ELSEVIER

Food Chemistry 80 (2003) 433–437

Food Chemistry

www.elsevier.com/locate/foodchem

Analytical, Nutritional and Clinical Methods Section

Evaluation of the 2-thiobarbituric acid method for the measurement of lipid oxidation in mechanically deboned gamma irradiated chicken meat

Heliana de Azevedo Gomes[a,b,*], Edir Nepomuceno da Silva[b], Marcos Roberto Lopes do Nascimento[a], Henrique Takuji Fukuma[a]

[a]*Poços de Caldas Laboratory, Brazilian Nuclear Energy Agency, Poços de Caldas, Minas Gerais State, 37701-970, PO Box 913, Brazil*
[b]*Department ot Food Technology, State University of Campinas, Campinas, São Paulo State, 13083-970, PO Box 6121, Brazil*

Received 27 May 2002; received in revised form 28 October 2002; accepted 28 October 2002

Abstract

This study evaluated the 2-thiobarbituric acid (TBA) method for the measurement of lipid oxidation in samples of mechanically deboned chicken meat (MDCM), both gamma-irradiated with doses of 4.0 and 5.0 kGy and non-irradiated. The values for the percent recuperation of 1,1,3,3-tetraethoxy-propane (TEP) and for the conversion factor K, for the samples irradiated with doses of 4.0 and 5.0 kGy and the non-irradiated samples, were, respectively, 88.6, 90.0 and 88.6% for the former and 4.98, 4.91 and 4.98 for K. Comparing the results obtained for the samples of MDCM irradiated with doses of 4.0 and 5.0 kGy and the non-irradiated samples, as evaluated by the TBA distillation method, it was observed that the values for percent recuperation of TEP, the values for K and the spectral curves, as also the linear equations of the standard curves for the distillation of malonaldehyde, were similar for all the samples, no specific interference in the TBA test, due to the process of irradiating the MDCM, being detected.

Keywords: Mechanically deboned chicken meat; 2-Thiobarbituric acid; Food irradiation; Lipid oxidation

1. Introduction

Lipid peroxidation is one of the major causes of quality deterioration in raw and cooked meat products during refrigerated or frozen storage. The 2-thiobarbituric acid (TBA) method is the most widely used test for measuring the extent of lipid peroxidation in red meat and poultry, due to its speed and simplicity (Raharjo & Sofos, 1993; Shahidi, 1997). The TBA test, when performed with the distillation procedure, is considered to be more sensitive and also more suitable for high fat samples (>10%) where turbidity may occur in the extracted samples (Férnandez, Pérez-Álvarez & Fernández-López, 1997; Shahidi & Hong, 1991). The 1,1,3,3-tetraethoxypropane has been used as the standard to assess recoveries in the TBA test (Calhoun, Gaebler, & Mandigo, 1999; Férnandez et al., 1997; Hoyland & Taylor, 1991; Lawlor, Sheehy, Kerry, Buckley, & Morrissey, 2000). The phospholipid fraction of mechanically deboned chicken meat (MDCM) is highly unsaturated, and thus very susceptible to oxidation, showing a great potential to produce 2-thiobarbituric acid reactive substances (TBARS) (Dawson & Gartner, 1983; Pikul & Kummerow, 1991). Autooxidation of lipids proceeds via a free-radical chain mechanism and is catalysed by many factors, such as ionizing radiations (Shahidi, 1997).

In France, the irradiation of frozen MDCM has been implemented on an industrial scale, with whole installations dedicated exclusively to this raw material. However, there is a lack of studies of microbiological quality and lipid oxidation during refrigerated and frozen storage. In addition to contributing to the evaluation of the viability of using irradiated MDCM in raw formulations, since the process could guarantee pathogen free raw material, such studies are of crucial importance to the safety of

* Corresponding author. Tel.: +55-21-35-37223266; fax: +55-21-35-37223622.
E-mail address: hgomes@cnen.gov.br (H. . A. Gomes).

0308-8146/03/$ - see front matter
PII: S0308-8146(02)00499-5

the industrial productive chain. They could forecast possible alterations in frozen stored irradiated MDCM, due to fluctuations in temperature as a result of failures during transport and/or storage, and in manufactured foods containing this raw material. In this context, the MDCM used in the present study was irradiated in the frozen state and, thereafter, stored frozen or under refrigeration to mimic the previous situations described.

Numerous studies have shown that gamma irradiation provokes alterations in the lipid components of fresh and frozen chicken meat (Gruiz & Kiss, 1987; Hansen, Chen, & Shieh, 1987; Kanatt, Paul, Souza, & Thomas, 1997; Lakritz & Schwartz, 1995). In addition, the process of irradiating meats has been associated with the production of TBA-reactive substances, not only malonaldehyde, at both 532 nm and other wavelengths (Janicek, Haseltine, & Henner, 1985; Raharjo & Sofos, 1993; Tarladgis, Watts, & Younathan, 1960).

Different types of sample and procedures for the determination of malonaldeyde recovery would also result in different values for the recovery and conversion factors used to calculate the TBA number. However, the same conversion factors obtained in previous studies by different workers have been used to calculate the TBA numbers. This could certainly lead to erroneous results (Raharjo & Sofos, 1993; Tarladgis et al., 1960). Thus this study aimed at evaluating the measurement of TBARS by the 2-thiobarbituric acid method in mechanically deboned gamma irradiated chicken meat.

2. Materials and methods

2.1. Experimental design

For the analyses, samples were taken from MDCM produced from the back, without skin, in a commercial chicken slaughterhouse. Each 200 g sample unit was removed from a 60 kg batch and heat sealed in a transparent low density polyethylene bag (Andrews & Read, 1984; Lee, Sebranek, Olson, & Dickson, 1996). The samples were then frozen in a tunnel to -18 ± 1 °C and maintained in this state until irradiated. The samples irradiated with doses of 4.0 and 5.0 kGy and the non-irradiated samples were thawed under refrigeration ($+2\pm1$ °C) for one night and evaluated by the TBA method for the measurement of lipid oxidation, aiming at determining the standard curves, absorption spectra, percent of recovery and K value. In addition, the K value was also determined for samples of MDCM irradiated with the dose of 4.0 kGy and non-irradiated samples stored under refrigeration ($+2\pm1$ °C) after 2 days frozen storage, stored refrigerated ($+2\pm1$ °C) after 90 days frozen storage and stored frozen (-18 ± 1 °C). The average proximate analysis for the meat samples was 66.6% moisture (Horwitz, 1980), 19.1% fat (Horwitz, 1980), 13.2% protein (Horwitz, 1980), and 1.0% ash (Horwitz, 1980). The proximate analyses were perfomed in triplicate.

2.2. Irradiation

The gamma radiation source was a ^{60}Co gammacell with an activity of 10,300 Ci, producing a dosage rate of 7.32 kGy/h. Routine dosimetry was conducted using Far West Technology dosimeters. The samples were placed in the uniform portion of the radiation field and arranged to minimize any differences in the radiation dose. The mean standard deviations of the absorbed doses from the target doses were ±0.28 and ±0.35 for the doses of 4.0 and 5.0 kGy, respectively.

2.3. TBA method analysis

The TBA distillation method was performed on the MDCM samples, using a modified version of the method of Tarladgis et al. (1960). The modification of this procedure was the addition of butylated hydroxytoluene before the blending step, to prevent autoxidation (Pikul, Leszczynski, & Kummerow, 1983). One hundred grammes of each MDCM sample were blended with 10 ml BHT (1g l^{-1}). Aliquots of MDCM (10 g) were transferred to a flat-bottomed flask and one drop of silicone anti-foaming agent (Merck, Germany) added plus 2.5 ml HCl (4N) and 97.5 ml distilled water. This sample was then distilled and the first 50 ml of distillate collected. Next 5 ml of the distillate were added to 5 ml 0.02 M thiobarbituric acid (Merck, Germany) and heated in a boiling water bath for 35 minutes for colour development. Malonaldehyde was distilled in triplicate from the MDCM and its absorbance was determined at 532 nm in a 1 cm glass cell. The results were reported as μg ml^{-1} of distilled malonaldehyde. The standard curves were prepared by making appropriate dilutions of the 1×10^{-4} mol l^{-1} 1,1,3,3-tetraethoxy-propane(TEP) (Sigma Corporation, USA) standard solutions, to give amounts ranging from 1×10^{-8} to 8×10^{-8} moles of malonaldehyde per 5 ml, using a UV/VIS-911A GBC spectrophotometer. Absorption spectra were prepared for samples in the range from 475 to 565 nm. The percent recovery of TEP was calculated from the standard as follows:

$$P(\%) = (A_3 - A_1)\cdot100/A_2$$

where P is the percent recovery, A_3 is the absorbance of the distilled sample containing TEP, A_1 is the absorbance of the sample, and A_2 is the absorbance of TEP. The K value was calculated from the standard curves and known dilutions as follows (Tarladgis et al., 1960):

$$K_{\text{distillation}} = S/A \times 72.063 \times 10^7/C \times 100/P$$

where S is the moles of the standard concentration of TEP in 5 ml of distillate, A is the absorbance of the standard, 72.063 is the molecular weight of malonaldehyde, C is the weight of the sample in grammes, and P is the percent recovery of TEP.

The equations for the standard curves were obtained from the linear regression of the absorbance results against the concentration of the standard.

2.4. Statistics

Each study was replicated three times. Statistical calculations were performed using the SAS statistical package, SAS Institute. The analyses of variance and regressions were carried out using the minimum squares method. The means were compared using the Student t-test.

3. Results and discussion

According to Fig. 1, it can be seen that the MDCM samples, irradiated or otherwise, presented similar spectral curves, no singular TBARS being detected in the irradiated samples, presenting a maximum absorption peak at a wavelength different from that observed for the non-irradiated samples, within the range evaluated in this study, that is, 475–565 nm. Maximum absorbance was shown at 531 nm for the MDCM samples irradiated with doses of 4.0 and 5.0 kGy and the non-irradiated samples. According to Hoyland and Taylor (1991), the distillation method eliminates the greater part of the interfering substances in the TBA test. According to Tarladgis et al. (1960), TBA reacts with other substances apart from malonaldehyde, such as glyoxal, in distillates from rancid foods, forming coloured complexes which absorb at 525 and 550 nm. This compound may be present in irradiated meats. In this study, no absorbance peak was detected in the irradiated samples, at the above cited wavelengths.

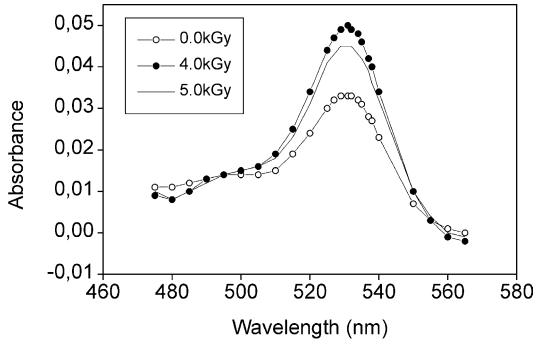

Fig. 1. Absorbance scans of the red pigment produced by the reaction of thiobarbituric acid (TBA) with the TBA-reactive substances in distilled MDCM.

Fig. 1 shows that the samples irradiated with doses of 4.0 kGy presented the greatest absorbance at 531 nm, followed by the sample irradiated with a dose of 5.0 kGy and the non-irradiated samples. Since the objective of this study was to evaluate the applicability of the 2-thiobarbituric acid method in the measurement of lipid oxidation in mechanically deboned gamma-irradiated chicken meat, no attempt was made to compare the TBA numbers within the samples submitted to different doses of gamma radiation.

The mean percent recuperations of TEP for the MDCM irradiated with doses of 4.0 and 5.0 kGy and for the non-irradiated samples were, respectively, 88.6 ± 1.9, 90 ± 1.6 and $88.6 \pm 2.5\%$, showing there was no significant difference ($P > 0.05$) in the recuperation of TEP between irradiated and non-irradiated samples. Similarly the values for K were: 4.98 ± 0.1, 4.91 ± 0.14, 4.98 ± 0.11 for the samples irradiated with 4.0 and 5.0 kGy and for the non-irradiated samples respectively, also showing no significant difference ($P > 0.05$). However it has been reported in the literature that, even with the same recovery, the conversion factor may be different (Pikul, Leszczynski, & Kummerow 1989; Salih, Smith, Price, & Dawson, 1987). This difference is likely to be due to differences in the molar absorptivity of malonaldehyde in each procedure or extraction medium, as reported in several studies (Raharjo & Sofos, 1993).

Wang, Zhu, and Brewer (1997) showed that, in terms of recovery, the results obtained from various methods for the determination of 2-thiobarbituric acid reactive substances (TBARS) were different from each other, as well as from other published data. According to these authors, the recovery rate, based on the method of Tarladgis et al. (1960), used for three distillation methods, was slightly lower (59%) than other published values (66–70%). These same authors argued that these differences in recovery rate could subsequently affect the conversion factors that are used in calculating TBARS in terms of their mg kg^{-1} concentration.

The results for recuperation and the K values obtained in this study are slightly inferior to those obtained by Pollonio (1994), who also used the TBA distillation method under conditions similar to those used in the present study, and detected a K value of 5.93 and a percent recuperation of 96% for MDCM samples. On the other hand, the results obtained in this study do not agree with those obtained by Moerck and Ball (1974), who used the distillation method and a standard of TEP to determine a K value of 8.6 for MDCM. Numerous studies have used a K value of 7.8, as determined by Tarladgis et al. (1960) in bovine meat, to determine the TBARS number in studies on lipid oxidation in MDCM and mechanically deboned turkey meat (Johnson, Cunningham, & Bowers, 1974; Uebersax, Dawson, & Uebersax, 1977, 1978). This value for K

(7.8) is considerably different from the values found for MDCM in this study and in other studies described in the literature (Moerck & Ball, 1974; Pollonio, 1994), confirming that, in order to obtain accurate results, it is necessary to run both standard curves to determine the recovery rate and K values for each method, type of food and work condition, rather than using those from the literature (Wang et al., 1997).

The linear equations of the standard curves for the distillation of malonaldehyde from the MDCM samples irradiated with doses of 4.0 and 5.0 kGy and non-irradiated samples were, respectively: $y_{4.0\ \mathrm{kGy}} = 0.00305 + 0.59128x$ and $y_{5.0\ \mathrm{kGy}} = 0.0045 + 0.5961x$ and $y_{0.0\ \mathrm{kGy}} = 0.00079 + 0.59668x$, where y = absorbance of sample and x = malonaldehyde ($\mu g.ml^{-1}$ distillate). Comparing these equations with each other, it can be seen that the relative mean deviation encountered was 0.5%. For the calculation in question, a value of $x = 1.3$ was determined, this being the greatest value in μg malonaldehyde per ml^{-1} of distillate encountered during the evaluation of these samples. Thus it can be considered that there was no significant difference in the recuperation curves of the standard when comparing the irradiated with the non-irradiated samples.

Studies have shown increases in the TBARS number up to a certain point during the storage period, followed by a decrease in these values (Babji, Chin, Chempaka, & Alina, 1998; Gokalp, Ockerman, Flimpton, & Harper, 1983). Igene and Pearson (1979) stated that, during the evaluation of lipid oxidation in stored foods, decreases in TBA values are probably due to interactions between malonaldehyde and proteins. Shahidi (1997) stated that this type of interaction also appears to occur during the storage of cooked meat, leading to a reduction in the TBARS values. Thus, in this study, K values were determined throughout the refrigerated ($+2\pm1$ °C) and frozen (-18 ± 1 °C) storage of the MDCM samples, aimed at testing whether the alterations produced in the meat during storage could interfere with the recuperation of the standard, and consequently effect the conversion factor K of this raw material.

Since the previously determined K values did not present significant differences ($P > 0.05$), a single point on the standard curve was chosen (addition of standard to obtain 2×10^{-8} moles of malonaldehyde) to follow the behaviour of the K value during the storage of MDCM in samples irradiated with 4.0 kGy and in non-irradiated samples. Fig. 2 shows the values of K for samples of MDCM during refrigerated and frozen storage. The statistical analysis showed there was no significant difference ($P > 0.05$) between the values for K obtained during the period of storage under the three storage conditions evaluated. The non-irradiated MDCM samples and those irradiated with a dose of 4.0 kGy presented the same pattern of the behaviour under the storage conditions analyzed. In addition, there was also

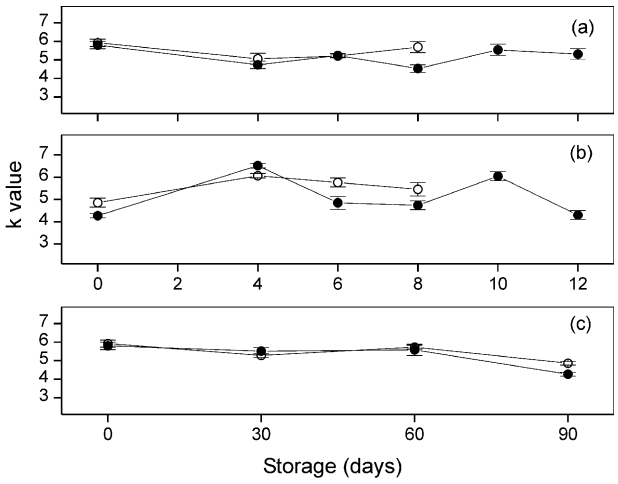

Fig. 2. K values for the MDCM samples (a) MDCM stored under refrigeration ($+2\pm1$ °C) after 2 days frozen storage (b) MDCM stored refrigerated ($+2\pm1$ °C) after 90 days frozen storage (c) MDCM stored frozen (-18 ± 1 °C). 0.0 kGy (○), 4.0 kGy (●). Vertical lines indicate standard deviation.

no significant difference ($P > 0.05$) between the values for K and the percent recuperation obtained for samples of MDCM stored under refrigeration ($+2\pm1$ °C) after being 2 days frozen, stored refrigerated ($+2\pm1$ °C) after 90 days frozen and stored frozen (-18 ± 1 °C). The mean values for K in MDCM samples irradiated with a dose of 4.0 kGy and in non-irradiated samples, stored under refrigeration ($+2\pm1$ °C), after 2 days frozen, stored refrigerated ($+2\pm1$ °C) after 90 days frozen and stored frozen (-18 ± 1 °C), were, respectively, 5.19 ± 0.48 and 5.47 ± 0.40, 5.13 ± 0.86 and 5.54 ± 0.51, 5.29 ± 0.69 and 5.45 ± 0.47.

According to the results, the values for K remained constant for the three storage conditions evaluated in this study, showing that alterations produced in the constituents of the MDCM during refrigerated and frozen storage, did not interfere with the recuperation of the standard. Thus the values for K, determined at the beginning of the storage period for the MDCM samples irradiated with doses of 4.0 kGy and for the non-irradiated samples, can be used for the conversion of sample absorbance into the TBARS number throughout the period of refrigerated or frozen storage under the conditions of the present study.

4. Conclusions

A comparison of the results obtained for the MDCM samples irradiated with doses of 4.0 and 5.0 kGy and the non-irradiated samples, as evaluated by the TBA distillation method, showed that the results for the percent recuperation of TEP, K values and spectral curves, as well as the standard absorption curve, were all similar, no particular interference being detected as a result

of the process of irradiation of the MDCM in the TBA test. The values of K determined at the beginning of the storage period in the non-irradiated MDCM samples and in those irradiated with doses of 4.0 kGy, can be used for the conversion of sample absorbance into the TBARS number throughout the period of refrigerated and frozen storage of this raw material.

Acknowledgements

The authors gratefully acknowledge the contribution of Wilson Custódio in the form of technical assistance.

References

Andrews, W. H. and Read, R. B. (1984). Food sampling plans and initial sample handling. In: *Bacteriological analytical manual* (6th ed) Arlington, AOAC.

Babji, A. S., Chin, S. Y., Chempaka, M. Y. S., & Alina, A. R. (1998). Quality of mechanically deboned chicken meat frankfurter incorporated with chicken skin. *International Jounal Food Sciences Nutrition*, *49*, 319–326.

Calhoun, C. M., Gaebler, D. M., & Mandigo, R. W. (1999). Storage stability of ground pork containing meat from na advanced meat recovery system. *Journal Food Science*, *64*(1), 69–75.

Dawson, L. E., & Gartner, R. (1983). Lipid oxidation in mechanically deboned poultry. *Food Technology*, *37*(7), 112–116.

Fernández, J., Pérez-Álvarez, J. A., & Fernández-López, J. A. (1997). Thiobarbituric acid test for monitoring lipid oxidation in meat. *Food Chemistry*, *59*(3), 345–353.

Gokalp, H. T., Ockerman, H. W., Plimpton, P. F., & Harper, W. J. (1983). Fatty acids of neutral and phospholipids, rancidity scores and TBA values as influenced by packaging and storage. *Journal Food Science*, *48*, 829–834.

Gruiz, K., & Kiss, I. (1987). Effect of ionizing radiation on the lipids in frozen poultry. *Acta Alimentaria*, *16*(2), 111–127.

Hansen, T. J., Chen, G-C., & Shieh, J. J. (1987). Volatiles in skin of low dose irradiated fresh chicken. *Journal Food Science*, *52*(5), 1180–1182.

Horwitz, W. (Ed.). (1980). *Official methods of analysis of the Association of Official Analytical Chemists*. Washington: AOAC.

Hoyland, D. V., & Taylor, A. J. (1991). A review of the methodology of the 2-thiobarbituric acid test. *Food Chemistry*, *40*, 271–291.

Igene, J. O., & Pearson, A. M. (1979). Role of phospholipids and triglycerides in warmed-over flavor development in meat model systems. *Journal Food Science*, *44*, 1285–1290.

Janicek, M. F., Haseltine, W. A., & Henner, W. D. (1985). Malondialdehyde precursors in gamma-irradiatied DNA, deoxynucleotides and deoxynucleosides. *Nucleic Acids Research*, *13*(24), 9011–9029.

Johnson, P. G., Cunningham, F. E., & Bowers, J. A. (1974). Quality of mechanically deboned turkey meat: effect of storage time and temperature. *Poultry Science*, *53*, 732–736.

Kanatt, S. R., Paul, P., Souza, S. F., & Thomas, P. (1997). Effect of gamma irradiation on lipid peroxidation in chicken lamb and buffalo meat during chilled storage. *Journal Food Safety*, *17*(4), 283–294.

Lakritz, l., & Schwartz, D. P. (1995). Formation of oxo- and hydroxyfatty acids in irradiated chickens. *Meat Science*, *40*(2), 279–285.

Lawlor, J. B., Sheehy, P. J. A., Kerry, J. P., Buckley, D. J., & Morrissey, P. A. (2000). Measuring oxidative stability of beef muscles obtained from animals supplemented with vitamin E using conventional and derivative spectrophotometry. *Journal Food Science*, *65*(6), 1138–1141.

Lee, M., Sebranek, J. G., Olson, D. G., & Dickson, J. S. (1996). Irradiation and packaging of fresh meat and poultry. *Journal Food Protection*, *59*(1), 62–72.

Moerck, K. E., & Ball, H. r. Jr. (1974). Lipid autoxidation in mechanically deboned chicken meat. *Journal Food Science*, *39*(5), 876–879.

Pikul, J., Leszczynski, D. E., & Kummerow, F. A. (1983). Elimination of sample autoxidation by butylated hydroxytoluene additions before thiobarbituric acid assay for malonaldehyde in fat from chicken meat. *Journal Agricultural Food Chemistry*, *31*, 1338–1342.

Pikul, J., Leszczynski, D. E., & Kummerow, F. A. (1989). Evaluation of three modified TBA methods for measuring lipid oxidation in chicken meat. *Journal Agricultural Food Chemistry*, *37*, 1309–1313.

Pikul, J., & Kummerow, F. A. (1991). Thiobarbituric acid reactive substance formation as affected by distribution of polyenoic fatty acids in individual phospholipids. *Journal Agricultural Food Chemistry*, *39*, 451–457.

Pollonio, M. A. R. (1994). *Estudo das propriedades funcionais das proteínas miofibrilares e oxidação lipídica de carne de frango mecanicamente desossada*, Doctor thesis Campinas, UNICAMP.

Raharjo, S., & Sofos, J. N. (1993). Methodology for measuring malonaldehyde as a product of lipid peroxidation in muscle tissues: a review. *Meat Science*, *35*, 145–169.

Salih, A. M., Smith, D. M., Price, J. F., & Dawson, L. E. (1987). *Poultry Science*, *68*, 754.

Shahidi, F. (1997). *Flavor of meat and meat products*. London: BAP.

Shahidi, F., & Hong, C. (1991). Evaluation of malonaldeyde as a marker of oxidative rancidity in meat products. *Journal Food Biochemistry*, *15*, 97–105.

Tarladgis, B. G., Watts, B. M., & Younathan, M. T. A. (1960). A destillation method for the quantitative determination of malonaldeyde in rancid foods. *Journal American Oil Chemists Society*, *37*, 44–48.

Uebersax, K. L., Dawson, L. E., & Uebersax, M. A. (1977). Influence of "CO_2 snow" chilling on TBA values in mechanically deboned chicken meat. *Poultry Science*, *56*(2), 707–709.

Uebersax, K. L., Dawson, L. E., & Uebersax, M. A. (1978). Storage stability(TBA) and color of MDCM and MDTM processed with CO_2 cooling. *Poultry Science*, *57*(3), 670–675.

Wang, C., Zhu, l., & Brewer, M. S. (1997). Comparison of 2-thiobarbituric acid reactive substances determination methods in various types of frozen, fresh meat. *Journal Food Lipids*, *4*, 87–96.