INVESTIGATION OF THE POTENTIAL UTILITY OF PERILLA ESSENTIAL OIL IN PRESERVATION OF FRESH PORK

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Received date: 12.04.2016 Accepted date: 10.08.2016

ABSTRACT

This study examined the effects of perilla essential oil treatments on pH, NH_3, and thiobarbituric acid (TBA), and microbiological indices, including total aerobic counts, *E. coli*, and *Staphylococcus aureus*, on the shelf-life of fresh pork. Fresh sirloin pork was sprayed with perilla oil at two concentrations of 1% (v/v) and 2% (v/v) and stored at 5°C. Results revealed that compared to the control samples stored in the same refrigerated cold condition, the shelf-life of the treated sirloin pork was extended to 6 and 9 days, respectively to the two concentrations of perilla oil applied. This indicates that perrila essential oil might play an important role as antioxidant and antibacterial agent in prolonging the shelf-life of refrigerated fresh pork. The treatment with 1% perilla oil had less effect on color and flavor of the fresh pork sample compared to concentration of 2%.

Keywords: Fresh pork, perilla essential oil, preservation.

Nghiên cứu khả năng sử dụng tinh dầu lá tía tô trong bảo quản thịt lợn

TÓM TẮT

Nghiên cứu này nhằm mục đích xác định ảnh hưởng việc xử lý tinh dầu lá tía tô đến các chỉ tiêu hóa lý (pH, NH_3, TBA) vi sinh (vi khuẩn hiếu khí tổng số, *E.coli, Staphylococcus aureus)* và thời hạn bảo quản thịt lợn tươi. Thịt thăn lợn tươi được tiến hành phun tinh dầu ở nồng độ 1%, 2%(v/v) và bảo quản ở điều kiện lạnh (5°C). Kết quả cho thấy tinh dầu lá tía tô có thể kéo dài thời gian bảo quản thịt lợn tươi ở điều kiện lạnh (5°C) đến 6 ngày ở nồng độ xử lý 1% và đến 9 ngày ở nồng độ xử lý 2%. Điều này chỉ ra rằng tinh dầu lá tía tô có khả năng chống oxy hóa và kháng khuẩn quan trọng trong việc kéo dài thời gian bảo quản của thịt lợn tươi. Xử lý tinh dầu tía tô 1 % ảnh hưởng ít hơn đến màu và mùi của thịt lợn so với nồng độ 2%.

Từ khóa: Bảo quản, tinh dầu lá tía tô, thịt lợn tươi.

1. INTRODUCTION

Meat and its products have experienced increasing popularity and have become widely enjoyed all over the world. However, meat products typically spoil during refrigeration due to two major causes: microbial growth and oxidative rancidity (Sebranek *et al.*, 2005). Lipid oxidation leads to the degradation of lipids and proteins, which in turn, contribute to reductions in nutritional quality as well as deterioration in flavor, color, and texture of displayed meat products (Aguirrezábal *et al.*, 2000), while bacterial contamination can potentially pose major public health hazards and economic losses in terms of food poisoning and meat spoilage (Fernández-López *et al.*, 2005). Lipid oxidation and microbial growth during storage can be reduced by applying antioxidant and antimicrobial agents to the

meat products, leading to a retardation of spoilage, extension of shelf-life, and maintenance of quality and safety (Devatkal and Naveena, 2010). Although several synthetic food additives have been widely used in the meat industry to extend food shelf-life, inhibit lipid oxidation, and delay or inhibit the growth of pathogenic microorganisms, the trend is to decrease their use because of the growing concern among consumers about such chemical additives. Consequently, the search for natural additives, especially of plant origin, has notably increased in recent years indicating that the application of natural food additives possessing both antioxidant and antimicrobial activities may be useful for maintaining meat quality, extending shelf-life, and preventing economic losses (Yin and Cheng, 2003; Mielnik *et al.*, 2008). Essential oils (EOs) are regarded as natural alternatives to chemical preservatives and their use in food meets the demands of consumers for mildly processed or natural products, since in modern food industries, mild processing is applied in order to obtain safe products that have a natural or "green" image. In this context, plant essential oils are gaining interest for their potential as preservative ingredients or decontaminating treatments, as they have GRAS (generally recognized as safe) status and a wide acceptance from consumers (Burt *et al.*, 2004). Researchers have also reported the efficacy of plant EOs as antimicrobial agents against foodborne pathogens and spoilage microflora in meat (Ouattara *et al.,* 1997; Busatta *et al.*, 2008).

A property of perilla oil that makes it unique among other essential oils is the fact that a small amount of perilla oil could offer significant antioxidant and antimicrobial activities while being safe for human consumption and being able to be applied to different foods with various pH values (Yu *et al.*, 1997). However, there has not been much research on the application of perilla essential oil in the preservation of meat. The objective of the present study was to investigate the antioxidant as well as the antimicrobial effectiveness of perilla essential oil on the quality of fresh pork during refrigerated storage at 5ºC.

2. MATERIALS AND METHODS

2.1. Materials

Fresh sirloin pork was sourced from Minh Hien Import and Export Company Ltd., Hanoi. Perilla leaves were purchased from Van Noi, Dong Anh. The perilla oil was extracted and distilled at the Centre of Essential Oils, Institute of Food Technology.

2.2. Methods

The preservation experiments were conducted using refrigerated storage at 5°C. Studied parameters and indices were monitored at 0, 3, 6, and 9 days from the start of preservation. The study was designed using four different experimental treatments as listed in Table 1. Each experimental treatment was repeated three times, using 100 g of fresh sirloin pork each time. For treated samples, the fresh sirloin pork was sprayed with 1% or 2% perilla oil at a rate of 3 mL per 100 g of pork. The samples were formed on plates and wrapped with plastic wrap (20 μm thickness).

Table 1. Experimental treatments used to test the preservation of sirloin pork

Samples	Experimental treatment details
M0 (Control 1)	Untreated samples
M1 (Control 2)	Sample sprayed with 10% propyleneglycol
M2	Sample sprayed with 1% perilla oil at 3 mL per 100 g pork
M3	Sample sprayed with 2% perilla oil at 3 mL per 100 g pork

Perilla oil at concentrations of 1% (v/v) and 2% (v/v) were prepared by diluting pure perilla essential oil in propyleneglycol 10%.

The chemical and microbial examinations of the pork samples were carried out following recommended techniques in TCVN:

+ pH: TCVN 4835:2002 (ISO 2917:1999)

+ NH_3: TCVN 3706:1990

+ Total aerobic counts: TCVN 4884:2002 (ISO 4833:2003)

+ *Escherichia coli*: TCVN 7924-2:2008 (ISO 16649-2:2001)

+ *Staphylococcus aureus*: TCVN 4830-1:2005 (ISO 6888-1-1999)

A thiobarbituric acid (TBA) analysis was performed as described by Pikul *et al.* (1989). A 10 g meat sample was homogenized with 35 mL of cold (4°C) extraction solution containing 4% perchloric acid and 1 ml of Butylated Hydroxyanisole (BHA). The blended sample was filtered through a Whatman No.4 filter paper into a 50 mL Erlenmeyer flask and washed with 5 mL of distilled water. The filtrate was adjusted to 50 mL with 4% perchloric acid, and 5 mL of the filtrate was added to 5 mL of 0.02 M TBA. Test tubes were heated in a thermostatically controlled water bath for 20, 30, 40, 50, and 60 min at 80 ± 2 °C to develop the malonaldehyde-TBA complex, and then cooled for 10 min with cold tap water. The absorbance was determined by a UV scanning spectrophotometer at 532 nm against a blank containing distilled water and 5 ml of 0.02 M TBA solution.

2.3. Statistical analysis

Each experiment was carried out in triplicate. The results were statistically analyzed using a one way analysis of variance (ANOVA) test with mean square error at 5% probability calculated with the Irristat 4.0 Software.

3. RESULTS AND DISCUSSION

3.1. Effects of perilla oil treatments on chemical indices of fresh pork during refrigerated storage at 5°C

3.1.1. Effects of perilla oil treatments on pH

pH has a high influence on water holding capacity, which is closely related to quality and microbial activity in meat. pH values play an important role in meat products because high pH is associated with high water holding capacity which facilitates microbial activities. On the other hand, low pH is often related to low water holding capacity, and pH acid often inhibits microbial growth. High pH gives meat a dark color while low pH causes pale meat. Both dark and pale colors are unattractive pork colors to consumers (Nguyễn and Nguyễn, 2008).

The pH values of the control and treated samples during storage at 5°C were monitored and measured. The results are shown in Table 2.

As can be seen in Table 2, the M0 and M1 control pork samples showed a decrease trend in their pH values during the first 3 (M0) and 6 (M1) days of storage, yet increased after that to a significantly higher pH value at the 9th day of storage. On the other hand, the pH values of the M2 and M3 treated pork samples gradually declined throughout the whole storage period. It is worthy to note that there were significant differences ($P \leq 0.05$) in the pH values of controls and treated pork samples at the 3rd and 9th days of storage. Changes in meat pH resulted from biochemical reactions and microbial activities in meat. The pH lowering during the first few days of storage is due to the meat muscle being at its rigor mortis state which produces lactic acid. Over time, meat proteins are denatured vigorously at various degrees, and together with microbial activities, continue leading to the formation of alkaline compounds which then increase meat pH. This may be attributed to the activation effect of the microbial load that causes protein hydrolysis with the appearance of alkyl groups (Yassin-Nessrien, 2003). The oil treated samples (M2 and M3) showed a declining direction in pH over time, compared to the increasing trend in pH of the untreated samples, indicating that the perilla oil treatments remarkably affected the growth of spoilage microbial organisms in meat, leading to a retardation of meat spoilage during storage.

Table 2. pH values of controls and treated fresh pork samples during cold storage at 5°C

Samples	Storage time			
	0 day	3rd day	6th day	9th day
M0	$5.77^{Ac} \pm 0.03$	$5.50^{Ad} \pm 0.02$	$5.61^{Ab} \pm 0,04$	$6.17^{Aa} \pm 0.06$
M1	$5.74^{ABa} \pm 0.03$	$5.57^{Bb} \pm 0.05$	$5.53^{Bc} \pm 0,03$	$5.72^{Ba} \pm 0.02$
M2	$5.73^{Ba} \pm 0.02$	$5.64^{Cb} \pm 0.05$	$5.55^{Bc} \pm 0,06$	$5.45^{Cd} \pm 0.03$
M3	$5.72^{Ba} \pm 0.03$	$5.69^{Da} \pm 0.03$	$5.61^{Ab} \pm 0,05$	$5.40^{Dc} \pm 0.04$

Note: A-D: Within a column, different letters indicate significant differences (P ≤ 0.05);
a-d: Within a row, different letters indicate significant differences (P ≤ 0.05)

Table 3. Ammonia concentration (mg/100 g pork) in controls and treated fresh pork samples during cold storage at 5°C

Samples	Storage time			
	0 day	3rd day	6th day	9th day
M0	$3.90^{Aa} \pm 0.59$	$26.04^{Ab} \pm 2.29$	$67.99^{Ac} \pm 1.75$	$95.98^{Ad} \pm 2.29$
M1	$3.18^{Ba} \pm 0.33$	$21.76^{Bb} \pm 1.75$	$47.79^{Bc} \pm 2.88$	$84.69^{Bd} \pm 4.57$
M2	$3.23^{Ba} \pm 0.25$	$15.94^{Cb} \pm 1.14$	$24.44^{Cc} \pm 2.88$	$50.51^{Cd} \pm 1.75$
M3	$3.27^{Ba} \pm 0.19$	$9.70^{Db} \pm 1.75$	$16.77^{Dc} \pm 2.88$	$35.57^{Dd} \pm 1.14$

Note: A-D: Within a column, different letters indicate significant differences (P ≤ 0.05);
a-d: Within a row, different letters indicate significant differences (P ≤ 0.05)

3.1.2. Effects of perilla oil treatments on ammonia (NH_3) concentration

Ammonia concentration is also an important criterion in accessing meat quality. Ammonia is the final product in the self-decomposition/denaturation of meat proteins. It is also a result of the activity of microbial organisms and proteolytic enzymes that breakdown meat proteins (Yassin-Nessrien, 2003). It was observed in our study that ammonia concentrations increased at different rates for different treatments (Table 3).

The oil treated samples had a slower rate of increase in ammonia concentration compared to a higher incremental rate of ammonia concentration over time in the non-oil treated samples (controls). The higher applied oil concentration (2%) led to a significantly slower rate ($P \leq 0.05$) of increase in ammonia concentration compared to that of the lower oil concentration (1%) treated sample. Ammonia concentrations of both oil treated samples were still lower than the acceptable limit of 35 mg per g of pork (TCVN 7046:2009) after six days, while it was over this level in the two controls. Our findings were in agreement with the study from Salem *et al.* (2010), in which garlic and lemon grass oils were used for beef preservation.

3.1.3. Effects of perilla oil treatments on lipid oxidation

Oxidation of lipids leading to rancidity is one of the most important changes during food storage and production (Melton, 1983; Rosmini *et al.*, 1996). Lipid oxidation gives rise to products that may have changes in the color, aroma, flavor, texture, and even the nutritive value of the food (Fernandez *et al.*, 1997). The thiobarbituric acid (TBA) value is routinely used as an index of lipid oxidation in meat products in stores (Raharjo and Sofos, 1999). This value was examined over the course of our study by measuring the absorbance of the malonaldehyde-TBA complex in the control and treated samples during cold storage at 5°C. Results are shown in Table 4.

Table 4. Absorbance of malonaldehyde-TBA complex of the control and treated fresh pork samples during cold storage at 5°C

Samples	Storage time			
	0 day	3rd day	6th day	9th day
M0	$0.0175^{Ad} \pm 0.0005$	$0.0271^{Ac} \pm 0.0013$	$0.0432^{Ab} \pm 0.0008$	$0.0671^{Aa} \pm 0.0015$
M1	$0.0174^{Ad} \pm 0.0005$	$0.0264^{Ac} \pm 0.0014$	$0.0424^{Ab} \pm 0.0016$	$0.0655^{Aa} \pm 0.0020$
M2	$0.0157^{Bbc} \pm 0.0007$	$0.0163^{Cb} \pm 0.0008$	$0.0238^{Cd} \pm 0.0011$	$0.0328^{Ca} \pm 0.0004$
M3	$0.0156^{Bcb} \pm 0.0007$	$0.0146^{Dd} \pm 0.0006$	$0.0158^{Db} \pm 0.0009$	$0.0204^{Da} \pm 0.0009$

Note: A-D: Within a column, different letters indicate significant differences ($P \leq 0.05$);
a-d: Within a row, different letters indicate significant differences ($P \leq 0.05$)

Table 5. Sensory evaluation of the control and treated fresh pork samples during cold storage at 5°C

Samples	Storage time			
	0 day	3rd day	6th day	9th day
M0	Bright red color, fresh meaty flavor, soft meat	Dark red color, fresh meaty flavor	Dark brown, slightly slimy on surface, stale odor	-
M1	Bright red color, fresh meaty flavor, soft meat	Dark red color, fresh meaty flavor	Reddish brown, slightly stale odor	-
M2	Slightly dark red color, slight perilla flavor	Slightly dark red color, slight perilla flavor	Slightly dark red color, slight perilla flavor	Dark red color, stale odor
M3	Dark red color, noticeable perilla flavor	Dark red color, noticeable perilla flavor	Dark red color, noticeable perilla flavor	Dark red color, noticeable perilla flavor

Note: "-": spoiled sample, unfit for use

Table 6. Total aerobic counts of the control and treated fresh pork samples during cold storage (logCFU/g)

Samples	Storage time			
	0	3rd day	6th day	9th day
M0	$4.59^{Ad} \pm 0.04$	$5.18^{Ac} \pm 0.06$	$5.72^{Ab} \pm 0.06$	$6.10^{Aa} \pm 0.03$
M1	$4.59^{Ad} \pm 0.04$	$4.81^{Bc} \pm 0.01$	$5.27^{Bb} \pm 0.03$	$5.56^{Ba} \pm 0.04$
M2	$4.59^{Ac} \pm 0.04$	$4.51^{Cc} \pm 0.03$	$4.75^{Cb} \pm 0.12$	$4.92^{Ca} \pm 0.04$
M3	$4.59^{Ab} \pm 0.04$	$4.50^{Cc} \pm 0.02$	$4.58^{Db} \pm 0.03$	$4.86^{Da} \pm 0.05$

Note: A-D: Within a column, different letters indicate significant differences ($P \leq 0.05$);
a-d: Within a row, different letters indicate significant differences ($P \leq 0.05$)

It can be seen in Table 4 that the highest incremental rate was recorded in the untreated samples (controls), while the sample treated with 2% perilla essential oil showed the lowest significant ($P \leq 0.05$) incremental rate of TBA values over the storage time. The incremental pattern in TBA values for all the stored samples throughout the chilling storage time may be due to the auto-oxidation of meat lipids, bacteriological, and/or oxidative rancidity. It is obvious that the perilla oil treatments had positive effects in retarding lipid oxidation in meat. The higher the oil concentration, the greater the effect of inhibiting lipid oxidation was observed.

3.2. Effects of perilla oil treatments on sensorial characteristics of fresh pork during cold storage at 5°C

Direct addition of essential oils to food may alter their sensory characteristics (Seydim and Sarikus, 2006). Lipid oxidation and other degradation reactions lead to the formation of low molecular compounds, which contribute to the sensory profile. Hydroperoxides and secondary oxidation products can react with proteins and amino acids during processing and storage, thus affecting the flavor, odor, and texture of meat products (Frankel, 1998). Sensory evaluations of the control and treated samples during storage at 5°C are shown in Table 5.

As can be seen in Table 5, it is clear that perilla oil treatments led to changes in the studied pork color and flavor. The treatment with 2% perilla oil resulted in dark red color and more noticeable flavor, while the treatment with 1% perilla oil had less effect on the color and flavor of the fresh pork samples. Lower concentrations of perilla oil can be combined with other antimicrobial compounds and/or other preservative technologies to improve the microbial stability and the sensory quality of meat.

3.3. Effects of perilla oil treatments on microbial indices

3.3.1. Effects of perilla oil treatments on total aerobic counts

A significant level of spoilage of meat and meat products takes place every year at different levels of the production chain including preparation, storage, and distribution. Besides lipid oxidation and autolytic enzymatic spoilage, microbial spoilage plays a significant role in this deterioration process leading to substantial economic and environmental impacts (Dave & Ghaly, 2011).

The mean values of total aerobic counts of untreated (controls) and treated pork meat samples during cold storage are shown in Table 6.

The results shown in Table 6 indicate that at the 3rd, 6th, and 9th days of storage, total aerobic counts differed significantly at $\alpha = 5\%$ among samples. According to TCVN, the acceptable limit of total aerobic counts is 10^5. This value was exceeded in the control M0 sample on the 3rd and 6th days of storage, whereas it was still lower than the limit in both perilla oil treated samples. It is also clear to note that total aerobic counts in the 2% oil treated pork (M3) were significantly lower ($\alpha = 5\%$) than in the 1% oil treated sample (M2). Similar results were also observed in studies by Salem *et al.* (2010) and Fratiani *et al.* (2010). Marino *et al.* (2001) found that as the concentration of oil decreased, total aerobic counts increased.

3.3.2. Effects of perilla oil treatments on Escherichia coli

Escherichia coli (*E. coli*) bacteria can be found in different foods such as meat, fish, ham, and fermented pork ham. *E. coli* is one of many pathogenic microorganisms associated with fresh meat and meat products (Dave and Ghaly, 2011; Lucera *et al.*, 2012). Meat that becomes contaminated with *E. coli* is a major concern because meat is a highly nutritive food that is commonly eaten.

Monitoring *E. coli* during cold storage in this study revealed that *E. coli* counts increased during storage, especially more rapidly towards the end of the storage period in all samples. *E. coli* counts in both oil treated samples were significantly ($\alpha = 5\%$) lower than in the controls (Table 7). It is also worthy to note that on the 9th day of storage, while *E. coli* counts in the 2% oil treated sample (M3) were still lower than the TCVN acceptance limit of 10^2, both controls and the 1% perilla oil treated fresh pork exceeded this limit. This agrees well with the findings of Ouattara *et al.* (1997) and Gutierrez *et al.* (2009) on the inhibitory action of thyme essential oils against *E. coli* in food as well as in *in vitro* models.

Table 7. *Escherichia coli* counts of control and treated fresh pork samples during cold storage (logCFU/g)

Samples	Storage time			
	0 day	3rd day	6th day	9th day
M0	$1.57^{Ad} \pm 0.04$	$1.78^{Ac} \pm 0.07$	$1.99^{Ab} \pm 0.04$	$2.38^{Aa} \pm 0.01$
M1	$1.57^{Ac} \pm 0.04$	$1.75^{Ab} \pm 0.13$	$1.89^{Bb} \pm 0.03$	$2.23^{Ba} \pm 0.01$
M2	$1.57^{Ac} \pm 0.04$	$1.64^{Bcb} \pm 0.03$	$1.83^{Cb} \pm 0.01$	$2.12^{Ca} \pm 0.11$
M3	$1.57^{Ac} \pm 0.04$	$1.55^{Bc} \pm 0.08$	$1.73^{Db} \pm 0.02$	$1.98^{Da} \pm 0.07$

Note: A-D: Within a column, different letters indicate significant differences (P ≤ 0.05);
a-d: Within a row, different letters indicate significant differences (P ≤ 0.05)

Table 8. *Staphylococcus aureus* counts of control and treated fresh pork samples during cold storage (logCFU/g)

Samples	Storage time			
	0 day	3rd day	6th day	9th day
M0	$1.34^{Ad} \pm 0.32$	$1.88^{Ac} \pm 0.08$	$2.23^{Ab} \pm 0.03$	$2.55^{Aa} \pm 0.13$
M1	$1.34^{Ad} \pm 0.32$	$1.67^{Ac} \pm 0.07$	$2.04^{Bb} \pm 0.15$	$2.39^{Ba} \pm 0.08$
M2	$1.34^{Ab} \pm 0.32$	$1.40^{Bb} \pm 0.08$	$1.64^{Cb} \pm 0.14$	$2.03^{Ca} \pm 0.07$
M3	$1.34^{Aa} \pm 0.32$	$1.36^{Bab} \pm 0.10$	$1.53^{Cb} \pm 0.11$	$1.80^{Da} \pm 0.04$

Note: A-D: Within a column, different letters indicate significant differences (P ≤ 0.05);
a-d: Within a row, different letters indicate significant differences (P ≤ 0.05)

3.3.3. Effect of perilla oil treatments on Staphylococcus aureus

Staphylococcus aureus, *Salmonella enterica*, and *Escherichia coli* are known as common foodborne pathogenic bacteria and are frequently isolated from meat and meat products (Borch and Arinder, 2002).

The results in Table 8 show that *Staphylococcus aureus* counts increased over time in all samples. At the end of storage period, *Staphylococcus aureus* counts reached the highest level in the M0 control sample. Interestingly, the 2% perilla oil treated pork had the least counts on the 9th day and the value was still lower than the TCVN acceptance limit of 10^2. The counts were over this acceptance limit on the 6th day for both controls and on the 9th day for the 1% perilla oil treated pork.

4. CONCLUSIONS

Perilla oil was found to be able to extend the shelf-life of studied fresh sirloin pork stored at a refrigerated temperature (5°C). A higher oil concentration led to more prolonged storage time. Fresh pork treated with 1% perilla oil had its shelf-life extended to 6 days when its pH values, ammonia, TBA, total aerobic counts, *Staphylococcus aureus,* and *E. coli* counts were all under TCVN acceptance limits. This was stretched up to 9 days for fresh pork treated with 2% perilla oil. Therefore, it is suggested that perilla essential oil can be used as a natural meat preservative with both antioxidant and antimicrobial activities against foodborne pathogens in maintaining meat quality, extending the shelf-life of meat products, preventing economic losses, and providing consumers with foods containing natural additives, which might be seen as more healthful than those of synthetic origin.

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