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Effects of mulberry powder on quality, texture, oxidative stability, and microbiological characteristics of white pork sausage (Moo Yor) during refrigerated storage

Viboon Pongkanpai,[1] Chatchawan Chotimarkorn,[1] Patima Permpoonpattana,[2] Saneeta Salam,[2] Umaporn Pastsart[2]

[1]Department of Food Technology, Faculty of Innovative Agriculture, Fisheries and Food, Prince of Songkla University, Surat Thani Campus; [2]Department of Agricultural Science and Technology, Faculty of Innovative Agriculture, Fisheries and Food, Prince of Songkla University, Surat Thani Campus, Thailand

Correspondence: Umaporn Pastsart, Department of Agricultural Science and Technology, Faculty of Innovative Agriculture, Fisheries and Food, Prince of Songkla University, Surat Thani Campus, Thailand 84000. Tel.: +66 77 278891. E-mail: umaporn.p@psu.ac.th

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Contributions: Umaporn Pastsart: study concept and design. Chatchawan Chotimarkorn, Patima Permpoonpattana, Saneeta Salam: experiments, data analysis, and draft of the manuscript. Viboon Pongkanpai: data analysis and manuscript revision. All authors read and approved the final manuscript.

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Abstract
The study evaluated the effects of mulberry (*Morus alba* L.) powder as a natural antioxidant on color, texture, antioxidant activity, lipid oxidation, and microbial quality of white pork sausage (Moo Yor) during refrigerated storage. Four treatment groups were prepared. Group 1 was formulated with 0% mulberry (control), group 2 was formulated with 1% mulberry, group 3 was formulated with 2% mulberry and group 4 was formulated with butylated hydroxytoluene (BHT) 0.02%. The results showed that the 2% mulberry group had significantly lower lightness (L*) values and higher redness (a*) values ($p<0.05$). Texture profile analysis showed that none of the texture parameters were significantly affected ($p>0.05$). Although microbial counts increased during storage, no significant differences in total plate counts were observed among the treatments ($p>0.05$). Moreover, the addition of mulberry led to a higher antioxidant activity and a lower lipid oxidation ($p<0.05$). These findings indicate that mulberry powder can effectively replace BHT as a natural antioxidant, helping to protect meat products from oxidative deterioration while maintaining product quality without negatively affecting the texture or microbial quality of white pork sausage during refrigerated storage.

Introduction
The white pork sausage, Moo Yor, is a traditional meat emulsion sausage and famous product in northern and northeastern region of Thailand. It is made by grinding and blending meat with fat, and various curing and flavoring agents, such as salt, sugar, pepper and tapioca starch. Normally, White pork sausage has a short shelf-life which is about 2 weeks in refrigerator (Klinjapo and Krasaekoopt, 2021). In processed meat products, one of the most important concerns is deterioration from the oxidation of fats and proteins. In particular, the oxidation of fats often results in changes in color, flavor, texture and nutritional values (Kanner, 1994). However, processed meat products are often loaded with synthetic antioxidant and antimicrobial additives to reduce oxidation and inhibit spoilage microorganisms such as butylated hydroxytoluene (BHT). These synthetic additives used in the meat processing industry have a detrimental effect on the consumer's health. Nowadays, there is an awareness of the consumption of these substances as it may remain and is unsafe for health (Saito *et al.*, 2003). Making processed meat products safer can be done by substituting synthetic substances that affect consumers with natural colorants and antioxidants to the products, *e.g.*, flavonoids, which help to prevent free radicals from oxidations and prolong shelf-life of products (Tsanova-Savova *et al.*, 2018).
Mulberry (*Morus alba* L.) belongs to the genus *Morus* and belongs to the family Moraceae and widely found in Southeast Asian countries. Mulberry fruit has delicious taste and high nutritional value as a source of polyphenols, especially flavonoid, flavanones and anthocyanin (Khalifa *et al.*, 2018). Mulberry fruits are also abundant in essential vitamins, minerals, and a diverse range of phenolic compounds such as cyanidin-3-O-glucoside, cyanidin-3-O-rutinoside, rutin, isoquercitrin, resveratrol, and caffeic acid (Xu *et al.*, 2018). These bioactive phenolic compounds play several important biological roles, including antioxidant, antimicrobial, anti-inflammatory, hepatoprotective, and anti-apoptotic activities. They can also enhance immune responses, which highlights their potential applications in the food, pharmaceutical, and cosmetic industries (Natić *et al.*, 2015). When incorporated into meat systems, mulberry can enhance antioxidant capacity and help maintain product quality during storage. Previous studies have demonstrated that the addition of mulberry to various meat products, such as minced pork, dried pork, and Cantonese sausage, effectively enhanced antioxidant potential and improved overall product stability during shelf life (Xu *et al.*, 2018; Xiang *et al.*, 2019; Cheng *et al.*, 2020). Moreover, the phenolic constituents of mulberries may interact with proteins through covalent and non-covalent mechanisms, thereby influencing structural stability, functional groups, solubility, and aggregation, which can ultimately modify protein functionalities in either beneficial or adverse ways (Cao *et al.*, 2016). Besides its antioxidant properties, mulberry contains anthocyanins that may influence the color characteristics of meat products. Therefore, mulberry powder (MP) was selected as a natural source of antioxidants

for white pork sausage, while its potential effects on product color were also evaluated.
Therefore, the objective of this study was to investigate the effect of MP as a natural substitute for BHT in white pork sausage, focusing on its influence on appearance, texture, antioxidant activity, lipid oxidation, and microbial quality during refrigerated storage.

Materials and Methods

Preparation of mulberry fruit powder

The ripe fruits of mulberry were washed and dried at 60°C in a hot air oven to prepare dried mulberry with approximately 22.3% of dry matter content. Subsequently, dried mulberry was ground to obtain mulberry fruit fine powder by passing through a 20-mesh sieve. Then mulberry fruit powder was placed in a vacuum bag and stored at -20°C until use.

Preparation of white pork sausage with the addition of mulberry fruit powder

The white pork sausage consisted of 63% lean pork, 25% pork back fat, 7% ice, 2% sugar, 1.5% salt, 1% tapioca flour and 0.5% pepper. All ingredients were obtained from local market in Surat Thani province, Thailand. Briefly, lean pork, all flavoring agents and half of ice were chopped for 1 min and then pork back fat and remaining ice were added and chopped until they were homogenous. While chopping, temperature of mixture was controlled below 15°C. Subsequently, the batter of sausage was divided into 4 groups (3 replicates in each group) as follows; batter mixed with 0%, 1%, 2% MP and 0.02% BHT (Loba Chemie, Mumbai, India). The addition was based on batter weight. All treated groups were wrapped with banana leaves in a cylindrical shape, about 100 g each cylinder, then steamed for about 30 minutes until the white pork sausage was well cooked.

Color value (CIE Lab)

Internal color of white pork sausage was measured in terms of the CIE-Lab coordinates L*, a* and b* with a Hunterlab Miniscan color meter (Reston, VA, USA) (D65 light source, 10° standard observer, 45°/0° geometry, 1-inch light surface, white standard) following standard procedures.

Texture profile analysis

Texture profile analysis (TPA) was performed in duplicate for each sample at day 0 of storage using a Brookfield CT3 texture analyzer (Middleboro, MA, USA) (10,000 g) equipped with a TA-SBA shear blade probe. Pork sausages were cut into pieces measuring 5 cm in length, 1 cm in width, and 1 cm in height. Samples were compressed to 75% of their original height in a double-cycle test at a speed of 2 mm/s. The TPA parameters, including hardness, adhesiveness, cohesiveness, springiness, gumminess, and chewiness, were recorded.

Antioxidant activity

Antioxidant capacity of white pork sausage was determined using the DPPH radical scavenging assay following the procedure of Wang *et al.* (2019), with minor modifications. Analyses were performed at 0, 5, and 10 days of storage after sausages were wrapped in oxygen-permeable film and kept at 4°C under fluorescent light. DPPH (2,2-diphenyl-1-picrylhydrazyl) dissolved in ethanol served as a free radical donor, and the decrease in absorbance at 517 nm was measured spectrophotometrically. For extraction, 2.5 g of sausage was homogenized with 7.5 mL ethanol, shaken for 10 min at room temperature, and centrifuged at 1,800 rpm for 10 min. The supernatant (0.5 mL) was then mixed with 3.5 mL of 0.1 mM DPPH solution and incubated in the dark for 20 min. Ethanol served as the blank. All measurements were performed in triplicate. DPPH scavenging activity (%) was calculated as [Eq. 1]:

$$\text{Scavenging activity (\%)} = (1 - As/Ac) \times 100 \qquad \text{[Eq. 1]}$$

where As is the absorbance of the sample and Ac is the absorbance of the control.

Determination of lipid oxidation
Lipid oxidation of white pork sausage was evaluated on days 0, 5, and 10 of storage using the thiobarbituric acid reactive substances (TBARS) assay as described by Tarladgis *et al*. (1960). Results were expressed as µg malondialdehyde (MDA) per g of meat. Briefly, 10 g of sample was homogenized with 40 mL of distilled water and 1 mL of BHT solution using an Ultra-Turrax homogenizer at 13,000 rpm. The homogenate was mixed with 3 mL of 4 M HCl and subjected to distillation. A 5 mL portion of the distillate was combined with 1 mL of TBA reagent, heated in a boiling water bath for 35 min, cooled to room temperature, and the absorbance was recorded at 532 nm.

Microbiological analysis
Total plate count (TPC) of white pork sausage was determined according to standard microbiological procedures. Samples were analyzed on days 0, 5, and 10 of refrigerated storage. For analysis, 1 g of sausage sample was aseptically transferred into a sterile tube containing 9 mL of sterile 0.9% NaCl and homogenized. Serial decimal dilutions were then prepared, and appropriate dilutions were spread in duplicate on plate count agar. Plates were incubated at 37°C for 24 h. Microbial counts were expressed as log CFU/g of sample.

Statistical analysis
Data were analyzed for the fixed effect of treatment using analysis of variance. Differences among means were compared by Duncan's multiple range test at a significance level of $p<0.05$. All statistical analyses were performed with SPSS software.

Results
Color
The color values of white pork sausage during refrigerated storage (0, 5, and 10 days) are presented in Table 1. Sausages supplemented with 1% and 2% MP exhibited significantly lower lightness (L*) and higher redness (a*) compared with the control and BHT treatments ($p<0.05$). In contrast, mulberry supplementation had no significant effect on yellowness (b*) throughout storage ($p>0.05$).

Texture profile analysis
TPA results of white pork sausage at day 0 of storage are summarized in Table 2. MP supplementation had no significant effect on hardness, adhesiveness, springiness, cohesiveness, gumminess, or chewiness ($p>0.05$). Although group 1 (control) and group 4 (BHT) tended to show higher values for hardness, cohesiveness, gumminess, and chewiness compared with groups 2 and 3 (1% and 2% mulberry), these differences were not statistically significant ($p>0.05$).

Antioxidant activity
Figure 1 illustrates the DPPH radical scavenging activity of white pork sausage during refrigerated storage. Sausages with MP 2% had the highest antioxidant activity on day 0, followed by MP 1%, BHT, and the control ($p<0.05$). On day five, a similar pattern was seen, with MP 2% > MP 1% > BHT > control ($p<0.05$) being the order of antioxidant activity. By day 10, the antioxidant capacity of sausages supplemented with mulberries was consistently higher than that of BHT and the control ($p<0.05$). These findings suggest that MP, especially at 2%, can effectively boost the antioxidant qualities of white pork sausage and could be used as a natural substitute for artificial antioxidants in cold storage.

Lipid oxidation
The lipid oxidation values of white pork sausage during cold storage are presented in Figure 2. On day 0, sausages supplemented with 1% and 2% MP showed significantly lower values ($p<0.05$), while the control group had the highest TBARS value ($p<0.05$), followed by the BHT group. By

day 5, lipid oxidation increased across all treatments, but the lowest TBARS values were consistently observed in the mulberry-supplemented groups, particularly at 2% ($p<0.05$). A similar trend was evident on day 10, where the control group had the greatest extent of lipid oxidation, followed by the BHT group, whereas sausages with 1% and 2% MP maintained significantly reduced MDA levels ($p<0.05$). Overall, the findings unequivocally show that adding MP to white pork sausage during chilled storage successfully prevented lipid oxidation, and the antioxidative effect increased as mulberry concentration increased.

Total plate count

Changes in TPC of white pork sausage during refrigerated storage are shown in Figure 3. At day 0, all treatments exhibited low microbial counts. As storage progressed, TPC values gradually increased in all treatment groups; however, no significant differences were detected among treatments throughout the storage period ($p>0.05$).

Discussion

The inclusion of MP into white pork sausage had a notable effect on product quality during refrigerated storage. In terms of appearance, sausages supplemented with mulberry (1-2%) exhibited lower lightness (L*) and higher redness (a*) compared to the control and BHT treatments. This increase in redness is mainly attributed to the presence of anthocyanins and other polyphenols in mulberry, which act as strong natural pigments (Khalifa *et al*., 2018). Mulberry extracts have been shown to considerably increase the redness and oxidative stability of Cantonese sausages and raw ground beef during refrigerated storage (Zhang *et al*., 2016; Xiang *et al*., 2019). These findings confirm the dual role of mulberry as both a natural antioxidant and colorant, increasing consumer appeal of meat products.

Conversely, the addition of mulberry had no significant effect on the texture parameters of the sausages, including adhesiveness, springiness, cohesiveness, gumminess, and chewiness ($p>0.05$). Although small numerical differences in hardness were observed, these were not statistically significant. The lack of texture modification suggests that mulberry polyphenols at the tested levels do not interfere with protein gelation or water-binding properties of meat emulsions. This observation aligns with previous studies suggesting that, while phenolic compounds may interact with proteins (Cao *et al*., 2016), moderate inclusion levels of plant-derived antioxidants generally do not compromise textural quality of meat products (Naveena *et al*., 2008). Regarding antioxidant capacity, mulberry supplementation markedly enhanced radical scavenging activity as determined by the DPPH assay. Sausages containing mulberry, especially at 2%, showed significantly higher antioxidant capacity than the control and BHT groups. This effect can be explained by the presence of phenolic compounds and anthocyanins that effectively donate hydrogen atoms or electrons to neutralize free radicals. These findings are consistent with earlier findings demonstrating that mulberry extracts suppressed lipid and protein oxidation in meat products (Xu *et al*., 2018; Cheng *et al*., 2020). The DPPH method itself is widely accepted for evaluating radical scavenging activity in food systems (Kedare and Singh, 2011).

The TBARS analysis further confirmed the antioxidative effect of mulberry during cold storage. Sausages formulated with MP had significantly lower MDA values compared with control samples across all storage times. The antioxidative protection was dose-dependent, with 2% mulberry showing the strongest effect, and in some cases performing better than BHT. This demonstrates the potential of mulberry to act as a natural substitute for synthetic antioxidants. These findings are in line with Xu *et al*. (2018) and Cheng *et al*. (2020), who reported that mulberry extracts effectively delayed lipid oxidation in pork products. Comparable effects of other plant-derived antioxidants such as pomegranate and grape extracts have also been reported in various meat systems (Ahn *et al*., 2002; Naveena *et al*., 2008).

The low TPC observed at day 0 in all treatments can be attributed to the steaming process, which effectively reduced the initial microbial load in white pork sausage (Jay *et al.*, 2005; ICMSF, 2011).

During refrigerated storage, TPC gradually increased in all treatments, which is commonly observed in cooked meat products due to post-processing contamination and favorable conditions for microbial growth during chilled storage (Toldrá, 2006; Zhou *et al*., 2010).
No significant differences in TPC were detected among treatments throughout storage (p>0.05), indicating that MP supplementation did not exert a strong antimicrobial effect under the conditions tested. This finding may be explained by the fact that plant-derived phenolic compounds, while exhibiting antimicrobial activity in vitro, often show limited effectiveness in complex meat systems due to interactions with proteins and lipids (Burt, 2004; Gyawali and Ibrahim, 2014). Similar observations have been reported in studies where natural antioxidants improved oxidative stability of cooked meat products without significantly altering microbial counts (Naveena *et al*., 2008).

Conclusions

Overall, this study demonstrated that MP is an effective natural additive for improving the quality and stability of white pork sausage. The addition of mulberry, particularly at 2%, enhanced product redness without negatively affecting texture, increased antioxidant activity, and significantly reduced lipid oxidation during refrigerated storage. These results suggest that mulberry can serve as a natural alternative to synthetic antioxidants such as BHT by enhancing oxidative stability and maintaining product quality during refrigerated storage. The findings also support the growing interest in using plant-based ingredients to meet consumer demand for healthier and clean-label food options.

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Table 1. Effects of mulberry (*Morus alba* L.) powder on color of white pork sausage (Moo Yor) during refrigerated storage (mean ± SD).

Item	Concentration of mulberry powder (%)			0.02% BHT	p
	0	1	2		
Day 0					
*L**	69.67±0.17[a]	60.20±0.39[b]	54.96±0.03[c]	69.67±0.23[a]	0.00
*a**	1.98±0.01[c]	4.21±0.03[b]	5.60±0.14[a]	2.07±0.30[c]	0.00
*b**	14.61±0.04	15.10±0.11	15.84±0.48	15.19±0.66	0.16
Day 5					
*L**	70.37±1.22[a]	62.21±0.24[b]	56.24±0.75[c]	69.94±0.16[a]	0.00
*a**	2.10±0.16[d]	3.65±0.14[b]	5.41±0.05[a]	2.56±0.07[c]	0.00
*b**	15.03±0.03	14.61±0.53	15.17±0.40	14.87±0.31	0.35
Day 10					
*L**	71.25±1.33[a]	64.60±2.09[b]	58.62±0.43[c]	70.85±1.05[a]	0.00
*a**	1.80±0.19[c]	3.00±0.43[b]	4.68±0.19[a]	1.86±0.41[c]	0.00
*b**	13.99±0.49	14.44±1.52	13.16±0.47	13.98±0.25	0.37

[a-d]Within a row, mean values with different superscripts differ significantly at $p<0.05$.

Table 2. Effects of mulberry (*Morus alba* L.) powder on texture profile parameters of white pork sausage (Moo Yor) at day 0 of refrigerated storage (mean ± SD).

Item	Concentration of mulberry powder (%)			0.02% BHT	p
	0	1	2		
Hardness (g)	426.00±60.95	304.50±153.29	294.20±119.43	375.80±61.53	0.09
Adhesiveness (mJ)	1.20±0.64	0.65±0.36	1.06±0.44	1.24±0.81	0.46
Springiness (mm)	19.25±10.90	10.71±5.42	10.08±1.18	19.46±12.90	0.22
Cohesiveness	0.27±0.21	0.12±0.09	0.08±0.06	0.42±0.40	0.16
Gumminess (g)	86.75±33.01	34.50±25.42	31.40±27.01	83.67±48.61	0.07
Chewiness (mJ)	15.30±11.85	3.55±3.07	9.43±6.78	9.43±5.25	0.08

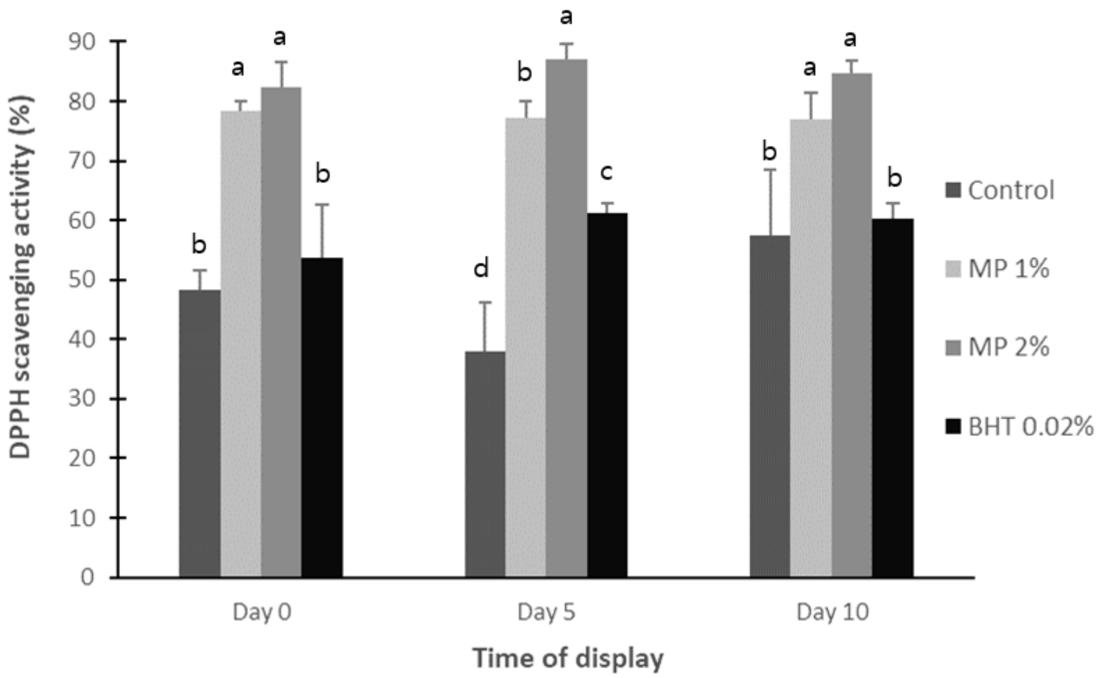

Figure 1. Mean values of DPPH radical scavenging activity (%) of white pork sausage (Moo Yor) supplemented with mulberry powder (MP) during refrigerated storage. Error bars indicate standard deviations. Different superscript letters (a–d) within the same storage day denote significant differences (p<0.05).

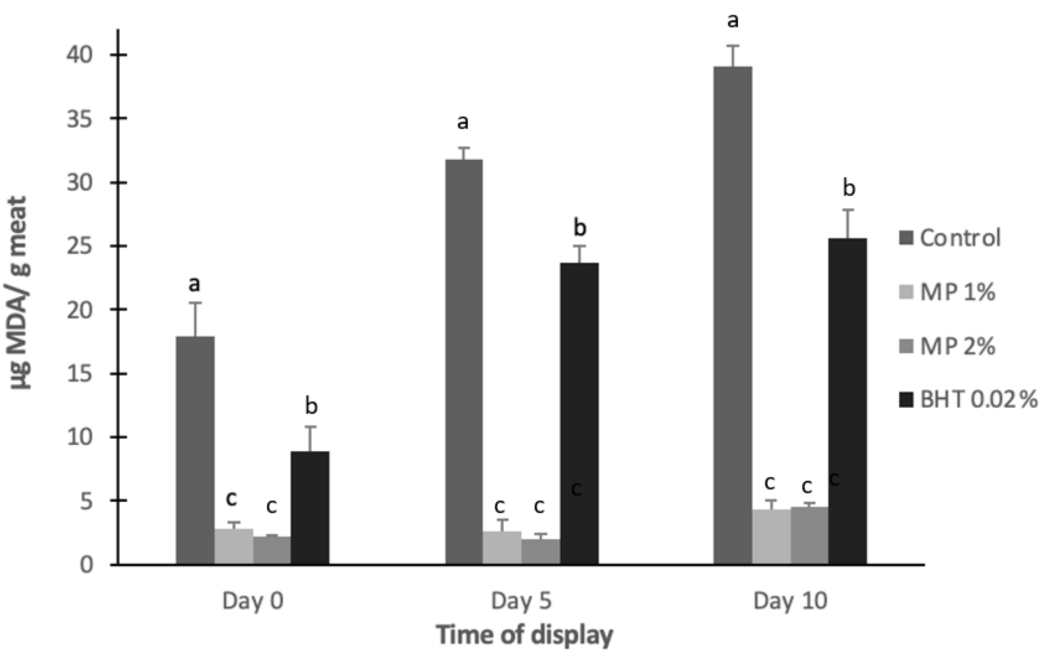

Figure 2. Mean values of lipid oxidation expressed as µg malondialdehyde (MDA) per g of meat in white pork sausage (Moo Yor) supplemented with mulberry powder (MP) during refrigerated storage. Error bars indicate standard deviations. Different superscript letters (a–c) within the same storage day denote significant differences (p<0.05).

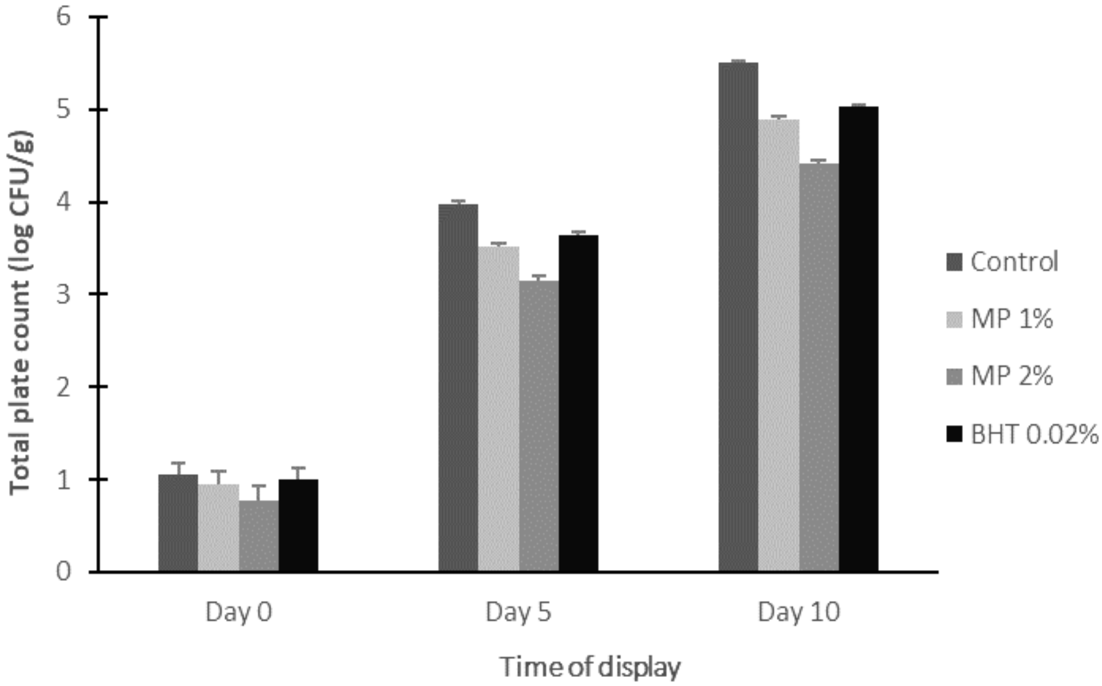

Figure 3. Mean values of total plate count (log CFU/g) of white pork sausage (Moo Yor) supplemented with mulberry powder (MP) during refrigerated storage. Error bars indicate standard deviations.