidation reactions. Unfortunately, to date they have not been as widely applied as synthetic antioxidants are. Despite high hopes connected with the application of natural antioxidants, problems have been encountered with the isolation of their pure forms from raw materials; moreover, they may turn out to be less readily soluble, or cause discolouration or changes in taste, aroma or colour [Bera et al. 2006, Formanek et al. 2001, Karwowska and Dolatowski 2007, Niki 2002]. Despite these obstacles, the application of natural antioxidants in food seems necessary and their use in combination with other technologies limiting adverse changes (e.g. modified atmosphere) is highly recommended and already implemented in different groups of foodstuffs [Klensporf-Pawlik and Jeleń 2009].

*Ginkgo biloba* (*Ginkgo biloba* L.), also called maidenhair tree, is one of the oldest plant species found on Earth. It has survived in an almost unchanged form for millions of years and for this reason it is referred to as "the living fossil". Initially this plant was found in China and it is estimated that it was brought to Europe around the 18th century. At present there are many commercially available preparations made from *Ginkgo biloba* leaves, which results from the broad spectrum of its advantageous action on the human organism. The antioxidant effect is determined by the presence of flavonoids, capable of free radical scavenging [Kobus et al. 2009]. Antioxidant potential of extracts from *Ginkgo biloba* leaves is comparable to that of ascorbic acid, glutathione or alpha-tocopherol [Kalisz et al. 2006].

So far Ginkgo extracts have not been used as additives of antioxidant character in food production. Thus the aim of this paper was to assess the effect of an addition of ethanol extracts from green and yellow leaves of *Ginkgo biloba* (*Ginkgo biloba* L.) on oxidative stability of lipids in the filling of meat dumplings in freeze stored in vacuum bags.

## MATERIAL AND METHODS

Experimental material comprised meat dumplings prepared from materials purchased in a retail store in the city of Poznań.

Batter was prepared from pork shoulder (22.2%), pork bacon (41.2%), onion (12.5%), eggs (9.5%), oil (6.7%), bread crumbs (6.9%), salt and pepper (1.0%). Batter was divided into four portions. One comprised the control, to which no antioxidant was added, while the others were supplemented with ethanol extracts from yellow and green leaves of *Ginkgo biloba* at 0.05% in relation to batter weight and a synthetic antioxidant BHT at 0.02%. Such prepared portions were divided into batches of approx. 20 g, from which dumplings were formed after it was combined with dough (dough formulation: 68.1% "wrocławska" wheat flour type 550, 13.9% eggs, 2.8% refined rapeseed oil, 15.2% water). Dumplings were cooked for 7 min in salted water with an addition of oil (until they floated).

After cooling dumplings were vacuum packaged in Multivac bags (150 × 250 mm and 150 × 350 mm) from PA/PE (thickness 75 µm, temperature range: -50/+90°C). Next samples were stored in -18°C for 180 days.

Extracts from ground green and yellow Ginkgo leaves were produced by single extraction with ethanol (96%) at a proportion of 2 g ground dried leaves per 100 ml solvent. The entire amount was shaken at 20°C for 16 h and next filtered and centrifuged for 5 min at 9000 × g (centrifuge type K 70, Janetzki, Germany). Clear solution collected above was evaporated on a rotational evaporator (RVO 200A, Ignos, the Czech Republic). Dry extracts were dissolved in a small amount of water and added to the batter.

Oxidation stability of meat dumpling filling was analysed using such indexes as anisidine number (AV) [PN-93/A-86926], peroxide value (PV) [PN-ISO 3960:2005], as well as thiobarbituric acid reactive substances (TBARS) [Pikul et al. 1989].

Additionally sensory examination of dumplings was conducted by the profile method at a sensory analysis laboratory meeting the requirements of standard PN-ISO 8589:1998, which aimed at the determination of the effect of used additives on changes in sensory attributes [Babicz-Zielińska et al. 2008]. The following attributes were determined: colour, consistency, juiciness, aroma, by differentiating the following descriptors: meaty, hay, rancid, as well as extrinsic, and taste (meaty, fatty, rancid, bitter, extrinsic and salty). Results were replaced with numerical values expressed in arbitrary units (from 0 to 10).

Antioxidant activity of Ginkgo extracts in meat dumplings were compared with sample with BHT addition and sample without any antioxidant – control sample.

Results were subjected to statistical analysis, taking into consideration basic statistical measures, which were calculated using Microsoft Excel software. Significance of differences was determined by Tukey's test at the significance level of 95% with the

application of Statistica ver. 8.0. software. Additionally Pearson's correlations between fat quality attributes were calculated.

Each determination was performed in two series and in at least three replications.

## RESULTS AND DISCUSSION

Contents of primary fat oxidation products already on the first day of analysis were determined in all samples of meat dumpling filling (Fig. 1 a). Throughout the entire frozen storage period the content of primary oxidation products was relatively the lowest in the sample with an addition of the extract made from yellow leaves (YE) – after 6 months it was 0.56 meq O<sub>2</sub>/kg fat, although fluctuations in the values of this index were observed during the whole period of analyses ( $p < 0.05$ ).

On the first day of in freeze storage no secondary oxidation products were detected in the sample with an addition of the green leaves extract (GE) or in the control (Fig. 1 b). In contrast, after storage the anisidine number was found to go up and down in the control and the sample with an addition of BHT, with the highest values of this index being recorded in these samples at 180 day of storage, amounting to 2.86 and 3.20, respectively ( $p < 0.05$ ).

A clearly effective action in the inhibition of the formation of secondary oxidation products was found for the extract from yellow Ginkgo leaves (sample YE) and a slightly inferior one for the extract from green leaves (GE;  $p < 0.05$ ).

In the filling of meat dumplings with an addition of the extract from yellow leaves (YE) the TBARS value initially was the highest ( $p < 0.05$ ) and it increased for the first 2 months of storage (Fig. 1 c). Starting from day 60 of measurements up to day 150, a slight decrease was recorded in the TBARS value for the sample with an addition of the yellow leaves extract (YE); however, at the last analysed time point (after 180 days of storage) an intensive increase was observed for the content of secondary oxidation products to the level of 1.00 mg/kg ( $p < 0.05$ ). Throughout the entire frozen storage period relatively the lowest amounts of these compounds were recorded in the sample with an addition of BHT ( $p < 0.05$ ).

In the control and in the sample with an addition of the extract from green leaves (GE) the TBARS values remained low in the first 3 months of storage in freeze dumplings. Only in the successive months a more intensive increase was observed in the value of this index in the control and a slower one in the sample with an addition of the extract made from green leaves (GE).

The investigated experimental design also included sensory examination using the profile method for meat dumplings in freeze stored for 180 days (Fig. 2 and 3).

It was found that an addition of the ethanol extracts as well as BHT did not have any effect on changes in the filling colour of dumplings, while cold storage did not contribute to changes in its intensity. Probably selected concentrations of extracts – particularly the extract from green leaves – containing chlorophyll were too low to have a disadvantageous effect on the colour. Moreover, as it was shown in the course of statistical analysis, tested samples over the entire period of analyses did not differ significantly in terms of consistency, which was given scores of 4.3-4.4, in a 10-point scale ( $p < 0.05$ ). Similarly no differences were found for juiciness, which decreased slightly during storage receiving scores of 6.1-7.2; however, these changes were not statistically significant ( $p < 0.05$ ).

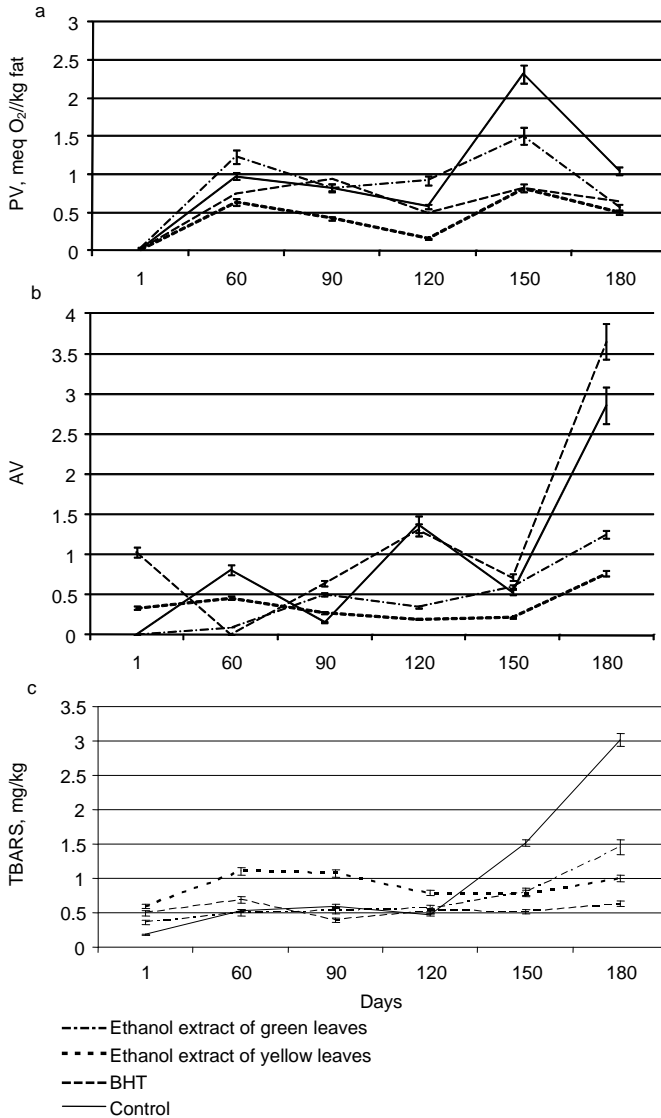


Fig. 1. The effect of *Ginkgo biloba* ethanol extracts on lipid oxidation measures in meat dumplings during 180 days of in freeze storage

Evaluation of aroma for in freeze stored samples indicated a lowering of the value in case of the meat descriptor – although the biggest drop of its value was recorded in case of samples with an addition of extracts from *Ginkgo* leaves, but the differences were statistically non-significant. In the last experimental stage (after 180 days) in case of in freeze stored samples the most off-flavour and hay aroma was found in samples with an addition of natural antioxidants.

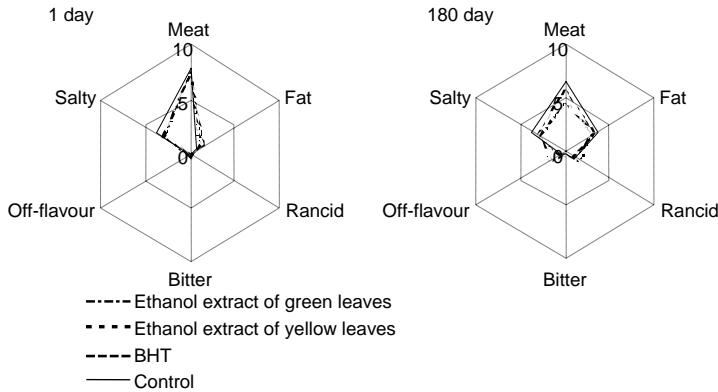


Fig. 2. The effect of *Ginkgo biloba* ethanol extracts on the sensory quality of meat dumplings evaluated after 180 days storage in freeze – taste

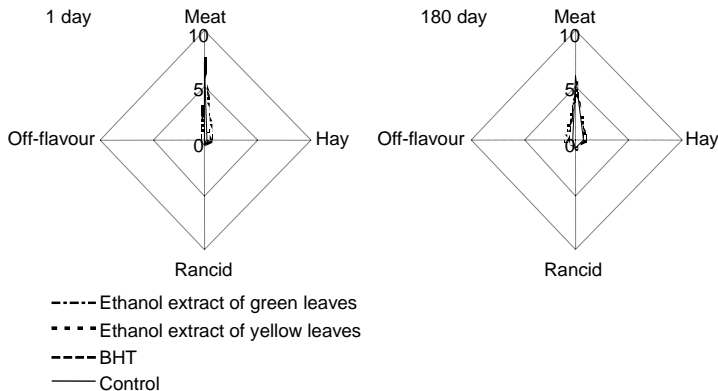


Fig. 3. The effect of *Ginkgo biloba* ethanol extracts on the sensory quality of meat dumplings evaluated after 180 days storage in freeze – odour

Moreover, a decrease was also observed in all samples for meat taste. In cold stored dumplings the highest level of fatty taste was reported in the control sample and that of rancid taste in the control sample and the sample with an addition of the yellow leaves extract. Samples with an addition of natural extracts after storage were evaluated as the most bitter and characterised by bitter taste.

During the sensory analysis it was observed that dumpling dough exhibited changes in fatty, as well as rancid aroma and taste to a higher degree than their filling.

Statistical analysis showed significant correlations between the anisidine number and TBARS content in the filling of in freeze stored meat dumplings. A positive correlation was found between secondary oxidation products of lipids reacting with p-anisidine and with thiobarbituric acid (Pearson's correlation coefficient  $R = 0.87$ ). Thus the more intensive rancid aroma and taste were reported in these samples, in which values of

these indexes were highest, since substances such as aldehydes, ketones or free fatty acids are responsible for the formation of the characteristic rancid taste and aroma. Moreover, a disproportion was observed between growth dynamics of secondary oxidation products expressed in the TBARS index and LA value and the dynamics of a decrease in peroxide contents. This resulted from the relatively low stability of hydroperoxides formed in the first stage, which underwent further multifaceted and complex changes. As a result secondary oxidation products were formed, which included both volatile and non-volatile substances.

There is many data in literature on the antioxidant action of natural antioxidants in food. Most experiments describe analyses of the stability of cold stored products. A definite minority of experiments concern the application of antioxidants in the production of in freeze foodstuffs, which results from the specific character of this technology, in which oxidation processes are considerably limited. An even smaller number of studies concern the application of antioxidants in products, in which meat is one of the components of the dish.

In a study by Derewiaka et al. [2008] a bigger increment was observed for the total content of cholesterol oxidation products during in freeze storage of dumplings, than it was during their cold storage. These changes are a consequence of fat changes such as polymerization, degradation or oxidation.

Nissen et al. [2004] stated that an addition of natural antioxidants in the form of extracts produced from rosemary, green tea, coffee or grape skins had a positive effect on the inhibition of lipid oxidation and the formation of secondary oxidation products in the cooked pork pate. Additionally it was shown that extracts may have a protective action towards vitamin E and that rosemary extract had an advantageous effect on the profile sensory evaluation of the product.

Jayathilakan et al. [2007] conducted experiments, which showed that in the inhibition of development of adverse sensory attributes resulting from the degradation of fat and defined as the *warmed-over-flavour* (undesirable, rancid, etc.) in samples of pork, beef and mutton, a considerable role is played by compounds with the antioxidant character, such as the Maillard reaction products, ascorbic acid, antioxidants from cloves and cinnamon, while among synthetic antioxidants it was the case for TBHQ, BHA and PG.

At the same time it needs to be stressed that an addition of table salt to processed products made from heat-processed meat has a pro-oxidation action, since it promotes the release of iron from haem molecules and disturbs the functioning of enzymatic systems scavenging free radicals. Thus processed meat products subjected to heat processing, which are next cold or in freeze stored, are more susceptible to oxidation than raw meat stored under identical conditions [Bartnikowska 2001].

In the discussed experiment it was attempted to apply an addition of natural antioxidants to meat batter in a product made from dough. Thus model experiments were conducted, which may have practical applications.

## CONCLUSIONS

1. Ethanol extract from yellow Ginkgo leaves and a synthetic antioxidant BHT were the most effective in the stabilization of lipids in the filling of meat dumplings in freeze stored for the period of 180 days.

2. Additions of extracts did not change significantly the odour and the taste of the dumplings, as well as other sensory factors.

3. During in freeze storage of dumplings in samples with an addition of extracts from Ginkgo leaves and BHT, aroma and taste of dumplings were observed to deteriorate; however, these changes were statistically non-significant.

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## **ZASTOSOWANIE EKSTRAKTÓW Z MIŁORZĘBU DWUKŁAPOWEGO JAKO PRZECIWUTLENIACZY W PIEROGACH MIĘSNYCH PRZECHOWYWANYCH W WARUNKACH ZAMRAŻALNICZYCH**

**Wstęp.** Celem pracy była ocena wpływu dodatku naturalnych przeciwutleniaczy, zastosowanych w postaci etanolowych ekstraktów z żółtych i zielonych liści *Ginkgo biloba* (0,05%) na tempo przemian oksydacyjnych w farszu pierogów mięsnych przechowywanych w stanie zamrożenia.

**Materiał i metody.** Przedmiotem badań były pierogi mięsne, przygotowane z surowców zakupionych w sieci detalicznej na terenie Poznania. Ekstrakty z rozdrobnionych liści zielonych i żółtych miłorzębu dwukłapowego otrzymano poprzez jednokrotną ekstrakcję alkoholem etylowym. Stabilność oksydacyjną farszu pierogów mięsnych badano za pomocą takich wskaźników, jak: liczba anizydynowa, liczba nadtlenczkowa oraz test z kwasem tiobarbiturowym.

**Wyniki.** Dodatek przeciwutleniaczy w połączeniu z próżniowym opakowaniem pierogów mięsnych przechowywanych w warunkach zamrażalniczych wpływały na hamowanie tempa przemian oksydacyjnych. Ekstrakty etanolowe z liści *Ginkgo biloba* ograniczały

tempo utleniania tłuszczu w produkcji, a ich skuteczność była porównywalna do efektu działania syntetycznego przeciwutleniacza BHT zastosowanego w celach porównawczych.

**Wnioski.** Dodane ekstrakty nie wywoływały negatywnych zmian we właściwościach sensorycznych mas mięsnych. Stwierdzono, że istnieje możliwość wykorzystania ekstraktów etanolowych sporządzonych z liści *Ginkgo biloba* jako przeciwutleniaczy przedłużających trwałość pierogów mięsnych.

**Słowa kluczowe:** *Ginkgo biloba* L., naturalne przeciwutleniacze, pierogi mięsne, lipidy, utlenianie

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