Lipid Peroxidation and Its Antimicrobial Effect in Foods

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Rancidity is lipid peroxidation process and an undesirable phenomenon occurred in foods having unsaturated fats and oils. It resulted in food deterioration. Therefore, there is a limitation of lipid peroxidation for food quality control as a value of TBA. Malonaldehyde (MDA) is the last product of lipid autoxidation and Thiobarbituric Acid (TBA) is the most widely used assay to detect lipid peroxidation in foods. Although rancidity is inevitable process, it can be controlled by some additives or processes. In this research, it was found that increasing lipid peroxidation level in Manti is inversely proportional to the microbiological load.

Keywords: Lipid Peroxidation, Antimicrobial Effect, TBA

1. Introduction

The oxidation of unsaturated fatty acids because of physical factors (temperature, pH) or biological reasons such as enzymatic degradation in foods called rancidity. Rancidity results in the formation of sensory and biological undesirable products, the result of which is a sign of deterioration in foods. After first step of autoxidation, lipid peroxidation shows geometric increase in foods. Keeping it under control therefore is important for the quality of food.

Lipid peroxidation is a chain reaction initiated by the formation of hydroperoxides. It occurs in two different ways. 1. Autoxidation a) free radical mechanism b) photo-oxidation and 2) mechanism of lipoxygenase, which is a biological reaction (food chemistry). It is the free radical mechanism that is best explained and studied. The initial phase of this mechanism is not fully explained, but it is believed that it is the cause of photo-oxidation. The first product in autoxidation reactions is hydroperoxide, which is more stable than other products. This is why it is used in the evaluation of lipid peroxidation in foods. Secondary products formed as a result of oxidation are variable. These include: aldehydes, ketones, epoxides, hydroxy compounds, even oligomers and polymers [1] (Figure 1).

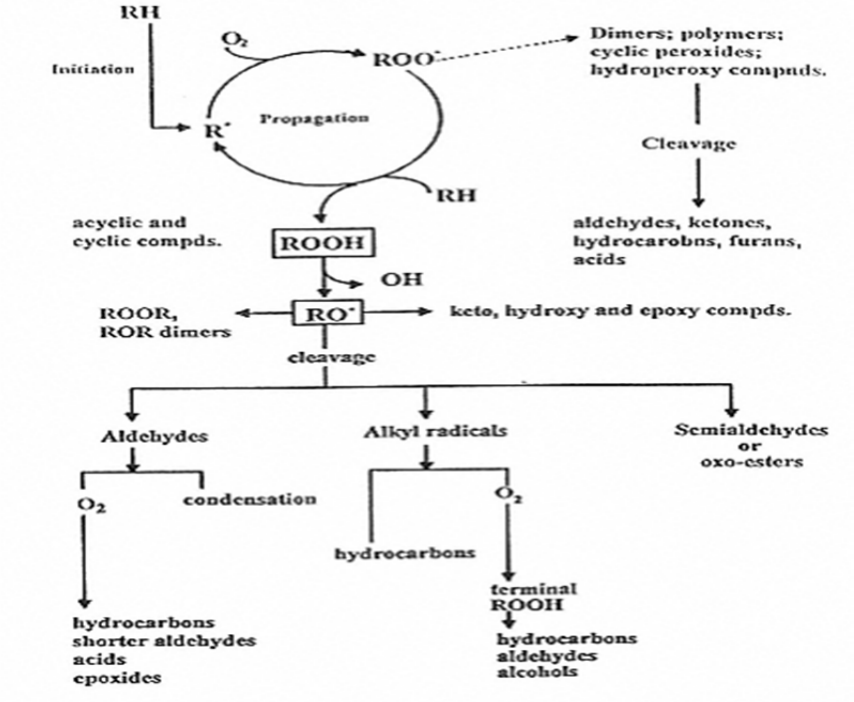


Fig. 1 Generalized scheme for autoxidation of lipids [2].

The methods used to measure lipid oxidation in foods are aimed at either primary products or secondary products. Therefore, the most commonly used methods are peroxide value, Thiobarbituric acid test, Kreis test and Iodine value, although there are many tests related to lipid oxidation measurement in the literature [3].

Among these, the costs of the highest specifity (NMR and EPR) are very high. On the other hand, there are methods (IR and Raman scattering) that require precision at the point of application and labor, even though the cost is low (Figure 2) [3]. Among all methods, TBA method is the most practical and widely used method at both cost and application point of view.

TBA assay first used for detection of oxidation products in animal tissue [4]. After that, its first usage in food was to detect oxidation of milk fat [5]. Patton and Kurtz [5] at this research found that malonaldehyde (MDA) was the product of rancidity process and can be used for detection and measuring of lipid peroxidation by TBA assay.

It is well known that MDA is produced in the last step of the autoxidation of unsaturated oils and fats. Besides, they indicated that TBA assay is more sensitive than the Kreis test and peroxide value. Dunkley and Jennings [6] were also tested the sensitivity of TBA assay. With respect to their research, TBA assay is reproducible and more sensitive than the Kreis test.

Traditional foods are special products having cultural aspects of the local regions of the countries. Manti (stuffed pasta) is one of the famous and nutritious traditional foods in Turkey and Middle Asia. Manti is sensitive to oxidation and microbial deterioration because it contains minced meat and dough [7]. Therefore, the aim of the study was to find correlation between lipid peroxidation and its antimicrobial effect. For this purpose *Salmonella typhimerium* and *E.coli* 0157: H7 were used as sample microorganisms.

METHOD	ANALYTE	SAMPLE PREPARATION	AMOUNT OF SAMPLE	SENSITIVITY	SPECIFICITY	COST	LIMITATIONS
Titration	Peroxides	Medium-Short	1 g	Medium-low	Medium-low	Low	Reagents susceptible to oxidation Absorption by UFA Dryness required
Uv-Vis[a] spectroscopy	Peroxides, *Conjugated dienes/trienes *MDA, aldehydes	Medium	500 mg	Medium	Medium	Low	High amount of solvents Low concentration range Variability depending on the dye *Insensitive to oleic acid
Chromatography	Peroxides, MDA, SOPs, volatiles, oligomers	Long	1-100 mg	High-very high (depending on the detector)	High-very high (depending on the detector)	High	Laborious experimental procedure and data processing
Chemiluminiscence	Peroxides	Short	1-200 mg	High	Medium	Low	Unknown mechanisms Light amplifiers required
Fluorescence	Aldehydes and volatiles	Very short	10-50 mm^2	Very high	High	Medium	Variability in wavelenghts
IR[b] spectroscopy	Peroxides, unsaturations, MDA	Very short-none	2-40 mg	Medium-high	High	Medium	Non-aqueous solutions required
Raman scattering	Peroxides, unsaturations, MDA	Very short-none	10-50 mm^2	Medium-high	High	Low	Some molecules are inactive
Nuclear magnetic resonance	Peroxides, aldehydes, dienes	Very short-none	10-200 mg	High	Very high	Very high	Complex data interpretation
Electron paramagnetic resonance	Radicals	Very short-none	100-900 mg	High	High	Very high	Complex data interpretation

[a] Ultraviolet-visible
[b] Infrared

Fig. 2 Characteristics of the different methods for analysis of lipid oxidation in foods reviewed in this article.

2. Material and Method

2.1 Thiobarbituric acid (TBA) analysis

Manti sample (10 g) was homogenized with water. The mixture was transferred to Kjeldahl flask and distilled by adding 2.5 mL 4 N HCl (Merck, Germany) and 1 mL antifoam chemical. After that, 5 mL of this distillate was mixed with equal volume of TBA (Merck, Germany) and incubated in water bath at 80-90 ^{o}C for 30 minutes. The measurement was made on a spectrophotometer at 538 nm and lipid peroxidation level was calculated according to below equation as equivalent of mg malondialdehit (MDA) per kg sample [8].

2.2 Antimicrobial activity

The manti sample distillates were used for their antimicrobial activity and evaluated by the help of disc diffusion method. The antimicrobial activity was tested against *E. coli O157:H7* ATCC 33150 and *S.typhimerium* as food-borne pathogens (kindly provided by Kayseri Agriculture Control Protection Management Center, Turkey). Strains first incubated overnight in Mueller-Hinton broth at 35 °C. After that, culture turbidity was adjusted to 0.5 McFarland standard and inoculated on to Mueller-Hinton agar by spread plate technique. Paper discs saturated with 20 µL of distillates were used for antimicrobial activity. The zones of inhibition on MH Agar were measured after 24 hour incubation at 35^{o}C [9].

3. Results and Discussion

Antimicrobial activity of lipid peroxidation was tested through this study. MDA determination as an indication of lipid peroxidation by the TBA assay can offer, at best, a narrow and somewhat empiric products of lipid peroxidation and the TBA assay is perhaps the most widely used assay for oxidative damage. Therefore, TBA assay was used for lipid autoxidation determination. The results showed that increase in lipid peroxidation level concentration resulted in increase in antibacterial activity (Figure 3). If the lipid peroxidation value in distillates was 1.5 mg/kg and above, it showed antimicrobial activity. Thus, it can be said that through storage, presence of TBA may inhibit bacterial growth and contamination.

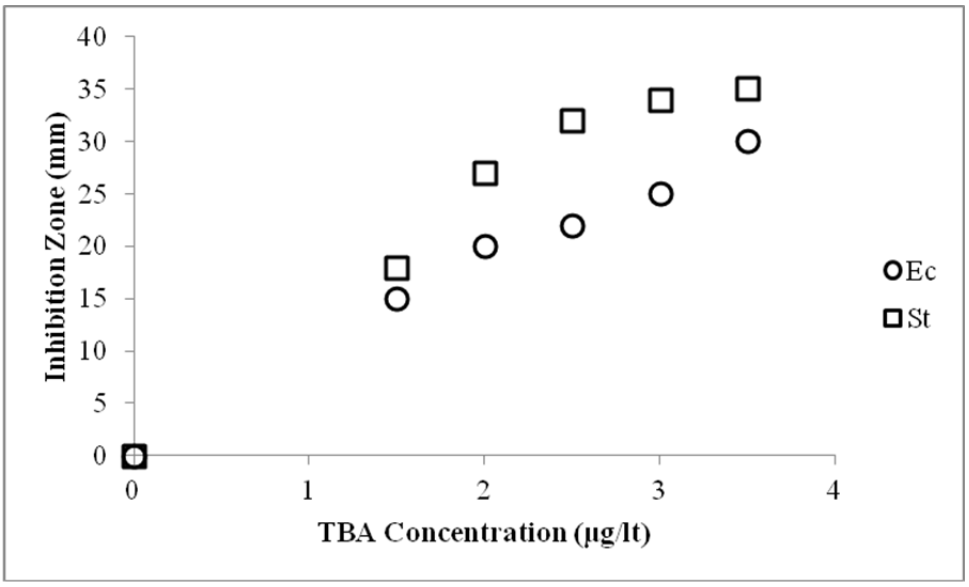
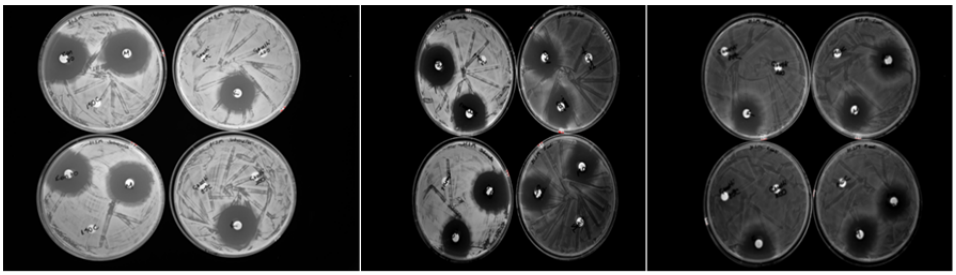


Fig. 3 Diameter zones for some pathogens related to TBA concentration [Ec: *E.coli* zone diameter(mm) St: *S.typhimerium* zone diameter(mm)]. *Control group was used in the study. **The zones formed by *S. typhimurium* and *E. coli* showed in Annex-1.

Although the TBA value of food products greater than 3 mg / kg is correlated with malodor and taste in foods [10,11], deterioration resulted from lipid peroxidation in Manti is not directly correlated with lipid peroxidation level. Some studies indicated that there was no reduction in food quality with respect to increasing lipid autoxidation. Besides, Yücetepe [12] found that sensory quality of manti increased with increasing lipid peroxidation level.

As a conclusion, lipid peroxidation level in Manti cannot express applicable value in the deterioration of manti. Therefore, there is a need for further research on this subject.



Annex 1 The zones formed by *S. typhimurium* and *E. coli*.

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