

# Potential of Piper betel Leaf Extract as Meat Preservative

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**Abstract:** *This study determined the potential of betel leaf extract as a meat-preserving agent. Specifically, it determined the physical, chemical, and sensory characteristics of the meaty and fatty parts of the extract-treated meat and its colony count after 15, 20, and 25 days.*

*The betel leaf extract was prepared by treating the pulverized dried leaves of the plant with 95% ethanol for 48 hours then evaporating the alcohol using the rotary evaporator. The resulting solution was used to treat the meat cubes at controlled environmental conditions. Results of the experiment revealed that the extract-treated meat was not as effective as the salt-treated meat in inhibiting the growth of bacteria, but this can be remedied by increasing the volume of the extract. Also, no significant difference between the experimental and the control groups was observed in terms of their internal and surface texture, and color but the contrary was observed in terms of their moisture content. The meat treated with the betel leaf extract has a higher moisture content than the salt-treated meat, and this is why it has higher bacterial count since moisture enhances the growth of bacteria.*

*Based on the findings of the study, the following are the recommendations forwarded for the future direction of related studies: (1) the meat should be sliced thinner (1 cm) to allow the extract to penetrate deeply into the tissues, (2) the meat should be immersed in boiling water before the application of salt/betel extract to lessen the bacterial load, (3) the proportion of extract to the meat should be increased while maintaining the ratio of the salt to the meat (example: 300 grams meat: 300 grams extract; 300 grams meat: 100 grams salt), and (4) the experiment should be performed to other meat samples like beef, chicken, fish to maximize the utilization of the extract.*

**Keywords:** *Piper betel, meat preservative, leaf extract, Piper betel leaf extract*

## 1. INTRODUCTION

The Philippines is a country endowed with abundant natural resources, both on seas and land. Plantlife is prosperous and diverse. A large portion of the country is covered with tropical forests. These forests can be found in varieties of trees, shrubs, vines, grass, ferns, orchids, and bushes. Many of these plants have known healing properties, while others are yet to be discovered.

Herbal medicine is considered the first type of medication that heal wounds and treat health issues encountered by the ancient inhabitants of the earth. Likewise, it is known that food preservation was very minimal and very simple, such as sun drying and application of plant extracts, like rosemary. Until this day, the plant is still a continuous study, and different plants are discovered for their medicinal and beneficial properties.

It is a known fact that herbal medicines in recent times are considered more efficient and more cost-friendly than chemically manufactured drugs. It is also proven that herbal treatments have fewer side effects and less negative impacts than drugs that are chemically manufactured.

Natural preservatives derived from individual plants can preserve products by preventing its decomposition (Russel & Gould, 2012). In terms of preservation, herbal plants could be the best alternative in keeping products or food due to its minimal adverse effects, rather than using chemically manufactured preservatives (Yardley, 2016). Herbal plants were also used as a preservative in some cases during the ancient time due to their antimicrobial activity.

Chemicals, on the other hand, are excellent food preservatives because aside from being bactericidal, they have the tendency to draw water out of the meat resulting to dehydration, which is detrimental to the growth of bacteria. However, despite these advantages, not all chemical preservatives are 100% safe. There are chemicals preservatives that are associated with harmful side effects, like sulfite and sodium benzoate. These chemical components of food additives may cause adverse reactions in certain sensitive people. These include, but not limited to, difficulty breathing, low blood pressure, diarrhea, flushing, abdominal pain, skin irritation, asthmatic reactions, and anaphylactic shock. Likewise, some chemical food additives, like nitrates and nitrites, may slightly increase one's risk for leukemia and other types of cancer.

Betel vine (*Piper betel* Linn.) belongs to the Piperaceae family. It is a shed-loving plant that takes leaves that are 4-7 inches long and 2-4 inches broad. It bears both male and female flowers. The betel plant is economically, medicinally, and traditionally essential to specific groups of people. It is cultivated by small farmers in the Cordilleras, and the leaves are sold in the market. It is used in combination with betel nut known as "momma" by the natives of the Cordillera as an alternative to cigarette. In India, the plant is cultivated for export in other countries like Bahrain, Canada, Great Britain, Nepal, Pakistan, and Saudi Arabia.

In other cultures, boiled betel leaves are utilized as a mouth wash. It is also known as a treatment remedy for cold, cough, bronchial asthma, rheumatism, bad breath, boils and abscesses, conjunctivitis, constipation, swelling of gums, cuts, and injuries (Gundala et al. 2014). The essential oil of betel leaves possesses antibacterial, anti-protozoan, and anti-fungal properties.

There are about 35 known chemical compounds present in *Piper betel* extract. Ten of these compounds are known to have antibacterial activity. These are  $\alpha$ -Pinene, Cis-Sabinene, Eugenol,  $\beta$ -Farnesene,  $\beta$ -Salenene, Eugenyl acetate, Garmacerene-B, Spathulenon, Globulol, and Allylpyrocatechol diacetate (Suryasnata et al. 2016).

Aside from its secondary active metabolites, the fresh betel leaf contains the following nutritional constituents: water (85-90%), protein (3-3.5%), fats (0.4-1.0%), minerals (2.3-3.3%), fiber (2.3%), chlorophyll (0.01-0.25%), carbohydrates (0.5-6.1%), nicotinic acid (0.63-0.89 mg/100g), Vitamin C (0.005-0.01%), Vitamin A (1.9-2.9 mg/100g), thiamine (10-70  $\mu$ g/100g), riboflavin (1.9-30  $\mu$ g/100g), tannins (0.1-1.3%), nitrogen (2.0-7.0%), phosphorus (0.05-0.6%), potassium (1.1-4.6%), calcium (0.2-0.5%), iron (0.005-0.007%), iodine (3.4 $\mu$ g/100g), essential oil (0.008-2.0%), and energy (44 kcal/100g).

Betel leaf was also stated by researches to contain an essential oil called chavicol that has a potential antiseptic and antioxidative properties that could aid in the preservation of food (Majundar et al. 2003). While there are beliefs that *Piper betel* has a carcinogenic effect, these have no scientific basis. According to Rai et al. (2011), it is not the betel leaf that has a carcinogenic property in the betel quid, but the areca nut (*Areca catechu* Linn), tobacco (*Nicotiana tabacum* Linn), and slaked lime. The areca nut and the tobacco are carcinogenic, while the slaked lime promotes the process of carcinogenesis. The betel leaf, on the other hand, possesses cancer-preventive effects. It has a very high antioxidant activity (DPPH method), high total phenolic content, and excellent cytoprotective activities, in vivo (Zazwi et al. 2013).

Meat is considered one, if not the most commonly consumed food products among Filipino household. However, fresh meat has been observed to last for only a day or less before it

becomes rotten. This chemical reaction can be curtailed by the different methods of processing and preserving the meat.

In this study, the researcher determined the ability of the betel leaf extract to preserve raw meat. In the mountain provinces, salted meat is considered to be a type of traditional meat that is kept by applying a generous amount of rock salt and smoking the meat or exposing it to direct sunlight for a minimum of 30 minutes to a maximum of 3 hours per day for at least two consecutive weeks (Prill-Brett & Tapang, 2007). Some provinces cure their salted meat by hanging the meat covered with salt over a raging fire (Caluza, 2012). This would preserve the meat giving it a long shelf life and allowing the convenience of long storage time.

In this study, the researcher would like to produce similarly preserved meat by exposing the meat to the same environment and procedure as that of the salted meat. However, instead of using salt, betel leaf extract would be applied. This will allow the researcher to find alternative benefits for the betel leaf and would be able to preserve the culture of the Cordilleras by using plants that are abundant in their community.

If the research is proven effective, then the community would be able to find alternative means to preserve meat, especially if salt is a commodity that should be avoided due to specific health issues. It also allows the preservation of the upland's culture of preserving meat by using plant extracts. Finally, it will encourage people to cultivate *Piper betle* vine, which is almost extinct nowadays.

## **2. Objectives of the Study**

This study determined the possibility of betel leaf extract as a meat preserving agent. Specifically, it determined:

1. the physical-sensory characteristics of the betel leaf extract-treated meat (both the meaty and fatty parts) in terms of texture, color, and moisture content;
2. if a significant difference exists between the extract-treated and salt-treated meat in terms of the texture, color, and moisture content;
3. the colony count of the betel leaf extract-treated meat after 15, 20, and 25 days; and
4. if significant variation exists in the colony count of the betel leaf extract-treated meat after 15, 20, and 25 days.

## **3. Scope and Delimitation**

The study was limited to the determination of the possibility of betel leaf extract as a meat preserving agent. The leaves were collected in Barang-ay Demo farm in San Juan, Ilocos Sur. The leaves were air-dried, pulverized and extracted using 95% ethyl alcohol.

Fatty and meaty portions of pork meat cubes were treated with the pure *Piper betel* leaf extract. This represents the experimental group. The meat portions treated with salt represents the control group. Both groups were sun-dried two weeks. The physical, sensory and bacterial assay were conducted on the 15<sup>th</sup>, 20<sup>th</sup> and 25<sup>th</sup> day of treatment.

## **4. Materials and Methods**

### **Design of the Study**

This study was experimental in design and aimed at determining the potential of the betel leaf extract as a meat preserving agent by preventing the growth of bacteria at 15, 20, and 25 days. The salted meat represented the researcher's control group and was compared to the meat processed with betel leaf extract, serving as the experimental group.

### The Locale of the Study

The extraction of the dried betel leaf was conducted at the University of Northern Philippines. At the same time, the experiment proper was performed at the Microbiology Laboratory of the College of Health Sciences. The analysis of the experimental and the control groups was done at the Societe Generale de Surveillance (SGS Philippines, Inc), Makati, Metro Manila, a food and health laboratory authorized by the Food and Drug Administration.

### Materials

- Betel leaf. It is also known as piper leaf. It is an East Indian herbal plant that is used to treat ailments. The role of betel leaf in this study would be an alternative preservative to salt.
- Pork belly meat. It is a lump of fresh meat from a pig that was used as part of the experimental group and control group.
- 95% ethanol. It is a reagent that aided in the extraction of the betel leaf.
- Rock salt. It is commonly used as a preservative of foods, especially meat products. This served as part of the control variable in this experiment.
- Distilled water. It is a form of water that has been purified by the process of distillation wherein the water was boiled and the vapor transferred to a container. This would decrease the tendency of contaminated water samples.
- Filter paper. It is a semi-permeable membrane that was used to strain the extract.
- Blender. It was used to grind the dried betel leaves into a fine powder.

### Procedure

#### A. Preparation of the betel leaf extract

In the preparation of the leaf extract, the researcher obtained fresh leaves of betel with no physical damage and in the best condition at the Barang-ay Demo Farm, San Juan, Ilocos Sur. The betel leaves were washed with tap water and finally with distilled water to remove contaminants. The fresh leaves were air-dried for seven days in the shade. After drying, the researcher crushed the leaves manually and then using a blender to produce a fine and powdery substance. Five hundred grams of the powder was soaked in an equal volume of 95% alcohol in a 1L beaker for 48 hours at room temperature. After two days, the ethanol was separated using a cheesecloth and finally with a Whatman filter paper. Then the alcohol was evaporated using a rotary evaporator. The resulting filtrate was subjected to further evaporation inside the oven maintained at 45<sup>0</sup>C until it became semi-solid. The prepared extract was placed in a sterile container, sealed, and kept at room temperature (Majumdar, et al. 2013).



Fresh *Piper betel* leaves.

Air-dried *Piper betel* leaves

### B. Preparation of the Meat

In the meat preparation, the researcher purchased one kilogram of freshly cut pork belly meat at the Vigan City Public Market to obtain fresh meat. The researcher cut equal bits of meaty and fatty parts of the pork into average slices of 1.5 cm. Then the meat cubes were then washed with distilled water to remove contaminants. The pork meat was divided into two equal parts. Each had the same meaty/fatty cuts and stored in sterile 1L beakers covered with clean aluminum foil.

### C. Experiment proper

The experiment was conducted in a sterile area to prevent contamination of the samples. The two beakers containing 300 grams of each prepared meat were labeled the control group and experimental group. In the control group, the pork belly cuts were treated with 200 grams of rock salt. The researcher ensured that the raw meat was covered with salt. In the experimental group, the pork belly cuts were treated with 200 grams of the concentrated betel leaf extract in the same way that the control group was treated with salt. The pork belly cuts from each group were tied separately to a thin iron rod (alambre) and sun-dried from 10 AM to 3 PM every day for 12 days. Adjustments on the exposure of the meat under the sun were made depending on the weather and environmental conditions. On the 13<sup>th</sup> day, the samples were transported in biohazard bags and submitted to the Societe Generale de Surveillance, Makati, Metro Manila for the microbiological, physical, chemical, and sensory analyses.

#### Treatment of the Data

The following descriptive rating was used to evaluate the color and texture of the pork belly meat:

Table 1  
Descriptive Rating of the Color and Texture of the Experimental and Control Meat

Scale	Color	Internal Texture	Surface Texture
4.21 – 5.00	Very dark yellow-brown	Very hard	Very rough
3.41 – 4.20	Dark yellow-brown	Hard	Rough
2.61 – 3.40	Yellow-brown	Slightly soft	Slightly rough
1.81 – 2.60	Light yellow-brown	Soft	Smooth
1.00 – 1.80	Very light yellow-brown	Very soft	Very smooth

Source: Societe Generale de Surveillance Philippines, Inc.

The scale above served as the basis for comparison between the control and experimental groups. It was provided by the Societe Generale de Surveillance Philippines, Inc., where the analysis was conducted. Likewise, the test for the ability of both the experimental and control meat to prevent bacterial growth was performed in the same laboratory using total plate counting.

The mean was used to determine the bacterial counts, while the standard deviation showed how varied the individual counts from the mean are. The significant differences in the colony count and preservation level of the betel leaf extract and salt-treated meat according to color, texture, moisture content utilized the use of t-test.

## 5. RESULTS AND DISCUSSION

This portion of the study presents, analyzes, and interprets the data gathered from the experiment. It includes physical-sensory as well as bacteriological evaluation of the fatty and meaty parts of the meat treated with the *Piper betel* extract.

Table 2 presents the result of the physical-sensory evaluation of the meat treated with the *Piper betel* leaf extract in terms of its internal and surface texture as well as its color after the fifteen-day evaluation period.

Table 2. Physical-sensory evaluation of the Piper betel leaf extract-treated meat

Pork Meat		Internal Texture		Surface Texture		Color	
		Result	Interpretation	Result	Interpretation	Result	Interpretation
Fatty Portion	Control Group	3.53	Hard	3.26	Slightly Rough	3.53	Yellow-brown
	Experimental Group	4.0	Hard	3.33	Slightly Rough	4.0	Dark yellow-brown
Meaty Portion	Control Group	4.46	Very hard	4.20	Rough	3.0	Yellow-brown
	Experimental Group	4.73	Very Hard	4.53	Very Rough	5.0	Dark yellow-brown

As observed from the table, both the experimental and the control groups show similar interpretations regarding the internal texture of the fatty portion of the meat. Both the control and experimental groups are interpreted as “hard”. Heinz & Hautzinger (2007), in a journal article entitled, “Meat Processing Technology,” states that if the meat is processed and dehydrated using heat, the meat’s texture changes from soft to firm. The hard texture of meat indicates that the meat is adequately dehydrated, and preservation could be possible. When the control and experimental values were subjected to statistical analysis to determine if there exists a significant difference between them, analysis shows no significant difference. This would indicate that both the experimental and control groups have reached the texture required for proper preservation (Heinz & Hautzinger, 2007).

With respect to the internal texture of the meaty portion, both the experimental and the control groups were interpreted as very hard. Likewise, statistical analysis of the results revealed no significant difference in the internal texture of the meaty portion. The journal entitled, “Meat Processing Technology” states that an adequately dehydrated meat should show a hard texture (Heinz & Hautzinger, 2007). The results indicate that the meaty portion has an internal texture harder than the fatty part of the meat. This is because the fatty part contains more moisture than the meaty part, requiring more time to attain the hard target ultimately.

In terms of their surface texture, both the experimental and the control groups show similar interpretations of the fatty portion of the meat. Both groups are interpreted as slightly rough. Statistically, there is no significant difference in the surface texture of the fatty portion of the experimental and control meat at 0.05 confidence level. With respect to the meaty portion, statistical analysis revealed no significant difference between the experimental and control meat,  $p = >0.05$  although the experimental group showed a rougher surface texture than the control group. The non-significance between the two groups indicated that both have reached the proper range in meat dehydration and have acquired the appropriate texture for preservation (Heinz & Hautzinger, 2007).

In terms of color, there is no significant difference in the color of the fatty portion of the experimental and control meat,  $p = >0.05$ . The control meat showed a yellow-brown color, while the experimental group showed a dark yellow-brown color. Basing on the expected color of traditional salted meat prepared by the Cordilleras, the color of the meat once treated and dried for several weeks should range from dark yellow to dark brown (Caluza, 2012). Therefore, the color of the meat for both the experimental and control groups reached the required range and is considered dried and preserved. The experimental group was much darker than the control group due to the extract (dark green) absorbed by the meat.

Aside from the internal and external texture as well as the color of the meat, its moisture content was also evaluated. Table 3 (below) shows the result of the moisture test. The test was conducted to determine the dryness of the control and experimental meat.

Table 3. Moisture content (in percent) of the control and experimental meat

Groups	Day 15	Day 20	Day 25
Experimental Group (Extract-treated meat)	15.68	14.39	13.42
Control Group (Salt-treated meat)	11.92	9.26	6.86

Table 4. T-test analysis for the comparison of the moisture content of the control and experimental meat using the air oven method

	T	Df	Sig. (2-tailed)	Interpretation
Day 15	22.649	4	0.002	Significant
Day 20	17.084	4	0.000	Significant
Day 25	7.221	4	0.000	Significant

The result in the moisture content determination shows a significant difference between the experimental and control groups,  $p = < 0.05$ . The control group was observed to be drier than the experimental group. According to Heinz (2007), the moisture content of treated meat after drying should be 5-10%, making them stable for an extended period.

Basing on the results provided in Tables 3, it would reflect that the control group is within the range of moisture content required for meat to have a stable shelf life, and the experimental group had higher moisture content. Therefore, there is a relevant significant difference between the control and the experimental groups in terms of their moisture content.

In addition to the physical-sensory evaluation of the meat utilized in the experiment, microbiological assay was also performed to determine the ability of both groups (control and experimental) to prevent bacterial growth.

Table 5. Total bacterial plate count in colony-forming unit/gram of the extract and salt-treated meat

	Day 15	Day 20	Day 25
Control Group (Salt-treated meat)	<b>435</b>	<b>207</b>	<b>235</b>
Experimental Group (Extract-treated meat)	<b>555</b>	<b>310</b>	<b>365</b>

The result of the microbial analysis in Table 5 shows that while both the control and the experimental groups have bacterial growth, the latter has greater colony count. Accordingly, the normal range for a suitable colony count is between 25 and 250 colony-forming units per gram (CFU/g), and it would be considered too numerous to count (TNTC) when the total plate count exceeds 250 (Weshoff et al., 1976).

More specifically, it was observed that on the 15<sup>th</sup> day, bacterial growth was at their peak, but this declined on the 20<sup>th</sup> day and rose again after the 25<sup>th</sup> day. This implies that even with the presence of preservative (salt), bacterial degradation continued. While utmost care was guaranteed in the preparation of meat cubes, still, there was bacterial contamination. These were perhaps contaminants from the environment and were salt-loving, so they tolerated a very high salt concentration. Likewise, bacterial growth was enhanced by the presence of water in the meat. However, as the meat was exposed to the sun for longer days, the meat was dehydrated, causing inhibition of bacterial growth and multiplication, and eventually, bacterial death.

The rise in the bacterial count after the 20<sup>th</sup> day indicated that the salt and the betel leaf extract could only preserve the meat for up to 20 days. After this, bacterial growth continues, and the meat will eventually get rotten.

## 6. Conclusions

Based on the data gathered, the researcher concluded that the extract-treated meat was not as effective as the salt-treated meat in inhibiting the growth of bacteria, but this can be remedied by increasing the quantity of the extract. Also, no significant difference between the experimental and the control groups was observed in terms of their internal and surface texture, and color. Still, the contrary was observed in their moisture content. The meat treated with the betel leaf extract has a higher moisture content than the salt-treated meat, and this is why it has higher bacterial count since moisture enhances the growth of bacteria.

## 7. Recommendations

Based on the findings of the study, the following are the recommendations forwarded for the future direction of related studies:

1. The meat should be sliced thinner (1 cm) to allow the extract to penetrate deeply into the tissues;
2. The meat should be immersed in boiling water before applying salt/betel extract to lessen the bacterial load.
3. To increase the proportion of the extract while maintaining the ratio of salt (example: 300 grams meat: 300 grams extract; 300 grams meat: 100 grams salt).
4. Perform the same experiment to other meat samples like beef, chicken, and even fish.

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