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Effects of Phytic Acid Content, Storage Time and Temperature on Lipid Peroxidation in Muscle Foods

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근육식품에서 지방산화에 대한 피틴산, 저장기간 및 온도의 영향

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ABSTRACT — Phytic acid, making up 1~5% of the composition of many plant seeds and cereals, is known to form iron-chelates and inhibit lipid peroxidation. Thiobarbituric acid reactive substances (TBARS), as an indication of lipid peroxidation, were measured in beef round, chicken breast, pork loin, and halibut muscle after the meats were stored for 0, 1, 3, 5, and 7 days at various temperatures [frozen (~20°C), refrigerator (4°C), and room temperature (25°C)]. Phytic acid effectively inhibited lipid peroxidation in beef round, chicken breast, halibut, and pork loin muscle ($p<0.05$). The inhibitory effect of phytic acid was dependent on concentration, storage time, and temperature. At frozen temperature, the inhibitory effect of phytic acid was minimal, whereas at room temperature, the inhibitory effect of phytic acid was maximal, probably due to the variation of the control TBARS values. At the concentration of 10 mM, phytic acid completely inhibited lipid peroxidation in all the muscle foods by maintaining TBARS values close to the level of the controls, regardless of storage time or temperature ($p<0.05$). The rate of lipid peroxidation was the highest in beef round muscle, although they had a close TBARS value at 0 day. Addition of phytic acid to lipid-containing foods such as meats, fish meal pastes, and canned seafoods may prevent lipid peroxidation, resulting in improvement of the sensory quality of many foods and prolonged shelf-life.

Key words □ Phytic acid, Lipid peroxidation, Beef round, Chicken breast, Halibut, Pork loin

Lipid peroxidation has received a great deal of attention in the field of food science. Lipid peroxidation in meats has become an important problem in restructured and precooked food products.[1,2] The peroxidative deterioration of lipid can not only produce toxic compounds but also change flavor, color, texture and nutritive value of foods.[3] Oxidation of muscle lipids involves the peroxidation of polyunsaturated fatty acids which are located in the membranes of muscle foods.[4]

Transition metals, especially iron, catalyze the production of hydroxyl radicals which can participate in the initiation event of lipid peroxidation.[5,6] Moreover, iron can promote lipid peroxidation by catalyzing the production of lipid radicals such as peroxyl and alkoxyl radicals.5 Both free iron and low molecular weight iron compounds play an important role in these reactions. Also, heme iron can catalyze lipid peroxidation by several actions; 1) formation of hypervalent heme iron (ferryl ion) complexes capable of directly oxidizing lipids;[7] 2) hydroxyl radical formation by autoxidation and autoreduction of heme iron;[8] and 3) free iron release from hemeproteins.[9] Food processing, such as freezing, refri-

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geration, thawing, and cooking, may also increase free and lower molecular weight iron compounds which can then initiate lipid peroxidation.[1,10,11]

Phytic acid is a natural plant inositol hexaphosphate constituting 1~5% of most cereals, nuts, legumes, oil seeds, pollen, and spores.[12] Phytic acid strongly inhibit lipid peroxidation in several model systems such as linoleic acid micelles, phospholipid liposomes, beef pattie, and cooked beef rolls.[1,2,13,14] The antioxidant properties of phytic acid may result from its ability to chelate transition metals and/or to enhance oxidation of ferrous ions to ferric ions. Phytic acid stabilizes meat color by decreasing metmyoglobin formation and it also enhances water-holding capacity of meat thereby increasing cook yield.[1,2]

The purpose of this study was to investigate the antioxidant effect of phytic acid on lipid peroxidation in beef round, chicken breast, pork loin, and halibut, when they were stored at various temperatures for one week. In stored muscle foods, phytic acid may play an important role in the prevention of deterioration of muscle foods by protecting against lipid peroxidation.

Materials and Methods

Materials

Phytic acid, 2-thiobarbituric acid, tetraethoxypropane (TEP), and Ferrozine [3-(2-pyridyl)-5,6-bis(4-phenylsulfonic acid)-1,2,4-triazine], were purchased from Sigma Chemical Company (St. Louis, MO). Trichloroacetic acid (TCA) were obtained from EM Science (Cherry Hill, NJ). Hydrogen peroxide, nitric acid, ammonium acetate, ascorbic acid, and pyrophosphate were obtained from Mallinckrodt Inc. (Paris, KY).

Methods

Sample preparation and treatment—This study was designed to use three concentrations of sodium phytate (0.1, 1, and 10 mM); three different storage temperatures (freezer, refrigerator, and room); five different storage periods (0, 1, 3, 5, and 7) for four muscle foods (beef round, chicken breast, pork loin, halibut muscle). The muscle foods were purchased from a local market. Visible fat and connective tissues were removed, and the muscle foods were chopped into small pieces with a stainless steel knife. Total fat from the muscle foods was extracted with chloroform-methanol solution (1: 2) and total fat content was determined gravimetrically on aliquots of the extract of the solvent removal. Muscle food samples (315 g) were blended with 35 ml test solutions (final concentration: 0.1, 1, 10 mM sodium phytate) using a food processor (Braun Multipractic MC 100, Braun Co., Lynnfield, MA). Aliquots were transferred into petri dish plates and stored in a plastic zip-lock storage bag for 7 days at various temperature. Sample preparation was performed in a cold room (4°C).

Chemical analysis—TBARS assay was performed as described by Buege and Aust.[15] Samples (0.5 g) were directly taken into a polyethylene test tube with screw cap (13×100 mm). To prevent spurious lipid peroxidation during heating, 20 μl of 0.2% butylated hydroxytoluene and 2.5 m*l* of 0.375% TBA-15% TCA-0.25 N HCl stock solution was added into the test tube and mixed with a vortex. The mixture was heated for 10 min in a boiling water bath (95~100°C) to develop a pink color, cooled with tap water, and centrifuged at 2500×g for 30 min. The supernatant was measured spectrophotometrically at 532 nm using a UV 2100 U spectrophotometer (Shimadzu Co., Kyoto, Japan). TBARS were calculated from a standard curve of malondialdehyde, a breakdown product of TEP.

Total iron concentration was determined in wet-ashed samples by using the ferrozine method.[16] Each homogenized sample (0.5 m*l*) was digested with 3 m*l* of concentrated nitric acid and 0.2 m*l* of 30% hydrogen peroxide on a hot plate until it formed a white ash. The white ash was dissolved in 0.2 m*l* of 1.0 N HCl and diluted with 0.8 m*l* deionized water. One m*l* of 0.5% ascorbic acid was added and the mixture was vortexed. After 20 min, 1 m*l* of 10% ammonium acetate buffer and 1 m*l* of 1 mM ferrozine color reagent were added and the mixture was again vortexed. The mixture was allowed to stand at room temperature for 45 min before the absorbance of each sample was determined at 562 nm.

Tissue nonheme iron was extracted by adding 0.5 m*l* of 25% trichloroacetic acid and 0.5 m*l* of 4% pyrophosphate to 0.8 m*l* of 20% tissue homogenate in a boiling waterbath for 20 min.[17] The mixture was then centrifuged at 4,000×g for 5 min, and the supernatant was saved. The extraction step was done three times on the same sample. One ml of the combined supernatant was used for determining nonheme iron using the ferrozine method.

Statistical analysis—Data were analyzed using SAS program on duplicate samples with three replications.[18] The least significant difference procedure was used to

determine significant differences at $p \leq 0.05$ between means of treatment groups.

Results

Muscle tissue contains a considerable amount of iron bound to proteins. Myoglobin is the most abundant hemeprotein in muscle tissue. Total iron content of muscle foods used in this study were 22.4, 8.3, 5.7 and 9.4 μg/g wet weight for beef round, chicken breast, halibut, and pork loin, respectively. Nonheme iron content were 7.5, 4.6, 3.9, and 3.4 μg/g wet weight for beef round, chicken breast, halibut, and pork lion, respectively (Table 1). Beef and pork had a similar percentage of nonheme iron but they showed a significant difference in nonheme iron content ($p<0.01$).

Total fat for beef, chicken, halibut, and pork was 5.4, 1.5, 2.3, and 3.6 g/100 g, respectively (Table 1). Beef contained the highest total fat content, whereas chicken breast had the lowest total fat content. There was no significant difference in total fat content between chicken breast and halibut muscle ($p>0.05$).

At day 0, the basal TBARS value of beef muscle (2.6 nmole/g) was similar to those of halibut (2.5 nmole/g) and chicken breast muscle (2.6 nmole/g) (Fig. 1). Among muscle foods used in this study, pork loin muscle (1.7 nmole/g) had the lowest TBARS value. There was no significant difference among phytic acid-treated groups in each muscle food ($p>0.05$).

In all the muscle foods, an increase in lipid peroxidation was strongly related to storage temperature and storage time at refrigerator or room temperature (Fig. 2~5). At freezing temperature, the increase in lipid peroxidation was minimal for all the muscle foods. The rate of lipid peroxidation in muscle foods followed the descending order: beef>halibut>chicken>pork. Beef round muscle was the most vulnerable to lipid peroxidation among muscle foods used in this study. When the beef muscle was stored upto 7 days either in a refrigerator (4°C) or at room temperature (25°C), TBARS formation was significantly increased ($p<0.01$). When the beef was stored at frozen temperature (-20°C) for 7 days, however, the TBARS value of the control was not significantly increased (Fig. 2). Phytic acid (0.1~10 mM) effectively and dose-dependently inhibited lipid peroxidation either

Table 1. Iron content in muscle foods

Meats	Total iron (μg/g)	Nonheme iron	Total fat (g/100 g)
Beef, round	22.4 ± 3.1^{a}	7.5 ± 0.5^{a}	5.4 ± 1.0^{a}
Chicken, breast	8.3 ± 1.5^{bc}	4.6 ± 0.5^{b}	1.6 ± 0.4^{b}
Fish (Halibut)	5.7 ± 1.3^{b}	3.9 ± 0.4^{bc}	2.3 ± 0.5^{b}
Pork, loin (lean)	9.4 ± 1.8^{c}	3.4 ± 0.7^{c}	3.6 ± 0.5^{c}

[abc] Means bearing the different superscripts within the same column are significantly different ($p<0.05$).

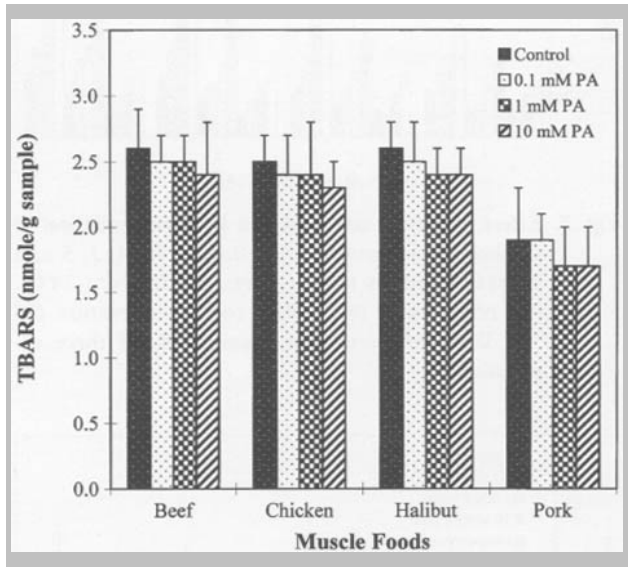

Fig. 1. Effect of phytic acid on lipid peroxidation in beef round, chicken breast, halibut and pork loin muscle at day 0. Data represents the mean ± S.E. of three replications. No significant difference between the treatment levels.

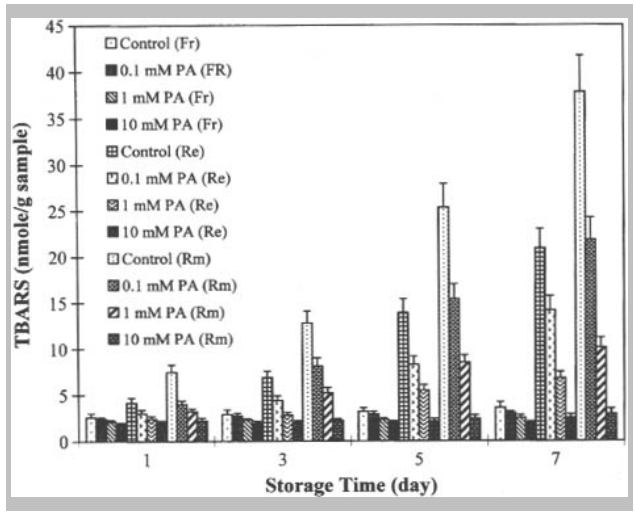

Fig. 2. Effect of phytic acid (PA) on lipid peroxidation in beef round muscle after storage for 1, 3, 5 and 7 days at various temperatures. Fr: freezer (-20°C), Re: refrigerator (4°C), Rm: room temperature (25°C). Data represents the mean ± S.E. of three replications.

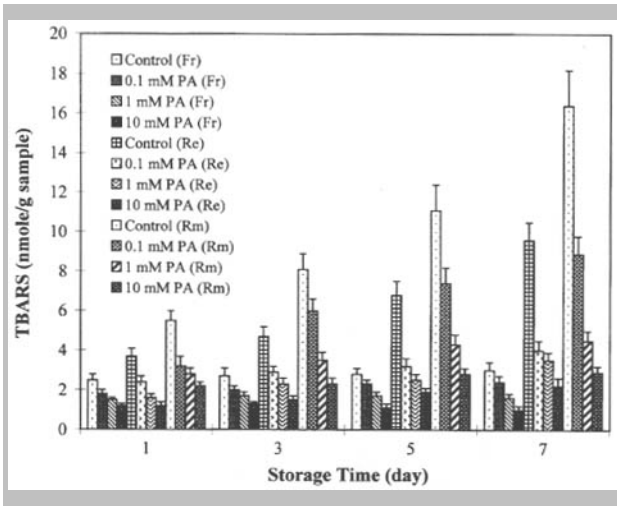

Fig. 3. Effect of phytic acid (PA) on lipid peroxidation in chicken breast muscle after storage for 1, 3, 5 and 7 days at various temperatures. Fr: freezer (-20°C), Re: refrigerator (4°C), Rm: room temperature (25 °C). Data represents the mean±S.E. of three replications.

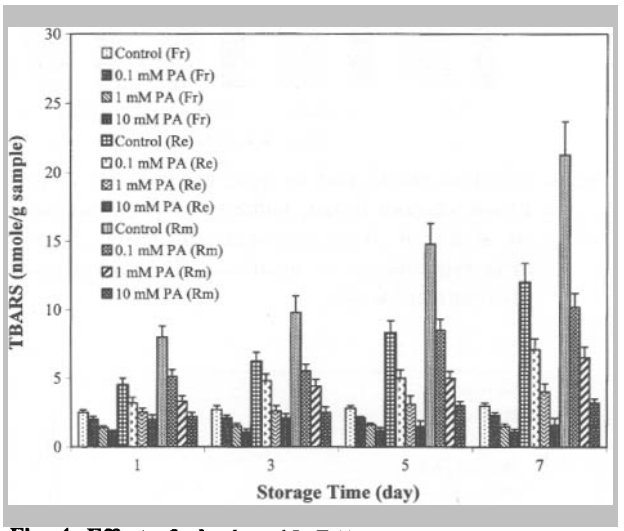

Fig. 4. Effect of phytic acid (PA) on lipid peroxidation in halibut after storage for 1, 3, 5 and 7 days at various temperatures. Fr: freezer (-20°C), Re: refrigerator (4°C), Rm: room temperature (25°C). Data represents the mean±S.E. of three replications.

in a refrigerator or at room temperature ($p<0.01$). After the beef was stored for 1, 3, 5, and 7 days at room temperature, the control TBARS value increased by 3-, 5-, 10-, and 15-fold, respectively, compared with that at day 0. Phytic acid (10 mM) completely inhibited lipid peroxidation by maintaining TBARS value to the initial TBARS value at day 0 (Fig. 2). The antioxidant effect of 10 mM phytic acid in beef muscle was very strong,

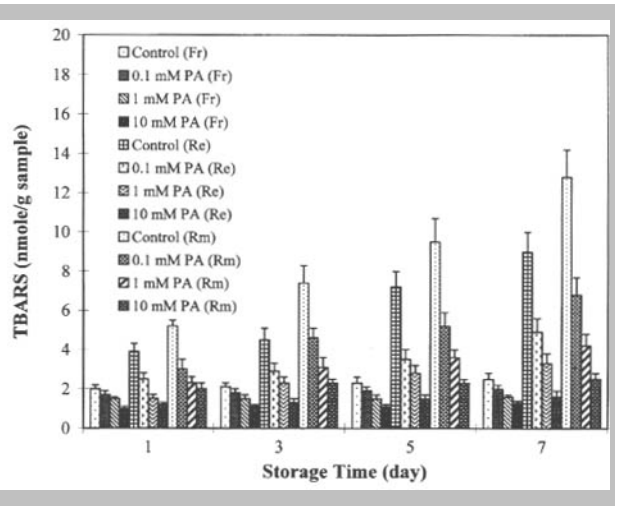

Fig. 5. Effect of phytic acid (PA) on lipid peroxidation in pork loin after storage for 1, 3, 5 and 7 days at various temperatures. Fr: freezer (-20°C), Re: refrigerator (4°C), Rm: room temperature (25°C). Data represents the mean±S.E. of three replications.

regardless of storage temperature and storage time.

As shown in beef muscle, the control TBARS value of chicken muscle significantly increased when it was stored upto 7 days either in a refrigerator or at room temperature ($p<0.01$). Phytic acid effectively inhibited lipid peroxidation of chicken muscle in a dose-dependent manner (Fig. 3). The TBARS value of chicken breast muscle at frozen temperature was not significantly increased with increasing storage time up to 7 days.

Lipid peroxidation in halibut muscle was also increased with increasing storage temperature and storage time (Fig. 4). Phytic acid (0.1~10 mM) significantly inhibited lipid peroxidation when the halibut muscle was stored in a refrigerator or at room temperature for 3~7 days ($p<0.05$). Similar to beef or chicken muscle, lipid peroxidation of halibut muscle at frozen temperature was not stimulated with increasing storage time. However, 10 mM phytic acid significantly inhibited TBARS formation frm halibut muscle regardless of storage temperature or time ($p<0.01$).

Lipid peroxidation rate in pork muscle was much slower than the other muscle foods (Fig. 5). However, phytic acid (0.1~10 mM) significantly inhibited lipid peroxidation of pork muscle, when the pork was stored at refrigerating or room temperature for 3~7 days ($p<0.01$).

Discussion

Meats contain high amounts of polyunsaturated fatty acid and transition metals which are strongly associated

with lipid peroxidation. Several processes, such as refrigeration and cooking, may increase degradation of heme compounds increasing free and lower molecular weight iron compounds.[10,11] Via the Fenton reaction, the free iron or low molecular weight iron compounds can catalyze the generation of hydroxyl radicals which can initiate lipid peroxidation. In addition, iron can propagate lipid peroxidation via metal-catalyzed decomposition of lipid peroxides into peroxyl and alkoxyl radicals.[5] Hemeproteins also play an important role in lipid peroxidation. The interaction of hydrogen peroxide with hemeproteins such as myoglobin, hemoglobin, and cytochrome releases free iron.9 Moreover, the heme moiety, both free and protein bound, not only initiates lipid peroxidation by producing reactive oxygen species but also enhances the rate of lipid peroxidation by decomposing the preformed lipid peroxides.[7,19]

Beef muscle was more vulnerable to lipid peroxidation than chicken, halibut, and pork muscle in this study. This might be due to a higher iron content; both nonheme and heme iron can catalyze lipid peroxidation. The especially high heme iron content in beef might be responsible for the high TBARS formation. Our laboratory showed that hydrogen peroxide-activated myoglobin effectively catalyzed lipid peroxidation in linoleic micelles and the hemeprotein-catalyzed system was stronger in catalytic activity on lipid peroxidation than nonheme iron-catalyzed systems.[14] Moreover, beef muscle has a higher phospholipid content than pork and chicken breast muscle.[20] The phospholipid in muscle membrane may provide a more ideal substrate for lipid peroxidation. Iron bound to negatively charged phospholipids promotes lipid peroxidation and consequently generates warmed-over flavor.[21] We have showed that phytic acid effectively inhibits iron- and hemeprotein-catalyzed lipid peroxidation in beef homogenate.[13]

Halibut (fish) is known to contain high amounts of polyunsaturated fatty acids, especially, n-3 fatty acids such as eicosapentaenoic acid and decosahexaenoic acid. These fatty acids are very vulnerable to lipid peroxidation since they have many carbonyl double bonds. Halibut also has a high P/S ratio (2.57) and high percent of nonheme iron (68%). These factors may contribute to the high TBARS value seen in halibut compared to chicken breast or pork loin. However, the TBARS value in halibut was much lower than beef muscle, which might be due to the lower iron content in halibut. Chicken breast muscle also has a high polyunsaturated/saturated fatty acid (P/S) ratio (0.85) compared with beef (0.15) or pork (0.25), but total fat (1.6%) is lowest among the muscle foods used in this study.20 In addition, chicken breast muscle has a high content of carnosine and anserine, dipeptides found in the skeletal muscle, which effectively inhibits lipid peroxidation.[22] Pork loin muscle has highest total fat but most of it is made up of saturated and monounsaturated fatty acids. Pork muscle has the lowest phospholipid content among the muscle foods used in this study.[20] Although the portion of nonheme iron to total iron is similar between pork and beef muscle, the total iron content in pork muscle is much lower than beef muscle. This difference may result in the lower TBARS value in pork loin muscle compared with beef round muscle.

The antioxidant effect of phytic acid may be due to chelation with transition metals such as iron and copper which can catalyze lipid peroxidation by several mechanisms. Phytic acid exhibits a high affinity for polyvalent cations and forms water-excluding chelates.[23] When all six coordination sites in Fe (III)-phytate chelate are occupied, the chelate cannot participate in the Fenton reaction. This explains how phytic acid inhibits generation of hydroxyl radical and subsequent lipid peroxidation.[23] The antioxidant effect of phytic acid is also partly due to its ferroxidase-like activity.[24] Phytic acid accelerates oxidation of Fe (II) to Fe (III) but does not affect reduction of Fe (III). The shift of the ratio of Fe (II)/Fe (III) might limit the lipid peroxidation in the muscle foods used in this study.[6]

The antioxidant or iron-chelating properties of phytic acid described above render this compound a unique and versatile food preservative. Therefore, phytic acid can be added to high lipid-containing foods to prevent both autoxidation and hydrolysis. Although phytate is known to inhibit nonheme iron absorption in the intestine of animals, it does not affect heme iron absorption. Moreover, red meat consumption is known to be positively associated with the risk factor of colon cancer, perhaps by increasing iron absorption and supplying substrates for lipid peroxidation. The use of phytic acid (or plant foods high in phytate) for meat processing or meat-containing diets may also be encouraged for the prevention of colon cancer.

국문요약

피틴산은 자연 항산화제로서 철분과 강력한 복합체를 형성하므로서 지방산화를 억제시킬 수 있다. 이 실험에서는 소고기, 닭고기, 돼지고기 및 생선을 사용하여 그 저장기간과 저장온도에 따른 피틴산의 항산화 효과를 관찰하고자 하였다. 세 종류의 저장온도(냉동, 냉장 및 상온)와 세 종류의 피틴산 농도가 사용되었으며, 피틴산으로 처리된 육류 및 생선이 7일간 저장되었다. 지방산화의 정도는 저장온도와 밀접한 관계를 보였다. 시료들이 냉동에서 보관되었을 때 지방산화가 잘 일어나지 않았으나, 냉장고나 상온에서 보관시 급격한 지방산화가 일어났다. 그러나 피틴산으로 처리된 식육 및 생선에서 현저히 낮은 지방산화를 보였다. 특히 10 mM 피틴산은 첨가는 모든 시료에서 저장온도 및 저장시간과 관계 없이 처음 시험을 시작할 때의 지방산화 수준으로 유의성 있게 억제시켰다. 사용된 육류 및 생선 중에서 소고기의 지방산화가 가장 빠르게 나타났으며 이것은 높은 철분 농도 및 세포막 인지질과 밀접한 관계에 기인된 것으로 사료된다. 이러한 결과로 부터 식육, 생선 및 캔류 식품 등에서 피틴산을 식품첨가제로서 사용하여 부패를 억제하며 저장 기간을 연장시킬 수 있을 것이다.

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