3-ハイドロキシ-2-ピロンの食用油脂に対する抗酸化効果

誌名	日本大學農獣医學部學術研究報告
ISSN	00780839
著者	滝沢, 正興
巻/号	41号
掲載ページ	p. 1-8
発行年月	1984年3月

Antioxidant Effects of 3-Hydroxy-2-pyrone on Edible Oils and Fat*

Masaoki TAKIZAWA, Tsunemi UEMATSU, Hidetoshi SAKURAI and Kenji ISHII**

Accepted October 13, 1983

Introduction

A number of natural and synthetic compounds have been used as antioxidants in foods. Recently, the natural antioxidant, tocopherol is preferable to chemical antioxidants since it has no problems of toxicity. There is, therefore, a continuing need for new agents which will be very effective without undesirable side effects.

3-Hydroxy-2-pyrone(HP) is a degradation product of ascorbic acid[1] and dehydroascorbic acid[2~3] and has been found in orange juice[4] and orange powder[5]. HP has a reducing activity and is readily soluble in vegetable oils. These properties of HP led to the present work which was undertaken to determine the efficacy of HP as an antioxidant.

The derivatives of ascorbic acid have been studied for their antioxidant effects by many investigators. The antioxidant activity of ascorbyl palmitate (AP) has been known and used as an antioxidant or a synergist for antioxidants[6~8]. However, no papers on the antioxidant activity of HP have been published until recently. The purpose of this paper is to compare the antioxidant effect of HP with those of other agents such as AP, butylated hydroxyanisol (BHA) and δ-tocopherol using vegetable oils and lard.

Materials and Methods

1. Vegetable oils and lard

Peanuts and soybeans were crushed and extracted with *n*-hexane in the cold. The extraction was repeated three times. The extracts (miscella) were heated in a rotary evaporator at 40°C to remove the hexane and crude peanut and soybean oils were obtained. Lard was obtained from a commercial supplier.

2. Antioxidants

HP was prepared from mucic acid by WILEY's method[9] as described previously[10]. BHA and AP were purchased from Wako Chemicals and were of reagent grade. δ-Tocopherol was kindly provided by Eisai & Co.

3. Florisil column chromatography

Crude peanut and soybean oils were purified by Florisil (magnesium silicate) column chromatography as described by TERAO et al.[11] to remove tocopherols. No tocopherols were detected in the Florisil-treated oils by thin layer chromatography using the procedure of TSUGO et al.[12].

4. Stability tests for the oils and lard

(1) **Active oxygen method (AOM)** 20 g test samples, to which HP, BHA, AP or δ-tocopherol was added at 0.01~0.08% concentrations by weight, were put into test tubes (25×200 mm). The test tubes were heated in a boiling water bath (98±1°C) with aeration at a flow rate of 2.33 ml/sec. The oxidized oil was withdrawn at appropriate intervals to determine peroxide value (POV) by the conventional titration procedure with thiosulfate[13].

(2) **Ultraviolet light irradiation test** Peanut oil (2.0 g), to which HP, BHA or δ-tocopherol was added at 0.04% concentration by weight were put into 100 ml-beakers and the beakers were placed in an incubator maintained at 45°C and UV light was irradiated from a 20 cm-distance by a UV lamp (20 W, Toshiba IXFL-15) for 12 hours. POV of the

Bull. Coll. Agr. & Vet. Med., Nihon Univ., No. 41, p. 1~8 (1984).

** Lab. Food Chemistry, Coll. Agr. & Vet. Med., Nihon Univ.

* 3-ハイドロキシ-2-ピロンの食用油脂に対する抗酸化効果.

** 滝沢正興・植松恒美・桜井英敏・石井謙二 (食品化学研究室).

oxidized oil was determined as described above.

5. Measurement of browning of the oxidized oil

Crude peanut oil with or without addition of HP, AP, BHA or δ-tocopherol at 0.04% concentration by weight was heated under the same conditions of AOM for 24 hours. The degree of browning caused by heating with antioxidants was measured at 480 nm with a Shimadzu Multipurpose Spectrophotometer MPS-5000 and was expressed as absorbance.

Results and Discussion

Figs. 1 and 2 show the antioxidant effects of 0.01～0.08% concentrations of HP on crude peanut and soybean oils comparing with BHA at 0.02% (the legal limit). HP at 0.08% was more active than BHA at 0.02%, and HP at 0.04% was as active as BHA at 0.02%. Tests on lard are illustrated in Fig. 3, where HP was added at 0.02～0.08% and δ-tocopherol (0.02%) and BHA (0.02%) were used for references. HP at 0.08% was more active than BHA and δ-tocopherol at 0.02%. δ-Tocopherol was least effective. With no addition of the antioxidants, POV reached 30 (meq/kg) after 26 hours of heating at 98°C and it rapidly increased to 100 in another 10 hours. The time required to attain 30 of POV was 28, 29, 30 and 32 hours with δ-tocopherol (0.02%), BHA (0.02%), HP (0.04%) and HP (0.08%), respectively. In Figs. 1～3, the antioxidant effect of HP increased gradually as the HP concentration increased.

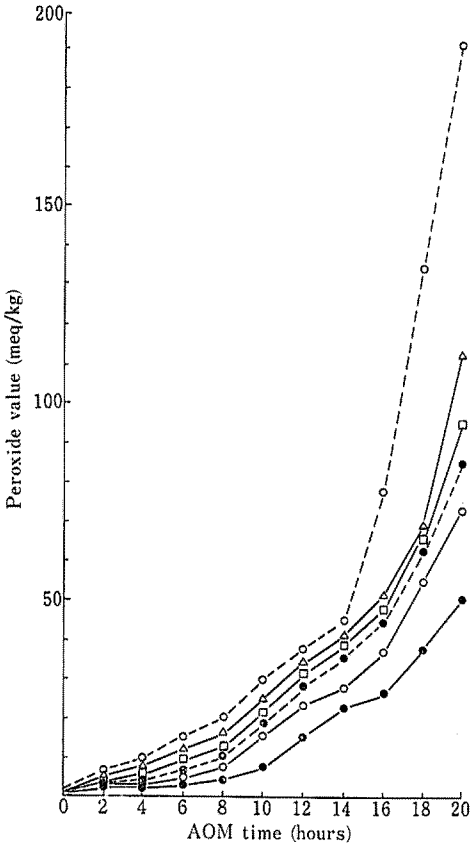


Fig. 1 Antioxidant effects of HP and BHA on crude peanut oil.

○---○ None △—△ HP(0.01%)
□—□ HP(0.02%) ○—○ HP(0.04%)
●—● HP(0.08%) ●---● BHA(0.02%)

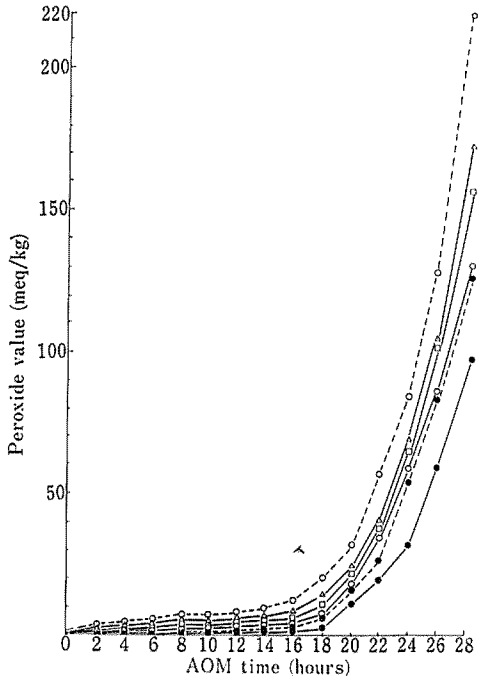


Fig. 2 Antioxidant effects of HP and BHA on crude soybean oil.

○---○ None △—△ HP (0.01%)
□—□ HP (0.02%) ○—○ HP (0.04%)
●—● HP (0.08%) ●---● BHA (0.02%)

Figs. 4～6 show the results in crude peanut and soybean oils and lard, to which HP, AP and δ-tocopherol were added at 0.04%. In Fig. 4, the POV increase in the presence of HP, AP and δ-tocopherol was slow in the first 12 hours of heating, but POV increased markedly thereafter excepting with AP. In Fig. 5, AP offered outstanding protection to peanut and soybean oils from autoxidation and HP was less effective than AP. POV in the oils with HP increased

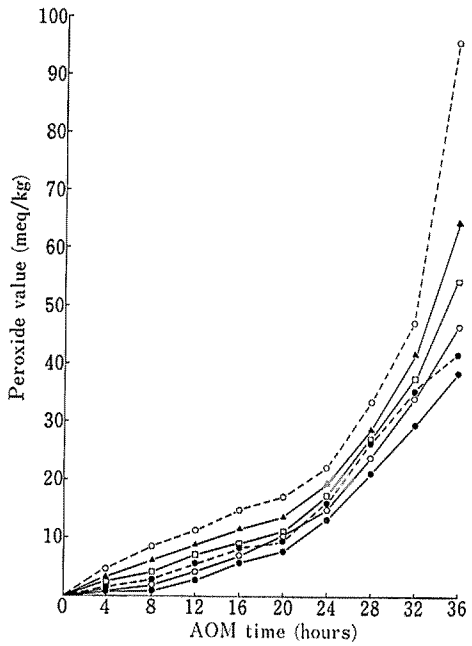


Fig. 3 Antioxidant effects of HP, BHA and δ-tocopherol on lard.
○---○ None　□—□ HP (0.02%)
○—○ HP(0.04%)　●—● HP(0.08%)
●---● BHA(0.02%)　▲—▲ δ-Toc(0.02%)

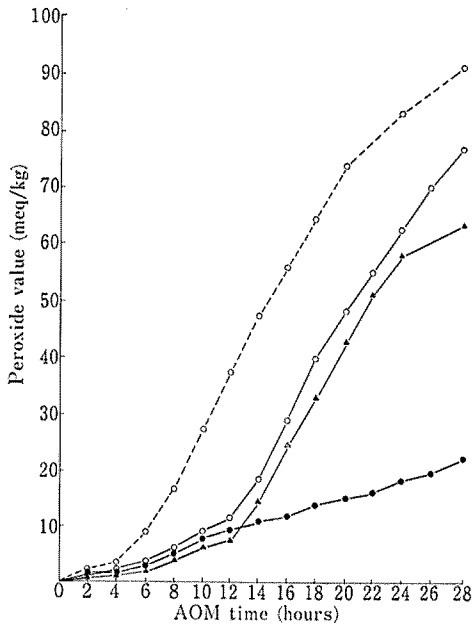


Fig. 4 Antioxidant effects of HP, AP and δ-tocopherol on crude peanut oil.
○---○ None　○—○ HP(0.04%)
●—● AP(0.04%)　▲—▲ δ-Toc(0.04%)

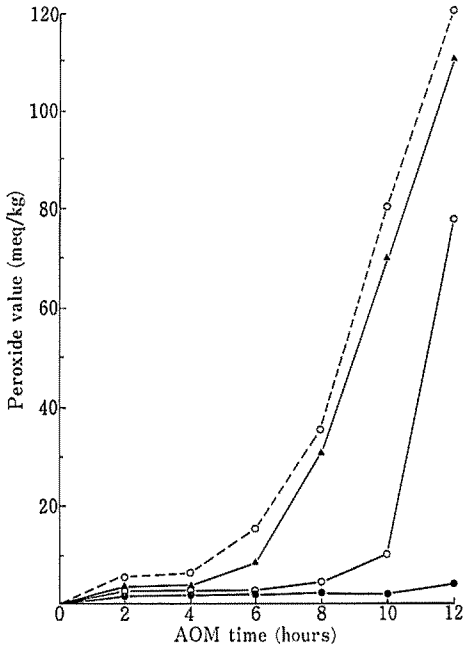


Fig. 5 Antioxidant effects of HP, AP and δ-tocopherol on crude soybean oil.
○---○ None　○—○ HP(0.04%)
●—● AP(0.04%)　▲—▲ δ-Toc(0.04%)

during the period of 10～12 hours of heating.

However, HP was more active than AP and δ-tocopherol in lard which contained no natural or added antioxidants (Fig. 6). AP and δ-tocopherol showed no antioxidant activity in the first 32 hours of heating. HP was shown to be effective in retarding oxidation of lard for at least 32 hours.

These findings suggest that naturally occurring tocopherols in the crude oils were synergized by AP. CORT[9] reported that when AP alone was added to animal fat, almost no antioxidant activity was observed, but AP accompanied with α- or δ-tocopherol showed a considerable antioxidant activity by synergism. KLÄUI[14] also noted that AP acts as a powerful synergist for tocopherols.

The results in Figs. 1～2 indicated that crude peanut and soybean oils even with no addition of antioxidants had resistance to autoxidation to some extent. The extent of prolongation of induction period was longer in soybean oil than in peanut oil. We believed that tocopherols naturally present were responsible for the crude oil's resistance to autoxidation and the difference in prolongation might be due to tocopherol content in the crude oils. Crude peanut

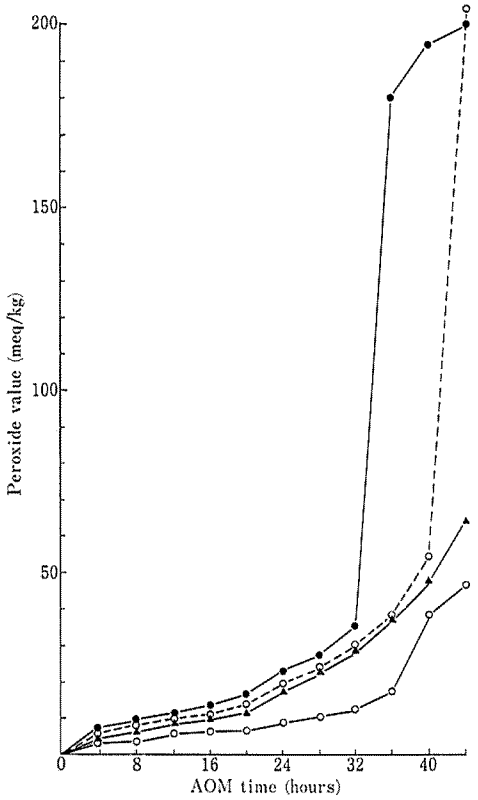


Fig. 6 Antioxidant effects of HP, AP and δ-tocopherol on lard.
○---○ None ○—○ HP(0.04%)
●—● AP(0.04%) ▲—▲ δ-Toc(0.04%)

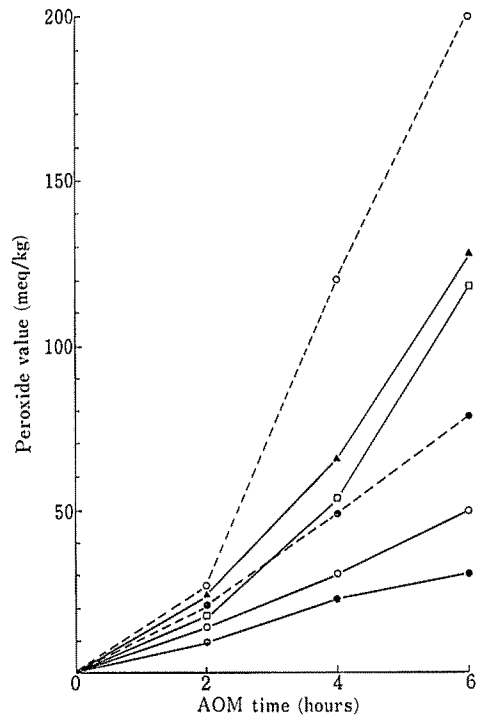


Fig. 7 Antioxidant effects of HP, BHA and δ-tocopherol on the Florisil-treated peanut oil.
○---○ None □—□ HP(0.02%)
○—○ HP(0.04%) ●—● HP(0.08%)
●---● BHA(0.02%) ▲—▲ δ-Toc(0.02%)

and soybean oils used in this work contained 15.7 and 75.2 mg% tocopherols, respectively. Consequently, these oils were purified by passage through a Florisil column to remove natural tocopherols.

Figs. 7 and 8 present the results in the Florisil-treated peanut and soybean oils with addition of 0.02 ~0.08% HP, 0.02% BHA and 0.02% δ-tocopherol. Autoxidation of the Florisil-treated oils proceeded very rapidly compared with that of the crude oils because of the removal of natural tocopherols. In the treated peanut oil (Fig. 7), POV attained 200 after 6 hours of heating (98°C) when no antioxidant was added. When HP was added at 0.04 or 0.08 %, POV did not increase to 50 after 6 hours of heating. HP at 0.08% was especially effective in retarding autoxidation of the purified peanut oil. Even at the 0.02% level of HP, there was some degree of protection in peanut oil. In Fig. 8, HP at 0.08% was most effective in protecting the purified soybean oil from autoxidation. BHA at 0.02% showed an antioxidant activity as strong as HP at 0.02 and 0.04%. δ-Tocopherol showed much less antioxidant effect on the purified soybean oil.

The antioxidant effect of HP, BHA and δ-tocopherol at 0.04% level on crude peanut oil exposed to UV light is shown in Fig. 9. The POV increase by UV irradiation was strongly inhibited with BHA but HP and δ-tocopherol offered much less protection to peanut oil, probably due to the susceptibility of both antioxidants to UV irradiation. From the results of Figs. 1 and 9, we found that HP was more susceptible to UV irradiation than to heat and, on the contrary, BHA was more susceptible to heat than to UV irradiation.

Strong heating of vegetable oils results in color development or browning as shown in frying and cooking oils. Effect of heating (98°C) on browning of peanut oil in the presence of HP, AP, BHA or

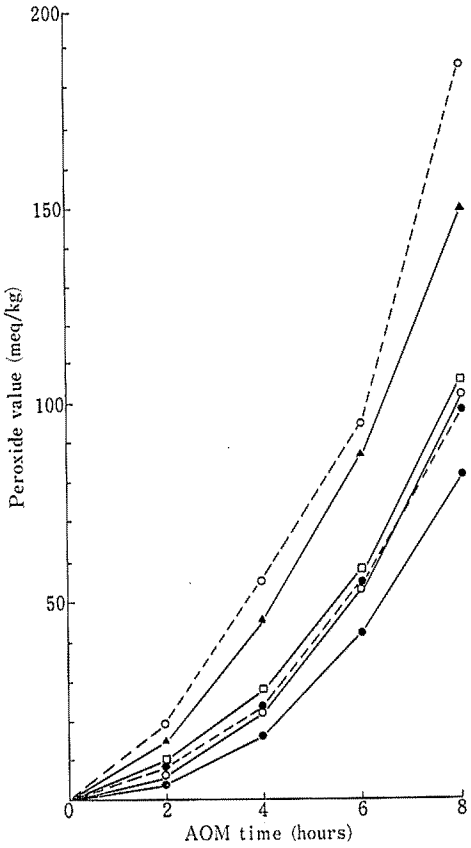


Fig. 8 Antioxidant effects of HP, BHA and δ-tocopherol on the Florisil-treated soybean oil.
○---○ None　□—□ HP(0.02%)
○—○ HP(0.04%)　●—● HP(0.08%)
●---● BHA(0.02%)　▲—▲ δ-Toc(0.02%)

Table 1 Browning of peanut oil in the presence or absence of antioxidants by heating

time (hrs)	None	HP	AP	BHA	δ-tocopherol
0	0.089	0.090	0.090	0.089	0.074
2	0.133	0.135	0.272	0.124	0.126
4	0.117	0.128	0.329	0.106	0.067
8	0.138	0.155	0.400	0.120	0.125
12	0.145	0.160	0.388	0.128	0.131
16	0.168	0.163	0.392	0.126	0.154
20	0.239	0.191	0.377	0.170	0.225
24	0.321	0.282	0.365	0.220	0.321

Peanut oil was heated under the same conditions of AOM. The antioxidants were added to peanut oil at 0.04% by weight. Browning was expressed as absorbance at 480 nm.

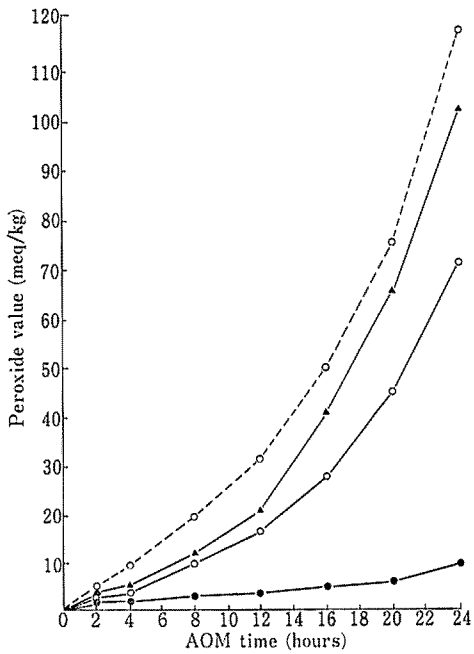
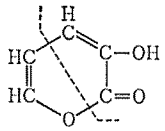


Fig. 9 Antioxidant effects of HP, BHA and δ-tocopherol on peanut oil exposed to UV irradiation.
○---○ None　○—○ HP(0.04%)
●—● BHA(0.04%)　▲—▲ δ-Toc(0.04%)

δ-tocopherol was investigated (Table 1). Browning occurred immediately in the first 2 hours when AP was present in peanut oil and proceeded until 8 hours of heating, but no further browning was observed thereafter. The intensities of browning in the oil alone and with HP, δ-tocopherol and BHA were similar in the first 12 hours of heating. Generally, AP gave a strong color development to peanut oil; browning was enhanced by interaction between the ascorbic acid moiety and carbonyls formed by autoxidation.

In determining the structure-activity relationship for HP, the works of RICHARDSON et al.[15] and HEIMANN[16] were very useful for us. RICHARDSON et al. reported the relationship between the chemical structure of flavonoids and their antioxidant activity and proposed that the antioxidant activity was due to the structure of -CO—C=C- in pyrone ring or open chalcone structure. HEIMANN also pointed out in studying flavonols that an enolic OH group in α, β-unsaturated ketone system is necessary for antioxidant activity and he mentioned the structure of



3-hydroxy-2-pyrone

kojic acid as an example. From these works, the antioxidant activity of HP is considered to be due to its pyrone ring structure. On the other hand, the antioxidant activity of AP depends on the 2 position in the ascorbic acid moiety[6].

In conclusion, HP (0.08%) was superior to BHA (0.02%) and δ-tocopherol (0.04%) and to AP (0.04%) for protecting peanut and soybean oils, and lard from autoxidation, respectively. But HP was susceptible to UV irradiation.

Summary

Evaluation of 3-hydroxy-2-pyrone (HP) as an antioxidant was carried out by comparing with ascorbyl palmitate (AP), butylated hydroxyanisol (BHA) and δ-tocopherol.

The addition of HP at 0.08% by weight to crude peanut and soybean oils and lard stabilized them from autoxidation. HP at 0.08% was more active than BHA at 0.02%. AP at 0.04% offered outstanding protection to crude peanut and soybean oils from autoxidation compared with HP and δ-tocopherol at 0.04%. In these cases, AP was considered to act as a synergist for natural tocopherols.

HP at 0.04～0.08% was very active in lard compared with AP and δ-tocopherol at 0.04% and BHA at 0.02%.

In Florisil-treated peanut and soybean oils which contained no appreciable amount of tocopherols, HP at 0.08% was more effective in retarding autoxidation of the purified oils than BHA and δ-tocopherol at 0.02%. HP at 0.04% was as active as BHA and δ-tocopherol at 0.02%.

HP (0.04%) as well as δ-tocopherol (0.04%) in peanut oil was susceptible to UV irradiation, whereas BHA (0.04%) was very stable and offered antioxidant effect on peanut oil exposed to UV irradiation.

The antioxidant activity of HP is considered to be due to its pyrone ring structure.

This paper was presented at the annual meeting of the Agricultural Chemical Society of Japan held in Tokyo on April 1, 1982.

We thank Eisai & Co. for kindly providing δ-tocopherol.

References

1) Tatum, J.H., P.E. Shaw and R.E. Berry: (1969) Degradation products from ascorbic acid. *J. Agric. Food Chem.*, **17**, 38～40.

2) Kurata, T. and M. Fujimaki: (1976) Formation of 3-keto-4-deoxypentosone and 3-hydroxy-2-pyrone by the degradation of dehydroascorbic acid. *Agric. Biol. Chem.*, **40**, 1287～1291.

3) Velisek, J., J. Davidek, V. Kubelka, Z. Zelinkova and J. Pokorny: (1976) Volatile degradation products of dehydroascorbic acid. *Z. Lebensm. -Unters. Forsch.*, **162**, 285～290.

4) Tatum, J.H., S. Nagy and R.E. Berry: (1975) Degradation products formed in canned single-strength orange juice during storage. *J. Food Sci.*, **40**, 707～709.

5) Shaw, P.E., J.H. Tatum, J. Kew, C.J. Wagner, Jr. and R.E. Berry: (1970) Taste evaluation of nonenzymic browning compounds from orange powder and use of inhibitors. *J. Agric. Food Chem.*, **18**, 343～345.

6) Cort, W. M.: (1974) Antioxidant activity of tocopherols, ascorbyl palmitate and ascorbic acid and their mode of action. *J. Am. Oil Chem. Soc.*, **51**, 321～325.

7) Imai, T., N. Arai and C. Inagaki: (1974) Antioxidant activity of various ascorbyl fatty acid esters. *Kaseigaku Zasshi*, **25**, 345～349.

8) Fujii, Y.: (1977) Effect of ascorbyl monopalmitate on the autoxidation of oils and fats. *Yamaguchi Daigaku Kyoikugakubu Ronso*, **26**, 189～195.

9) Wiley, R.H. and C.H. Jarboe: (1956) 2-Pyrones. XIX. 3-Hydroxy-2-pyrone and 4-arylhydrazone-2,3-pyranones. *J. Am. Chem. Soc.*, **78**, 2398～2401.

10) Ishizuka, S., H. Sakurai and K. Ishii: (1978) Analysis of 3-hydroxy-2-pyrone by amino acid analyzer. *Nippon Nogeikagaku Kaishi*, **52**, 549～551.

11) Terao, J., R. Yamauchi, H. Murakami and S. Matsushita: (1980) $^1O_2$ Quenchers on photoxidation of oils. *J. Food Process. Preserv.*, **4**, 479～493.

12) Tsugo, T., K. Yamanouchi and C. Kanno: (1968) Isolation and determination of tocopherols in foods by thin layer chromatography. *Nippon Nogeikagaku Kaishi*, **42**, 367～377.

13) AOAC: (1980) Official Methods of Analysis of the Association of Official Analytical Chemists, Association of Official Analytical Chemists, Washington, D.C., U.S.A., **13** th ed., p. 440.

14) Kläui, H.: (1976) Tocopherol, carotene and ascorbyl palmitate. *Int. Flavours Food Addit.*, **7**, 165～171.

15) Richardson, G.A., M.S. El-Rafey and M.L. Long: (1947) Flavones and flavone derivatives as antioxidant. *J. Dairy Sci.*, **6**, 397～413.

16) Heimann, W. und F. Reiff: (1953) Zusammenhänge zwischen chemischer Konstitution und antioxygener Wirkung bei Flavonolen. *Fette, Seifen, Anstrichmittel*, **55**, 451～454.

3-ハイドロキシ-2-ピロンの食用油脂に対する抗酸化効果

滝沢正興・植松恒美・桜井英敏・石井謙二

（食品化学研究室）

（昭和58年10月13日受理）

3-ハイドロキシ-2-ピロン（HP）は，アスコルビン酸の分解産物であり，オレンジ果汁，オレンジ粉末から分離されている。

HP は還元力を有し，水，エタノール，エーテル，クロロホルムに溶け，植物油にも容易に溶解する。

このような事実から，HP の食用油脂に対する抗酸化効果を落花生油，大豆油およびラードを用いて，アスコルビン酸パルミテート（AP），ブチルハイドロキシアニソール（BHA）および δ-トコフエロールと比較した。

上記の油脂に HP, AP, BHA および δ-トコフエロールを0.01～0.08% レベルで添加し，AOM 法で加熱処理し，過酸化物価（POV）を経時的に測定して自動酸化の程度を判定した。

落花生および大豆各粗原油に対して，0.08% HP 添加は0.02% BHA 添加（法的規制基準）より強い酸化防止効果を示した。また，0.04% AP 添加は，HP および δ-トコフエロール各0.04%添加に比べ，落花生および大豆各粗原油に対し，著しい抗酸化効果を示した。これは AP がシネルギスト（相乗剤）として粗原油中の天然トコフエロールと相乗作用を示したためと思われる。

抗酸化剤を含まないラードに対しては，0.04～0.08% HP 添加は0.04%AP 添加，0.04% δ-トコフエロール添加および0.02% BHA 添加より遙かに酸化防止効果があった。

フロリシル（ケイ酸マグネシウム）処理で天然トコフエロールを除いた落花生油および大豆油に対して，0.08%HP 添加が BHA および δ-トコフエロールの各0.02%添加より抗酸化効果が強かった。

紫外線照射下の落花生油中では，0.04%添加した HP および δ-トコフエロールは不安定であったが，0.04%添加した BHA は安定で強い酸化防止効果を示した。

以上の結果から，0.08%添加した HP は0.04%添加の AP および δ-トコフエロール，および0.02% 添加の BHA より優れた酸化防止効果を示すことを認めた。

HP の抗酸化力はそのピロン環の -CO—C=C- 構造に由来するものと思われる。