ACTA ICHTHYOLOGICA ET PISCATORIA
Vol. XXVII, Fasc. 2 Szczecin 1997

Ludmiła *STODOLNIK,* Rafał *ANDRZEJEWSKI*

Fish technology

IMPACT OF SELECTED MUSHROOMS ON INHIBITION OF LIPID OXIDATION OF THE MUSCLE TISSUE OF HERRING DURING FROZEN STORAGE

WPŁYW WYBRANYCH GRZYBÓW NA INHIBITOWANIE UTLENIANIA LIPIDÓW TKANKI MIĘŚNIOWEJ ŚLEDZI W CZASIE ZAMRAŻALNICZEGO PRZECHOWYWANIA

Chair of Fish Preservation, Agricultural University of Szczecin, Poland

Oxidative changes of the lipids in the minced muscle tissue of herring containing two mushroom species *Agaricus bitorquis* and *Xerocomus badius* were analysed in the course of 3-month-long frozen storage at –25°C. A good inhibition of the lipid oxidation of the muscle tissue of herring to hydroperoxides and dienes using the mushrooms was observed. The mushrooms were also active in slowing down the accumulation of the secondary oxidation products. The overall antioxidative activity of the mushrooms was higher than that of BHA in the respect of lipids of the muscle tissue of herring stored in the frozen conditions.

INTRODUCTION

Limitations in usage of the synthetic antioxidants such as BHA, BHT, and gallates in foodstuffs, encourage the quest for naturally occurring substances which are able to protect lipids. An abundant sources of antioxidative compounds are plant-origin materials. The basic lipid oxidation inhibitors of plant origin are: ascorbic acid, α-tocopherol, β-carotene, glutatione, chlorogenic acid, quercetin, and other flavonoids (Larson 1988; Munshi and Mondy 1989; Papadopulos and Boskou 1991). According to Al-Seikhan et al. (1995) one of the strong antioxidants is patatine (glycoprotein) present in potatoes and accounting to 40% of all soluble proteins (Park 1983). Its antioxidative activity is many times stronger than that of chlorogenic acid and quercetin and glutathione (Al-Seikhan et al. 1995).

Kasuga et al. (1988) revealed that some species of edible mushrooms growing in Japan have antioxidative properties. Extracts of the "cows ear" mushroom *Suillus bovinus* (known in Poland as "maślak sitarz") show strong antioxidative properties (Kasuga et al. 1993). The main compound inhibiting lipid oxidation in this mushroom is variegatic acid (3,3',4,4'-tetra-hydroxy puluvinic acid) and its antioxidative activity is not only stronger than α-tocopherol but also stronger than BHA (Kasuga et al. 1995). The second important compound showing antioxidative properties, but less active than variegatic acid, was diboviquinone-4,4.

It is evident from the above-mentioned studies, that mushrooms may contain compounds effectively inhibiting lipid oxidation. It prompted the present authors to study the impact of the two mushrooms commonly eaten in Poland (common champignon, *Agaricus bitorquis* (alternative English name – Torq) and "podgrzybek brunatny", *Xerocomus badius*) on the ability of inhibition of lipid oxidation of the minced muscle tissue of the herring kept under frozen storage conditions, compared to a synthetic antioxidant BHA.

MATERIAL AND METHODS

Material

Present study was based on the muscle tissue of the Baltic herring (*Clupea harengus* L.) caught in the early March of 1996. They represented the spring spawning stock, their gonads were at IV/V stage of Maier's maturity scale and their flesh was at fading post-mortem stage. The fish were filleted, skinned, and their dorsal muscles were removed. The muscles were minced in a grinding machine with 2.5-mm strainer. The minced meat obtained was mixed and divided into 350-g portions. The mushrooms, previously dried at 40°C and ground were added in the following amounts: champignon—0.5%, "podgrzybek"—0.5%, champignon—0.25% + "podgrzybek"—0.25%. Buthylated hydroxyanisole (BHA) was added in the amount of 0.01%. Minced meat without additives constituted a control sample. After thorough mixing of each experimental alternative, the minced meat was divided into 55-g samples in a form of 1-cm-thick rectangular prisms. They were wrapped in a polyethylene foil, frozen at –25°C, and subsequently stored also at –25°C for a three-month period. The minced meat was subjected to chemical analysis after 2, 4, 6, 9, 12 weeks of the frozen storage following their air defrosting at 4°C. The results obtained constitute arithmetic means of three parallel repetitions.

Methods

Lipids from the muscle tissue of the fish were extracted using a chloroform-methanol mixture (Linko 1967). Hydroperoxides were determined in the chloroform layer of the extracts using an indirect method of their oxidation to malonal-

dehyde and its reaction with TBA (Schmedes and Hølmer 1989). The content of secondary oxidation products was determined in the methanol-water layer using TBA (Schmedes and Hølmer 1989). Content of dienes and trienes was determined spectrophotometrically through absorbance readings of diluted chloroform extracts in the ratio of 1:10 within ultraviolet spectrum. Intensive band for dienes was at 243 nm and for trienes—at 278 nm. The results were expressed as absorption coefficient (Paquot 1982). Lipid content was determined using a weight method based on the chloroform layer, following distilling out the solvent and drying the reminder at 80°C within 1 h. Antioxidative activity of the additives used expressed in percents calculated according to a formula developed by Al-Saikhan et al. (1995). Solubility of the proteins in 5% solution of NaCl was determined based on a method described earlier (Stodolnik 1994).

RESULTS

The muscle tissue of herring sampled for the present study contained 4.3% of lipids. It showed the presence of hydroperoxides in the amounts equal to 6.5 mg/kg of malondialdehyde, secondary products of oxidation (5.3 mg/kg of the tissue), and also dienes and trienes, expressed as the absorption coefficients—5.4 and 0.91 respectively (Figs. 1-4).

Table 1

Antioxidative activity (%) of the additives used to the muscle tissue of herring during its frozen storage

Additive	Hydro-peroxides	Secondary oxidation products	Dienes	Trienes	Total
Champinion 0.5%	43.6	2.0	17.9	–3.9	14.9
"Podgrzybek" 0.5%	42.9	13.4	15.6	–7.3	16.2
Champinion 0.25% + "podgrzybek" 0.25%	42.1	8.3	15.9	–19.0	11.8
BHA 0.01%	38.3	26.0	12.7	–17.8	14.8

The addition of the mushrooms to the muscle tissue slowed down oxidation of lipids, but they acted in variable ways on the dynamics of deposition of individual products of lipid changes during frozen storage. Both mushroom species, used also jointly in a mixture inhibited formation of hydroperoxides on a similar level of activity. Antioxidative activity of the champignon and "podgrzybek", calculated based on hydroperoxides were higher than that of BHA (Tab. 1).

All additives slowed down oxidation of the muscle tissue of the herring to aldehydes, although less actively than to hydroperoxides. In this case the best inhibitor was BHA followed by about two times less active—"podgrzybek", and three times less active—mushroom mixture. The champignon addition inhibited oxidation only slightly. The mushrooms were good inhibitors of fatty acids changes to diene groups, much better than BHA. None of the mushroom used inhibited formation of trienes. Neither BHA prevented the conjugation of the acids to trienes.

It is evident from the calculations of the activity of the additives in relation to triene groups of the fatty acids, that each of them catalysed triene build-up in the lipids of the muscle tissue of herring at the time of frozen storage. Particularly active were the mushroom mixture and BHA (Tab. 1).

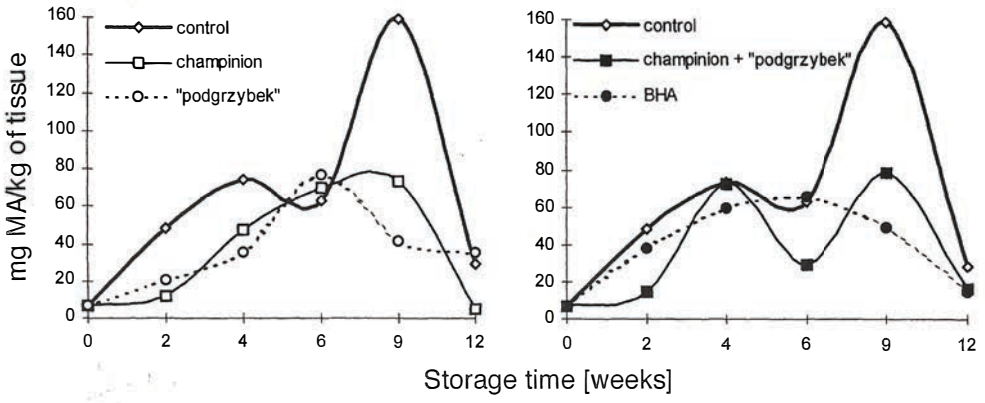

Fig. 1. Changes in the content of hydroperoxides in the muscle tissue of herring during frozen storage (chloroform layer of the extracts)

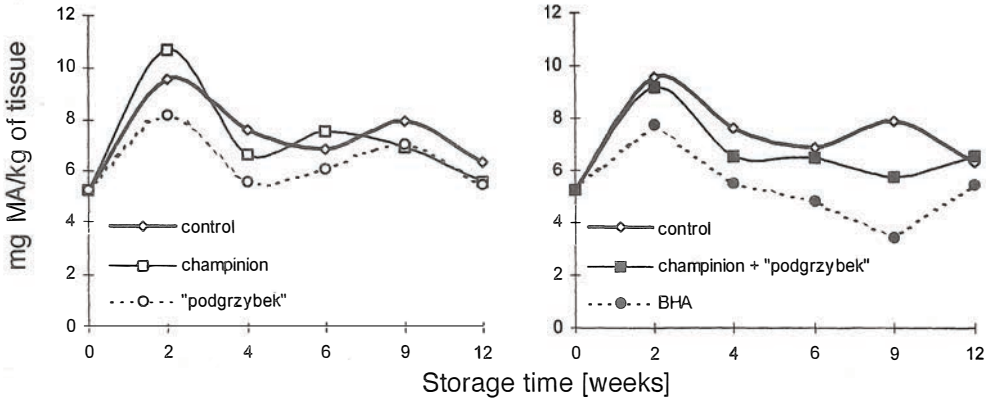

Fig. 2. Changes in the content of secondary oxidation products in the muscle tissue of herring during frozen storage (methanol-water layer of the extracts)

The overall antioxidative activity of both mushroom species and BHA, calculated based on the content of all used parameters of oxidation of lipids of the herring muscle tissue during three-month frozen storage at –25°C was as follows: "podgrzybek" > champignon > BHA > champignon + "podgrzybek" (Tab. 1).

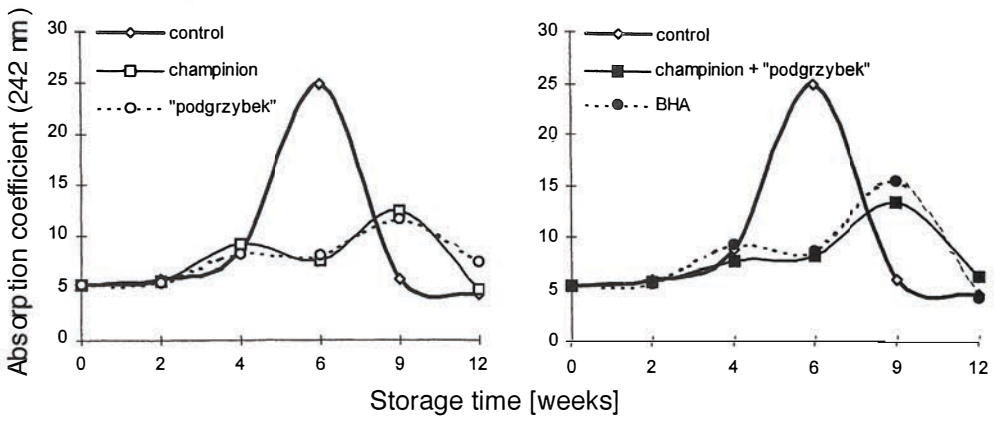

Fig. 3. Changes of the absorption coefficient of the lipids of the muscle tissue of herring during frozen storage

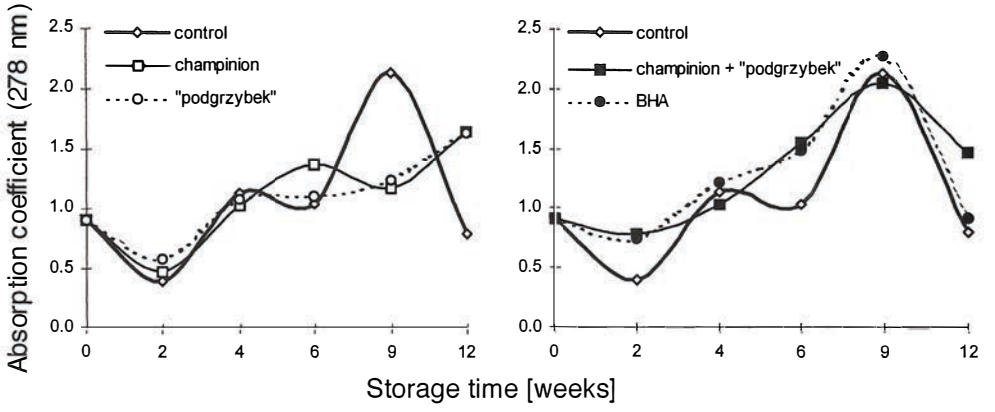

Fig. 4. Changes of the absorption coefficient of the lipids of the muscle tissue of herring during frozen storage

Antioxidative properties of champignon and "podgrzybek" may be a result of their high content of free amino acids, which constitute in average 20% of their weight. The dominant amino acids are L-glutaminic acid, L-asparagine, α-alanine, proline, and arginine (Łobaszewski et al. 1990). Antioxidative properties of glutaminic acid in relation to the lipids of the muscle tissue of the fish were noticed in an earlier study (Stodolnik and Podsiadło 1983). Certain antioxidative role in the dried mushrooms can be attributed to the products of non-enzymatic browning. Good antioxidative activity of the latter were observed by Dubravskaja (1989). Possibility of formation of such compounds is indicated by a high content of carbohydrates, free amino acids, and lipids in dried mushrooms. In champignons the carbohydrates amount to 37.4% (Szczygieł et al. 1974), while lipids

constitute 1.7% (Szczygieł et al. 1974). Free amino acids from dried mushrooms were recovered by Łobaszewski et al. 1990. The principal volatile compound giving mushroom aroma 1-octen-3-ol constituted 84% of the entire volatile fraction in a fresh, unprocessed champignon (Sułkowska and Kamiński 1977). In "podgrzybek" it ammounted to 67.5% (Woźniak and Gapiński 1988). The above-mentioned compound cannot, however, play a role in inhibition of lipid oxidation as its content declines by 90-100% in the process of drying (Sułkowska and Kamiński 1977). The principal volatile compounds of the dried champignons are benzyl alcohol (27–67%) and benzaldehyde (6–44%) (Sułkowska and Kamiński 1977). Their antioxidative role has been hitherto unknown. A synergetic action of some other non-volatile taste components of mushrooms in inhibition of lipid oxidation cannot be ruled out. Such components as glutaminic acid, uridine, and their phosphate compounds constitute 4.3 μM/g of dry weight of the button mushroom *(Agaricus bisporus)*, while guanosine compounds (5-GNP) and adenosine (5-AMP) constitute 0.63 μM and 2.00 μM respectively (Krickij and Kulaev 1963; Łobaszewski et al. 1990).

It has not been known whether champignon and "podgrzybek" contain veriegatic acid and diboviquinone described by Kasuga et al. (1995) as active inhibitors of lipid oxidation present in the cow ear mushroom. On the other hand the present study revealed that both the champignon and "podgrzybek" are a source of compounds that inhibit lipid oxidation.

Table 2

Protein solubility (g/100 g) in the muscle tissue of herring in 5% NaCl solution during frozen storage

Sample	Storage time (weeks)					
	0	2	4	6	9	12
Control	7.00	6.88	6.42	7.68	7.83	7.10
Additive:						
Champinion 0.5%	8.30	7.82	6.42	6.75	7.64	8.00
"Podgrzybek" 0.5%	9.30	7.75	6.36	6.77	8.33	7.10
Champinion 0.25% + "podgrzybek" 0.25%	8.10	7.24	6.48	6.34	7.45	6.86
BHA 0.01%	7.68	7.23	6.67	6.63	7.28	6.76

The present study revealed that the addition of mushrooms to the muscle tissue of herring caused increase in the amounts of proteins extracted from a tissue that had not been frozen. This increase was higher that it could be expected from the protein content in the mushrooms used (Tab. 2). In the conditions of frozen storage, however, quick lowering of protein solubility within the first month of the storage was observed and it coincided with the highest concentrations of secondary oxidation products in the muscle tissue of herring. The observed tendency of solubility changes of the proteins and changes in the content of malondialdehyde may suggest high sensitivity of mushroom protein structure at the time of storage at –25°C in the environment of muscle tissue of herring.

CONCLUSIONS

1. The addition of the mushrooms to the muscle tissue of herring more successfully inhibited oxidation of lipids to hydroperoxides, than BHA. The champignon was more active than "podgrzybek".
2. The activity of mushrooms in inhibiting oxidation of lipids of the muscle tissue of herring to secondary products was lower, than that of BHA. In this case "podgrzybek" was more active than champignon.
3. The champignon, out of all additives used, the most efficiently inhibited the conjugation of the fatty acids to dienes.
4. The overall antioxidative activity of all additives used, calculated based on the content of hydroperoxides, secondary oxidation products, dienes and trienes was higher for the mushrooms than for BHA. The "podgrzybek" represented the highest activity.

REFERENCES

Al-Saikhan M. S., L. R. Howard, J. C. Miller jr., 1995: Antioxidant activity and total phenolics in different genotypes in potato (*Solanum tuberosum* L.). J Food Sci., **60**, 2: 341–343, 347.

Dubrovskaja T. A., 1989: Ispolzovanie antiokislitelnych svojstv produktov reakcii Majjara [Usage of antioxidative substances, products of the Maiilard reaction]. Express-informacija, Rybn. Choz., Ser. Obrabotka ryby i moreproduktov, **7**: 1–11. (In Russian).

Kasuga A., Y. Aoyagi, T. Sugahara, 1988: Antioxidant activities of edible plants. J. Jap. Soc. Food Sci. Technol., **35**, 12: 828–834.

Kasuga A., Y. Aoyagi, T. Sugahara, 1993: Antioxidant activities of mushrooms extracts. J. Jap. Soc. Food Sci. Technol., **40**, 1: 56–63.

Kasuga A., Y. Aoyagi, T. Sugahara, 1995: Antioxidant activity of fungus *Suillus bovinus* (L:Fr.)". Kuntze. J. Food Sci., **60**, 5: 1113–1115, 1142.

Krickij M. S., I. S. Kulaev, 1963: Kislotorastvorimyje nukleotidy plodovych tel sampiniona dvusporovogo (*Agaricus bisporus* L.) [Acid-soluble nucletides of the reproductive organs of the bottom mushrooms (*Agaricus bisporus* L.)]. Biochimja, **28**, 4: 694–699. (In Russian).

Larson R. A., 1988: Review article number 30: The antioxidants of higher plants. Phytochemistry, **27**: 969–978.

Linko R. R., 1967: Fatty acids and other components of Baltic herring flesh lipids. Ann. Univ. Turku, Ser. A, **101**: 7–121.

Łobaszewski E., B. Paczyńska, S. Zalewski, 1990: Charakterystyka substancji zapachowo-smakowych grzybów [Characteristics of smell and taste substances of muschrooms]. Przem. Spoż., **10**: 244–245. (In Polish).

Munshi C. B., N. I. Mondy, 1989: Ascorbic acid and protein content of potatoes in relation to tuber anatomy. J. Food Sci., **54**: 220–221.

Papadopoulos G., D. Boskou, 1991: Antioxidant effect of natural phenols on olive oil. J. Am. Oil Chem. Soc., **68**, 9: 669–671.

Park W. D., 1983: Tuber proteins of potato – a new and suprising molecular system. Plant Mol. Biol. Rep., **1**: 61–66.

Paquot C., 1979: Standard methods for the analysis of oils, fats and derivatives. Pergamon Press, Oxford: 71–74.

Stodolnik L., L. Podsiadło, 1983: The antioxidant effect of glutamic acid and glycerine on cod mince in frozen storage. 16th Int. Congr. Refrig. Paris, Comm. C–2. Preprints Int. Inst. Refrig., Paris: 312–317.

Stodolnik L., 1994: Zastosowanie naturalnych antyoksydantów do rozdrobnionej tkanki mięśniowej śledzi bałtyckich przechowywanej w warunkach zamrażalniczych [Usage of natural antioxidants for minced muscle tissue of the Baltic herring kept in conditions in frozen storage]. Chłodnictwo, 10: 21–25. (In Polish).

Sułkowska J., E. Kamińska, 1977: Effects of different drying methods on quality and content of aromatic volatiles in dried mushrooms Agaricus bisporus. Acta Alim. Pol., **3**, 4: 409–425.

Szczygieł A., J. Piekarska, Z. Klimczak, 1974: Skrócone tabele wartości odżywczej produktów spożywczych [Shortened tables of nutritive values of food products]. PZWL, Warszawa: 18–35. (In Polish).

Woźniak W., M. Gapiński, 1988: Grzyby [Mushrooms].: 2–17. (In Polish).

Ludmiła *STODOLNIK,* Rafał *ANDRZEJEWSKI*

WPŁYW WYBRANYCH GRZYBÓW NA INHIBITOWANIE UTLENIANIA LIPIDÓW TKANKI MIĘŚNIOWEJ ŚLEDZI W CZASIE ZAMRAŻALNICZEGO PRZECHOWYWANIA

STRESZCZENIE

W badaniach zastosowano zmieloną tkankę mięśniową śledzi bałtyckich z dodatkiem suszonych grzybów: pieczarka – 0,5%, podgrzybek – 0,5%, ich mieszaniny po 0,25% przechowywaną w temperaturze –25°C. Analizowano aktywność antyoksydacyjną dodatków na podstawie zawartości hydronadtlenków, wtórnych produktów utleniania, dienów i trienów w okresie trzymiesięcznego zamrażalniczego przechowywania. Stwierdzono, że zastosowane grzyby mają dobre właściwości hamowania utleniania do hydronadtlenków. Również znacznie inhibowały kumulowanie wtórnych produktów utleniania lipidów tkanki mięśniowej śledzi. Ogólna aktywność antyoksydacyjna zastosowanych grzybów była większa niż BHA, największą stwierdzono dla podgrzybka w środowisku tkanki mięśniowej śledzi, przechowywanej w temperaturze –25°C.

Received: 27 October 1997

Authors' address:

Ludmiła Stodolnik PhD DSc ProfTit
Chair of Fish Preservation
Agricultural University of Szczecin,
Kazimierza Królewicza 4, 71-550 Szczecin, Poland