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ANTIOXIDANT EFFECT OF PROTEIN HYDROLYZATES IN A FREEZE-DRIED MODEL SYSTEM

INTRODUCTION

RECENT YEARS have seen the introduction of a veritable cornucopia of prepared and convenience foods for institutional feeding and for the retail consumer market. Many of these, especially the dehydrated foods, would become unacceptable very rapidly were it not for the addition of antioxidants.

Only a few antioxidant materials are approved for food use. The primary

antioxidants are phenolic substances, most of these synthetic. Their use is being questioned by consumers at a rapidly increasing rate. The removal of nordihydroguaiaretic acid (NDGA) from approval (Federal Register, 1968) may fore-token other delistings. This possibility should act to spur the search for antioxidant substance from natural sources, and particularly for nonphenolic materials.

Antioxidant activity in proteinaceous materials has been reported by many

workers. Bishov et al. (1960) Bishov and Henick (1961, 1962) observed stabilization by intact protein in dehydrated model systems. The effects of amino acids as antioxidants have been reported by Marcuse (1962), Bishov and Henick (1964) and Karel and Tannenbaum (1966). The latter workers found that the amino acids acted only to prolong the induction period of autoxidizing lipids, whereas phenolic antioxidants also reduced the rate of the rapid oxidation

Table 1—Gel fractionation of protein hydrolyzates

Fraction	Recovered %	
	AYP	HVP
On G-10	85.5	37.8
On G-15	6.0	26.3
Thru G-15	8.3	39.6
Total	99.8	99.7

Table 2—Antioxidant effects of protein hydrolyzates^a

Sample	Induction period, ^b hr	
	10% ^c	25% ^c
AYP-I	145	147
AYP-II	190	143
AYP-III	140	140
HVP-I	120	120
HVP-II	97	107
HVP-III	60	93
Control	24	24

^aFreeze-dried model systems containing 1g CMC, 1g tocopherol-free corn oil and protein hydrolyzate as indicated

^bTime to consume 50% of oxygen from air in headspace at 65.5°C

^cOil basis

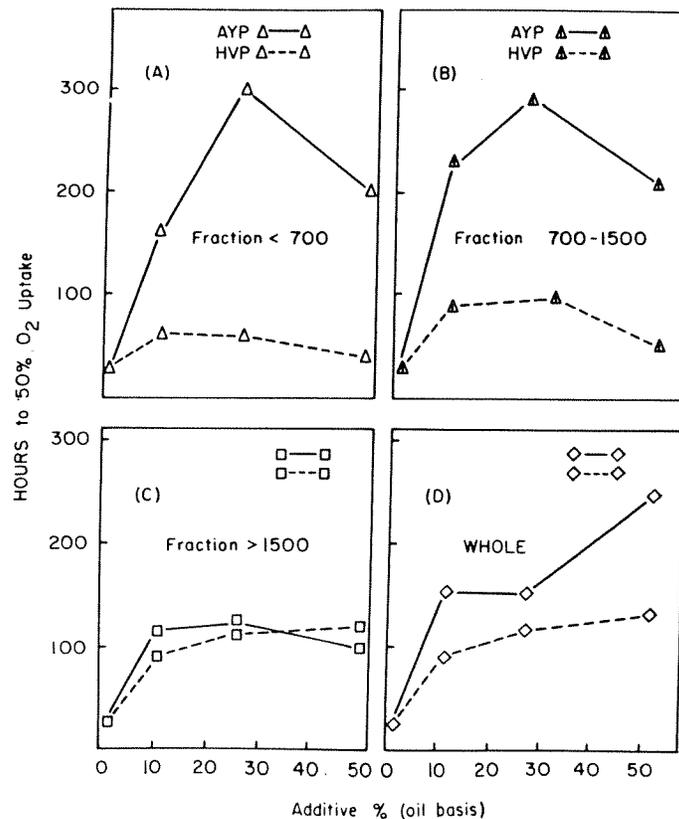


Fig. 1—Antioxidant activity of molecular sized fractions of AYP and HVP in freeze-dried emulsions.

Table 3—Antioxidant effects of nucleotides^a

Sample	Induction period, ^b hr	
	10% ^c	25% ^c
AYP	145	147
Nucleotide ^d	107	138
AMP	58	68
IMP	24	47
Control	24	24

^aFreeze-dried model system containing 1g CMC, 1g tocopherol-free corn oil and hydrolyzate fraction as indicated

^bTime to consume 50% oxygen from air in headspace at 65.5°C

^cOil basis

^dLaboratory separation from AYP

phase. A most interesting, but still unexplained, resistance to antioxidation was shown in linoleate salts of lysine and arginine synthesized by Chang and Linn (1964). This phenomenon was studied at length by Koch et al. (1971). In a study of factors affecting the acceptance and stability of a soup and gravy base, Bishov et al. (1967) observed an apparent antioxidant activity in the hydrolyzed vegetable protein (HVP) component.

These findings led directly to the present work which was undertaken to determine the effectiveness of protein hydrolyzates as antioxidants and to identify, if possible, the active agents. Because of their availability, autolyzed yeast proteins (AYP) and HVP were chosen for initial investigation.

EXPERIMENTAL

Materials

Commercial AYP and commercial HVP (Vico-Asmus Products Div., A.E. Staley Manufacturing Co. and Food Ingredients Division, The Nestle Co., Inc.) were used as received and after the fractionations described in "Methods." Carboxy-methyl-cellulose (CMC) (Hercules Manufacturing Co.), tocopherol-free corn oil and butyl hydroxy anisole (BHA) (Distillation Products Industries Div., Eastman Chemical Products, Inc.), adenosine monophosphate (AMP) and inosine monophosphate (IMP) (Nutritional Biochemical Corp.) and dextran gels (Sephadex G-10 and G-15) (Pharmacia Fine Chemicals A.B.) were used as received.

Methods

Fractionation of AYP and HVP by molecular size was accomplished in a batch procedure successively on Sephadex G-10 and G-15. Typically 100g of Sephadex G-10 was added, with stirring, to 100g of the hydrolyzate dissolved in 300 ml of water. After additional stirring the mixture was held overnight at 4°C and then filtered with suction. Fresh Sephadex G-10 (100g) was added to filtrate and the treatment was repeated for a total of four cycles. The final filtrate was treated in a similar manner with Sephadex G-15.

The final filtrate after treatment with both grades of Sephadex contained molecules larger than about 1500 molecular weight. The mate-

Table 4—Synergistic effects of protein hydrolyzates with phenolic antioxidants^a

% ^b	0		10		25		50	
	I.P. ^c hr	I.P. hr	Syn% ^d	I.P. hr	Syn%	I.P. hr	Syn%	
BHA	AYP% ^b							
0	39	131		140		200		
.005	59	221	38	267	47	326	37	
.010	64	255	46	305	53	370	74	
.020	98	307	44	447	61	498	52	
	Mean = 50.2							
<u>α-tocopherol</u>								
0	24	78		116		132		
.02	48	156	56	198	33	200	25	
.04	42	137	54	177	28	236	40	
.06	55	178	58	227	39	254	40	
.08	59	184	57	224	36	240	38	
	Mean = 42.0							
BHA	HVP% ^b							
0	14	90		123		158		
.005	42	189	40	275	48	373	52	
.010	96	253	34	333	40	440	47	
.020	116	386	52	458	53	495	49	
	Mean = 46.1							
<u>α-tocopherol</u>								
0	14	90		123		158		
.02	27	160	39	207	33	238	30	
.04	33	195	47	227	40	277	38	
.06	52	237	49	257	40	260	26	
.08	34	162	35	204	32	242	28	
	Mean = 36.4							

^aFreeze-dried model systems containing 1g CMC, 1g tocopherol-free corn oil and antioxidant materials as indicated

^bOil basis

^cTime to consume 50% of oxygen from air in headspace at 65.5°C; average of duplicate determinations

^dPercent of the observed effect due to synergism: $Syn \% = 100 [(M-C) - (P-C) - (A-C)] / (M-C)$ where M = I.P. of combined treatment; P = I.P. of phenolic antioxidant; A = I.P. of AYP; and C = I.P. of control

rial included by the gels was eluted, from each batch separately, by successive water washings with a total volume of 1100 ml/100g of gel. Final elution was with three 100 ml portions of 95% ethanol/100g of gel. Eluates and washings from each grade of gel were combined and the volume reduced to about 250 ml on a vacuum rotary evaporator. The eluate from G-10 contained molecules smaller than about 700 molecular weight and from G-15 those between about 700 and 1500. These solids were recovered by freeze drying.

A nucleotide-rich fraction was separated from AYP by an alcohol extraction, ion-exchange procedure described by Mabrouk (1972).

Model systems for oxidation studies were prepared individually by adding (in succession) to a small blender cup, 50 ml water, the antioxidant material (AYP, HVP, their fractions, BHA, tocopherol) as required (see Tables and Figures for amounts), 1g CMC and dropwise, with intermittent mixing, 1g of tocopherol-free corn oil. The entire mixture was blended for 2 min, transferred to a 250 ml round bottom flask, shell frozen and freeze dried (Bishov et al., 1960).

Oxidation of the model system was carried

out in the drying flask under an atmosphere of air at 65.5°C. The flasks were closed with rubber serum stoppers. Oxygen in the headspace was determined periodically by gas chromatography (Bishov and Henick, 1966). The end of the induction period was taken, arbitrarily, when 50% of the original oxygen had been consumed.

RESULTS & DISCUSSION

Fractionation of protein hydrolyzates

Typical recovery of fractions from the Sephadex separation of the hydrolyzates is shown in Table 1.

Antioxidant effects of protein hydrolyzates

A number of commercially-available hydrolyzates, both AYP and HVP, were screened for antioxidant effect using the freeze-dried model system. Results of a run are shown in Table 2. It can be seen that all samples show an effect and that in some the effect is concentration dependent. Sample AYP-II exhibits a bi-

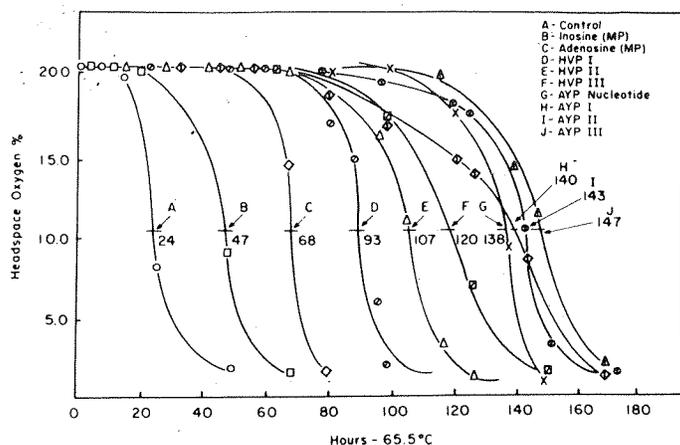


Fig. 2—Oxygen uptake by corn-oil, CMC freeze-dried emulsions with added protein hydrolyzate.

phasic behavior, decreased effect with increasing concentration, often seen in other antioxidants.

The molecular size (separated on Sephadex G-10 and G-15) fractions from one AYP and one HVP were similarly screened as seen in Figure 1. The smaller sizes, up to 1500, of AYP were more effective than the entire material.

The differences in the effectiveness of AYP and HVP are noticeable. It was thought that the difference might be due to the high salt content of HVP. Tests using salt-free HVP, however, showed that this was not the case. The milder conditions of autolysis compared to acid hydrolysis suggested that in AYP production the nucleotides might be better preserved. The effects of a nucleotide fraction isolated from AYP and of commercial AMP and IMP are seen in Table 3.

An autoxidation rate study is summarized in Figure 2. It can be seen that the sole effect of these experimental stabilizing agents is to prolong the induction period. No differences are noted,

except for one sample of AYP (Curve H, Fig. 2) in the rapid rate of autoxidation after the end of the induction period.

Synergism with phenolic antioxidants

The effects of AYP and of HVP in concert with BHA and with α -tocopherol are seen in Table 4. In this system BHA is a much more effective antioxidant than is α -tocopherol. Its effect is also much more concentration dependent. These hydrolyzates are synergistic with both of these phenolic antioxidants. The extent of the combined effect due to synergism is shown in Table 4, as is the method by which it was calculated. Although the values appear to vary widely and do not follow any consistent trend, the effect is significant, reproducible and above experimental error.

This synergism may be used to good advantage in foods. Alone, 10% AYP (oil basis) appears to be the equivalent of 0.02% (oil basis) of BHA. But when combined with only 0.005% of BHA, the

total effect is as great as that of 50% AYP or twice that of 0.02% BHA. Evaluation of these effects in typical foods is now underway and preliminary results appear to be promising.

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