https://dergipark.org.tr/tr/pub/ataunivbd
2021; 16(1): 73-79

Research Article/Araştırma Makalesi
DOI: 10.17094/ataunivbd.836612

Atatürk Üniversitesi Veteriner Bilimleri Dergisi
Atatürk University Journal of Veterinary Sciences

Lipid Peroxidation Risk in White and Kashar Cheese

Alper Kursat DEMIRKAYA[1a]✉, Nursah GOLUKSUZ SAHIN[2b]

1. Bilecik Seyh Edebali University, Vocational School, Department of Food Processing, Bilecik, TURKEY.
2. Bilecik Seyh Edebali University, Institute of Graduate, Department of Biotechnology, Bilecik, TURKEY.
ORCID: 0000-0002-7994-7832[a], 0000-0003-0511-4472[b]

Geliş Tarihi/Received	Kabul Tarihi/Accepted	Yayın Tarihi/Published
06.12.2020	05.04.2021	26.04.2021

Bu makaleye atıfta bulunmak için/To cite this article:
Demirkaya AK, Goluksuz Sahin N: Lipid Peroxidation Risk in White and Kashar Cheese. Atatürk University J. Vet. Sci., 16(1): 73-79, 2021. DOI: 10.17094/ataunivbd.836612

Abstract: Lipid oxidation is responsible for the development of many diseases with the loss of characteristics of foods and therefore by leading the loss of quality and formation of toxic compounds. Dairy products are also very susceptible to oxidative degradation because they contain polyunsaturated fatty acids. In this study, the oxidative deterioration levels of 50 Kashar cheese and 50 White cheese marketed in Bilecik city were evaluated using Thiobarbituric Acid (TBA), Total Antioxidant Status (TAS) and Total Oxidant Status (TOS) tests. While TBA, TAS and TOS values of the kashar cheese samples were found as 0.057-1.552 µgMA/g (0.526±0.299 µgMA/g), 0.020-1.522 µmol Trolox equiv/L (0.853±0.222 µmol Trolox equiv/L) and between 1.322-4.140 µmol H_2O_2 equiv/L (2.691±0.626 µmol H_2O_2 equiv/L), respectively, TBA, TAS and TOS values of the white cheese samples were found as 0.047-1.427 µgMA/g (0.475±0.352 µgMA/g), 0.029-0.311 µmol Trolox equiv/L (0.094±0.062 µmol Trolox equiv/L) and between 2.028-3.937 µmol H_2O_2 equiv/L (3.180±0.503 µmol H_2O_2 equiv/L), respectively.

Keywords: Kashar cheese, Lipid peroxidation, White cheese.

Beyaz ve Kaşar Peyniri'nde Lipid Peroksidasyon Riski

Öz: Lipid oksidasyonu, gıdaların has özelliklerini kaybederek kalite kaybına ve toksik bileşiklerin oluşumuna yol açarak birçok hastalığın gelişmesinden sorumludur. Süt ürünleri de çoklu doymamış yağ asitlerini fazla miktarda içermesinden dolayı oksidatif bozulmaya çok duyarlıdır. Bu araştırmada, Bilecik'te tüketime sunulan 50 adet kaşar peyniri ve 50 adet beyaz peynirin oksidatif bozulma düzeyleri Tiyobarbiturik Asit (TBA), Toplam Antioksidan Seviyesi (TAS) ve Toplam Oksidan Seviyesi (TOS) testi kullanılarak değerlendirildi. Kaşar peyniri örneklerinin TBA, TAS ve TOS değerleri sırasıyla 0.057-1.552 µgMA/g (0.526±0.299 µgMA/g), 0.020-1.522 µmol Trolox equiv/L (0.853±0.222 µmol Trolox equiv/L) ve 1.322-4.140 µmol H2O2 equiv/L (2.691±0.626 µmol H2O2 equiv/L) arasında tespit edilirken, beyaz peynir örneklerinin TBA, TAS ve TOS değerleri sırasıyla 0.047-1.427 µgMA/g (0.475±0.352 µgMA/g), 0.029-0.311 µmol Trolox equiv/L (0.094±0.062 µmol Trolox equiv/L) ve 2.028-3.937 µmol H2O2 equiv/L (3.180±0.503 µmol H2O2 equiv/L) arasında tespit edildi.

Anahtar Kelimeler: Beyaz peynir, Kaşar peyniri, Lipid peroksidasyonu.

✉ Alper Kursat Demirkaya
Bilecik Seyh Edebali University, Vocational School, Department of Food Processing, Bilecik, TURKEY.
e-mail: alperkursat.demirkaya@bilecik.edu.tr

INTRODUCTION

Lipid oxidation is a free radical complex chain reaction in which the formation of free radicals (initiation), the formation of hydroperoxides (diffusion), and the formation of non-radical degradation products (termination) (1,2). The most important free oxygen radicals are superoxide radical, hydrogen peroxide, hydroxyl radical and singlet oxygen, Hydroperoxyl radical is lipid radical (alkyl radical), lipid peroxyl radicals (active peroxide), lipid alkoxyl radicals (active oxide) (3). The most important effects of free radicals are lipid peroxidation. Hydroperoxides which occur in the chain phase of lipid oxidation and are not stable compounds cause the oxidation of pigments and vitamins and therefore dark organic polymers occur by polymerization. They turn into various metabolic forms, such as aldehydes, ketones, alkanes, alkenes, alcohols, carboxylic acids and polymerization products by facing structural degradation in chain openings (3-5). These aldehydes, which are the result of the oxidation of fatty acids, are considered as the main cause of undesirable odour and taste formation in food. Texture change, damage of vitamin and essential fatty acids lead to decrease in nutritional value and shelf life of the products and toxic compounds occur in advanced oxidation formation (3,6,7). Initial oxidation products in lipid-containing foods usually emerge at later stages of storage. As oxidation progresses, deterioration accelerates and oxidation becomes unstoppable (8,9). Products such as malondialdehyde, which predominantly result from the degradation of three or more double-linked fatty acids as a result of this reaction, are substances presenting mutagenic and carcinogenic effects determining the severity of oxidation (3,10-12). Malonaldehyde (MDA) and free radicals are toxic biological substances that cause lipid, protein, carbohydrate oxidation and DNA (deoxyribonucleic acid) damage in living organisms (2,13). As a result; pathologies such as diabetes mellitus, necrotizing enterocolitis (NEC), patent ductus arteriosus (PDA), hypoxic ischemic encephalopathy (HIE), premature retinopathy (ROP), bronchopulmonary dysplasia (BPD), periventricular leukomalacia (PVL), intraventricular haemorrhage (IVH) and many other diseases develop (2,3,14). For this reason, many analytical methods have been developed to determine lipid oxidation. These analytical methods have been characterized based on the principle of measuring secondary products such as aldehydes and polymers and the measurement of oxidant or primary peroxide products (15). Currently, the most widely accepted methods for measuring free radicals are the use of thiobarbituric acid reactive substance malondialdehyde (MDA) or ABTS (2,2-azino-bis-3-ethylbenzthiazoline-6-sulfonic acid) markers (3,16). In most studies the Thiobarbituric Acid (TBA) reaction is generally used because determination of MDA level is the most preferred way used to determine the oxidation in foods (11,17-20). Procedures involving thiobarbituric acid (TBA) which is an analytical reagent are widely used to determine the progress of oxidation in dairy products since lipid oxidation is one of the important quality parameters for dairy products (21-23). Liang (9) notes that the TBA test has shown positive results only in oils containing oleic and linoleic acid to a certain extent. For this reason, lipid oxidation level can be determined by measuring Total Antioxidant Status (TAS) and Total Oxidant Status (TOS) besides TBA test in oils containing polyunsaturated fatty acids. Kashar cheese and white cheese are more affected by chemical, microbiological and physical changes in terms of their structural properties. In particular, lipid oxidation can be easily observed due to the presence of polyunsaturated fatty acids in their structure at high level and therefore reaction products that can cause human health can occur. Nevertheless, there was no study on literature in which lipid oxidation level in food was determined by determining TAS and TOS. For this purpose, in this study lipid oxidation levels of white cheese and

kashar cheese marketed in Bilecik city were determined by TBA, TAS and TOS tests.

MATERIALS and METHODS

Materials

In this study, 50 kashar cheese and 50 white cheese samples which are marketed at the existing stores in Bilecik city, were provided according to random sampling method without threatening to the routine sales procedure and packing material brought to the laboratory under the cold chain and kept at +4°C until the analyses were completed.

Methods

Thiobarbituric Acid (TBA) Measurement

10 g of kashar cheese and white cheese sample were subjected to maceration for 2 minutes with 50 mL distilled water and then transferred to a distillation flask by being washed with 47.5 mL of water. 2.5 mL of 4 M HCI was added to the distillation apparatus and distilled in a way that 50 mL of distillate was obtained within 10 minutes. 5 mL is taken from distillation and transferred to a tube and a 5 mL of TBA solution (in 90% glacial acetic acid) was added and it was waited in boiling water bath for 35 minutes. At the end of this duration, the tubes were cooled and the absorbance values were read against the standard solution in the spectrophotometer at 538 nm wavelength and the results were given as µgMA/g (24, 25).

Total Oxidant Status (TOS) Measurement

Total oxidant status measurement was determined by the method developed by Erel (26), which depends on the bases that oxidants oxidize Fe2-o-dianisidine (ferrous ion chelator) complex to Fe^{+3} (ferric ion) and cause an increase in absorbance by reacting with a chromogen (xyleneol ratio) in an acidic environment. The measurement was calibrated with hydrogen peroxide and the results were reported as µmol H_2O_2 equiv/L.

Total Antioxidant Status (TAS) Measurement

The total antioxidant status measurement was determined by the method developed by Erel (16), which depends on the bases that the characteristic color formed by of 2,2'-Azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) radical bleaches by the antioxidants in the sample added to the setting. The results are given in µmol Trolox equiv/L.

RESULTS and DISCUSSION

The obtained mean values of TBA, TAS and TOS amounts of the examined 50 white cheese samples are given in Table 1 and the percentage distribution and frequency numbers are given in Figure 1.

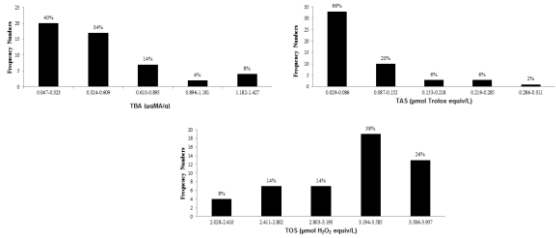

Figure 1. Percentage distribution and frequency numbers of TBA, TAS and TOS values determined in white cheese samples.
Şekil 1. Beyaz peynir örneklerinde belirlenen TBA, TAS ve TOS değerlerinin yüzde dağılımı ve frekans sayıları.

The mean values of TBA, TAS and TOS amounts of the examined 50 kashar cheese samples are given in Table 2 and the percentage distribution and frequency numbers are given in Figure 2.

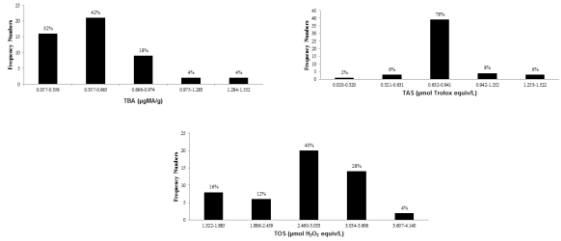

Figure 2. Percentage distribution and frequency numbers of TBA, TAS and TOS values determined in kashar cheese samples.
Şekil 2. Kaşar peyniri örneklerinde belirlenen TBA, TAS ve TOS değerlerinin yüzde dağılımı ve frekans sayıları.

In the study TBA values of the white cheese samples were found as 0.475±0.352 µgMAg^{-1} on average between 0.047-1.427 µgMA/g, TAS values were found as 0.094±0.062 µmol Trolox equiv/L on average between 0.029-0.311 µmol Trolox equiv/L and TOS values were found as 3.180±0.503 H_2O_2 equiv/L on average between 2.028-3.937 µmol H_2O_2 equiv/L (Table 1). On the other hand, TBA values of the kashar cheese samples were found as 0.526±0.299 µgMA/g on average between 0.057-1.552 µgMA/g, TAS values were found as 0.853±0.222 µmol Trolox equiv/L on average between 0.020-1.522 µmol Trolox equiv/L and TOS values were found as 2.691±0.626 µmol H_2O_2 equiv/L on average between 1.322-4.140 µmol H_2O_2 equiv/L (Table 2).

Table 1. TAS, TOS and TBA amounts in white cheese samples.

Tablo 1. Beyaz peynir örneklerinde TAS, TOS ve TBA miktarları.

Properties	N	Minimum	Maximum	Mean
TBA (µgMA/g)	50	0.047	1.427	0.475±0.352
TAS (µmol Trolox equiv/L)		0.029	0.311	0.094±0.062
TOS (µmol H_2O_2 equiv/L)		2.028	3.937	3.180±0.503

Table 2. TAS, TOS and TBA amounts in kashar cheese samples.

Tablo 2. Kaşar peyniri örneklerinde TAS, TOS ve TBA miktarları.

	N	Minimum	Maximum	Mean
TBA (µgMA/g)	50	0.057	1.552	0.526±0.299
TAS (µmol Trolox equiv/L)		0.020	1.522	0.853±0.222
TOS (µmol H_2O_2 equiv/L)		1.322	4.140	2.691±0.626

According to the obtained findings, TBA values were different in kashar cheese and white cheese samples. The slightly higher TBA value in kashar cheese compared to white cheese samples may be due to the difference in the composition of fatty acids. The TBA values of kashar cheese and white cheese samples were found to be lower than TBA values of studies conducted using different types of cheese samples (19,20,24,27,28). Lipid oxidation occurs at low levels in cheese products (23,25,29,30). Hedegaard et al. (31) found that TBA values increased by the duration of storage and Chen et al. (32) reported that variations may occur in TBA values and that this is due to the unstable structure of malonaldehyde which presents in the structure of hydrogen peroxides. Meanwhile, it can be said that the different TBA values found in the study may result from the differences in the raw materials and production techniques in cheese production and the storage conditions. There is no restriction on the number of TBA (µgMA/g) in the white cheese standard and the kashar cheese standard (33,34). Excessive and uncontrolled lipid oxidation causes undesirable rancid taste although the formation of the appropriate amounts of free fatty acids leads to desired flavor (35,36). Jenq et al. (37) stated that when the amount of malondialdehyde (MDA) is above 0.055 µg/g, the oxide aroma can be realized in milk. Especially secondary oxidation products, especially aldehydes, are the most important compounds causing the change of taste in milk and dairy products (38). However lipolytic agents such as lipase and lipoprotein lipase which goes to milk from blood cause the acceleration of lipid oxidation in cheese and undesirable taste changes due to mechanical damage in milk fat globules such as homegeneration (39-41). Storage conditions such as gas permeability of the packaging material, relative humidity and temperature are also effective in this situation (31,42). Although significant changes are observed in MDA content, it has been stated that there is a correlation between TBA values and the taste of the oxides in milk and dairy products (25,43).

Nevertheless, Frankel and Neff (44) stated that the TBA reaction is not specific for MDA and many lipid oxidation products and reacts with other biological elements. Therefore, it can be stated that low TBA values in samples do not indicate the risk of oxidation because malonaldehyde reacts with other compounds, especially with proteins, and measurement of TAS and TOS values conducted in this study on dairy products is important in determining the lipid oxidation. Gutierrez (45) stated that the presence of Pro-oxidants significantly reduced the total antioxidant capacity and increased the oxidation products in the milk during storage. The obtained data in the study showing that TAS values are low and TOS values are high in cheese samples with high TBA values confirm the results obtained by Gutierrez (45) and Tripaldi et al. (23).

As a result, the formation of toxic compounds and undesirable taste development occur since foods containing polyunsaturated fatty acids at a high level such as dairy products are susceptible to oxidation. The risk of oxidative deterioration in dairy products should be considered in terms of standard quality production and public health. Lipid oxidation can be controlled by taking the necessary precautions such as the use of good technology in production, packaging and proper storage conditions. However, since there is no study similar to this study in which the lipid oxidation level was determined in cheese samples using TAS and TOS measurements, the obtained results will contribute to the field providing more data.

Conflict of interest

The authors declare that they have no conflict of interest.

REFERENCES

1. Dominguez R., Pateiro M., Gagaoua M., Barba FJ., Zhang W., Lorenzo JM., 2019. A comprehensive review on lipid oxidation in meat and meat products. Antioxidants, 8, 429.
2. Estevez M., Li Z., Soladoye OP., Van-Hecke T., 2017. Health risks of food oxidation. In "Advances in food and nutrition research", Vol. 82, 45-81, Academic Press, United States.
3. Sousa BC., Pitt AR., Spickett CM., 2017. Chemistry and analysis of HNE and other prominent carbonyl-containing lipid oxidation compounds. Free Radic Biol Med, 111, 294-308.
4. Liao H., Zhu M., Chen Y., 2020. 4-Hydroxy-2-nonenal in food products: A review of the toxicity, occurrence, mitigation strategies and analysis methods. Trends Food Sci Technol, 96, 188-198.
5. Amaral AB., Silva MVD., Lannes SCDS., 2018. Lipid oxidation in meat: mechanisms and protective factors–a review. Food Sci and Technol, 38, 1-15.
6. McSweeney PLH., 2004. Biochemistry of cheese ripening. Int J Dairy Technol, 57, 127-144.
7. Daoud S., Bou-Maroun E., Waschatko G., Cayot P., 2020. Lipid oxidation in oil-in-water emulsions: Iron complexation by buffer ions and transfer on the interface as a possible mechanism. Food Chem, 128273.
8. Kamal-Eldin A., Makinen M., Lampi AM., 2003. The Challenging Contribution of Hydroperoxides to the Lipid Oxidation Mechanism. In: "Lipid Oxidation Pathways", Ed., Kamal-Eldin, A., 1-36, AOCS Press, New York.
9. Liang JH., 2000. Kinetics of fluorescence formation in whole milk powders during oxidation. Food Chem, 71, 459-463.
10. Olivecrona T., Vilaro S., Olivecron, G., 2003. Lipases in milk. In: "Advanced Dairy Chemistry I. Proteins", Ed., Fox P.F, McSweeney P.L.H., 473-488, Kluwer, New York, USA.
11. Phillips RW., 1998. Fat-soluble vitamins. In: "Veterinary Pharmacology and Therapeutics Booth", Ed., N.H., McDonald, L.E., 6th, 928-949, Iowa State University Press, Ames, USA.
12. Unalan IU., Arcan İ., Korel F., Yemenicioğlu A., 2013. Application of active zein-based films with controlled release properties to control *Listeria monocytogenes* growth and lipid oxidation in

fresh kashar cheese. Innov Food Sci Emerg, 20, 208-214.

13. Tarakci Z., Kucukoner E., 2006. Changes on physicochemical, lipolysis and proteolysis of vacuum-packed Turkish kashar cheese during ripening. J Cent Eur Agric, 7, 459-464.
14. Mercier Y., Gatellier P., Viau M., Remignon Renerre M., 1998. Effect of dietary fat and vitamin e on lipid and protein oxidation in turkey meat during storage. Meat Sci, 48, 301-317.
15. Kristensen D., Skibsted LH., 1999. Comparison of three methods based on electron spin resonance spectrometry for evaluation of oxidative stability of processed cheese. J Agric Food Chem, 47, 3099-3104.
16. Erel O., 2004. A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable ABTS radical cation. Clin Biochem, 37, 277-285.
17. Demirkaya AK., 2013. Evaluation of the lipid oxidation with thiobarbituric acid (TBA) test in butter. Atatürk University J Vet Sci, 8, 237-240.
18. Demirkaya AK., 2014. Lipid oxidation in minced meat and chicken meats consumed in Bilecik, Turkey. A Food J, 12, 26-29.
19. Holm VK., Mortensen G., Risbo J., 2006. Quality changes in semi-hard cheese packaged in a poly (lactic acid) material. Food Chem, 97, 401-410.
20. Thibeault DW., 2000. The precarious antioxidant defences of the preterm infant. Am J Perinatol, 17, 167-181.
21. Kayahan M., 2003. Oil Chemistry. 105-118. METU Press. Ankara, Turkey.
22. Kistrup HV., Mortensen G., Vishart M., Agerlin PM., 2006. Impact of poly-lactic acid packaging material on semi-hard cheese. Int Dairy J, 16, 931-939.
23. Tripaldi C., Rinaldi S., Palocci G., Di Giovanni S., Campagna MC., Di Russo C., Zottola T., 2020. Chemical and microbiological characteristics of homogenised ricotta cheese produced from buffalo whey. Ital J Food Sci, 32.
24. Tarladgis BG., Watts BM., Younathan MT., Dugan LR., 1960. A distillation method for the quantitative determination of malonaldehyde in rancid foods. J Am Oil Chem Soc, 37, 44-48.
25. Rossell JB., 1994. Measurement of rancidity. In: "Rancidity in foods". Ed., J.C. Allen, R.C. Hamilton, 22-53, Chapman and Hall, New York.
26. Erel O., 2005. A new automated colorimetric method for measuring total oxidant status. Clin Biochem, 38, 1103-1111.
27. Nawar WW., 1996. Lipids. In: "Food Chemistry". Ed., Fennema, O.R., 225-319, Marcel Dekker Inc, New York, USA.
28. Ayodeji AA., Ahure D., Efiong EE., Acham IO., 2020. Production and quality evaluation of cheese from soy and coconut milk using selected coagulants. Eur J Nutr Food Saf, 17, 1-12.
29. Collins YF., McSweeney PLH., Wilkinson MG.,2003. Evidence of a relationship between autolysis of starter bacteria and lipolysis in Cheddar cheese during ripening. J Dairy Res, 70, 105-113.
30. Lin SS., 1991. Fats and Oils Oxidation. In: "Introduction to Fats and Oils Technology". Ed., Wan, P.J., 211-222, AOCS, Champaign, Illinois.
31. Hedegaard RM., Kristensen D., Nielsen JH., Frost MB., Ostdal H., Hermansen JE., Kröger-Ohlsen M., Skibsted LH., 2006. Comparison of descriptive sensory analysis and chemical analysis for oxidative changes in milk. J Dairy Sci, 89, 495-504.
32. Chen MC., Yeh GHC., Chiang BH., 1996. Antimicrobial and physicochemical properties of methylcellulose and chitosan films containing a preservative. J Food Process Preserv, 20, 379-390.
33. TS, 2006. TS 591 White Cheese. The Institute of Turkish Standards, Ankara, Turkey.
34. TS, 2006. TS 3272 Kashar Cheese. The Institute of Turkish Standards, Ankara, Turkey.
35. Walstra P., Jenness R., 1984. Dairy Chemistry and Physics. 58-96. Wiley, New York, USA.
36. Abdel-Ghany IHI., Sakr SS., Sleem MM., Shaaban HA., 2020. The effect of milk fat replacement by

some edible oils on chemical composition, antioxidant activity and oxidative stability of spreadable processed cheese analogues. Int Res J Food Nutr, 2, 6-14.

37. Jenq W., Bassette R., Crang RE., 1988. Effects of light and copper ions on volatile aldehydes of milk and milk fractions. J Dairy Sci, 71, 2366-2372.
38. Badings HT., 1984. Flavor and off-Flavors. In: "Dairy Chemistry and Physics". Ed., Walstra, P., Jenness, R., 336-357, Wiley, New York, USA.
39. Fox PF., Guinee TP., Cogan T., McSweeney PLH., 2000. Fundamentals of Cheese Science. Aspen Publishers, Gaithersburg, MD, USA.
40. Okur ÖD., 2010. Determination of traditional dolaz cheese characteristics and standardization of the production. Süleyman Demirel University, Instıtute of Science, Isparta, Turkey.
41. Shapovalov S., Mikhaylov S., Skryl A., Chereshneva Y., Tsomartova D., Ivanova M., Pavlova, M., 2019. Free radical oxidation and antioxidant status of milk from different cow breeds. Biomed J Sci Tech Res, *23*, 17242-17247.
42. Zhang L., Zhang Z., Chen Y., Ma X., Xia M., 2021. Chitosan and procyanidin composite films with high antioxidant activity and pH responsivity for cheese packaging. Food Chem, 338, 128013.
43. Fenaille F., Mottier P., Turesky RJ., Ali S., Guy PA., 2001. Comparison of analytical techniques to quantify malondialdehyde in milk powders. J Chromatogr, 921, 237-245.
44. Frankel EN., Neff WE., 1983. Formation of malondialdehyde from lipid oxidation products. Biochim Biophys Acta, 754, 264-270.
45. Gutierrez AM., 2014. Effects of lipid oxidation initiators and antioxidants on the total antioxidant capacity of milk and oxidation products during storage. Iowa State University, Instıtute of Science, Ames, Iowa.