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EFFECT OF THE ESSENTIAL OILS OF "ROMERO" ROSMARINUS OFFICINALIS AND "PEREJIL" PETROSELINUM CRISPUM ON THE MICROBIOLOGICAL QUALITY OF "ALPACA" HAMBURGER VICUGNA PACOS WITH MACHINE LEARNING

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Abstract

The meat industry produces raw materials with healthy ingredients by modifying the diet and formulating meat products based on meat from other species. The objective of this research was to formulate a hamburger using alpaca (Vicugna pacos) and essential oils of rosemary (Rosmarinus officinalis) and parsley (Petroselinum crispum) in concentrations of 0.5% and 1.0%. For this, the essential oils were extracted, for each essential oil a yield was obtained, where 0.734% for the essential oil of rosemary and 0.634%, for the essential oil of parsley. Microbiological analyzes were carried out on the hamburgers to determine Escherichia coli, Salmonella sp. and Staphylococcus for seven days. Sensory attributes (color, smell, taste, and texture) were characterized by 30 untrained panelists (university students). After 7 days of follow-up, the hamburgers did not present the presence of microbiological parameters. Significant differences ($p<0.001$) were observed for concentrations and attributes. However, 0.5% parsley essential oil had higher odor, flavor, and texture scores compared to rosemary. It was concluded that our applied methodology allows to improve the useful life of the product, the antimicrobial effect and the acceptability, guaranteeing a good quality and nutritious product. Deep learning is one kind of machine learning with the overarching goal of making it easier to organize and use human knowledge across many domains, at scale. The field of microbiology benefits greatly from the application of mathematical principles to the maximization of variation functions. Multiple data types are used.

1. Introduction

One of the most consumed fresh raw products is hamburger meat, which undergoes rapid microbial deterioration, shortening its shelf life (Alirezau et al., 2020), increasing consumer demand for natural foods without chemical preservatives, as a consequence of some synthetic preservatives. used in the food industry, carcinogenic and teratogenic effects have been attributed, such as nitrites and monosodium glutamate and this has led to more research on new preservation techniques to promote food safety, maintaining their nutritional and sensory properties (Amadio et al., 2019) the deterioration of fresh meat is due to lipid oxidation, which causes the appearance of unpleasant odors and flavors and the alteration of color, generally producing a decrease in the organoleptic quality of the product. They also lead to a decrease in the nutritional value of meat and the generation of compounds that are potentially harmful to health, related to the risk of various pathologies (Aziz & Karboune, 2018)

Meat is a good substrate for the growth of spoilage and pathogenic microorganisms, since it has a high-water activity (close to 1) and is a source of proteins, lipids, various low molecular weight soluble components, vitamins, minerals and a small carbohydrate concentration (0 - 1.2%). Contamination can occur during slaughter, processing, and storage operations. To delay all these processes of alteration of the meat during these phases, refrigeration (Arrioja-Bretón et al., 2020) is used, to preserve the polyunsaturated fatty acids from oxidative deterioration, and antioxidants are used. Due to their low cost, high stability, and effectiveness, synthetic antioxidants such as hydroxyanisole butylated (BHA), hydroxytoluene butylated (BHT), propyl gallate (GP) and tert - butylhydroxyquinone (TBQH) are mainly used for this purpose. Due to the toxic and carcinogenic effects on human health, the current trend of consumers is to reject the use of synthetic additives and demand natural and quick-preparation foods (Asioli, 2017).

In recent years, many vegetables and their essential oils have been studied as sources of antioxidants and natural preservatives. In this sense, the alternative use of natural resources has received increasing attention, since many of these products have additional functional properties, (Jonberg et al., 2017) in this context essential oils have been gaining interest, thanks to their antioxidant, antibacterial, antiviral and antifungal properties (Aziz & Karboune, 2018) and because they are generally recognized as safe substances (GRAS), having a wide acceptance by consumers. , (Mutlu-Ingok et al., 2020), its effect is produced by its content of phenolic compounds and organosulfur, (Falleh et al., 2020) in meats and meat products, the essential oils of rosemary, parsley, oregano, thyme, clove, garlic, ginger, coriander, and basil have shown great potential to be used as antimicrobial and antioxidant agents (Chouhan et al., 2017) although the In vitro studies support these functional properties, it has been found that a higher concentration is generally necessary to achieve the same effect in food, and it can alter the flavor of food and exceed the limit accepted by consumers because the oils essential are mixtures of different components, one of the alternatives to achieve the desired conservation effect in lower concentrations is to use combinations of different plant extracts and thus increase their bioactivity, (Alirezalu et al., 2020) However, the acceptability and shelf life that could be obtained in a hamburger by adding a mixture of essential oils in its preparation is unknown (Contreras-López et al., 2021)

As an alternative to the traditional sugar cane burger, alpaca meat is lean meat with minimal fat and almost no cholesterol, which is sold in the local markets of Huancavelica. However, its consumption is low because its nutritional and nutritional benefits are not widely known. This situation means that a wide variety of fresh raw meat products, salted meats, sausages, among others, are marketed more frequently in the region (Xiong, 2023).

Characterization of microbial communities may be used for a wide variety of purposes, including the prediction of human disease states, the detection of environmental contaminants, and the use of trace evidence in forensic science.

Intriguingly, machine learning has the potential to help us better understand microbial communities by allowing us to recognize patterns in the data we collect about them. However, machine learning models are often used as "black boxes," with little to no transparency into how the algorithms arrived at their predictions. The complexity of modern machine learning models is to blame. It is typical practice when working with sophisticated machine learning algorithms to put accuracy and performance ahead of interpretability.

For this reason, the objective of this research was to formulate a hamburger based on alpaca meat (Vicugna pacos) using essential oils of rosemary (Rosmarinus ofjicinalis) and parsley (Petroselinum crispum), evaluating its effect on the microbial load and its acceptability in the region of Huancavelica, Peru.

2. Materials and methods

2.1. Vegetal material

Flowering rosemary and parsley samples were collected during May 2022 in the Acobamba-Huancavelica province (Latitude: -12.8428, Longitude: -74.5694 12° 50′ 34″ South, 74° 34′ 10″ West). Only the aerial part of the plant was used; the leaves and the apical parts, before extracting the essential oil, were dried in the shade for seven days at room temperature (18 °C).

2.2. Extraction of essential oil by hydrodistillation

For the extraction of rosemary and parsley essential oil, the hydrodistillation method proposed by (Elyemni et al., 2019), for this, 200 g of the aromatic herb were added to 1600 ml of distilled water in a 2-liter spherical flask. This flask was adapted to a refrigeration tube containing cold water and brought to a boil by adding heat in an oven, to obtain essential oil by entrainment of water vapor. Finally, two phases were obtained, an aqueous phase (aromatic water) and an organic phase (essential oil). Then, the essential oil is separated by means of a decanting pear, it is packaged in an amber-colored bottle to avoid its oxidation, and later it is stored at 4 °C, until its use. To calculate the yield (R), the following formula proposed by (Semerdjieva et al., 2019)

$$R\,(\%) = \frac{\text{quantity of oil (g)}}{\text{Quantity of plant material (g)}} \text{ x } 100 \qquad (1)$$

2.3. Hamburger preparation

The hamburgers were made from alpaca meat of the Huacaya breed, at the age of milk teeth (12 to 18 months), raised under a traditional grazing system. The carcass was transported in a cooler at a freezing temperature - 4.0 °C, from the local market of the Castrovirreyna district to the Laboratory of Agroindustrial Processes 01 of the Professional School of Agroindustrial Engineering of the National University of Huancavelica (UNH), located in the district and province of Acobamba, where it was stored at -18 °C until its respective use.

To obtain the alpaca meat pieces, part of the carcass was thawed at room temperature for 6 hours, deboned and ground with a BOXA: Mci-32plus mill with the meat pieces that presented the greatest amount of meaty tissues (Kaur et al., 2021)

The preparation of the hamburger was done manually, mixing the following ingredients (Linares et al., 2020) (see Table 1). as well as the essential oils under study in concentrations ranging from (0.5 - 1.0) %. All the ingredients were mixed with the help of a MAINCA: CM-14 cutter for 8 minutes, obtaining a dough, from which hamburgers are obtained with an approximate dimension of 7.0 cm in diameter, 0.8 cm thick, and 45 g of weight.

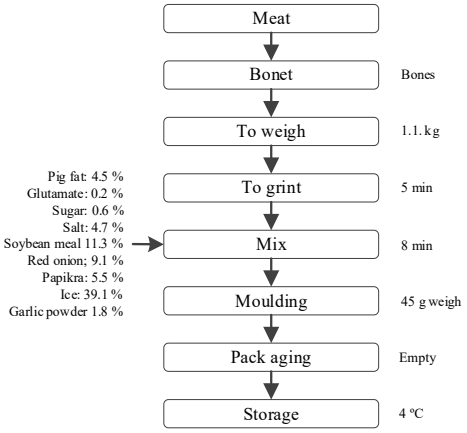

Figure 1. Flowchart to obtain the hamburger

Table 1. Formulation of the hamburger based on alpaca meat.

Ingredients	Quantity (g)
Alpaca meat	1100.0
Bacon (pork fat)	50.0
Glutamate*	23
Sugar	7.0
Salt	52.0
Soybean flour	125.0
Red onion	100.0
Peppers	60.0
Ice	430.0
Garlic powder	20. 1
Oil rosemary essential _	0.5 and 1%
Oil parsley essential _	0.5 and 1%

Note: (*): According to the maximum permissible limits proposed by NTP 201.007:1999 (revised 2019).

2.4. Analysis microbiological

Microbial counts of E. coli, Salmonella sp. and Staphylococcus aureus were determined according to the methodology described by (Arrioja-Bretón et al., 2020), for this, samples of 10 g of alpaca hamburger were weighed and 90 ml of sterile 1% peptone water were mixed for 3 minutes at room temperature. Serial decimal dilutions were prepared by taking 1 mL of the above solution in 9 mL of peptone water in duplicate, pouring and scattering for total count on Plate. Count Agar, then this mixture was incubated at 30 °C for 48 hours in an incubator (HH.B 11.300- BY:GREETMED). Finally, the microbial count was performed using a microbial colony counter (TECNAL: CP600).

Table 2. Percentage of essential oil added for each treatment.

Formulation	Addition of essential oil
HAAER5	0.5% rosemary essential oil
HAAER1	1.0% rosemary essential oil
HAAEP5	0.5% parsley essential oil
HAAEP1	1.0% parsley essential oil

2.5. Profile sensory

The sensory properties of the alpaca meat patty sample with Rosmarinus essential oil Officinalis and Petroselinum crispum were classified through the following attributes: color, smell, flavor, and texture as recommended by (Anzaldua, 1994). The attributes were determined with 30 untrained panelists who were recruited from the Professional School of Agro-industrial Engineering of the National University of Huancavelica, 15 were male and 15 females, with ages ranging from (19 - 25) years, used the hedonic scale; poor (1 point) to excellent (5 points) (Forghani et al., 2017). Here 4 formulations (essential oil of rosemary and parsley in concentrations of 0.5 and 1.0) % were tested. In order not to alter the appreciation due to hunger or satiety, the tasting time was held in the morning from 10 to 11 hours. Prior to this, the patties were grilled to improve sensory characteristics and presentation (Hartmann et al., 2020) Likewise, they were cut into small pieces of uniform sizes. Finally, these hamburger pieces were coded according to the essential oil added (see Table 2) and these were delivered in order to the panelists, seeking their

qualification to later determine the degree of acceptability

2.6. Static analysis

The data obtained in the sensory evaluation were processed using the FRIEDMAN test. Finally, the Student's t-test was applied to establish the best formulation of alpaca meat hamburger with the essential oil of Rosmarinus. Officinalis and Petroselinum crispum, using SPSS version 25 software, to determine acceptability in terms of color, odor, flavor and texture (Das, 2019).

2.7. Machine Learning in Microbiology

There is no way to conceive of molecular and cellular processes in nature without microbes. Many kinds of standard biological study cannot be carried out without the use of microorganisms. Machine learning is currently being applied to a wide variety of disciplines. Machine learning (which encompasses deep learning) is a field of study that applies mathematical ideas to optimize variation functions for use in the study of microbes. The final result is supposed to be a system that facilitates group efforts to categories and implement broad bodies of knowledge across many disciplines. (Jongberg et al., 2017) Improvements in categorization and forecast are just two examples of the fruitful outcomes of computational biology's embrace of microbial community analysis. This review paper provides an introduction to the applications of machine learning and deep learning in microbiology, with specific attention paid to the microbiome and categorization, microbial ecology, illness and epidemiology, and drug discovery. The most recent findings and theories in these fields are also covered (see figure 2).

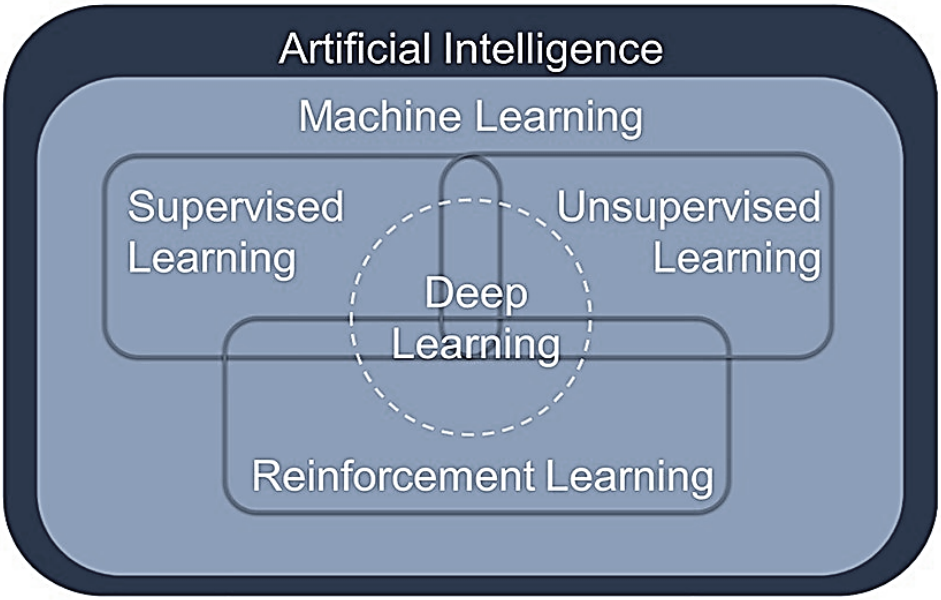

Figure 2. Analyzing the connections between AI, ML, and DL (Aziz & Karboune, 2018)

2.8. Artificial Intelligence and hamburger preparation

A machine with the ability to make hamburger preparation is essentially an AI-enhanced robot chef. The Moley Robotics kitchen is the first fully autonomous kitchen and the first fully automatic artificial intelligence device for the kitchen argued by, (Bhavaniramya et al., 2019). As such, it is a relatively new addition to the field of artificial intelligence robot cooking. This device, installed on the roof, is a component of a fully automated cooking setup. With the help of its two arms, which travel along a train placed in the ceiling, it is able to adjust the thermostat, use the washbasin, mix and pour ingredients, and shake containers. Moley Robotics has a completely working kitchen that can cook and clean up after 5,100 meals at once.

The perfect hamburger can now be made by robots thanks to the integration of sensors into everyday kitchen appliances. More than 1,200

factors are monitored by their senses of contact, respiration, sight, and hearing every single microsecond. Similar to how the human brain stores and retrieves information, the operating system receives and processes these memories (OS). These capabilities help them learn new skills over time and handle a wide variety of cooking tasks. Devices that detect touch, contact, and closeness are used in artificially intelligent robot kitchens so that chores can be recorded, motions can be recorded, and directions can be followed. By storing relevant information in its brain and retrieving it when needed, the AI robot chef can teach itself new skills.

The restaurant preparation industry is increasing its expenditures on technology. Sales in China alone totaled more than 124 million dollars in 2018, and it is anticipated that the food and beverage business around the world will have committed more than 510 million dollars in robots by the year 2025.

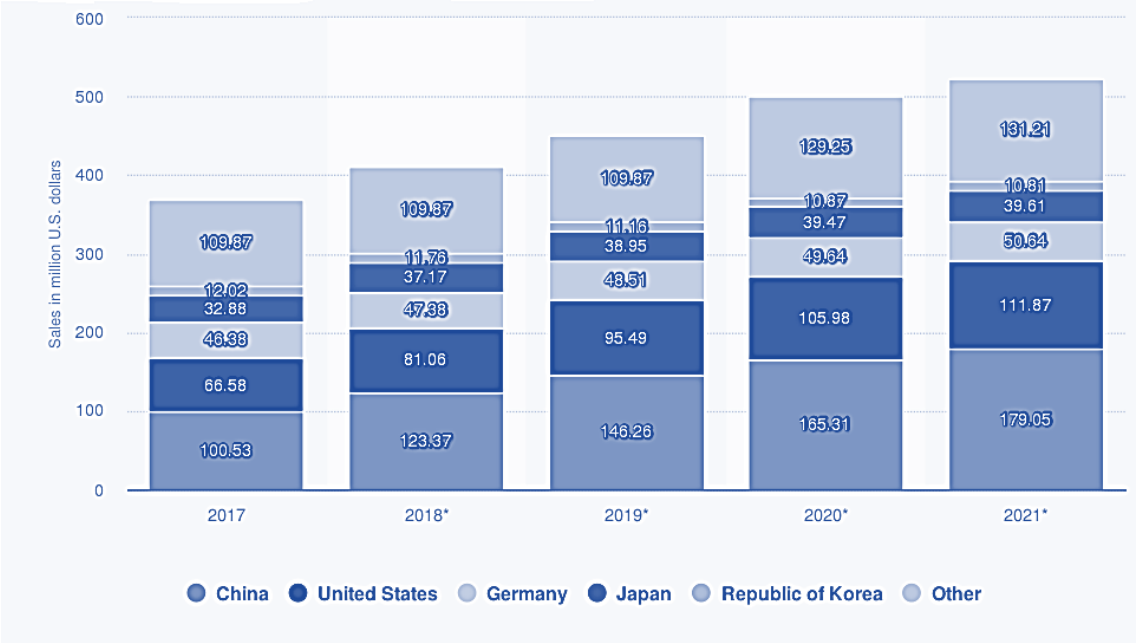

Figure 3. Sales value of new AI robot Installation to make burgers

3. Results and discussions:

3.1. Extraction yield of essential oils.

The yield including, maximum, minimum, range, standard deviation and error together with the results (see Table 3) showed that an extraction yield in rosemary essential oil of 0.734 % was obtained, while the parsley essential oil yield was of 0.634%, with a confidence interval of 95% ($p<0.05$).

(Elyemni et al., 2019) reported 0.347 % extraction yield of essential oil from Rosmarinus officinalis L. using the hydrodistillation methodology, this value was much lower than that reported by this research because a decanter pear was used to separate the essential oil from the aromatic water. Likewise, 3.44% was obtained for the extraction yield of essential oil from dry seeds of petroselinum. crispum using the hydrodistillation methodology, due to the difference in the raw material used, parsley leaves versus parsley seeds, (Dong et al., 2017).

Table 3. Rosemary and parsley essential oil extraction yield.

	Minimum (%)	Max (%)	Scope (%)	Mean (%)	South Dakota	I know
Rosemary	0.69	0.78	0.08	0.73	0.037	0.022
Parsley	0.61	0.66	0.09	0.63	0.041	0.014

E Min= minimum; Max=maximum, SD =standard deviation; SE = standard error

3.2.Evaluation proximal chemistry

The results are shown (see Table 4) corresponding to the formulation of the alpaca meat hamburger with the addition of HAAEP5 (0.5% parsley essential oil), which was accepted in the sensory characteristics of color, smell, flavor and texture.

Table 4. Proximal chemical composition of artisan alpaca meat burger with 0.5% parsley essential oil.

Parameter	Quantity (%)
total protein	45.16
Fat	10.12
Ashes	6.20
Humidity	63.10

The moisture concentration in the alpaca meat patty was lower than that reported for the lamb meat patty, resulting in an increase in the other proximal properties. However, the opposite happened in the rest of the proximal composition, there was a higher concentration of protein, ash and fat (see Table 4), for the alpaca meat hamburger versus the lamb meat hamburger, since the proximal composition of lamb meat burgers with rosemary spice with different packaging under aerobic conditions reported values of moisture (72. 10 ± 0.34) %, protein (18.64 ± 0.31) %, ash (1.92 ± 0.02) % and fat (4.68 ± 0.49) % (Linares et al., 2020). Thus, the proximal composition of the lamb burger with origanum extract was also evaluated. vulgare, reporting moisture values (62.06 ± 0.49) %, protein (15.21 ± 0.21) %, ash (2.52 ± 0.05) % and fat (22.62 ± 0.79) % (Fernandez et al., 2017) There, fore, the concentration of moisture, ash and protein in the alpaca meat patty was higher than that reported for the lamb meat patty, however, the fat concentration was higher in the lamb patty. This is due to the different raw materials and spices used in the preparation of the hamburgers.

3.3.Evaluation microbiological

The alpaca meat burger with the addition of essential oils of rosemary and parsley at a concentration of 0.5 - 1.0 %, in a time of 0 to 7 days at 4 °C, did not report the presence of Escherichia coli, Salmonella sp. nor Staphylococcus aureus, from the beginning to the end of the study period (see Table 5). Many of today's most useful tools rely on machine learning, from web search and email spam filtering to product suggestion and mobile banking fraud detection.

Table 5. Evaluation Escherichia coli Salmonella sp. and Staphylococcus aureus, in the alpaca meat hamburger with the addition of rosemary and parsley essential oil at different concentrations.

Day	Rosemary essential oil		Parsley essential oil	
	0.5%	1.0%	0.5%	1.0%
	Escherichia coli (cfu /g)			
0	<50	<50	<50	<50
7	<50	<50	<50	<50
	Salmonella sp. (cfu /g)			
0	A	A	A	A
7	A	A	A	A
	Staphylococcus aureus (cfu /g)			
0	< 100	< 100	< 100	< 100
7	< 100	< 100	< 100	< 100

Note: A = absence of microorganisms

The data obtained are within the maximum permissible limit, as indicated by the Peruvian Technical Standard (Dwivedi et al., 2017), as we can see in other studies, the presence of fecal coli and E. coli has not been reported. (Semerdjieva et al., 2019) several investigations affirm that essential oils have the ability to alter and penetrate the lipid structure of the cell membrane of the microorganism, which causes the denaturation of the cell membrane, therefore, the death of the microorganism (Xiong, 2023).

3.4. Sensory profile

Sensory profile analysis of alpaca meat hamburger with different percentages of Rosmarinus essential oil ofjicinalis and Petroselinum crispum was evaluated using the Friedman method (see Table 6).

Table 6. Friedman test results between treatments for each sensory attribute

attributes	Treatments	Add (Ranks)	Mean (Ranks)	T2 -	p-value
Colour	HAAER5	62.00	2.07[b]	53.49	<0.0001
	HAAER1	41.00	1.37[c]		
	HAAEP5	104.00	3.47[a]		
	HAAEP1	93.00	3.10[a]		
Odor	HAAER5	58.00	1.87[c]	73.40	<0.0001
	HAAER1	46.00	1.50[d]		
	HAAEP5	116.00	3.73[a]		
	HAAEP1	90.00	2.90[b]		
Flavor	HAAER5	54.00	1.80[c]	223.95	<0.0001
	HAAER1	39.00	1.30[d]		
	HAAEP5	112.00	3.73[a]		
	HAAEP1	95.00	3.17[b]		
Texture	HAAER5	49.00	1.63[d]	71.81	<0.0001
	HAAER1	50.00	1.67[c]		
	HAAEP5	108.00	3.60[a]		
	HAAEP1	93.00	3.10[b]		

A significant difference p<0.001 (see Table 6) was found for the attributes of color, smell, flavor, and texture. For the color attribute, the highest score was found in HAAEP5 and HAAEP1 with an average score of 4.07 and 3.73 (good) and F1 has the lowest score with 2.07 (very regular).

Regarding (see Figure 3) the odor, flavor, and texture attributes, the highest score is found in HAAEP5 with scores of 4.27; 4.20, and 4.04 showing a good rating.

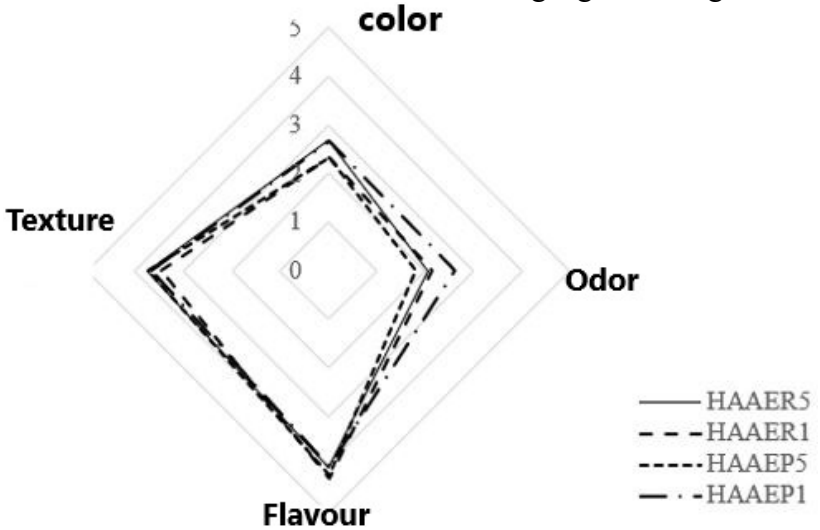

Figure 4. Evaluation of sensory attributes of alpaca meat hamburger with essential oil of Rosmarinus Officinalis and Petroselinum crispum.

For the preparation of hamburgers, the type of meat to be used is important; For example, the sensory properties and color parameters were evaluated in the hamburger made from llama meat with the addition of sanky husk powder, reporting means for each attribute in color, smell, flavor and texture 4.45, 2.99, 6.10 and 7.84, respectively (Yousefi et al., 2014) these results are different from those reported in Figure 2 and this is due to the difference in the raw material used, llama meat versus alpaca meat. Likewise, they determined the sensory evaluation of a hamburger made from beef coated with chitosan plus oregano essential oil, reporting the following mean values for the sensory attributes of color, smell, flavor, and texture: 2.2, 2.5, 2.5, and 2.8, respectively (Holley & Patel, 2005) values that differ compared to those reported in Figure 2. The color attribute in the sensory evaluation of meat is the most important factor when customers wish to purchase a meat derivative, since color is related to freshness, (Ngapo et al., 2017) Likewise, the color of the surface of the raw hamburger is an organoleptic characteristic that is taken into account at the time of purchase. However, the color does not define its proximal chemical composition, (Yousefi et al., 2014)

4.Conclusions

Rosemary essential oil extraction yielded 0.734%, whereas parsley essential oil extraction yielded 0.634%. Rosemary and parsley oil were used to season the alpaca meat patties. Antibacterial action was confirmed after seven days of testing, and the product was found to be free of Escherichia coli, Salmonella sp., and Staphylococcus. When comparing college students based on sensory attributes including color, smell, taste, and texture, statistically significant differences (p 0.001) were discovered.

There were over 32,217 alpacas in the Huancavelica Region, each having more protein and vitamins than a pound of red meat. The plants used in the production of essential oils may also be found in the research domains. One big drawback, however, is that adding too much essential oil might dilute the pleasant sensory attributes (taste and scent) that have contributed to the product's success. Similar to how the traditional method of extracting essential oil from aromatic plants (hydrodistillation) results in a poor yield, modern technology may be used to increase this rate of success.

We've developed and tried a recipe for alpaca burgers that incorporates the usage of Rosmarinus essential oils. Preventing meat by-products from spoiling too quickly is explored, and the use of essential oils like Rosmarinus officinalis and Petroselinum crispum is mentioned. The protein, ash, and fat content of alpaca meat are all higher than those of lamb (for a more in-depth comparison, see (Rios-Mera et al., 2020). In a blind tasting test, alpaca meat burgers outperformed beef patties coated with chitosan (oregano essential oil) (Peruvian, 2006) in terms of overall flavor and texture. The recent research verified that consumers preferred hamburgers that had 0.5% parsley essential oil. After then, microbiological testing was done, and no signs of E. coli, Salmonella, or Staphylococcus aureus were discovered until day 7. These cutoffs are the upper limits that the NTP will tolerate (Mariutti & Bragagnolo, 2017). The goal was to develop a healthy recipe for alpaca burgers.

Image recognition is a popular application of machine learning. It may be used to identify objects or people in photos. It is common practice to utilize image and facial recognition software to automatically suggest persons to tag in images. Facebook may soon suggest people you already know to automatically tag in your images. Facebook uses machine learning to identify people in photographs and propose names to put in the captions. Deep Face is Facebook's proprietary system for automatically identifying and naming individuals in photographs.

Essential oils, which are extracted from aromatic herbs and used in many manufactured products for their antibacterial and antioxidant properties, are supported by current scientific evidence. Understanding its bioactive impact on meat breakdown is important for the marketability of artisan hamburgers and their offspring. Although there is a large amount of

literature showing the bioactive influence of essential oils on meat, further research is needed to establish the consequences of consuming artisan burgers cooked with essential oils. For example, new methodological approaches that include essential oil mixtures that express a synergistic antimicrobial effect through the accumulation of active components. It is just one of the examples that allows a more detailed study of the application of essential oils, providing greater reliability and validity to the reader.

Our applied methodology allowed to improve the useful life of the product, antimicrobial effect and acceptability of hamburgers made from alpaca meat and which guarantees a good quality and nutritional product.

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