



# The comparison of quality and microbiological characteristic of hamburger patties enriched with green tea extract using three techniques: Direct addition, edible coating and encapsulation



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## ABSTRACT

This study was aimed at the comparison of the effects of green tea extract (GTE) added with different techniques (direct addition, edible coating and encapsulation) on quality (particularly oxidative) and microbiological properties of hamburger patties. High molecular weight (1000 kDa) chitosan was utilized as the coating material and chitosan-TPP solution was formed for encapsulation of the extract. At the beginning (day 0) and in the middle of storage (day 4), control patty and the patties including GTE had higher pH values than the coated patties ( $p < 0.05$ ) probably due to the inhibition effect of coating materials as a barrier on microbiological growth. TBARS value of control patty was higher than the other treatments in each measuring time (on the days 0, 4 and 8). This showed that enrichment of treatments by adding or coating with encapsulated green tea extract solution led to attenuation effect against lipid oxidation. However, TBARS value of all the treatments increased throughout storage, except the treatment coated with 1% (w/w) chitosan solution ( $p > 0.05$ ). According to microbiological results, there were significant differences among the treatments.

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## 1. Introduction

Meat products are very popular among food items (Lee et al., 2005). Although synthetic additives have been widely used in the meat industry to inhibit both the process of lipid oxidation and microbial growth, natural additives are preferred in place of them in recent years because of the growing concern among consumers about such chemical additives. Compounds obtained from natural sources such as grains, oilseeds, spices, fruit and vegetables have been investigated. Therefore, the development and application of natural products with both antioxidants and antibacterial activities in meat products may be necessary and useful to prolong their storage shelf life and potential for preventing food diseases (Fernández-López, Zhi, Aleson-Carbonell, Pérez-Alvarez, & Kuri, 2005).

Although use of edible coatings and films to preserve food quality is not a novel concept, research in this field at academic,

government, and private industry laboratories has intensified recently. The action of these coatings as a barrier to the passage of oxygen and water, thereby slowing oxidation reactions and retaining moisture, is the main mechanism used by coatings to enhance quality and extend storage life. Furthermore, adding plant extracts gives the coatings antimicrobial and antioxidant properties (Gómez-Estaca, Montero, Giménez, & Gómez-Guillén, 2007). As there is a demand for convenient “value added” meat products of good acceptability and low price, much effort has been devoted to improving the quality of precooked meat products using appropriate coatings and/or the addition of antioxidants (Biswas, Keshri, & Bisht, 2004).

Encapsulation and controlled-release of active food ingredients are important applications in food and nutrition that can be attained with nanotechnological approaches (Huang, Yu, & Ru, 2010). Encapsulation involves the incorporation of food ingredients, enzymes, cells or other materials in small capsules. Applications for this technique have increased in the food industry since the encapsulated materials can be protected from moisture, heat or other extreme conditions, thus enhancing their stability and

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maintaining viability. Reducing the size of the encapsulates offers opportunities related with prolonged gastrointestinal retention time caused by bio-adhesive improvements in the mucus covering the intestinal epithelium (Bouwmeester et al., 2009; Ferreira, Rocha, & Coelho, 2007; Garcia, Forbe, & Gonzales, 2010; Gibbs, Kermasha, Alli, & Mulligan, 1999; Neethirajan & Jayas, 2011).

Green tea is a popular beverage in Asia for many years and believed to have more beneficial effects on health than other tea beverages. Many studies showed that green tea extracts, which are rich in polyphenols and catechins, have antibacterial, antiviral and radical scavenging activities (Chiu & Lai, 2010; Manea, Vasile, & Meghea, 2014).

Green tea catechins are the predominant group of polyphenolic compounds present in unfermented dried leaves of the plant. Biological and pharmacological effects (anti-inflammatory, antimicrobial, antitumour, anti-oxidative and anti ageing) of green tea leaf extract have been attributed to green tea catechins. Green tea catechins are comprised of four compounds which are epicatechin, epicatechin gallate, epigallocatechin and epigallocatechin gallate. All the catechins have synergistic effect, therefore the crude green tea leaf extract exhibits higher antimicrobial activity than do isolated green tea catechins (Sharma, Gupta, Sarethy, Dang, & Gabrani, 2012; Wanasundara & Shahidi, 1998).

The aim of the study is to incorporate various antioxidative substances into the hamburger patties by using different techniques in order to retard the deterioration of the patties during storage and to find the healthiest products in terms of lipid oxidation and microbial quality.

## 2. Materials and methods

### 2.1. Preparation of edible coating solution by using chitosan

A 1% (w/w) chitosan solution (95% deacetylated, MW: 1000 kDa, obtained from Golden-Shell Pharmaceutical Co., Ltd., China) was dispersed in acetic acid solution (10 ml glacial acetic acid (99.8%)/1L distilled water) with addition of 25% glycerol. The film forming chitosan solution was stirred until dissolution (Park, Daeschel, & Zhao, 2004).

### 2.2. Preparation of solution including microparticles

Microparticles were achieved by the cross linking of chitosan (95% deacetylated, MW: 1000 kDa) - sodium tripolyphosphate (CS-TPP) solution (5:1 mass ratio). Chitosan (1%) was dissolved in 1% acetic acid solution to form chitosan solution. TPP (85%, technical grade, Acros Organics, Morris Plains, NJ) solution (0.2%, w/v) dissolved in Milli-Q water were dropped into 1% chitosan, in order to form CS-TPP microparticles. After that, ultrasonication (3.75 W/mL energy output) was applied to the solutions for 3 min.

The solution including 0.5% green tea extract (98%) was also prepared. The green tea extract (98% pure) used in the study mainly contained 63.73% (–)-epigallocatechin gallate (EGCG), 21.58% (–)-epicatechin gallate (ECG), 3.76% caffeine, 3.44% epicatechin (EC), 1.24% (–)-epigallocatechin (EGC). This extract was added into the chitosan solution (1%) and then the dropwise addition of TPP (0.2%) was performed to form microparticles loaded with green tea extract.

### 2.3. Particle size of the solutions

Particle size of the solutions were measured by dynamic light scattering instrument (Brookhaven BIC 90 plus) equipped with a Brookhaven BI-9000AT digital correlator (Brookhaven Instrument Corp, New York, NY). All measurements were performed in

triplicate with the detection angle of 90 at  $25 \pm 1$  °C.

### 2.4. Formulation of hamburger patties

Hamburger patties of the formulations with GTE were prepared with minced meat from a local supermarket (80% lean beef + 20% fat), 0.80% salt and 0.20% black pepper. All the hamburger patties were formed by using a steel mould made for hamburger preparation in order to maintain the size of the treatments totally same.

In this study, 8 different treatments of hamburger patties were prepared (Table 1). First treatment was the control and prepared as given above. In treatment 2, 5% GTE was added into the patty out of total weight of the product. In treatments 3 and 4, the solution including encapsulated GTE (CS + 0.5% GTE + TPP) was incorporated into the patties as 1% and 5% out of total weight, respectively. The other treatments (5–8) were coated by dipping method. The hamburger patty samples were immersed into the solutions given in Table 1 at room temperature, held 5 s and then left to dry 30 s. This process was repeated 3 times. After dipping process, patties were dried in biological safety cabinet for 1 h.

All the treatments were stored in an aluminium plate covered with cling film at 4 °C for 8 days and analyzed on the 0, 4 and 8 days of storage. The treatments were not stored more than 8 days, because they began to spoil and stink.

### 2.5. Moisture analysis

Oven drying method was used to find the total moisture contents of the treatments. Moisture was calculated according to the weight difference of the sample before and after drying for 2 h at 125 °C and given as percent value (AOAC, 1990). Three repetitions were implemented for each treatment.

### 2.6. pH values

The pH values of the treatments was conducted by immersing a common pH-electrode into the sample and measuring the value. The experiments were attained in quadruplicate for each treatment.

### 2.7. TBARS (2-thiobarbituric acid) analysis

TBA method was performed as given by Pikul, Leszczynski, and Kummerow (1989) and Ulu (2004). Aqueous extraction method was preferred and perchloric acid (4% perchloric acid in distilled water), butylated hydroxyanisole (7.2% BHA in 98% ethanol), and TBA solution (0.02M in distilled water) were used as reagents. The heating part of the experiment was done for 40 min at 80 °C. TBA values were determined as amount of malonaldehyde. Absorbance values at 532 nm were used to calculate the TBA value as mg malonaldehyde/kg sample. Four repetitions were applied at each experimental group.

**Table 1**  
Formulation of Treatments with green tea extract.

Green tea extract (GTE) added hamburger patties	Coated hamburger patties
1- Control	5- CS
2- 5% GTE	6- CS + 0.5% GTE
3- 1% (CS + 0.5% GTE + TPP)	7- CS + TPP
4- 5% (CS + 0.5% GTE + TPP)	8- CS + 0.5% GTE + TPP

GTE: Green tea extract, CS: Chitosan, TPP: Sodium tripolyphosphate.

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