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学 位 論 文 題 名

Electrical Effects on Inactivation of Microorganisms during Ohmic Heating and Effect of Aluminium on Inactivation of *Escherichia coli*

(通電加熱における微生物の不活化に与える電気的影響および大腸菌の不活化に与えるアルミニウムの影響)

学位論文内容の要旨

Food safety has become an increasing concern of consumers. Pasteurization is a process used in the food industry for destroying pathogenic bacteria. Attempts have been made in many studies to apply ohmic heating to food pasteurization. The obvious advantage of ohmic heating over external heating is rapid internal heating that results in less heat damage and overcooking in food. Most of the previous studies on microbial inactivation suggested that the applied electricity under ohmic heating causes electroporation of cells membranes. However, the effects of electrical parameters on microbial inactivation produced by ohmic heating are not completely clarified. Therefore, the objectives of this study were to examine the effects of electrical frequency and current on inactivation of microorganisms as well as the electrical damage of bacteria under lethal and sub-lethal ohmic heating conditions. There has also been concern about the material of containers used in external heating, and this issue was also examined in the present study.

1. Effects of Electrical Frequency of Ohmic Heating on Heating Rate and Inactivation of Microorganisms

Samples of suspended *E. coli (ATCC® 12435™ and ATCC® 25922™)* and *K. oxytoca* in Tryptic soy broth (TSB) were treated by ohmic heating heating. An alternating current (AC) at 15-3 A with a frequency of 20, 10 or 1 kHz was applied during ohmic heating. For each frequency, samples were heated from 10°C to 63°C, held at 63°C for 6 min, and from 10°C to 65°C and held at 65°C for 5 min. Samples were collected every minute. Results showed that inactivation of *E. coli* and *K. oxytoca* increased as the frequency increased. Moreover, decimal reduction times (*D-values*) of samples treated at 20 kHz were lower than those of samples treated at 1 kHz. Considering that the electrical energy applied during high frequency was higher than that applied during low frequency, these results suggest that high electrical energy at high frequency results in the effective inactivation of microorganisms.

2. Electrical Effects on Death Kinetic Properties of Microorganisms during Ohmic Heating

The electrical effects of ohmic heating on microorganisms were studied in suspended *E. coli* and *K. oxytoca* cells in TSB. Samples were heated from 10°C to 70°C by setting a constant current at 15 A of 20 kHz, with an electric field range of 6.1-15.5 V/cm. Samples were collected at temperatures of 55°C, 60°C, 63°C, 65°C, 67°C, 69°C and 70°C. Results obtained by the ohmic heating showed obviously greater inactivation than that obtained by external heating, especially at high temperatures of 65°C, 67°C, 69°C and 70°C. However, there was no significant difference between the results obtained by ohmic heating and external heating at 55°C and 60°C. The electrical energy was 76.8 kJ with an average electric field of 8.6 V/cm. The results suggested that the electrical

energy to which the samples were exposed caused bacteria to become weak; however, an adequate temperature was required to injure bacteria during treatment. In conclusion, the combination of high electrical energy and high temperature is critical for microbial inactivation by ohmic heating.

3. Electrical Damage of *Escherichia coli* under Lethal and Sub-Lethal Treatment Conditions

Electrical damage of suspended *E. coli* in nutrient broth under lethal and sub-lethal ohmic heating conditions was investigated. Samples were heated under a lethal treatment condition from 10°C to 55°C and held at 55°C for 30 min by setting the electrical current at 18-2 A of 20 kHz. Sub-lethal treatment was performed by setting the electrical current at 10 A of 20 kHz. Samples were heated from 20°C to 40°C and cooled down to 20°C; the heating and cooling processes were repeated 10 times. Cells that survived after treatment were cultured in Tryptic soy agar (TSA: a nutrient-rich medium) and Desoxycholate (DESO: a selection medium containing an inhibitor). The percentage of bacterial damage was calculated and the results were compared with those obtained by external heating. The ratio of damaged cells of *E. coli* subjected to lethal ohmic heating was greater than that of *E. coli* subjected to lethal external heating, especially at the early stage of treatment (0-10 min). This result suggests that the damage to bacteria was caused by electrical heating. However, at a retention time of more than 15 min, there was no difference between the percentages of damaged cells under ohmic heating and external heating conditions. In the case of later retention time, the current of ohmic heating decreased and became lower than the current during the increasing phase and early stage of treatment. It can be concluded that damage to cells during this period was caused only by heating stress. The ratio of damaged cells under the sub-lethal ohmic heating condition was 92%. This percentage was much higher than that of cells subjected to external heating (32%). These results prove that damage to *E. coli* was caused by the current under the sub-lethal ohmic heating conditions.

4. Effect of Aluminium on Inactivation of *Escherichia coli* during Pasteurization of Milk

Suspended cells of *E. coli* in commercial milk were heated in aluminium and stainless-steel cups at 60°C, 63°C, 65°C and 67°C and held at those temperatures for 25, 5, 3 and 2 min, respectively. Results obtained under the temperature conditions of 65°C and 67°C clearly showed that cells of *E. coli* were killed more rapidly in aluminium cups than in stainless-steel cups. Furthermore, *D-values* in the aluminium cups were significantly shorter than those in stainless-steel cups, especially at higher temperatures. *D-values* in aluminium cups were lower when compared in stainless-steel cups at 60°C and 63°C, but there were no significant difference between two treatments. Considering the temperature-dependency factor in thermal inactivation kinetics (*z-values*), results obtained by using aluminium cups were slightly lower than those obtained by using stainless-steel cups. These results indicate that an aluminium utensil has an inactivating effect on *E. coli* during pasteurization and that the rate of inactivation increases as temperature increases.

5. Conclusion

The electrical frequency, electrical current and electrical energy to which a sample is exposed are critical parameters for inactivation and damage of bacteria. Concerning on the result has been confirmed that electrical energy given to samples caused bacteria become weak. However, a sufficient temperature was required for complete inactivation during treatment.

学位論文審査の要旨

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学位論文題名

Electrical Effects on Inactivation of Microorganisms during Ohmic Heating and Effect of Aluminium on Inactivation of *Escherichia coli*

(通電加熱における微生物の不活化に与える電気的影響および
大腸菌の不活化に与えるアルミニウムの影響)

本論文は，全7章からなる総頁数118の英文論文である。
論文には図61，表6，引用文献93が含まれ，別に参考論文1編が添えられている。

食品の安全は消費者にとって重要な問題である。殺菌は食品中の有害微生物を不活化する操作である。食品の殺菌法の一つに加熱殺菌があり，その加熱法に外部加熱や通電加熱（内部加熱の一種）がある。通電加熱は迅速で均一な加熱ができるとの利点があり，加えて熱による殺菌以外の非熱的殺菌効果の可能性もあるとされる。すなわち，既往の研究によれば，通電加熱は電気穿孔(electroporation)により細胞膜を損傷することで微生物の不活化を促すとの報告もある。しかしながら，通電加熱が微生物の不活化に与える電気的影響については不明確な点が多い。そこで本研究は通電加熱の周波数や電流が微生物に与える影響について検討し，また，外部加熱における加熱容器の材質（アルミニウム）が大腸菌に与える影響について明らかにしたものでる。
本研究の主要な結果を以下に述べる。

1．通電加熱の周波数が微生物の不活化に与える影響

Escherichia coli と *Klebsiella oxytoca* を対象菌として，通電周波数を1，10，20kHzとし，電流を3～15Aに制御して10 ℃から63 ℃への加熱と10 ℃から65 ℃への加熱をそれぞれおこなった。その結果，周波数が高くなると殺菌効果が大きくなった。*E. coli* の Decimal reduction times（*D-values*：菌数が1/10に減少するための時間）は1kHzより20kHzが短くなり，周波数が高いと殺菌効果が向上することが示された。

２．加熱殺菌における電気的影響

E. coli と *K. oxytoca* を対象菌として，周波数を 20kHz とし，電流を 15A に制御して 10 ℃から 70 ℃まで加熱し，55℃, 60℃, 63℃, 65℃, 67℃, 69℃ および 70℃の各温度で試料を採取し菌数を測定した。同一の温度履歴で外部加熱をおこない，同様に各温度で試料を採取し菌数を測定し，通電加熱と外部加熱との殺菌効果を比較した。その結果，同じ温度履歴の外部加熱と比較して，65℃から 70℃の温度範囲での通電加熱の殺菌効果が高かった。この結果は，電流の効果により菌の耐熱性が弱まり，熱による殺菌効果が高くなることを示唆していた。

３．通電加熱における損傷菌の挙動

損傷菌は，外部からのストレスにより半致死的状態となった菌である。*E. coli* の損傷菌は，tryptic soy agar（TSA: nutrient-rich な培地）と desoxycholate（DESO: inhibitor を含む培地）により判定することができる。

E. coli を対象菌として，周波数を 20kHz とし，電流を 10A に制御して，*E. coli* が熱殺菌されない温度範囲（20 から 40 ℃）で通電加熱と冷却を反復し，*E. coli* に与える電気効果として損傷菌を調べた。同一の温度履歴で外部加熱と冷却を反復し，損傷菌を調べた。その結果，通電加熱における損傷菌は 92%に達し，外部加熱（32%）より損傷菌が多かった。この結果は通電加熱による微生物への不活化の電気的効果を示していた。

４．アルミニウムが *Escherichia coli* の不活化に与える影響

E. coli を対象菌として，アルミニウム容器を用いた外部加熱で 10 から 60℃，10 から 63℃，10 から 65℃および 10 から 67 ℃への加熱をそれぞれおこない，菌数を測定した。同一の温度履歴でステンレス容器を用いて外部加熱を行い比較対照とした。その結果，アルミニウム容器の *D-values* がステンレス容器より短く，アルミニウムの殺菌効果が高いことが明らかとなった。この結果はアルミニウムに殺菌（抗菌）作用があることを示していた。

以上の結果は，加熱による熱的殺菌効果に加えて，通電加熱やアルミニウムに非熱的な殺菌効果があることを示唆するものであり，食品の有害微生物の不活化にこれらが活用できることを明らかにした。

よって，審査員一同は，Ruangthip Nareethep が博士（農学）の学位を受けるのに十分な資格を有するものと認めた。