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SYNERGISTIC EFFECT OF ANTIOXIDANT ACTIVITY OF CASEIN AND ITS ENZYMATIC HYDROLYSATE IN COMBINATION WITH ASCORBIC ACID AND β-CAROTENE IN MODEL OXIDATION SYSTEMS

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Abstract. The experiment studied the synergetic effect of casein and its enzymatic hydrolysate in combination with ascorbic acid and β-carotene in slowing down the oxidation of linoleic acid in model oxidation systems. The obtained results indicate that the antioxidant efficiency of applied antioxidants depends on the type of the system in which the lipid oxidation occurs and from the mechanism of oxidation (autooxidation, photosensitization, oxidation by lipooxygenase). For the applied preparations the best antioxidant activities were obtained for emulsion system of linoleic acid in the reaction of the autooxidation, where the co-operation between antioxidants of different physical properties and mechanisms of the antioxidant action in various emulsion phases was possible.

Key words: emulsion, casein, ascorbic acid, β-carotene, autooxidation, lipooxygenase, photosensibilization

INTRODUCTION

Apart from the activity of microorganisms oxidation reactions proceeding in food are one of principal reasons for limiting the food stability and the usefulness to consumption. The most common substrates of the oxidative changes are unsaturated fatty acids since double bonds weaken the C-H bond of adjacent atoms of carbon in aliphatic chains of unsaturated fatty acids, which facilitates oxidation [Madhavi et al. 1995]. Process of lipid oxidation mostly consists in the free radical chain reaction (autooxidation), although lipid peroxides are also created in the reaction of unsaturated fats oxidation with singlet oxygen in the presence of sensitizer (e.g. chlorophyll) or during enzymatic oxidation, e.g. with lipooxygenase as a catalyst [Robinson et al. 1995]. The formed peroxides are subject to self-transformations to secondary compounds which are responsible for undesirable flavor changes, losing of nutritive quality of products and even reducing of food safety [Addis and Warner 1991, Belitz and Grosch 1999].

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Inhibition of oxidation changes is reached through the control of food storage temperature, the application of packages providing a physical barrier for the access of oxygen and light, as well as through the elimination of prooxidant factors like transient metal ions of (mainly iron and copper) and addition of antioxidant components [Frankel 1996].

Lipids in food are often present in the form of multicomponent colloids, mainly as emulsions [Schwarz et al. 2000]. Decker [1998] says that oxidation of emulsified lipids differs from that occurring in bulk fats. It results from the distribution and the division of lipid molecules and other components among emulsion regions, the opportunities of interactions between emulsion chemical components and the presence of interfacial surface around lipid drops. Lipid peroxides themselves are located on the surface of lipid drops in emulsions. The oil/water interface is the place of interaction between lipid peroxides and prooxidant factors (e.g. ions of trace metal) which migrate from the water phase. Therefore the membrane of interfacial surface has a significant influence on the mechanisms of lipid oxidation in emulsion systems, determining, among other things, the physical barrier, that makes the diffusion of components from various emulsion regions difficult [Coupland and McClements 1996]. It was stated that increasing of the oxidative stability of emulsion may be reached by the appropriate selection of the interface components – the application of specific emulsifiers having antioxidant properties [Osborn and Akoh 2004]. It was also stated that they are also able to prove antioxidant activity of other compounds about similar properties due to synergetic effect. Emulsifiers determine the electric charge of lipid drops and that is how they can influence the rate of oxidation, mainly catalyzed by ions of metal like iron or copper [Decker 1998, Mei et al. 1998]. The charge has also a significant influence on some antioxidants efficiency, e.g. negatively charged molecule of the ascorbic acid works better as an antioxidant in the presence of positive charged lipid drops. In food technology proteins are often applied as emulsifiers (milk casein, β-lactoglobulin, whey proteins, proteins of soya bean, egg's protein). The division of protein molecules between membrane of lipid drops and the water phase, the composition of the membrane and the conformation of the protein may influence, apart from physical stability of the emulsion, on efficiency of oxidation delaying [Coupland and McClements 1996]. Protein hydrolysis may increase the protein ability to prevent oxidation in emulsions, presumably thanks to improving the contact with the lipid phase because of an exposure of hydrophobic amino acids residues, which are moreover able to operate as antioxidants [Saiga et al. 2003].

Considering complexity of food systems in which oxidation reactions proceed, one should suppose that single antioxidant operation will not bring the expected effect of food protecting against undesirable oxidation changes. No antioxidant is capable of controlling all factors influencing oxidative food stability. Basing on biological systems of the antioxidant defence it was observed that they consist of many components of various chemical and physical characteristics which fulfill more effectively their antioxidant protection by cooperation. It seems, that antioxidants in food should be applied as a systems containing components operating according to various mechanisms, e.g. inhibiting different prooxidants (chelators of metal ions, scavengers of free radicals or other reactive forms of oxygen, compounds deactivating enzymes), active in various regions of the system (water phase, interface, lipid phase), because they would be able to prove synergistic effect increasing antioxidant property of other antioxidants [Decker 1998, Zhang and Omaye 2000].

The mechanism of the synergistic reactions between antioxidants is not known exactly but the most common hypotheses are: regeneration of primary antioxidant through the transfer of hydrogen from the synergist's molecule, reducing of oxidation initialization by metal ions complexing, scavenging of singlet oxygen, reducing of lipid peroxides to stable non-radical products (e.g. alcohols), improving stability of primary antioxidants by environment acidifying, operating in various locations where oxidation proceeds (water phase, lipid phase, interface) [Madhavi et al. 1995, Frankel 1996, Schwarz et al. 2000].

The aim of this study was the examination of antioxidant properties and synergism of casein and its enzymatic hydrolysate in combination with β-carotene and ascorbic acid in the reaction of autooxidation, enzymatic oxidation and photosensibilized oxidation, led in the emulsion system and in the ethanol solution of linoleic acid, as well as the examining of the antioxidant activity for water soluble antioxidants towards hydroxyradicals.

Moreover the chemical and physicochemical characteristic of casein and its hydrolysate was made consisting of the quantitative analysis of various forms of nitrogen, the surface aromatic hydrophobicity determination and the electrophoretic separation of the studied preparations.

MATERIALS AND METHODS

Materials

Antioxidant preparations used at work: dephosphorylated preparation of casein (SIGMA C-5890), casein enzymatic hydrolysate (SIGMA C-6835), ascorbic acid (SIGMA A-5960), β-carotene (ICN BIOMEDICALS 5636E). Model oxidation systems were created using linoleic acid (SIGMA L 1268). Emulsion systems were stabilized with the Tween 20 emulsifier (SIGMA D-8955).

Methods

1. Characteristics of preparations of casein and casein enzymatic hydrolysate

1.1. Determination of the content of nitrogen forms with Kjeldahl method. To determine the amount of the total nitrogen content, of the nitrogen soluble in 0.01 M phosphate buffer pH 7 and of non-protein nitrogen (protein was precipitated with 12.5% TCA), samples were subjected to mineralization and ammonia distillation using BÜCHI distillation equipment. Titration of distillates was run with 0.1 M HCl solution using TITROLINE EASY SCHOTT apparatus. Content of nitrogen was converted to protein content using the 6.38 coefficient.

1.2. Surface aromatic hydrophobicity of protein preparations determined by spectrophotometric method with ANSA reagent [Hayakawa and Nakai 1995, Di Lollo et al. 1993]. 0.1% solutions of casein and its hydrolysate in 0.01 M phosphoric buffer pH 7 were prepared. In amber glass phials there were prepared: *blank* – 5 ml of protein solution without ANSA (ANSA: 8-anilino-1-naphtalenosulfonic acid), *samples* – with addition

of 10 µl ANSA. All phials were treated with the stream of nitrogen and kept in darkness for 45 min, next the measurement of fluorescence intensity was performed (SHIMADZU RF-1501 spectrofluorimeter) in the following conditions: excitation 390 nm, emission 470 nm. Hydrophobicity of studied preparations were calculated according to the formula:

$$H = (FI_{ANSA} - FI)/B$$

where: H – surface aromatic hydrophobicity, FI_{ANSA} – fluorescence intensity of the proper sample, FI – fluorescence intensity of the blank.

1.3. SDS-PAGE of protein fractions [Laemmli 1970]. Separating (10.0%) polyacrylamide gel with SDS pH 8.8 and stacking (3.0%) polyacrylamide gel with SDS pH 6.8 were prepared. Protein preparation for the separation:

– extraction in denaturing conditions: to weighted preparations 1 ml of sample buffer with SDS and 50 µl 2-mercaptoethanol were added. Samples were mixed, heated (100°C/3 min) and cooled immediately,

– extraction in non-denaturating conditions: to weighted samples of preparations 1 ml of sample buffer without SDS was added and samples were shaken for 30 min,

– electrophoretic separation in vertical system: plates with gels were subjected to 25 mV electrical current for 15 min. After turning off the power supply suitable compartments caches were loaded with 12 µl of prepared solutions of preparations (10 µl of the marker, SIGMA SDS-6). Stacking was led with 20 mA current. Amperage was then increased to 40 mA. Separation was fixed in 20% TCA for 24 hours. The proteins were stained with Coomassie Brillant Blue.

2. Determination of the antioxidant properties of the studied preparations and synergistic effects of their combinations

The preparations were used in the following additions: casein (20 $\mu g \cdot ml^{-1}$), casein hydrolysate (20 $\mu g \cdot ml^{-1}$), ascorbic acid (1 $\mu g \cdot ml^{-1}$), β-carotene (1 $\mu g \cdot ml^{-1}$). Applied combinations of the antioxidants were as follows: **A)** casein, **B)** casein hydrolysate, **C)** ascorbic acid, **D)** β-carotene, **E)** ascorbic acid + casein, **F)** ascorbic acid + hydrolysate, **G)** β-carotene + ascorbic acid, **H)** β-carotene + casein, **I)** β-carotene + hydrolysate, **J)** β-carotene + ascorbic acid + casein, **K)** β-carotene + ascorbic acid + hydrolysate. Solutions of casein, hydrolysate and ascorbic acid were prepared in 0.01 M phosphate buffer pH 7. If any combination did not contain buffer- soluble antioxidants they were replaced with the suitable quantity of the buffer. Control samples without the antioxidants addition and samples with suitable antioxidants were prepared, according to mentioned combinations. For all antioxidant's combinations blanks not subjected to oxidation were prepared.

2.1. Determination of antioxidant efficiency of the preparations studied in the autoxidization of the linoleic acid solution – determination of peroxides content according to Kuo et al. [1999]. *Preparation of the linoleic acid solution without β-carotene:* 500 µl of dichloromethane and 100 µl of linoleic acid were diluted to 50 ml with ethanol (99.8%). *Preparations of the linoleic acid solution with β-carotene*: 500 µl of the β-carotene solution in dichloromethane and 100 µl of linoleic acid were diluted to 50 ml

with ethanol. Both mixtures were then diluted six times with 0.1 M phosphate buffer pH 8. Oxidation r*eaction*: 500 µl of samples were taken (with or without β-carotene) and shaken in the water bath (37°C/3 min). Oxidation was started by 100 µl of 4 mM $FeCl_2$ addition. The reaction was stopped after 10 min by adding 4 ml of 0.6% HCl solution in ethanol. Then, in order to determine the linoleic acid peroxides content, 100 µl of 30% ammonium thiocyanate and 100 µl of 20 mM $FeCl_2$ were added to the samples. After 5 minutes from adding the iron(II) chloride the absorbance at 480 nm was measured (SCHIMADZU UV-1201V) against blank. Antioxidant activities were calculated on the basis of the following formula:

$$A = [(A_{con} - A_{pr})/AK] \cdot 100\%$$

where: A – antioxidant activity, A_{con} – absorbance of control, A_{pr} – absorbance of the sample.

2.2. Determination of antioxidant efficiency of the preparations studied in the autoxidization of the linoleic acid emulsion [Kuo et al. 1999]. *Emulsion preparation:* 1 ml of the β-carotene solution in chloroform (or 1 ml of chloroform for the emulsion without β-carotene) was added to 50 mg of Tween 20 and 112 mg of linoleic acid. Chloroform was evaporated under nitrogen following addition of 90 ml of 0.05 M phosphoric buffer pH 7 and the mixture was homogenizated (20 500 rpm/30 s). *Reaction of oxidation:* 333 µl of the proper emulsion, 40 µl of protein solution and 37 µl of ascorbic acid were mixed and shaken in water bath (37°C) through 3 min. Reaction was started by 20 µl of the water solution of hemoglobin (0.035%). The process was stopped after 10 min by the addition of 5 ml HCl solution in ethanol. Determination of the antioxidant activities according to point 2.1.

2.3. Determination of antioxidant efficiency of the preparations studied in the oxidation catalyzed by lipoxygenase in the linoleic acid solution. The solution of linoleic acid without/with β-carotene – according to the point 2.1. For diluting 0.2 M borate buffer pH 9 was used. *Enzymatic oxidation:* 200 µl of linoleic acid solution, 350 µl of ascorbic acid and 500 µl of protein solution was mixed following addition of 150 µl of the enzyme solution (SIGMA L-7127) in highly chilled 0.2 M borate buffer pH 9. After 7 min of oxidizing the measurement of the conjugated diens was performed at 234 nm (SCHIMADZU UV-160) against blank. Antioxidant activity was calculated according to the formula given in the point 2.1.

2.4. Determination of antioxidant efficiency of the preparations studied in the oxidation catalyzed by lipoxygenase in the linoleic acid emulsion. *Emulsion preparation*: 66 mg of Tween 20 and 4 ml of the linoleic acid were taken. To the emulsion without β-carotene 4 ml of chloroform were added (if with β-carotene – 4 ml of its solution in chloroform). Then chloroform was evaporated under nitrogen; 100 ml of 0.2 M borate buffer pH 9 were added to the mixture following homogenization (20 500 rpm/30 s). *Enzymatic oxidation:* 2.5 ml of linoleic acid solution, 250 µl of protein solution and 250 µl ascorbic acid solution was taken. Reaction was started with addition of 50 µl of enzyme solution (p. 2.3). After 3 min of the oxidation the measurement of the conjugated diens was performed at 234 nm against blank (SCHIMADZU UV-160). Antioxidant activity was calculated according to formula in point 2.1.

2.5. Determination the retarding of photosensibilized oxidation reaction in the emulsion of linoleic acid by the antioxidants studied. *Emulsion preparation*: according to point 2.2 but 1 ml of the chlorophyll in acetone (1.1 $\mu g \cdot ml^{-1}$) was added to the mixture before buffer addition and the solvents were evaporated under nitrogen. Beaker was wrapped with aluminium foil to protect against light. *Reaction of* p*hotosensibilization*: the samples were exposed to 100 W bulb light for 15 min at 45 cm distance from the source of the light. The reaction was stopped through adding 5 ml of HCl solution in ethanol and remove of the light source. The measurement of the linoleic acid peroxides content was performed as in point 2.2.

2.6. Determination of the ability of water soluble preparations to inactivate of hydroxide radicals [Hunt et al. 1998]. *To the test tubes were taken*: 10 ml of sodium benzoate and copper sulphate solution (3 mg $CuSO_4 \cdot 5\ H_2O$ and 17.3 mg benzoate solution was diluted in 100 ml of 0.1 M phosphate buffer pH 7.2), 0.5 ml of antioxidants solutions and 1 ml of DTET solution (SIGMA D-8255: 18.5 mg in 100 ml of deionized water). *Generation of hydroxide radicals:* in samples and control it was started through the addition of 3.2 µl of 30% H_2O_2. There was no addition of H_2O_2 to blank samples. All samples were incubated in the water bath with shaking (37°C/30 min). The fluorescence was measured with SCHIMADZU RF-1501 in the following conditions: excitation 308 nm, emission 410 nm. Antioxidant activities **(A)** were calculated for the preparation and their combinations using the formula:

$$A = [(FI_{con} - FI_{pr})/FI_{con}] \cdot 100\%$$

where: FI_{con} – intensity of the fluorescence for the control, FI_{pr} – intensity of the fluorescence of sample.

2.7. Data analysis: electrophoretograms obtained for casein and for its enzymatic hydrolysate were processed using the Gelscan 1.45 application. In case of the oxidation reactions in model systems measurements of antioxidant activities were made for all samples in four replications. Statistical significance of differences between antioxidant activities of the substances applied and their combinations were determined using the Statgraphics Plus 2.1 application with LSD test for $p < 0.05$ (table 3). The synergistic effects between antioxidants in studied combinations were calculated on the basis of the formula:

$$\%\ SYNERGISMS = [A_M - (A_1 + A_2 + ... + A_n)] / (A_1 + A_2 + ... + A_n)] \cdot 100\%$$

where: A_M – antioxidant activity of all antioxidants in the system, $A_1 + A_2 + ... + A_n$ – sum of individual antioxidant activities of preparations. Obtained values of synergism between applied antioxidants in the model systems are shown in Table 2.

RESULTS AND DISCUSSION

In the first part of our study we characterized casein and casein enzymatic hydrolysate preparations. For both preparations contents of total, soluble and non-protein nitrogen were determined with the Kjeldahl method. Contents of each forms of nitrogen for both preparations are shown in Table 1.

Table 1. Characterization of casein and casein enzymatic hydrolysate
Tabela 1. Charakterystyka preparatów kazeiny i hydrolizatu enzymatycznego kazeiny

	Casein Kazeina	Casein hydrolysate Hydrolizat kazeiny
Total protein, g/100 g of preparation Białko ogółem, g/100 g preparatu	97.8 ±1.02	–
Total nitrogen, g/100 g preparation Azot ogółem, g/100 g preparatu	15.3 ±0.16	13.5 ±0.11
Soluble nitrogen, g/100 g of total nitrogen Azot rozpuszczalny, g/100 g azotu ogółem	65.4 ±1.26	96.9 ±0.45
Non-protein nitrogen, g/100 g of soluble nitrogen Azot niebiałkowy, g/100 g azotu rozpuszczalnego	not detected nie stwierdzono	100.0
Aromatic hydrophobicity, FI units Hydrofobowość aromatyczna, j.u. FI	89.9 ±3.77	4.9 ±1.66

Casein preparation contained 15.3% of total nitrogen what corresponds to 98% of the total protein. Enzymatic hydrolysate contained 13.5% of total nitrogen. Soluble nitrogen in casein was 65.4% of total nitrogen; however casein hydrolysate provided to contain approximately 97% of this form of nitrogen.

Non-protein nitrogen, coming from polypeptides built by less than 100 amino acids, was not detected for the casein. For hydrolysate non-protein nitrogen was 100% quantity of the soluble nitrogen. Resulting from the difference of total and non-protein nitrogen content expressing as total nitrogen, the hydrolysate contained below than 0.5% of protein nitrogen.

Webb et al. [2002] pointed that the solubility of proteins is bound with the charge density amassed on the surface of their molecules and grows with the charge density. Probably for protein hydrolysates high solubility (close to 100%) is caused by the presence of soluble protein fractions of low molecular masses which are typical of the high degree hydrolysis (fractions below 5 kDa).

The next stage of this study included denotation of aromatic hydrophobicity with fluorescent method and the ANSA reagent. Casein was distinguished by high aromatic hydrophobicity – 90 FI units (Table 1), whereas its enzymatic hydrolysis had an effect on decreasing hydrophobicity significantly, which for hydrolysate equated 5 FI units. It could mean that the small quantity of hydrophobic aromatic amino acids residues was subjected to the surface exhibition. Dalgleish [1996] pointed that the N-termined part of the polypeptide chain of β-casein (hydrophilic fragment) is more susceptible to the protease's operation comparing to the C-termined fragment. Perhaps, hydrophobicity reduction could result from the proportionally considerable quantity of amino acids residues with charge which were subjected to expose. The hydrophobicity of enzymatic hydrolysates influence the kind of enzyme applied for protein hydrolysis, the time of the hydrolysis and its degree [Saiga et al. 2003]. Similarly like in our work the enzymatic hydrolysis of pea's proteins caused substantial hydrophobicity reduction to degree dependent on the enzyme applied. Hydrolysate obtained by the pepsin application possessed 5-times smaller hydrophobicity than pea proteins and hydrophobicity 7.5 times smaller – those obtained using proteinase N [Wołosiak and Worobiej 1999]. Webb et al. [2002] pointed after Keshav and Nakai [1979] that there is a negative correlation be-

tween surface properties and surface hydrophobicity of proteins. Surface hydrophobicity increase influences lessening interface and surface tension what effects with better emulsifying and stabilizing properties in emulsion systems. Aromatic hydrophobicity increase exposes more non-polar fragments in the molecule which are able to locate themselves in the lipid phase of emulsion [Chen et al. 1995, Webb et al. 2002, Saiga et al. 2003], so higher hydrophobicity may improve antioxidant properties of preparation because of their better contact with the aliphatic phase. However Chen et al. [1998] did not make a note that there is dependence between hydrophobicity and antioxidant properties for some peptides. Taking into account low hydrophobicity of the studied hydrolysate, we are able to suppose that its functional properties in the emulsion system were deteriorated in comparison with non-modified protein, however it does not necessarily have to influence antioxidant operations [Chen et al. 1998].

The last stage of characteristic of above-mentioned preparations was the electrophoresis on the polyacrylamide gel with sodium dodecyl sulphate (SDS). Graphs presenting optical density corresponding to each of the polypeptide fractions are shown on Figures 1, 2, 3. For casein in partly denaturing conditions (the only denaturant factor was SDS as a component of gels) 11 fractions of protein were obtained with masses from 13 to approx. 92 kDa (Fig. 1). The highest percentage participation among all fractions occurred in case of fraction 5 (27%) and mass of 30.5 kDa, then fraction 4 – 38.5 kDa (about 19%) and 3 – 66.3 kDa which stated over 18% of proteins of the preparation.

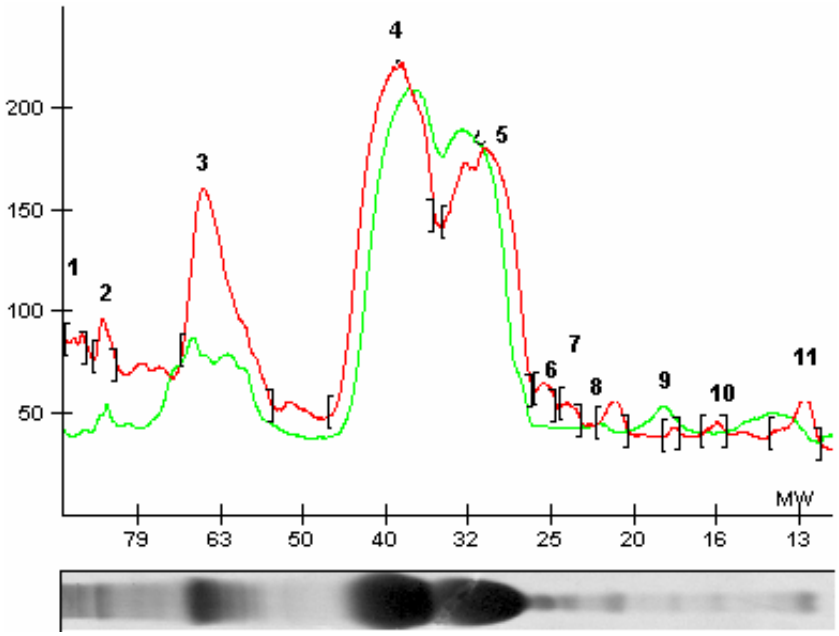

Fig. 1. Electrophoretic separation of casein fractions after subjecting to partial denaturation (line with marked numbers of the fractions)
Rys. 1. Rozdział elektroforetyczny białka kazeiny poddanego częściowej denaturacji (linia z oznaczonymi numerami frakcji)

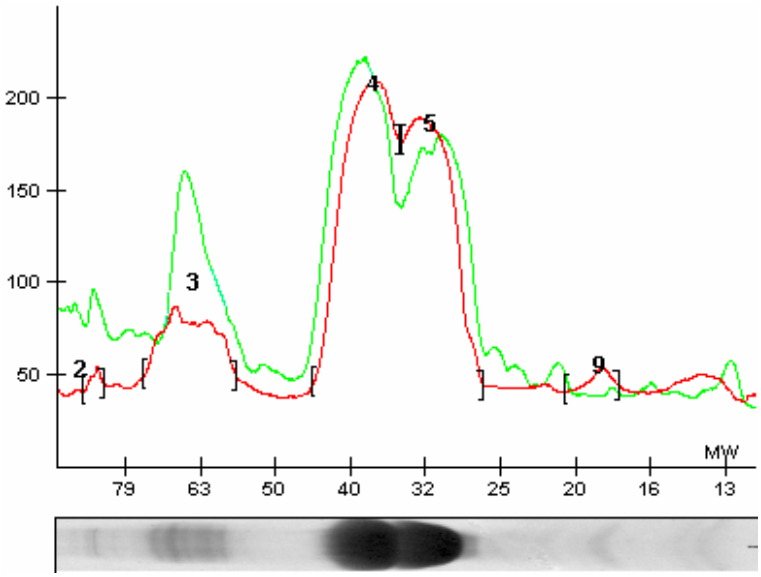

Fig. 2. Electrophoretic separation of casein fractions after subjecting to denaturation (line with marked numbers of the fractions)
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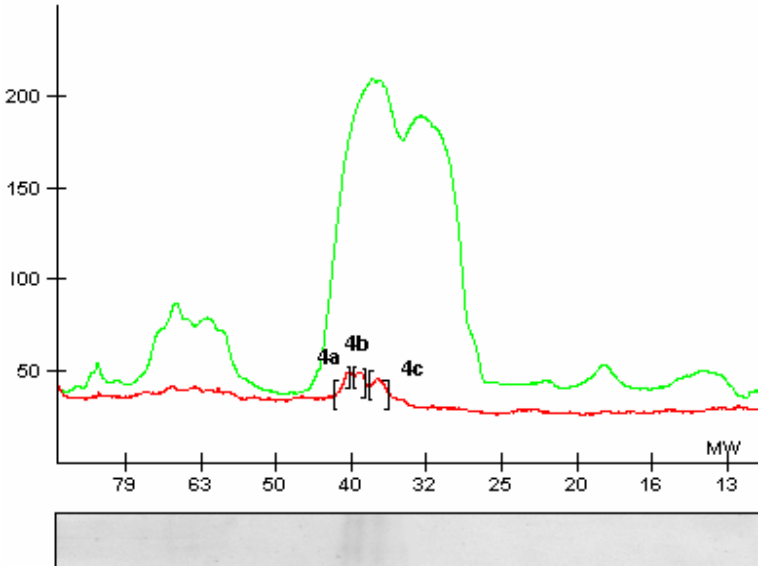

Fig. 3. Electrophoretic separation of protein fractions of casein hydrolysate after subjecting to denaturation (line with marked numbers of the fractions)
Rys. 3. Rozdział elektroforetyczny dla hydrolizatu kazeiny poddanego denaturacji (linia z oznaczonymi numerami frakcji)

For casein subjected to complete denaturation (Fig. 2), we noticed that due to destruction of the higher degree structure of protein under influence of dodecyl sulphate, mercaptoethanol and temperature, fraction 1 (above 92 kDa), as well as fractions approx. 21 to 26 kDa (fractions 6, 7, 8) and from 10 to 18 kDa (fractions 10, 11) were decomposed. The number of bands increased obviously within fractions number 3. For denaturated casein the main fractions were 4 and 5 with masses approx. 32.5 kDa and 37.5 kDa present respectively as 45% and 33% of protein preparation, comparing with casein extracted in non-denaturating conditions an increase in percentage participation of such fractions can be seen. Fractions 6 – 11, having small participation, are components of γ-casein (product of casein enzymatic degradation) and fraction 6 (about 27 kDa) was identified as fragments of α_{s2}-casein, fraction 7 as fragments of β-casein (app. 24 kDa), whereas 9 as fragments of κ-casein (about 18-19 kDa). Within 29 kDa there probably were located undivided bands of β-casein and κ-casein (5). According to literature, masses from app. 25 to 30-40 kDa correspond to α_{s1}- and α_{s2}-casein, that is why they probably build fraction 4 [Dziuba and Mioduszewska 1997, Jahaniaval et al. 2000, Webb et al. 2002]. Fractions 1, 2, 3 and 4 were represented by polymers of casein proteins.

For casein hydrolysate, in consequence enzymatic hydrolysis and denaturation conditions, polypeptides above masses 40 kDa and below 37 kDa were disintegrated. Three new fractions (4a, 4b, 4c) from 37.0 kDa (45%) to 40.0 kDa (29%) were observed, which were separated inside fraction number 4 corresponding to casein (Fig. 3). Fractions about low molecular masses, formed as a result of hydrolysis, probably were not detected in the consequence of washout from the gel during its treatment. For casein hydrolysate subjected to extraction in non-denaturate condition before electrophoresis the image of separation was not obtained.

The goal of the second part of this paper was to analyse the antioxidant activity of the antioxinats studied (ascorbic acid, β-carotene, casein and casein enzymatic hydrolysate) during the oxidation of linoleic acid, in model oxidation systems. Letter of designation of antioxidants combination used is given in Table 2.

Table 2. Letter of designation of used combination of antioxidants
Tabela 2. Oznaczenia literowe stosowanych kombinacji przeciwutleniaczy

A	Ascorbic acid – Kwas askorbinowy
B	Casein – Kazeina
C	Casein hydrolysate – Hydrolizat kazeiny
D	β-carotene – β-karoten
E	Ascorbic acid + casein – Kwas askorbinowy + kazeina
F	Ascorbic acid + casein hydrolysate – Kwas askorbinowy + hydrolizat
G	β-carotene + ascorbic acid – β-karoten + kwas askorbinowy
H	β-carotene + casein – β-karoten + kazeina
I	β-carotene + casein hydrolysate – β-karoten + hydrolizat
J	β-carotene + ascorbic acid + casein – β-karoten + kwas askorbinowy+ kazeina
K	β-carotene + ascorbic acid + hydrolysate – β-karoten + kwas askorbinowy + hydrolizat

Antioxidative properties in the reaction of autooxidization and oxidation in the presence of lipooxygenase were investigated in single-phase system (solution of linoleic acid in ethanol) and in emulsion of linoleic acid. Reaction of photosensibilized oxidation was run only in emulsion system. Additionally, for the ascorbic acid, casein and hydrolysate antioxidant activities were studied towards hydroxyradicals ($^{\bullet}OH$). Average antioxidant activities of the tested preparations were shown in Figures 4-9 in the text.

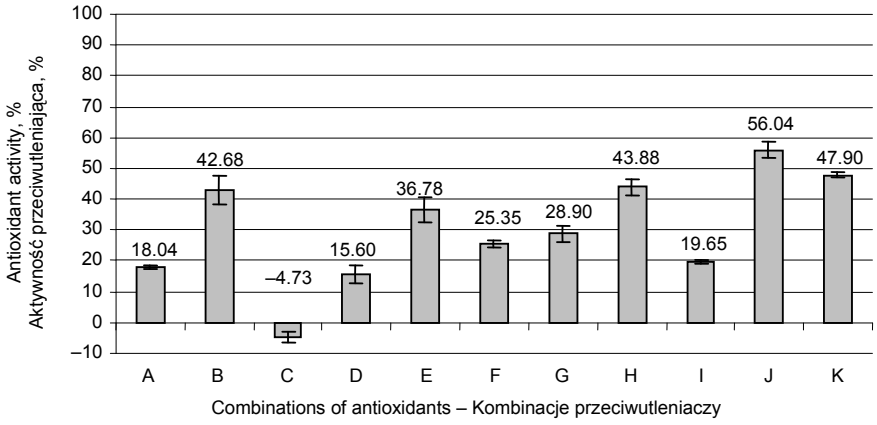

Fig. 4. Antioxidant activities of the preparations studied in the autooxidization of linoleic acid solution. Symbols A, B, C..., K – combinations of antioxidants explained in Table 2
Rys. 4. Aktywność przeciwutleniająca badanych preparatów w reakcji autooksydacji w roztworze kwasu linolowego. Oznaczenia A, B, C..., K – kombinacje przeciwutleniaczy podane w tabeli 2

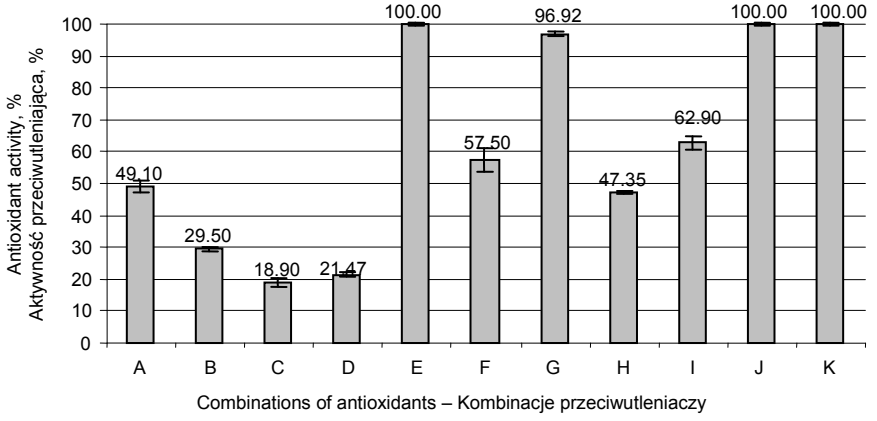

Fig. 5. Antioxidant activities of the preparations studied in the autooxidization of linoleic acid emulsion. Symbols A, B, C..., K – combinations of antioxidants compatible explained in Table 2
Rys. 5. Aktywność przeciwutleniająca badanych preparatów w reakcji autooksydacji w układzie emulsyjnym kwasu linolowego. Oznaczenia A, B, C..., K – kombinacje przeciwutleniaczy podane w tabeli 2

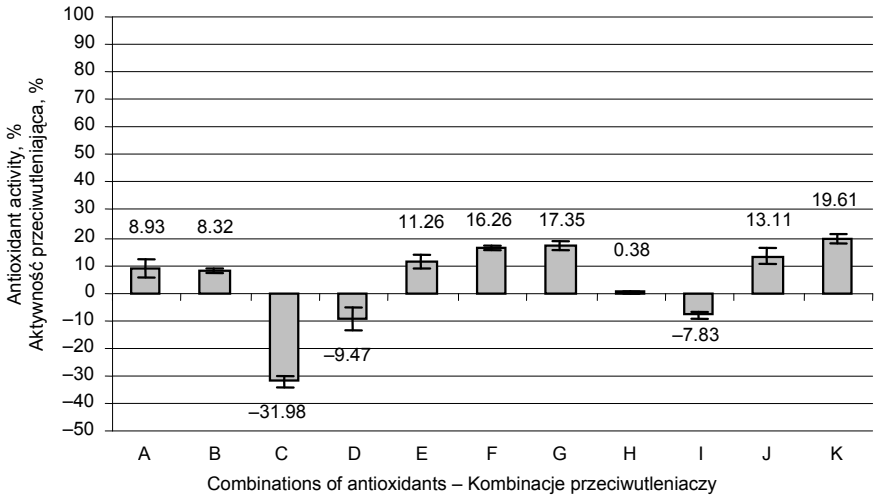

Fig. 6. Antioxidant activities of the preparations studied in of linoleic acid solution oxidized by lipoxygenase. Symbols A, B, C..., K – combinations of antioxidants explained in Table 2

Rys. 6. Aktywność przeciwutleniająca badanych preparatów w reakcji z lipooksygenazą w roztworze kwasu linolowego. Oznaczenia A, B, C..., K – kombinacje przeciwutleniaczy podane w tabeli 2

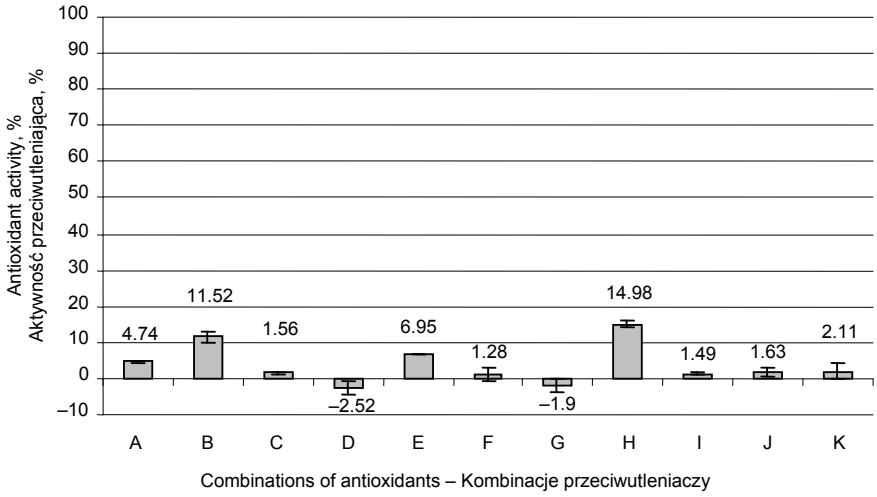

Fig. 7. Antioxidant activities of the preparations studied linoleic acid emulsion oxidized by lipoxygenase. Symbols A, B, C..., K – combinations of antioxidants given in Table 2

Rys. 7. Aktywność przeciwutleniająca badanych preparatów w reakcji z lipooksygenazą w emulsji kwasu linolowego. Oznaczenia A, B, C..., K – kombinacje przeciwutleniaczy podane w tabeli 2

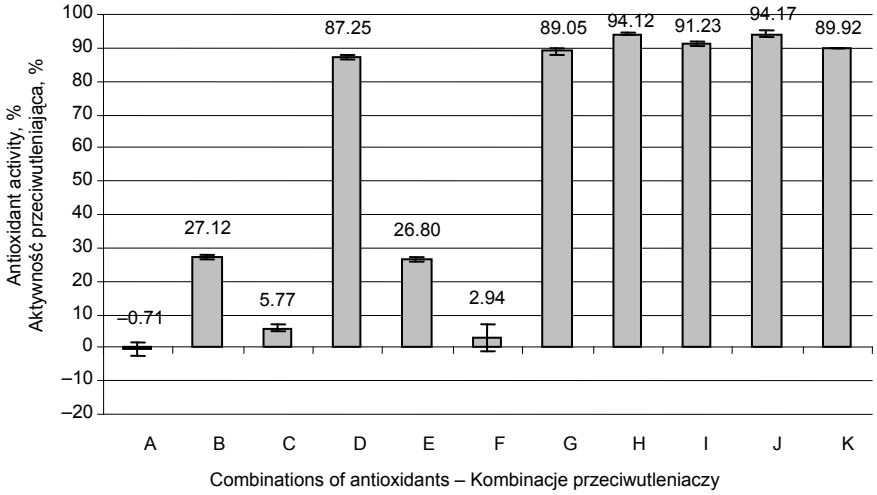

Fig. 8. Antioxidant activities of the preparations studied in photosensibilized oxidation of linoleic acid emulsion. Symbols A, B, C..., K – combinations of antioxidants explained in Table 2

Rys. 8. Aktywność przeciwutleniająca badanych preparatów w reakcji utleniania fotosensybilizowanego emulsji kwasu linolowego. Oznaczenia A, B, C..., K – kombinacje przeciwutleniaczy podane w tabeli 2

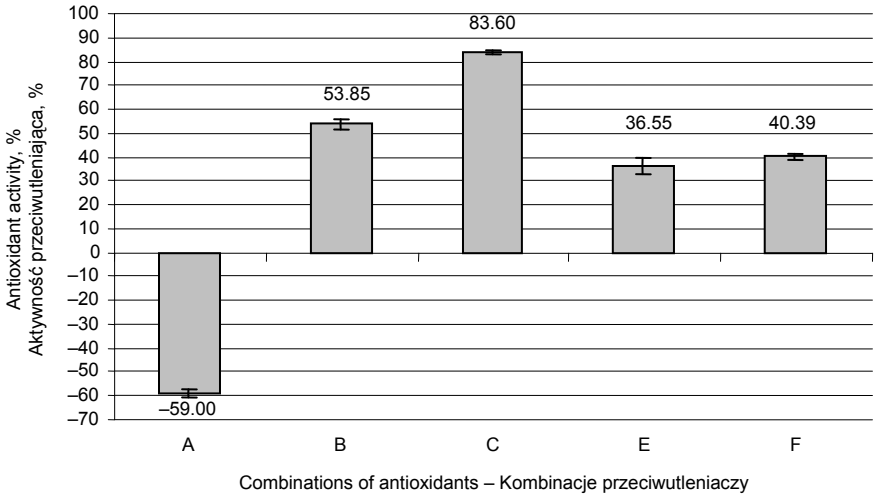

Fig. 9. Antioxidant activities of water-soluble preparations in the reaction with hydroxyradicals. Symbols A, B, C, E and F – water soluble antioxidants and their combinations explained in Table 2

Rys. 9. Aktywność przeciwutleniająca preparatów rozpuszczalnych w wodzie wobec rodników wodorotlenowych. Oznaczenia A, B, C, E i F – kombinacje przeciwutleniaczy podane w tabeli 2

Autooxidization in the solution of linoleic acid was catalyzed with Fe(II) ions, which promoted oxidation of substrate by decomposition of lipid hydroperoxides to free radicals, because iron of hemoglobin (used in emulsions) showed very low activity of oxidation in this system (solution of linoleic acid in ethanol). Operating in the absence of other antioxidants in the solution of linoleic acid the best antioxidant properties showed casein, which activity was about 43% (Fig. 4). Considerably weaker properties possessed β-carotene and ascorbic acid (16-18%), while enzymatic hydrolysis of casein caused the disappearance of its antioxidant efficiency and slight prooxidant activity in effect (about 5%).

Replacement of casein with β-carotene did not significantly alter the speed of oxidation in the system. In case of combination with ascorbic acid there was observed a reduction of activity to 37%. In the analysed system the most effective combination turned out the employment of all three preparations, which showed 56% of activity. Relatively high activity (48%) proved also ascorbic acid in combination with β-carotene and the hydrolysate.

On the basis of the obtained antioxidant activities of the studied antioxidants combinations the synergistic effects were calculated (%). Received synergistic values were compiled in Table 3. In the discussed system the antioxidant force of ascorbic acid with β-carotene and casein hydrolysate combination was higher from the sum of individual antioxidant activities (66% synergism), but such effect was not detected for combination with the best antioxidant properties in this system (casein, ascorbic acid and β-carotene). Combination of casein hydrolysate with ascorbic acid proved 90% synergism, but hydrolysate and β-carotene – 81%.

Good casein operation in the analysed system was probably owed to its strong properties of transitory metal ion chelating [McClements and Decker 2000]. The amino and carboxyl groups of the amino acids residues in the protein molecule are able to participate in such process, as well as nitrogen of histidine imidazol ring [Saiga et al. 2003] and phosphoserine groups, from which not all could became dephosphorylated. Comparing the activity of casein to hydrolysate, we could suppose that not only the characteristic composition of amino acids and their sequence in the protein chain, but also presence of peptides of particular length and structure of the chain is necessary to possess good properties of the metal ion binding by compounds of protein origin [Chen et al. 1995, Saiga et al. 2003]. Antioxidant activity of casein may also result from the capability to free radicals capturing (histidine residues) or reducing them on the way of making the hydrogen atom available, e.g. by tyrosine, phenylalanine [Chen et al. 1995, 1998]. It seems that inactivation of free radicals by casein was supported by β-carotene here [Subagio and Morita 2001] and with free radicals reduction by ascorbic acid, which moreover is able to chelate metal ions [Kitts 1997]. Ascorbic acid and casein could also regenerate each other.

Change of the system character from the linoleic acid solution to the emulsion influenced the antioxidant action of preparations. In the emulsion their antioxidant activities in the process catalyzed by Fe(II) ions of hemoglobin in most cases turned out significantly higher. We noticed important change for ascorbic acid and casein hydrolysate. In emulsion ascorbic acid was slowing the autooxidization of linoleic acid down in 49%, operating more effectively than hydrophobic β-carotene (approx. 21%). Casein retarded oxidation in 30%, but in the connection with β-carotene in 48%. Hydrolysate was still the weakest antioxidant, despite improving the activity to 19% comparatively with that

in solution system. In combination with β-carotene, two not very effective antioxidants in the studied emulsion, showed a significant growth of activity (63%) in braking peroxides formation. Hydrolysate co-operated also with ascorbic acid (resulting activity about 58%), however casein maintained much better in such a combination (100% of activity). Only combination of casein hydrolysate with ascorbic acid and β-carotene equalized their activity with analogous combination for casein. It is worth mentioning that also two preparations characterized by extreme range of hydrophobicity (ascorbic acid and β-carotene) collaborated very well in the reaction of oxidation (97%). Combination of preparations acting in different emulsion phases resulted in much higher activity comparing to continuous systems. The highest synergistic effects (Table 3) were found in this setup for β-carotene with hydrolysate (55% synergism), ascorbic acid with β-carotene (37%) and ascorbic acid with casein (27%).

Casein, as a protein exhibiting emulsifying properties, locates on the o/w interface surface and creates a protective physical barrier for lipid drops. It makes the exchange of components between emulsion phases to be difficult, like diffusion of oxygen to the lipid drops interior, moving of prooxidative ions as well free radicals rising in the water phase [Coupland and McClements 1996].

The capacity of casein to create the thick surface interface (12 nm), where a bigger part of its mass is adsorbed, is an important property of this protein contributing to the oxidative stability of emulsion system [Hu et al. 2003, Dalgleish 1996]. Moreover, the ability to reduce or to pick free radicals out is equally important [McClements and Decker 2000]. With the background of other applied antioxidants the complex properties of casein caused also quite good individual prevention against oxidation. Ascorbic acid counteracts for lipid oxidation through direct reacting with factors initiating this process present in the water phase and the interface, primarily deactivating the free radicals rising in the water phase [Kiokias and Gordon 2003] and binding of oxygen [Kitts 1997]. Also the regeneration of oxidized ascorbic acid is possible in the system by combining of two monodehydroascorbic radicals. Probably ascorbic acid may reduce hydroperoxides to stable alcohols, through non-radical transformations as well [Frankel 1996], reducing on this way the amount of oxidation process substrates. It could prevent also oxidation of Fe(II) to Fe(III), which in the porphyryl system of hemoglobin is very active as prooxidant [Richards and Dettmann 2003]. Presumably, the fact that the casein preparation was dephosphorylated contributed good co-operation effect of casein and ascorbic acid. Removing phosphoric groups from phosphoserine residues led to reducing the density of negative charge in N-termined casein fragment [Dalgleish 1996, Husband et al. 1997]. This hydrophilic part of the casein molecule is able to localize in the water phase of the interface. Reduction of the repelling force between this fragment of the protein and ascorbic acid molecules, which are negatively charged in water, was able to influence improving their antioxidant operation [Husband et al. 1997, Decker 1998].

β-carotene is not very good free radicals scavenger and presumably therefore possesses relatively low antioxidant activity against lipid autooxidation catalyzed with metal ions [Kiokias and Gordon 2003]. In accordance to literature [Zhang and Omaye 2000, Kiokias and Gordon 2003] applying β-carotene to the system with ascorbic acid proved co-operation. Probably, hydrophilic ascorbic acid prevents for rising reactive carotene radicals or regenerates already formed, what makes β-carotene keeping its antioxidant properties [Kiokias and Gordon 2003]. Hydrolysate, possessing hydropho-

bic fragments of aromatic amino acids in structure as well as exposed residues of polar amino acids, being very well soluble in the water phase, was also able to partial locating in the emulsion interface and to counteract there for oxidizing. At the same time it prevents carotene present inside lipid drops. Hydrolysate weaker activity comparing with casein perhaps resulted from low hydrophobicity and, because of that, smaller participation in the interface.

In the reactions catalyzed by lipoxygenase, the degree of oxidation of linoleic acid was measured on the basis of the quantity of conjugated diens rising during such transformations. In the linoleic acid solution (Fig. 6) β-carotene operated faintly as a prooxidant. Combination with ascorbic acid gave a clear (but little) synergistic effect – antioxidant activity of such combination was above 17% and differed significantly from the ascorbic acid activity (about 9%). Ascorbic acid also had better activities in merging with hydrolysate – above 16%. In the studied system the hydrolysate proved high (32%) prooxidant activity. These properties were changed in the combination with ascorbic acid and β-carotene. It allowed obtaining the best antioxidant activity, retarding oxidation due to lipoxygenase in about 20%. The biggest synergistic effect (Table 3) was reached in the system analysed in setting the ascorbic acid with β-carotene and casein (68%).

Table 3. The synergistic effects between the studied antioxidants combinations in model oxidation systems
Tabela 3. Efekty synergistyczne pomiędzy połączeniami badanych przeciwutleniaczy w modelowych układach oksydacyjnych

Synergistic effects, % – Efekty synergistyczne, %							
	E	F	G	H	I	J	K
Autooxidation of the linoleic acid solution Autooksydacja w roztworze kwasu linolowego	nd	90.0	nd	nd	81.0	nd	66.0
Autooxidation of the linoleic acid emulsion Autooksydacja w emulsji kwasu linolowego	27.0	nd	37.0	nd	55.0	nd	11.5
Enzymatic oxidation in the linoleic acid solution Utlenianie enzymatyczne w roztworze kwasu linolowego	nd	nd	nd	nd	nd	68.5	nd
Enzymatic oxidation in the linoleic acid emulsion Utlenianie enzymatyczne w emulsji kwasu linolowego	nd	nd	nd	66.4	nd	nd	nd
Photosensibilized oxidation in the linoleic acid emulsion Utlenianie fotosensybilizowane w emulsji kwasu linolowego	1.5	nd	2.9	nd	nd	nd	nd

nd – not detected.
nd – brak.

In the emulsion of linoleic acid (Fig. 7) subjected for lipoxygenase action the prooxidant character of β-carotene was weaker than in the solution (–2.5%). In the system discussed, casein combined with β-carotene prevented oxidization in almost 15% making a better solution than other antioxidants combinations, although it strengthened casein activity (11%) only slightly. Other combinations of the studied substances turned out ineffective. Considering the synergistic effect most effective combination was casein and β-carotene (66%) – Table 3.

Table 4. The statistical differences significance between antioxidant activities of the combinations studied in different oxidation systems
Tabela 4. Istotność statystyczna różnic aktywności przeciwutleniających stosowanych kombinacji przeciwutleniaczy w różnych układach oksydacyjnych

System of oxidation Układ oksydacyjny	A	B	C	D	E	F	G	H	I	J	K
	the significance of statistical differences istotność różnic statystycznych aktywności przeciwutleniających porównywanych kombinacji przeciwutleniaczy w danym układzie oksydacyjnym										
Autooxidation of the linoleic acid solution Autooksydacja w roztworze kwasu linolowego	b	e	a	d	c	b	c	e	b	f	e
Autooxidation of the linoleic acid emulsion Autooksydacja w emulsji kwasu linolowego	c	b	a	g	d	a	f	c	e	g	g
Enzymatic oxidation in the linoleic acid solution Utlenianie enzymatyczne w roztworze kwasu linolowego	d, e	d	a	d, e	f, g	b	f, g	c	b	e, f	g
Enzymatic oxidation in the linoleic acid emulsion Utlenianie enzymatyczne w emulsji kwasu linolowego	c, d	e	b	d	b	a	a	f	b	b	b, c
Photosensibilized oxidation in the linoleic acid emulsion Utlenianie fotosensybilizowane w emulsji kwasu linolowego	a	c	b	c	b	d	d, e	f	e, f	f	d, e
Formation of hydroxyradicals in the presence of Cu(II) Utlenianie katalizowane rodnikami wodorotlenowymi w obecności Cu(II)	a	c	d	–	b	b	–	–	–	–	–

A, B, C..., K – applied antioxidants and their combinations presented in Table 2.
a, b, c..., g – the same letters indicate lack of statistical differences significance ($p < 0.05$).
A, B, C..., K – stosowane przeciwutleniacze i ich kombinacje podane w tabeli 2.
a, b, c..., g – te same oznaczenia literowe oznaczają brak istotności statystycznej różnic ($p < 0,05$).

Both in the solution of linoleic acid and in the emulsion, preparations reduced oxidation catalyzed by lipoxygenase in a slight degree. Perhaps it was the consequence of different mechanism of oxidation with lipoxygenase, because the enzyme creates complexes with substrate which is oxidation, without free radicals release to the environment of the reaction [Robinson et al. 1995]. Such proceeding of oxidation makes antioxidants action to be difficult. Besides, lipoxygenase, what literature indicates [Whitaker et al. 1991, Robinson et al. 1995], oxidizes other components, like carotenoids, depriving them of the antioxidant properties. This process takes place at the same time with lipid compounds oxidation [Robinson et al. 1995, Aziz et al. 1999]. We could assume that ascorbic acid regenerated oxidized β-carotene molecules [Kiokias and Gordon 2003]. It was also possible that it reduced lipid hydroxyperoxides essential for the lipoxygenase activation.

Mechanism of the lipooxygenase operation on the surface of lipid drops in emulsion has not been known well [Saka et al. 2000]. Authors suggested that fractions of proteins, of proper size and applied as emulsifiers are able to protect effectively from lipooxygenase activity creating space hindrance. They noticed lower lipoxygenase activity in emulsions stabilized with proteins presumably because of the physical barrier created on the oil-water surface but not due to direct antioxidant operation of proteins. It is possible that the fact described above influenced the better casein operation comparing to its hydrolysate. It also seems that by locating in the interface, casein was able to regenerate β-carotene being inside the drops, which then remained active. Proteins subjected for enzymatic modification change their functional properties depending on the degree of hydrolysis. The high hydrolysis degree brings to form low-molecular peptides what deteriorates emulsifying properties. It was suggested that to possess emulsifying properties the hydrolysate should not contain peptides smaller than 5 kDa [Crowley et al. 2002].

The reaction of photosensibilization oxidation was led in the emulsion system. The degree of oxidization was determined by the measurement of hydroperoxides formed during linoleic acid oxidation the in presence of chlorophyll as a sensitizer. In accordance to literature β-carotene confirmed very good properties in this system probably by single oxygen scavenging as well blanking of activated sensitizers [Munné-Bosch and Alegre 2002], showing 87% of antioxidant activity. Activity of carotene did not change in the combination with ascorbic acid (89%) and with ascorbic acid and hydrolysate (app. 90%). All combinations containing β-carotene showed the activity of 90% and higher. Casein exhibited the antioxidant activity of 27%, confirming that some amino acids possess the ability to scavenge singlet oxygen [Chen et al. 1998].

Casein combined with β-carotene operated on the level of 94%, but the hydrolysate retarded photosensibilization only in 6%. Ascorbic acid did not show ability to blank singlet oxygen what is not consistent with the literature data. This probably resulted from the fact that both sensitizer (chlorophyll) and the substrate of oxidization (linoleic acid) were located in the lipid phase of emulsion where ascorbic acid did not have any access. In this model oxidation system there was no significant synergistic effect in antioxidants combinations.

Antioxidative activities of water soluble preparations (ascorbic acid, casein, casein enzymatic hydrolysate and their combinations) were checked also towards hydroxy radicals ($^{\bullet}$OH), in the system without linoleic acid as a substrate for oxidation. Casein hydrolysate operating in 84% showed the best hydroxy radicals deactivation effect (Fig. 9). Also casein was a good antioxidant here (54%). Hydrolysis of the protein improved casein action, presumably making more residues of amino acids accessible for picking out this most reactive form of oxygen. An exposure of peptide fragments containing tyrosine and proline was also able to bind copper ions – catalysts of the reaction [Chruścińska et al. 1997]. Ascorbic acid accelerated rising of hydroxy free radicals in the very high degree. It was prooxidant in 59%, what confirms the ascorbic acid ability of participating in the Haber-Weiss reaction, during which ascorbic acid reduces ions of transitory metal (in this case Cu(II) ions) to more reactive forms, which participate in $^{\bullet}$OH radicals forming [Kitts 1997]. Beside that, addition of ascorbic acid to casein and the hydrolysate lowered their antioxidant properties significantly.

CONCLUSIONS

1. Casein hydrolysate differed from casein preparation in the significant way possessing 100% of soluble nitrogen in the form of non-protein nitrogen and eighteen times smaller aromatic surface hydrophobicity.

2. An antioxidant efficiency of the applied antioxidants depends on the character of the system and on the mechanism of oxidation reaction.

3. Casein possessed the most stable antioxidant character in all the systems applied showing the antioxidant activity up to 54% and not showing any prooxidant properties.

4. Deeply advanced enzymatic hydrolysis of casein caused a decrease of antioxidant activity for oxidation process lead in the solution and the emulsion of linoleic acid.

5. Ascorbic acid and β-carotene applied separately showed diverse properties – from operating as a prooxidants to antioxidant during autooxidization (ascorbic acid) and for photosensibilized oxidation (β-carotene) in the linoleic acid emulsion.

6. In non-enzymatic systems the strongest antioxidant activity (56-100%) showed combinations of casein with ascorbic acid, β-carotene and both of them, whereas the highest synergistic effects were obtained for combinations of the hydrolysate with ascorbic acid or β-carotene (55-90%).

7. Different mechanism of reaction catalyzed by lipoxygenase caused considerable decrease of the antioxidant efficiency.

REFERENCES

Addis P.B., Warner G.J., 1991. The potential health aspects of lipid oxidation productives. „Free radicals and food additives". Taylor and Francis London, 77-119.

Aziz S., Wu Z., Robinson D.S., 1999. Potato lipoxygenase catalysed co-oxidation of β-carotene. Food Chem. 64, 227-230.

Belitz H.D, Grosh W., 1999. Food chemistry. Springer Berlin, 181-213.

Chen H.M., Muramoto K., Yamauchi F., 1995. Structural analysis of antioxidative peptides from soybean. J. Agric. Food Chem. 43, 574-578.

Chen H.M., Muramoto K., Yamauchi, Fujimoto K., Nokihara K., 1998. Antioxidative properties of histidine containing peptides designed from peptide fragments found in the digests of a soybean protein. J. Agric. Food Chem. 46, 49-53.

Chruścińska E., Dyba M., Micera G., Ambroziak W., Olczak J., Zabrocki J., Kozłowski H., 1997. Binding ability of Cu^{2+} ions by optiate – like fragments of bovine casein. J. Inorg. Biochem. 66, 19-22.

Crowley P., O'Brien C.M., Slattery H., Chapman D., Arend E.K., Stanton C., 2002. Functional properties of casein hydrolysates in bakery applications. Eur. Food Res. Technol. 1-10.

Coupland J.N., McClements D.J., 1996. Lipid oxidation in food emulsions. Trends Food Sci. Technol. 7, 83-91.

Dalgleish D., 1996. Conformations and structures of milk proteins adsorbed to oil-water interfaces. Food Res. Int. 29, 541-547.

Decker E.A., 1998. Strategies for manipulating the prooxidative/ antioxidative balance of food to maximize oxidative stability. Trends Food Sci. Technol. 9, 241-248.

Di Lollo A., Alli J., Biliarderis C., Barthakur N., 1993. Thermal and surface active properties of citric acid-extracted and alkali-extracted proteins from Phaseolus beans. J. Agric. Food Chem. 41, 24-29.

Dziuba J., Mioduszewska H., 1997. Quantitive analysis of milk proteins by SDS page method. J. Food Nutr. Sci. 6, 91-96.
Frankel E.N., 1996. Antioxidants in lipid food and their impact on food quality. Food Chem. 57, 51-55.
Hayakawa S., Nakai S., 1985. Relationship of hydrophobicity and net charge to the solubility of milk and soy proteins. J. Food Sci. 50, 486-491.
Hu Min, McClements D.J., Decker E.A., 2003. Lipid oxidation in corn oil-in-water emulsions stabilized by casein, whey protein isolate, and soy protein isolate. J. Agric. Food Chem. 51, 1696-1700.
Hunt J.V., Simpson J.A., Dean R.T., 1988. Hydroperoxide-mediated fragmentation of proteins. Biochem. J. 250, 87-93.
Husband F.A., Wilde P.J., Mackie A.R., Garrood M.J., 1997. A comparison of the functional and interfacial properties of β-casein. J. Coll. Interf. Sci. 195, 77-85.
Jacob R.A., 1995. The integrated antioxidant system. Nutr. Res. 15 (5), 755-766.
Jahaniaval F., Kakuda Y., Abrahahm V., Marcone M.F., 2000. Soluble protein fractions from pH and heat treated sodium caseinate: physicochemical and functional properties. Food Res. Int. 33, 637-647.
Keshavarz E., Nakai S., 1979. The relationship between hydrophobicity and interfacial tension of proteins. Biochim. Biophys. 576 (2), 269-279.
Kiokias S., Gordon M.H., 2003. Antioxidant properties of annato carotenoids. Food Chem. 83, 523-529.
Kitts D.D., 1997. An evaluation of the multiple effects of the antioxidant vitamins. Trends Food Sci. Technol. 8, 198-203.
Kuo J.M., Yeh D.B., Pan B.S., 1999. Rapid photometric assay evaluating antioxidative activity in edoble plant material. J. Agric. Food Chem. 47, 3206-3209.
Laemmli U.K., 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227, 680-685.
Madhavi D.L., Deshpande S.S., Salunke D.K., 1995. Food antioxidants. Technological, toxicological and health perspectives. Marcel Dekker New York, 5-65, 66-159, 267-361, 361-471.
McClements D.J., Decker E.A., 2000. Lipid oxidation in oil-in-water emulsions: Impact of molecular environment on chemical reactions in heterogeneous food systems. J. Food Sci. 65, 1270-1281.
Mei L.Y., McClements D.J., Decker E.A., 1999. Lipid oxidation in emulsions as affected by charge status of antioxidants and emulsion droplets. J. Agric Food Chem. 47, 2267-2273.
Munné-Bosch S., Alegre L., 2002. Interplay between ascorbic acid and lipophilic antioxidants defences in chloroplasts of water stressed Arabidopsis plansts. Fed. Eur. Biochem. Soc. Lett. 524, 145-148.
Osborn H.T., Akoh C.C., 2004. Effect of emulsifier type, droplet size and oil concentration on lipid oxidation in structured lipid- based oil-in-water emulsions. Food Chem. 84, 451-456.
Richards M.P., Dettmann M.A., 2003. Comparative analysis of different homoglobins: autooxidation, reaction with peroxide and lipid oxidation. J. Agric. Food Chem. 51, 3886-3891.
Robinson D.S., Wu Z., Domoney C., Casey R., 1995. Lipoxygenases and the quality of foods. Food Chem. 54, 33-43.
Saiga Ai, Tanabe S., Nishimura T., 2003. Antioxidant activity of peptides obtained from porcine myofibryllar proteins by protease treatment. J. Agric. Food Chem. 51, 3661-3667.
Saka Y., Mori T., Matsumura Y., 2000. Reaction of soybean lipoxygenase-3 in emulsions as affected by emulsifiers, salts and phospholipids. Coll. Surf. B: Biointerfaces, 19, 187-196.
Schwarz K., Huang S.W., German J.B., Tiersch B., Hertmann J., Frankel N., 2000. Activities of antioxidants are affected by colloidal properties of oil-in-water and water-in-oil emulsions and bulk oils. J. Agric. Food Chem. 48, 4874-4882.
Subagio A., Morita N., 2001. Instability of carotenoids in a reason for their promotion on lipid oxidation. Food Res. Int. 34, 183-188.

Whitaker J.R., Robinson D.S., Eskin N.A.M., 1991. Lipoxygenases. Oxidative enzymes in food. Elsevier London, 176-207.

Webb M.F., Naeem H.A., Schmidt K.A., 2002. Food protein functionality in a liquid system: a comparison of deaminated wheat protein with dairy and soy proteins. Food Chem. Toxicol. 67, 2896-2902.

Wołosiak R., Worobiej E., 1999. Aktywność antyoksydacyjna izolatów i hydrolizatów białek grochu [Antioxidant activity of peas protein isolate and hydrolysate]. Żywn. Nauka Technol. Jakość. Sup., 3, 105-111 [in Polish].

Zhang P., Omaye S.T., 2000. β-carotene and protein oxidation; effect of ascorbic acid and α--tocopherol. Toxicology 146, 37-47.

SYNERGIZM DZIAŁANIA KAZEINY I JEJ ENZYMATYCZNEGO HYDROLIZATU W POŁĄCZENIU Z KWASEM ASKORBINOWYM I β-KAROTENEM W MODELOWYCH UKŁADACH OKSYDACYJNYCH

Streszczenie. W pracy badano synergizm działania kazeiny i jej hydrolizatu enzymatycznego w połączeniu z kwasem askorbinowym i β-karotenem w hamowaniu reakcji utleniania kwasu linolowego w modelowych układach oksydacyjnych. Uzyskane wyniki badań wskazują, że efektywność antyoksydacyjna stosowanych przeciwutleniaczy zależy od rodzaju układu, w którym zachodzi reakcja utleniania składników lipidowych oraz od mechanizmu tych reakcji. Najlepsze aktywności przeciwutleniające stosowanych preparatów uzyskano dla układu emulsyjnego w reakcji autooksydacji kwasu linolowego, gdzie możliwe było współdziałanie przeciwutleniaczy o odmiennych właściwościach fizycznych i mechanizmach działania antyoksydacyjnego w różnych fazach emulsji. Najmniejszą efektywność hamowania utleniania zastosowane dodatki przeciwutleniaczy wykazały w reakcji katalizowanej lipooksygenazą.

Słowa kluczowe: emulsja, kazeina, kwas askorbinowy, β-karoten, autooksydacja, lipooksygenaza, utlenianie fotosensybilizowane

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