

## Effect of *Ganoderma lucidum* Powder on Oxidative Stability, Microbial and Sensory Properties of Emulsion Type Sausage

### Abstract

**Background:** *Ganoderma lucidum* from *Ganodermataceae* family is a kind of mushroom known to have various therapeutic properties such as lowering high blood sugar and high blood pressure, boosting the immune system as well as its antibacterial and antioxidant effects. **Materials and Methods:** this study investigated the oxidative stability, microbial and sensory properties of sausage at three different treatments; (i) 1% w/w *Ganoderma lucidum* powder (GLP) without nitrite as a food preservative (P), (ii) 0.5% w/w GLP with 80 ppm nitrite (N + P), and (iii) sausage with 120 ppm nitrate (N). Lipid oxidation was evaluated using peroxide value (PV) and thiobarbituric acid reactive species. Antimicrobial properties were assessed by total plate count (TPC), yeasts and molds, coliforms, *Clostridium perfringens*, and *Staphylococcus aureus*. Sensory assessment was evaluated by nine-point hedonic procedure. **Results:** Samples in N + P treatment showed lower PV than other treatments at the storage period with no significant difference in 2-thiobarbituric acid (TBA) between N and N + P. The P group showed the highest TBA value ( $P < 0.01$ ). TPC remained below maximal permissible limit recommended by ISIRI during 30 days of storage in all sausage formulations (6.9798 log CFU). There was not found any coliforms bacteria, *Clostridium perfringens*, and *S. aureus*. The sensory evaluation indicated that there is no significant difference between samples in texture, taste, and smell. The color and overall acceptability of N group were higher and N + P group was closer to N group. **Conclusion:** The results suggest that *G. lucidum* powder might be considered as a potential natural preservative for meat products.

**Keywords:** Antimicrobial, antioxidant, *Ganoderma lucidum*, sausage

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### Introduction

Sausage is one of the oldest forms of processed food which is usually made from minced meat, animal fat, salt, spices, and sometimes aromatic herbs.<sup>[1]</sup> It often forms a significant part of diet and its consumption is increasing because of several reasons (i) increase in young population, (ii) increase in women's employment, (iii) decrease in purchasing power of families, (iv) need for an appropriate substitute for meat, (v) a gradual tendency toward simple living, and (vi) simple, fast, and cheap industry of sausage production.<sup>[2]</sup>

Nitrate/nitrite is a common ingredient in sausage formulation with clear and distinct functions; it is a microbial preservative particularly against anaerobic ones such as *Clostridium*s also it acts in the development of specific pink color, taste, and smell of sausage.<sup>[3]</sup> However, its usage in sausage formulation is controversial as carcinogenic.

Nitrosamines can be created as a consequence of acid nitrous with secondary amines. This reaction may take place in both foods or stomach.<sup>[4]</sup> Production of nitrosamines could be accelerated through the activity of intestine flora, acidic conditions, and high heat over the storage period.<sup>[5]</sup> Accordingly, minimizing the use of sodium nitrate as a preservative and replacing it with natural and less toxic alternatives is one of the interested areas of research in food safety.

*Ganoderma lucidum* is a mushroom in red, light yellow, or brownish-red colors and is used in Far East countries specially Japan, China, and Korea for pharmaceutical purposes.<sup>[6-8]</sup> It is known as "Reishi" in Japan, "Linshi" in China, and "Yeongji" in Korea. Traditionally, it is used to treat stomach cancer, hepatitis A, B, and C, osteoarthritis, insomnia, bronchitis, nervous disorders, asthma, stomach ulcer, high blood pressure, and high cholesterol.<sup>[9]</sup> Its fruit body and mycelia contain different

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groups of compounds including sterols, steroids, peptides, lactones, alkaloids, polysaccharides, triterpenoids, and proteins with proven medicinal effects.<sup>[10-12]</sup> Polysaccharides are the most significant because of their anticancer features.<sup>[13]</sup> Sa-Ard *et al.* showed that *G. lucidum* protein extracts have antioxidant and antibacterial activities.<sup>[14]</sup> Wannasupchue *et al.* showed that application of *G. lucidum* in the production of smoked fish sausage delayed lipid oxidation during the storage period.<sup>[15]</sup> More lactic acid bacteria and polysaccharide with higher viscosity and acetaldehyde content were observed in *Ganoderma* yogurt than control sample (yogurt without *G. lucidum*).<sup>[16]</sup>

The present study evaluates the possible use of *G. lucidum* as a natural preservative in cooked sausage production. Furthermore, its antioxidant and antibacterial effect in emulsion type sausage is investigated for the first time.

## Materials and Methods

### Sample collection and identification

*G. lucidum* grown on *Carpinus betulus* L. (*Corylaceae*) was collected by hand from Behshahr and Neka forests in the north of Iran and transferred in plastic bags to the laboratory. They were identified using keys and morphological characters provided by Hallenberg.<sup>[17]</sup>

### Sample preparation

*G. lucidum* fruiting bodies were oven-dried at 40°C for 2–3 days. Then, oven-dried mushrooms were grinded using a blender and stored in plastic bags in a freezer at –20°C for later analyses.<sup>[18]</sup>

### Sausage preparation

Beef was purchased from a local store. It was grinded using a mincer (Pars Khazar Co., Iran) with 3 mm hole. Emulsion type sausage was made following this recipe: 600 g of minced meat mixed in a food maker (Moulinex Co., France) with ingredients including 200 g shattered ice, 25 g milk powder, 60 g flour, 25 g starch, 20 g gluten, 5 g soya, 50 g sunflower oil, 15 g salt, 20 g garlic, 3 g phosphate, 0.5 g ascorbic acid, 1 g coriander powder, 1 g red pepper, 1 g white pepper, 1.5 g nutmeg, and 1.5 g ginger. This mixture was used to make three different treatments of the study; (i) 1% w/w *Ganoderma lucidum* powder (GLP) without nitrite (P), (ii) 0.5% w/w GLP with 80 ppm nitrite (N + P), and (iii) sausage with 120 ppm nitrate (N). Then, the prepared sausages packed using a commercial artificial casing and cooked in a water bath at 72°C for 2.5 h. Sausages were stored in a fridge at 3°C–4°C for 45 days.

### Sensory evaluations

Sensory assessment including six attributes; color, flavor, taste, texture, appearance, and overall acceptability were evaluated by twenty volunteered panelists (staff and students) from the Department of Nutrition and Food Science, Isfahan University of Medical Sciences,

Isfahan. Panelists were asked to present their idea using a nine-point hedonic scales, in which 1 was “extremely dislike” and 9 was “extremely like.”<sup>[19]</sup>

### Measurement of peroxide value

Peroxide value (PV) was determined according to the AOAC International 1991.<sup>[20]</sup> Five grams of each sample was weighed in a 100 ml glass stoppered Erlenmeyer flask and heated in a water bath at 60°C for 3 min to melt the fat. Then, samples were mixed with 30 ml acetic acid: chloroform solution (3:2 v/v) for 3 min to dissolve the fat. Samples were then filtered through a Whatman filter paper using vacuum. Saturated potassium iodide solution (0.5 ml) was added to the filtrate. The titration was allowed to run against a standard solution of sodium thiosulfate (25 g/l). PV was calculated and expressed as milliequivalent peroxide per kilogram of sample:<sup>[20]</sup>

$$PV \text{ (meq/kg)} = (S \times N) / W \times 1000$$

Where S is the volume of titration (ml), N is the normality of sodium thiosulfate solution ( $n = 0.01$ ), and W is the sample weight (kg).

### Measurement of thiobarbituric acid value

2-thiobarbituric acid (TBA) assays were conducted following the procedure described by Buege and Aust.<sup>[21]</sup> A composition of 0.5 g of each sample was mixed with 2.5 ml of 0.375% TBA - 15% TCA - 0.25 N HCl stock solution. The mixture was heated for 10 min in a water bath at 95°C–100°C to develop a pink color, then was cooled with tap water and centrifuged at 5500 rpm for 25 min in a ultracentrifuge (Sigma 3K30). The absorbance was measured at 532 nm using a UV/visible spectrophotometer (Cecil Instruments Ltd., United Kingdom). TBA number was calculated as mg malondialdehyde (MDA)/kg sample and calculated based on the following formula:<sup>[21]</sup>

$$TBA \text{ value} = \text{Absorbance at } 532 \text{ nm} \times 2.77$$

### Microbiological analysis

The samples of three treatment sausages were analyzed for microbiological quality during storage using standard procedures of Iran.<sup>[22-25]</sup> Ten grams of each sausage sample was homogenized with 90 ml sterile Ringer’s solution and after that serial dilutions were prepared. One milliliter of each dilution was transferred to the agar medium plates for bacterial total count (37°C, 48 h), YGC agar medium for yeast and molds (25°C, 5 d), VRBA medium for coliforms (30°C, 24 h), and SPS agar for *Clostridium perfringens* (37°C, 48 h).

A volume of 0.1 ml of each dilution was spread with a bent sterile glass rod on triplicate plates of pre-poured and dried on Baird-Parker agar for *Staphylococcus aureus* (37°C, 48 h). Colonies were counted after incubation and results were expressed as CFU/g of sausage sample.

### Statistical analyses

Each experiment was conducted in triplicate. The difference between treatments was assessed using analysis of variance (one-way ANOVA) followed by means comparison using either Tukey's HSD test, if variance was homogeneous, or Dunnett's T3 test, if variance was not homogeneous. The data were analyzed by analysis of variance (ANOVA) using IBM statistic SPSS for windows, version 22.0. Armonk, NY: IBM Corp.  $P < 0.05$  was considered statistically significant.

### Results

#### Sensory evaluations

The sensory panels collected for the different treatments are presented in Table 1. There was no statistically significant difference between N, P, and N + P treatments in texture, taste, smell, and color. The overall acceptability of N group was higher and N + P group was closer to N group. Sausage with 1% w/w GLP (P group) had the lowest score color.

#### Measurement of peroxide value

Changes occurring in the PV of different treatment groups during storage time were shown in Figure 1. In day 0, PV of P group was  $0.833 \pm 0.0577$ , and it was the highest ( $P < 0.01$ ) among treatments analyzed. In days 14, 21, 30, and 45, there was no significant difference between P and N samples, but the P + N samples showed lower PV at the storage period. PVs of all samples were below 25 meq of active  $O_2/kg$ , which is considered as acceptable limit for PV of fatty foods.

#### Measurement of 2-thiobarbituric acid value

The TBA values of sausage groups during 45 days of chilled storage are shown in Figure 2. There is no significant difference between N and N + P groups while P group shows the highest TBA value ( $P < 0.01$ ).

#### Microbiological analysis

According to Figure 3, total bacterial count increased with storage time. There was no significant difference between N and N + P during the first 14 days and day 30. There was a significant difference between N and P just on day 7 ( $P < 0.05$ ). Changes in microbial count during 30 days of storage in all sausage formulations remained below 6.98 Log CFU maximal permissible limit for total plate count recommended by ISIRI.

Based on microbiological results, no growth of *C. perfringens*, *Coliforms*, and *S. aureus* was detected

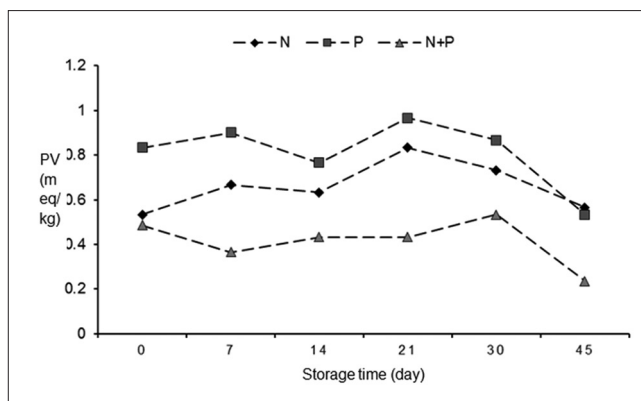


Figure 1: Peroxide value during storage at 4°C. N: Sausage with 120 ppm nitrite, P: Sausage with 1% *Ganoderma lucidum* powder, N + P: Sausage with 0.5% *Ganoderma lucidum* powder and 80 ppm nitrite

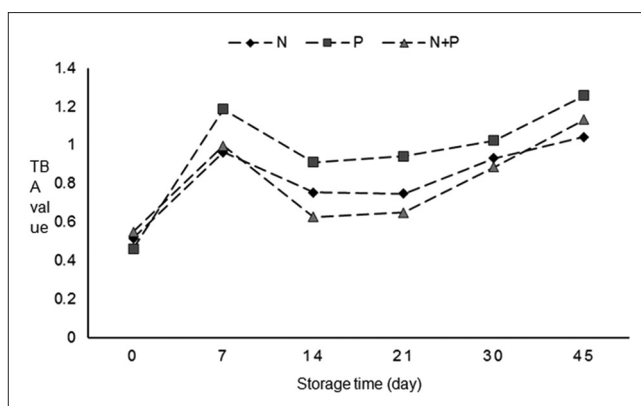


Figure 2: The 2-thiobarbituric acid during storage at 4°C. N: Sausage with 120 ppm nitrite, P: Sausage with 1% *Ganoderma lucidum* powder, N + P: Sausage with 0.5% *Ganoderma lucidum* powder and 80 ppm nitrite

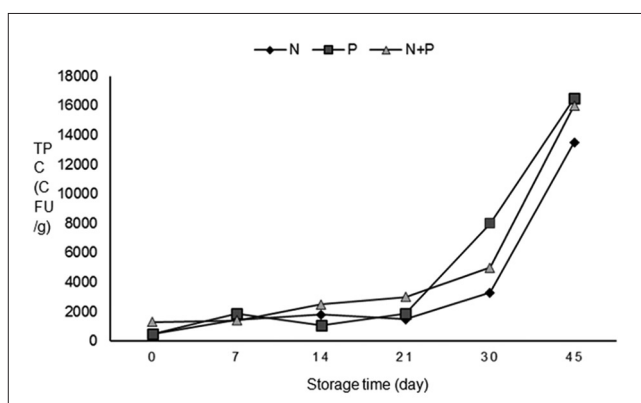


Figure 3: Total plate count during storage at 4°C. N: Sausage with 120 ppm nitrite, P: Sausage with 1% *Ganoderma lucidum* powder, N + P: Sausage with 0.5% *Ganoderma lucidum* powder and 80 ppm nitrite

Table 1: Means of sensory evaluations of sausage with/without *Ganoderma lucidum* powder

Sample	Texture	Taste	Color	Smell	Acceptability
N	6.33±1.054 <sup>a</sup>	7.00±2.070 <sup>a</sup>	7.06±1.907 <sup>a</sup>	7.06±1.709 <sup>a</sup>	6.93±1.830 <sup>a</sup>
P	6.33±1.759 <sup>a</sup>	5.86±2.030 <sup>a</sup>	4.93±2.016 <sup>a*</sup>	6.00±1.463 <sup>a</sup>	5.73±1.830 <sup>a*</sup>
N + P	6.33±1.046 <sup>a</sup>	6.26±1.099 <sup>a</sup>	6.26±1.099 <sup>a</sup>	7.06±1.486 <sup>a</sup>	6.66±0.975 <sup>a</sup>

\*\*Means parameters in the same column followed by different superscripts are significantly different ( $P < 0.05$ )



during 30 days of storage. In days 30 and 45, we observed 90 and 220 CFU/g of molds and yeasts, respectively, on the sausage samples in treatment P which was lower than which is recommended by ISIRI.

## Discussion

Recently, substitution of natural preservatives for chemicals in food formulation has received special attention. Some studies have reported equal and/or even higher antioxidant effects from natural preservatives than chemical. The results of this study showed that GLP as a natural ingredient in sausage formulation could be used to reduce the amount of nitrite without significant adverse effects on sausage characters.

*G. lucidum* previously showed antioxidant activity *in vitro* and *in vivo* studies.<sup>[26,27]</sup> Tao *et al.* showed that two polysaccharides from *G. lucidum* (GLP1 from fermentation broth and GLP2 from fruiting body) have strong antioxidant activities.<sup>[28]</sup>

Measurement of MDA that produced in the process of lipid peroxidation is used to evaluate lipid peroxidation to assess oxidative stress. TBA value was increased in the first stage of storage because of the MDA decomposition and polymerization. The gradual decrease in thiobarbituric acid reactive species between days 7 and 21 could be caused by microbial-mediated decay of MDA and its oxidation to other products such as alcohol and acid.<sup>[29,30]</sup> Wannasupchue *et al.* showed that increasing of TBA value can be controlled using water extract and spore of *G. lucidum* in smoked fish sausages.<sup>[15]</sup>

The data are in agreement with what reported earlier by Quereshi *et al.* and Yoon *et al.* who claimed that the different *G. lucidum* extracts can prevent bacterial growth.<sup>[31,32]</sup> Keypour *et al.* showed that chloroform extract of *G. lucidum* has antibacterial effect against *Bacillus subtilis* and *S. aureus* in all concentrations.<sup>[33]</sup> After pasteurization (at 72°C), no sign of *Coliforms* bacteria was observed in any samples mainly owing to their heat sensitivity. Moreover, the lack of oxygen in packed sausage further inhibited their growth. This confirms the appropriate hygiene quality of the product and production environment. To date, although the antimicrobial activity of *G. lucidum* compounds was widely reported in culture media, few reports are available on its effect in processed foods.

## Conclusion

Based on the results obtained in the present study, sausage samples with 0.5% w/w GLP which reduced in nitrite were quite acceptable. Addition of GLP provides antioxidant and antimicrobial benefits during the storage, but GLP at 1% w/w did not result in high acceptance of color, so the use of nitrite was required. Therefore, it is suggested that GLP as a natural medical herb, could be used to reduce the usage of synthetic additives.

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## Conflicts of interest

There are no conflicts of interest.

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