嘉南藥理科技大學專題研究計畫成果報告

陰極電解液特性及對脂質乳化液氧化安定性之作用

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計畫編號：90-FH-05

執行期間：90 年 1 月 1 日至 90 年 12 月 31 日

計畫主持人：梁哲豪

共同主持人：

計畫參與人員：

執行單位：食品衛生系

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Abstract

High pH, low dissolved-oxygen, and negative redox-potential values were demonstrated for cathodic solutions immediately after electrolysis. The effects of cathodic solutions on the oxidation of methyl linoleate were investigated in emulsion systems under different reaction conditions. When the reaction was incubated at 37℃ with a radical initiator (2,2′-azobis (2-amidinopropane) dihydrochloride), the oxidation of methyl linoleate was inhibited in cathodic NaCl solutions. When methyl linoleate was incubated at the same temperature without catalysts, the oxidation was retarded in either cathodic water or cathodic NaCl solutions. The use of cathodic solutions as the aqueous phase of emulsions may inhibit the formation of fluorescent products resulting from the interaction of oxidizing methyl linoleate and lysine in the emulsion systems.

Key words: cathodic solution; fluorescence; linoleate; oxidation; redox potential

Materials and methods

1. Electrolysis of water and NaCl solutions

Distilled water and NaCl solutions (0.5, 1.0, and 2.0 mM) were electrolyzed with an electrolyzing device (AM-2; Alken Ind., Kochi, Japan). This device consists of a cathode and an anode separated by an ion-exchange diaphragm (Type PP-33). The solutions produced in the cathode compartment during electrolysis are termed cathodic solutions. The pH and redox-potential values of these solutions were measured using a pH meter (Basic model; Denver Instruments, Arvada, CO, USA) and a redox-potential meter (CG 840; Schott, Hofheim, Germany), respectively. The oxygen dissolved in the cathodic solution was measured at 30℃ with a dissolved-oxygen meter (Oxi 320; WTW, Weilheim, Germany).

2. Oxidation of methyl linoleate in cathodic solutions

Methyl linoleate was mixed homogeneously with Triton X-100. Aliquots of the this mixture were used for the preparation of methyl linoleate emulsions by adding distilled water, NaCl or cathodic solutions. Nine milliliters of each methyl linoleate emulsion was pipetted into a flat-bottomed glass bottle (30 mL, 2.6 cm i.d.) with a screw cap. When oxidation was induced using AAPH, 1 mL of this solution was added to each of the emulsions. For oxidation in the presence of lysine, 1 mL of a lysine solution was added to each of the bottles. The final concentrations of methyl linoleate, Triton X-100, AAPH, and lysine were 1.0 mM, 0.1% (w/v), 0.25 mM, and 2.0 mM, respectively. The reaction mixture was incubated in the dark at 37 ℃. At regular intervals, the samples were withdrawn and subjected to assays for peroxide value, conjugated diene, and fluorescence.

3. Peroxide value

Peroxide values were determined using the ferric thiocyanate method of Mitsuda et al., (1966). Each 0.1 mL of sample solution was mixed with 0.1 mL 30% ammonium thiocyanate and 0.1 mL ferrous chloride (0.02 M in 3.5% HCl), and then diluted with 4.7 mL 75% ethanol. The absorbance at 500 nm was determined with a Hitachi U-2000 spectrophotometer (Hitachi, Tokyo, Japan).

4. Conjugate dienes measurement

A 0.1 mL aliquot of the sample solution was diluted with 4.9 mL of distilled water. The absorbance of the mixture was determined at 235 nm.

5. Transmission-fluorescence measurement

Each 0.5 mL of sample solution was mixed with 0.5 mL of 0.1 M phosphate buffer (pH 6.5), and then diluted with 4.0 mL distilled water. The fluorescence of the solution was determined by a Hitachi F-2000 spectrofluorometer (Hitachi, Tokyo, Japan). The excitation spectra of the mixtures were scanned from 220 to 400 nm with the emission wavelength fixed at 430 nm. The emission spectra were scanned from 400 to 600 nm with the excitation wavelength fixed at 340 nm. The fluorescence intensity was determined at an excitation wavelength of 340 nm and an emission wavelength of 430 nm. The fluorescence was measured under the following conditions: scan speed, 240 nm/min; response, 0.5 sec; bandpass, 20 nm; photomultiplier voltage, 700 V. The fluorescence intensity was expressed as a ratio, relative to a standard solution of 0.01 ppm quinine sulfate in 0.1 N H_2SO_4 (Gillespie, 1985).

Results and discussion

Fig. 1A illustrates the change in pH values for cathodic solutions during electrolysis of distilled water and NaCl solutions. Electrolysis of these solutions produces alkaline conditions at the cathode. The increase in pH values for cathodic NaCl solutions was greater than for cathodic water. In addition, pH values were increased as concentrations of NaCl increased, which suggests that electrolysis was more pronounced in solutions where electrolyte concentrations were higher. The pH values remained the same for the cathodic solutions throughout two weeks of storage at 37℃, demonstrating that electrolysis of water or NaCl solution can produce alkaline solutions at the cathode with relatively stable pH values.

Electrolysis of water or NaCl solutions results in decreased redox potential for cathodic solutions (Fig. 1B). A negative redox-potential value means that the solution at the cathode can serve as a reducing agent, however, the redox-potential value of the cathodic solution has a tendency to increase during storage. For our samples, this value increased and stabilized to about 50-100 mV after being incubated for five days at 37℃, remaining lower than before electrolysis (about 300 mV). For example, electrolysis of 2.0 mM NaCl solution for 30 min resulted in a remarkable change in redox potential from 302 (± 35) mV to −852 (± 3) mV. This value increased again to around 72 (± 5) mV after five days of storage at 37℃. Fig. 2 depicts the relationship for redox-potential and dissolved-oxygen values for the cathodic solutions. The decreased redox-potential value during electrolysis is accompanied by a drop in the dissolved-oxygen value. Comparatively, changes in the redox-potential and dissolved-oxygen values were more pronounced during electrolysis of NaCl solutions than during electrolysis of distilled water. A more pronounced tendency to act as reducing agents was demonstrated by the cathodic solutions, with greater negative-redox potentials and lower dissolved-oxygen values noted. These cathodic solutions may be useful in the aqueous phase for preparing lipid emulsions, and may prove beneficial for improving the oxidative stability of lipids. During storage, however, both the dissolved-oxygen and redox-potential values increased to nearly the same levels as those demonstrated for the solutions before electrolysis.

The effect of cathodic solutions on AAPH-catalyzed oxidation of methyl linoleate was evaluated by determining the formation of conjugated dienes and hydroperoxides (Fig. 3). In the distilled-water control, the initial rate of conjugated dienes and hydroperoxide formation increased steeply to a maximum at 20 hrs and then decomposed gradually with time. No

significant difference was demonstrated for the oxidation rate in NaCl solution (2.0 mM) compared with that in distilled water. For the cathodic NaCl solutions, the initial rates for conjugated dienes and hydroperoxide formation were significantly lower than those for cathodic water. The antioxidative activity was more pronounced using cathodic NaCl solution containing higher concentrations of NaCl. These results demonstrate that the antioxidative activity of the cathodic NaCl solutions might have been a reflection of scavenging ability for free radicals. Shirahata et al., (1997) have demonstrated that cathodic NaCl solution (0.1 g/L) exhibited superoxide dismutase (SOD)-like activity. They attributed this activity to the amount of dissolved atomic hydrogen in the cathodic solution. It is plausible that the cathodic NaCl solutions, with their greater negative-redox potential, are capable of scavenging free radicals, subsequently resulting in the inhibition of methyl linoleate oxidation. Conversely, the cathodic water, being relatively lower in negative redox potential, exerts a lesser antioxidant effect on the AAPH-catalyzed oxidation of methyl linoleate.

In the absence of a catalyst, the effects of the cathodic solution on the oxidation of methyl linoleate-lysine were determined by measuring hydroperoxide and fluorescence formation. As presented in Fig. 4A, the rate of hydroperoxide formation for cathodic water was significantly lower than that for distilled water. The cathodic NaCl solution (2.0 mM) significantly inhibited hydroperoxide formation,while the NaCl solution exhibited no antioxidant activity for methyl linoleate. Further, the antioxidant activity of cathodic water was similar to that of the cathodic NaCl solution. These results demonstrate an antioxidant effect on methyl linoleate oxidation in the absence of catalysts for the cathodic water, but none for AAPH-catalyzed lipid oxidation. In the absence of catalysts, it is anticipated that the rate of oxidation mainly depends on the dissolved oxygen in the system. Hence, the cathodic water and cathodic NaCl solution, having lower dissolved-oxygen values, exhibit an antioxidant effect for methyl linoleate.

Many studies have suggested that oxidative degradation of hydroperoxides is necessary for formation of fluorescent substances through reaction with various amino compounds (Fukuzawa et al., 1985; Lio and Yoden, 1988a). In this study, methyl linoleate was oxidized in an aqueous solution containing lysine, in order to study the effect of cathodic solutions on fluorescence formation during this oxidation. In distilled water, the interaction of oxidizing methyl linoleate and lysine produced fluorescent substances, exhibiting an excitation wavelength at 340 nm and an emission wavelength at 430 nm. Lio and Yoden (1988b) have reported that the degradation products of methyl linoleate hydroperoxides, upon reaction with 1-aminopentane as a model amino compound, produce identical fluorescence spectra, exhibiting excitation and emission maxima at 340-366 nm and 408-442 nm, respectively. In the distilled-water control, and in NaCl solution (2.0 mM), the rate of fluorescence formation increased steeply during incubation at 37℃ (Fig. 4B). Conversely, fluorescence formation was inhibited in either cathodic water or cathodic NaCl solution. Hidalgo and Zamora (1993) have investigated the influence of pH on fluorescence development in a model system of oxidized lipid and lysine. Their results revealed a higher rate of fluorescence formation where pHs were in the range 9-12. By contrast, cathodic water and cathodic NaCl (2.0 mM) with pH values in the range 9-11, may inhibit the development of fluorescence. This may be due to the fact that cathodic water and cathodic NaCl solutions are capable of preventing the formation of hydroperoxides, subsequently resulting in the inhibition of fluorescence development.

Based on our results, we have determined that the

cathodic solutions could be used for preparing lipid emulsions, and for inhibiting the oxidation of methyl linoleate, however, this antioxidative effect depends on their redox-potential values, and the presence of catalysts. The use of cathodic solutions, in an aqueous phase, could inhibit the development of fluorescent compounds derived from the interaction of oxidizing lipids and lysine in the emulsion system.

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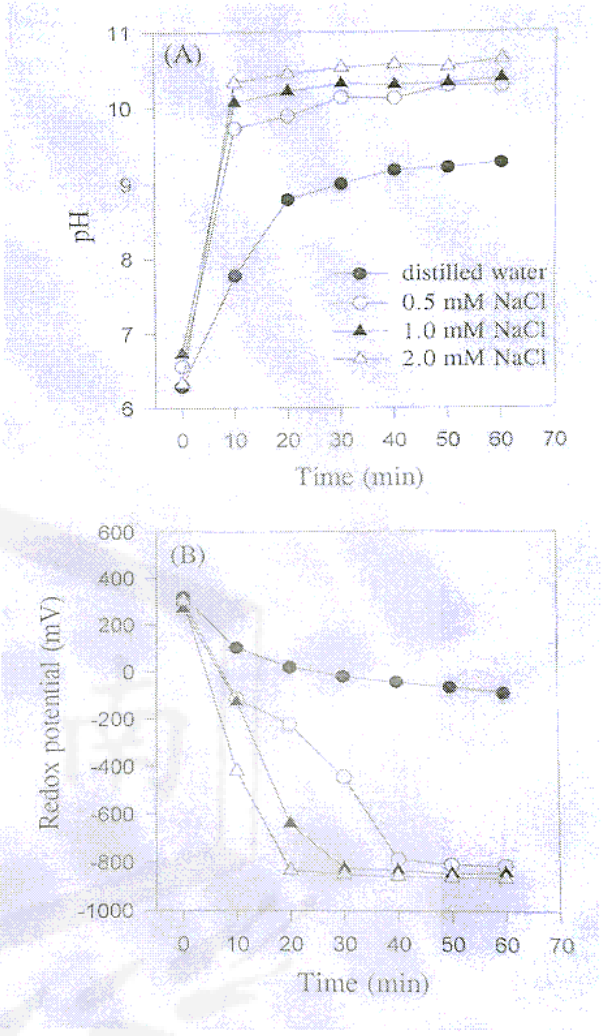

Fig. 1: (A) Changes in pH value for the cathodic solutions during electrolysis. (B) Decreases in redox-potential values for the cathodic solutions during electrolysis. Results represent the mean of three separate experiments.

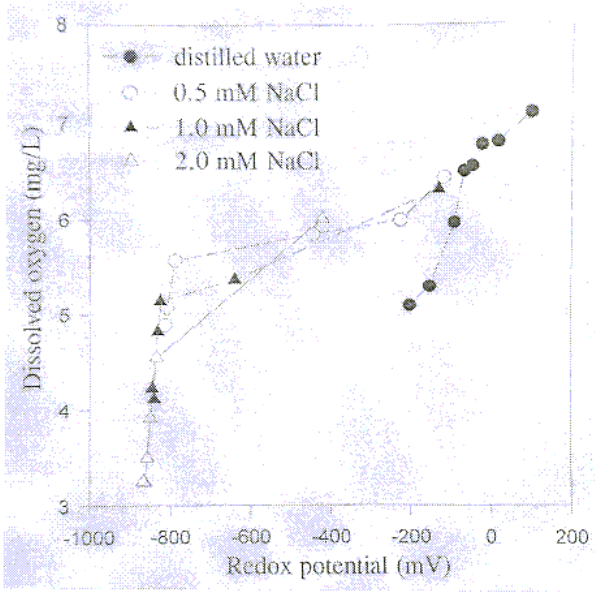

Fig. 2: Relationship of redox-potential values with dissolved-oxygen values in cathodic solutions during electrolysis. The values are the means of three separate experiments.

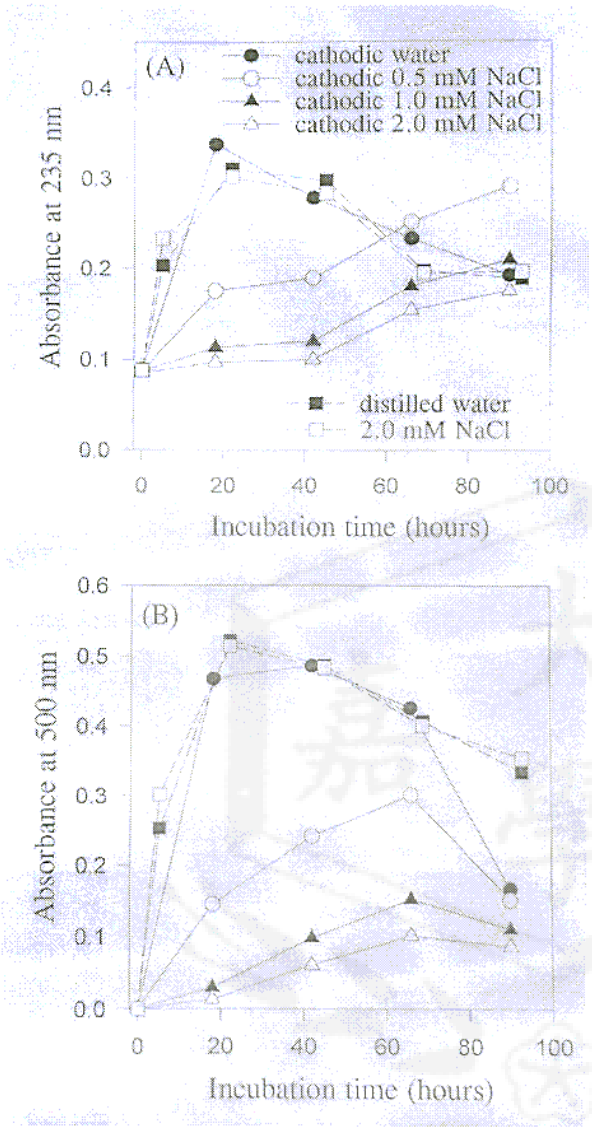

Fig. 3: Effect of cathodic solutions on oxidative stability of methyl linoleate emulsions in the presence of a radical initiator (AAPH) at 37 : (A) conjugated dienes formation; (B) hydroperoxides formation. The cathodic solutions were obtained at the cathode by 60 minutes of electrolysis. Results represent the mean of two separate experiments (triplicates for each experiment).

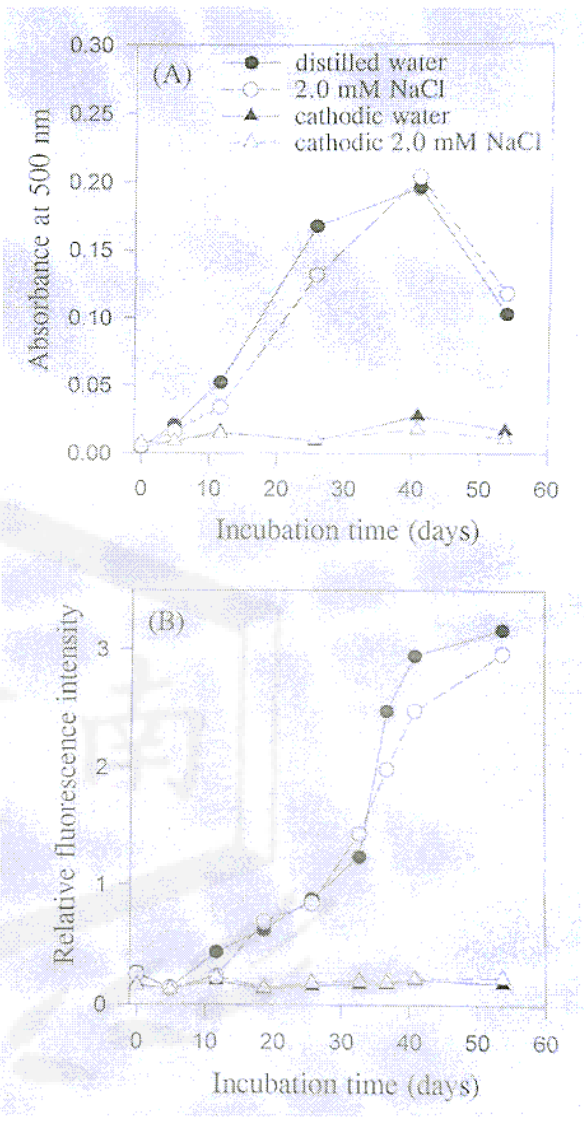

Fig. 4: Effect of cathodic solutions on oxidative stability of methyl linoleate-lysine emulsions at 37 without catalysts: (A) hydroperoxide formation; (B) fluorescence formation. The cathodic solutions were obtained at the cathode by 60 min of electrolysis. Results represent the mean of two separate experiments (triplicates for each experiment).