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EFFECT OF PROPOLIS AS A PRESERVATIVE APPLIED TO ALPACA (VICUGNA PACOS) MEAT HAMBURGER, HUANCAVELICA, PERU

ALFONSO RUIZ RODRIGUEZ[1], FRANKLIN ORE ARECHE[1]*, OLIVER TAYPE LANDEO[1], OLGA VICENTINA PACOVILCA-ALEJO[1], CESAR CIPRIANO ZEA MONTESINOS[1], RAFAEL JULIAN MALPARTIDA YAPIAS[2], JENY YANET MARQUEZ SUL CA[1], TANIA JAKELINE CHOQUE RIVERA[3], DENIS DANTE CORILLA FLORES[1], FLOR BEATRIZ LIZÁRRAGA GAMARRA[1], OLIVIA MAGALY LUQUE VILCA[3], JIMMY PABLO ECHEVARRÍA VICTORIO[2], BECQUER FRAUBERTH CAMAYO-LAPA[4], CECILIA YANETT REATEGUI VALLADOLID[5], RUSSBELT YAULILAHUA-HUACHO[1] and SAMI ULLAH[6]

[1]National University of Huancavelica, Huancavelica - Peru.
[2]Altoandina National Autonomous University of Tarma, Tarma - Peru.
[3]National University of Juliaca, Juliaca - Peru.
[4]National University of Central Peru, Huancayo – Perú.
[5]Alas Peruanas University, Peru
[6]PARC Adaptive Research cum Demonstraion Institute, Tank.

Abstract

Natural preservatives have less of a negative effect on human health and other systems, making them the superior choice over conventional preservatives. The purpose of this study was to evaluate the impact of propolis, a natural preservative, affected the flavour and texture of an artisanal hamburger made with alpaca meat (*Vicugna pacos*). An experimental design with 5 treatments was established, a control treatment of alpaca hamburger with artificial preservative (T1) and four experimental treatments with the addition of propolis (0.25 ml, 0.50 ml, 0.75 ml and 1.0 ml in 100 g of meat mixture). The Kruskal-Wallis non-parametric variance test and the Mann-Whitney non-parametric test were utilised to examine the data collected from the randomised full blocks design of the experiment. Thirty semi-trained tasters evaluated propolis on its general look, smell, taste, colour, and texture, in addition to its antibacterial effects on *Staphylococcus aureus*. The results showed that the bacterial count of S. *aureus* was kept at 1.5x10 CFU/g, which is between the allowable ranges of 102 and 103, suggesting that

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CONTACT Franklin Ore Areche franklin.ore@unh.edu.pe National University of Huancavelica, Huancavelica - Peru.

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propolis has the ability to inhibit this bacteria. The sensory tests showed that the addition of propolis does not produce negative effects on the general appearance, smell, taste and texture compared to the control treatment ($p > 0.05$), only the color was affected ($p < 0.05$) only in doses greater than 0.5 ml per 100 g of meat mixture. In conclusion, the applicability of propolis as a preservative in the alpaca meat hamburger is verified.

Introduction

The current tendency in the food industry is to make food of a high enough quality that consumers would buy it. This calls for the addition of goods that keep the food fresh for longer so that it may be marketed and consumed. Pasteurization, freezing, drying, and the use of chemical compounds are just some of the methods used to preserve food for longer periods of time, not only by preventing spoilage due to microorganisms but also by keeping the antioxidant potential high enough to satisfy consumers.[1] Preservatives are used to keep food fresh, and while most of them are harmless, some can cause serious health problems. Preservatives must meet specific conditions and be used in measured amounts. An ideal chemical addition would be non-toxic, water-soluble, flavor-neutral, pH-stable, inexpensive, simple to implement, and effective against pathogens in the food's specific pH range.[2] Food can be preserved in either a natural or chemical way. Refrigerating and freezing, pasteurising, drying, smoking, and pickling are all examples of natural food preservation methods. Food preservation also makes heavy use of sugar, salt, alcohol, and vinegar. The sugar in jam prevents or slows the growth of germs, moulds, and yeasts, and the sugar in many other foods helps to prolong their shelf life by regulating the water content of foods. When added to food, salt (NaCl) quickly increases osmotic pressure, making it impossible for bacteria to survive and multiply. The exterior appearance and coloration of a specimen can be preserved from bacterial or fungal destruction by soaking it in ethanol.[2]

The use of synthetic preservatives in food has been questioned due to their safety[3,4,5,6,7,8, 9,10,11] which has generated a preference for natural products, which has increased research on antioxidants and other preservatives derived from natural sources such as cocoa, rice, apple, red onion, oregano, rosemary, honey and propolis.[3] In chemical preservation, products such as vinegar (acetic acid), lactic acid, citric acid and its salts are used, which when added to food lower the pH to levels that are unfavorable for the growth of spoilage organisms and also, they are used as flavorings.[2] In this context, propolis hav been highlighted for its use in food, for its therapeutic and nutritional uses. Propolis is a resinous product produced by bees, commonly used in folk medicine. It is produced by bees (*Apis mellifera* L.) for different uses in the hive such as filling holes, reducing the entrance and exit of hive openings, lining the internal walls of the hive and interior cells, the repair of damaged combs and the consolidation of mobile combs.[12,13,14] One of the most important properties of propolis is its antimicrobial activity against *Staphylococcus aureus, Escherichia coli, Candida albicans, Salmonella typhimurium* and *Listeria monocytogenes*.[15,16] Quite a lot of research data on the antibacterial capabilities of propolis extract and its use in livestock products has been published on both a national and worldwide scale. Research findings on propolis extract's antibacterial capabilities have been widely published, and many of these studies have practical implications. Propolis has antibacterial characteristics that are effective against gram-positive and -negative bacteria, moulds, and fungi. The aim of this research was to test the effect of propolis as a preservative and antimicrobial on the susceptibility to taste, aroma and texture of artisan hamburger made from alpaca (*V. pacos*) meat.

Materials and Methods

The research was carried out in the province of Acobamba, department of Huancavelica, Peru, located at an altitude of 3417 m a.s.l. n. m. The process of making alpaca meat burgers and the sensory analyzes were carried out in the Agroindustrial Processing Laboratory of the Faculty of Agricultural Sciences of the National University

of Huancavelica. The Proximal Chemical Analysis of the raw material and hamburgers with alpaca meat, as well as the Microbiological Analysis of the treatments were carried out in the FIIA Quality Control laboratory of the National University of Central Peru —Huancayo.

Raw Material

The meats were from the market of the District of Huancavelica Province of Huancavelica, Peru and they were received verifying their freshness. In total there was 4.5 kg of meat. The propolis, as well as the other ingredients used for the preparation of the hamburger and the tests carried out, was obtained from stores located in the market of the District of Huancavelica, Province of Huancavelica, Peru.

Preparation of the Hamburger with Alpaca Meat

The fresh alpaca meat, after being received and inspected, was subjected to a grinding process at a temperature of 8 °C to achieve the appropriate dimensions for the process. This reduction was done with a meat grinder. Then it was refrigerated at a temperature of -4 °C for 6 hours in order to preserve and maintain its organoleptic properties until its respective operation. Subsequently, the ingredients for the preparation were weighed, as detailed in Table 1.

Table 1: Formulation of the alpaca burger

Ingredients	Weight (kg)	Percentage
Alpaca meat	2.25	45.00%
Alpaca fat	0.75	15.00%
Wheat flour	0.41	8.21%
Soy flour	0.1000	2.00%
Salt	0.05	1.00%
Ground oregano	0.0025	0.05%
Pepper	0.005	0.10%
Cumin	0.0015	0.03%
Minced garlic	0.015	0.30%
Ajinomoto	0.0005	0.01%
Chopped onion	0.005	0.10%
Total ingredients	**3.5895**	**71.5%**
Water	1.425	28.50%
Grand total	**5.0145**	**100.00%**

The ground alpaca meat and the previously weighed inputs were mixed in a container (pyrex), at a temperature of 4 °C and left to rest for approximately 2 min, as indicated by Rodríguez Pérez *et al.*[17]

After the elaboration of the meat for hamburger, the addition of propolis was proceeded, with which 5 treatments were established, which are detailed in Table 2.

Table 2: Formulation of treatments with propolis

Treatment	Description	Type
T1	100 g meat mixture with a chemical preservative (nitrite)	Control
T2	Propolis dose of 0.25 ml in 100 g of meat mixture	Experimental
T3	Propolis dose of 0.50 ml in 100 g of meat mixture	Experimental
T4	Propolis dose of 0.75 ml in 100 g of meat mixture	Experimental
T5	Propolis dose of 1.00 ml in 100 g of meat mixture	Experimental

The elaborated products were packed in a Teknopor tray sealed with polyethylene

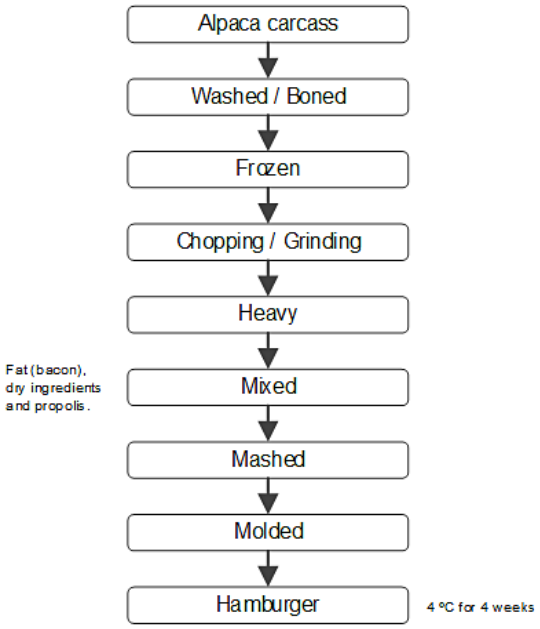

Fig. 1: Flow chart for making alpaca meat burgers

between the pieces to prevent adhesion between them, and then they were cooked at 90 °C for 2 minutes on each side and subjected to sensory analysis. Finally, they were stored at a temperature of 4 °C for 28 days. Figure 1 schematizes the process of making the alpaca hamburger with propolis.

Proximal Chemical Analysis

Proximal chemical analysis was performed on fresh alpaca meat and meat prepared for hamburger according to the methods described in Table 3.

Table 3: Applied methods for proximal chemical analysis

Parameter	Applied norm
Protein	AOAC Official Method 2011.04
Humidity	NTP -ISO.1442-2006
Fat	NTP 201.016-2002
Ashes	NTP 201.022-2002

Microbiological Analysis

The microbiological analysis was performed according to the Sanitary Standard of Microbiological Criteria for Food and Beverages (R.M. No. 591-2008/ MINSA). The analysis was performed every 7 days for a month. The test method was for *Staphylococcus aureus*, according to the AOAC Performance Tested MethodSM 052101 standard.

Sensory Evaluation

The evaluation of the acceptability was carried out by a panel of semi-acting tasters made up of 30 students from the Professional Academic School of Agroindustry of the National University of Huancavelica, evaluating different attributes such as: general appearance, color, taste, smell and texture. For sensory analysis, a 9-point hedonic scale test was applied, in accordance with that indicated by Areche *et al.*[20]

Experimental Design

The study was structured under an experimental scheme of a Completely Randomized Block design with 4 experimental treatments and a control treatment. This design was evaluated through Kruskal-Wallis non-parametric analysis of variance with a significance level of p = 0.05. The statistical design was evaluated with the use of the statistical program MINITAB v.16.

Statistical Analysis

The Kruskal-Wallis non-parametric variance test and the Mann-Whitney non-parametric test were used for comparing the mean values. In this case, the statistical hypotheses evaluated were: Ho: the medians of the treatments are not statistically different with $p \geq 0.05$. Ha: the medians of the treatments are statistically different with $p < 0.05$. For the case of hypothesis tests where Ha is verified, the Mann-Whitney Nonparametric test was applied to two independent samples, in the same way with significance p = 0.05.

Results

Proximal Chemical Analysis

Table 4 shows the results of the composition of the fresh alpaca meat and the elaborated meat for hamburgers.

Table 4: Proximal chemical composition of alpaca meat and alpaca hamburger meat

Features	Percentage	
	Alpaca meat	Hamburguer meat
Humidity	69.01	65.50
Ash	1.36	4.80
Protein	23.30	32.30
Fat	2.36	8.31

It is observed that the hamburger meat presented lower humidity and higher percentages of ash, protein and fat due to the addition of the ingredients and the process of making the hamburger meat itself.

Table 5: Microbiological analysis of hamburgers made with alpaca meat

Time	Staphylococcus aureus count (CFU/g)
Week 1	< 10
Week 2	1.0x10
Week 3	1.2x10
Week 4	1.5x10

Microbiological Analysis

The result of the microbiological analysis was carried out according to the sample of the T2 treatment with a dose of 0.50 ml of propolis from hamburgers made with alpaca meat obtained, which are shown in table 5.

These values are within the limits established in the Technical Health Standard NTS No. 071-MINSAI DIGESA V.01, which establishes the microbiological criteria of sanitary quality and safety for food and beverages for human consumption. According to the standard, in the case of Staphylococcus aureus (CFU/g), the minimum is 10^2 and the maximum is 10^3.

General Appearance of the Hamburger with Alpaca Meat

Table 6 shows the results obtained from the hedonic test for the general appearance test of the hamburger with alpaca meat.

Table 6: Results of the general appearance test of the hamburger with alpaca meat

Treatments	N	Median	Average mark	Z
T1	30	3.00	79.6	0.87
T2	30	3.00	75.2	0.09
T3	30	3.50	77.6	0.42
T4	30	3.00	70.5	-0.84
T5	30	3.00	74.6	-0.54
H parameter = 1.55	GL = 4		p = 0.819	
H parameter adjusted for ties = 1.73	GL = 4		p = 0.786	

According to the Kruskal-Wallis test, both the general p-value and the one adjusted for ties are greater than significance ($p > 0.05$), so it can be said that there are no significant differences between the medians of the 5 treatments at a level confidence of 95%, that is, the addition of propolis does not affect the general appearance of the alpaca meat burger compared to the control T1 without propolis.

Flavor of the Hamburger with Alpaca Meat

The results of the sensory analysis of the flavor of the alpaca meat hamburger are detailed in table 7.

Table 7: Alpaca beef burger taste test results

Treatments	N	Median	Average mark	Z
T1	30	4.50	87.7	1.88
T2	30	4.50	72.6	-0.25
T3	30	4.00	80.3	0.97
T4	30	3.00	62.5	-1,70
T5	30	3.50	73.3	-0.89
H parameter = 6.58	GL = 4		p = 0.160	
H parameter adjusted for ties = 7.67	GL = 4		p = 0.104	

The values of significance $p > 0.05$ for both the general calculation and the adjusted for ties show that there are no statistically significant differences between the treatments regarding the sensation of taste of the hamburger based on the analysis by Kruskal-Wallis, which indicates that the addition of propolis does not modify or affect the flavor of the hamburger compared to the control without propolis.

Color of the Hamburguer with Alpaca Meat

The color sensory test provided the results shown in table 8.

Table 8: Alpaca meat burger color test results

Treatments	N	Median	Average mark	Z
T1	30	4.50	93.8	2.28
T2	30	3.00	66.7	-1.15
T3	30	4.50	89.1	1.63
T4	30	3.50	70.1	-0.89
T5	30	3.50	63.3	-1.87
H parameter = 10.75	GL = 4		p = 0.030	
H parameter adjusted for ties = 12.00	GL = 4		p = 0.017	

In the case of the color of the alpaca meat hamburger, it is observed that the significance $p < 0.05$ according to the Kruskal-Wallis test, both for the general case and for the one adjusted for ties, indicates a significant difference between the treatments, so it can be said that the addition of propolis affects the color of the hamburger. To identify the pairs of treatments that showed significant differences, the Mann-Whitney test was used. The result is shown in figure 2.

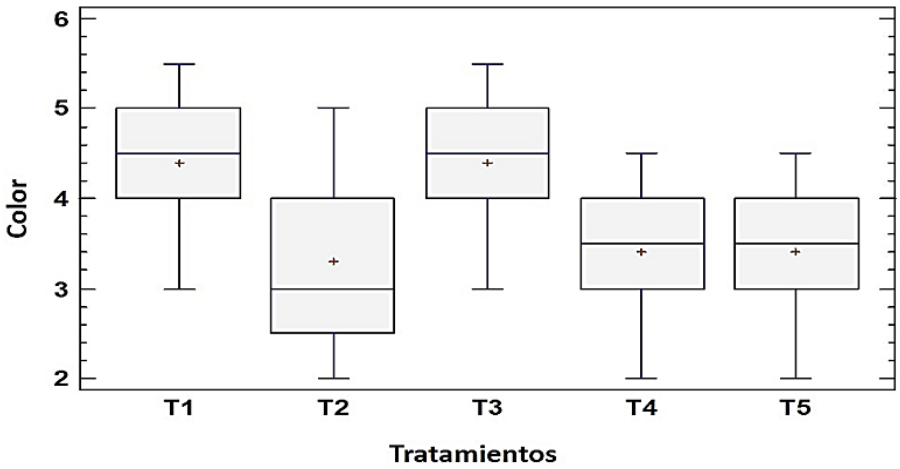

Fig. 2: Boxplot diagram of color results

Figure 2 shows that treatments T2, T4 and T5 do not present significant differences ($p > 0.05$) because their medians overlap between a range of values that coincide. On the other hand, treatments T1 and T3 present medians that are statistically equal and different from the rest. It can be considered that the T3 treatment was the one that preserved color similar to the treatment without propolis (T1). From the above it follows that propolis can be added to the hamburger up to a propolis dose of 0.50 ml in 100 g of meat mixture without affecting the color.

Smell of the Hamburger with Alpaca Meat

The sensory test for the smell of the alpaca meat hamburger provided the results shown in table 9.

Regarding the results of the sensory smelling test, the Kruskal-Wallis p-values ($p > 0.05$), both overall and adjusted for ties, indicate that there are no significant differences between the treatments, which refers to the fact that the smelling is not is affected by the addition of propolis compared to the control treatment without propolis (T1).

Texture of the Hamburguer with Alpaca Meat

The Kruskal-Wallis test for the sensory texture test showed the results detailed in table 10.

The texture of the alpaca meat burger did not present significant differences between treatments, according to the Kruskal-Wallis test ($p > 0.05$). It can be said that the propolis added to the hamburger did not affect the texture, being the same for the treatments with propolis and the one that did not have it.

Table 9: Results of the smell test of the hamburger with alpaca meat

Treatments	N	Median	Average mark	Z
T1	30	4.00	79.8	0.60
T2	30	4.00	74.7	-0.12
T3	30	4.00	77.2	0.23
T4	30	4.00	73.2	-0.33
T5	30	4.00	75.5	-0.39
H parameter = 0.55	GL = 4		p = 0.968	
H parameter adjusted for ties = 0.65	GL = 4		p = 0.958	

Table 10: Results of the texture test of the hamburger with alpaca meat

Treatments	N	Median	Average mark	Z
T1	30	4.50	80.0	1.12
T2	30	3.00	76.4	-0.14
T3	30	4.50	82.3	0.72
T4	30	3.50	65.6	-1.07
T5	30	4.50	73.9	-0.62
H parameter = 2.67	GL = 4		p = 0.615	
H parameter adjusted for ties = 3.05	GL = 4		p = 0.550	

Discussion

The preservative additives of foods such as processed meat are very important not only to maintain their freshness, but also so that their organoleptic characteristics are adequate for the consumer. Hence, the use of natural products derived from plants has become an important research topic, especially in the case of essential oils and extracts such as cinnamon extract,[19] garlic extract,[20] clove extract,[21] thyme essential oil.[22] The foregoing demonstrates the potential of additives of natural origin in the preservation of processed meat. Regarding the composition of alpaca meat, it differs from that of other meats usually used for hamburgers, according to Cobos and Díaz *et al.*[23] moisture is within the range of 72 to 76% in meats of different species, therefore that the humidity determined for the alpaca meat is out of the range with a lower value (69.01%), being closer to the pork meat that presents approximately 72% humidity. Ash from alpaca meat is higher than that from other species (1.36%), with lamb meat reporting a value closer to 1.12%. Likewise, a higher protein value was reported compared to other meats (23.30%) with a similar value in turkey meat (22.64%). The fat in alpaca meat is within the range reported for other meats (1.9 – 5.5%) and the value of 2.36% is between those reported for turkey meat (1.93%) and veal meat (2.87%).

Regarding the processed meat for hamburger, the composition reported by Radünz *et al.*[24] shows

a decrease in moisture and protein of the meat when processed, which corresponds to what was obtained in the case of alpaca meat, which when being processed for hamburger decreased its moisture to 65.50%, however in the case of protein an increase from 23.30 to 32.30% (38.63% increase) was observed, which is not consistent with the reference, although this increase may be due to the addition of propolis, which has variable amounts of protein depending on its production source, that is, the type of plant from which the birds have extracted the raw material for its production, hence values between 7 and 10 have been reported,[25] likewise, added soy flour is also a potential source of protein since it has been reported that it presents typical values of around 45.00% protein[26] and even values greater than 46.00% have been reported.[27] The antibacterial activity of propolis was verified based on its effect on *Staphylococcus aureus*, which is consistent with what was observed by Shariatifar *et al.*[28] who, when evaluating the antimicrobial, antifungal and antioxidant activities of propolis, concluded that it can be a good alternative to the artificial preservatives used in the current food industry. The organoleptic analysis showed that the addition of propolis as a preservative in alpaca hamburger meat does not produce significant effects on the sensory parameters evaluated, with the exception of color, which was affected only when more than 0.50 ml was added to 100 g of meat. meat mixture., so this value can be considered as the limit of addition of propolis in the alpaca hamburger. Similar results, using propolis as a preservative for beef hamburger meat, were obtained by Shavis *et al.*[29] who concluded that, in general, propolis at different concentrations had no adverse effect on the sensory properties of the treated samples compared to those of the untreated control sample during storage time.

Despite showing that the addition of propolis as a preservative to the alpaca burger does not produce negative effects on the organoleptic properties, which remain similar to the burger with an artificial preservative, Pobiega *et al.*[30] indicate, after the results of their study, that a disadvantage of propolis is that it has a unique flavor and aroma, which could negatively influence the sensory properties of the foods to which it is added. Similarly, Özer[31] in his review work reports that in relation to the influence of propolis on the sensory properties of foods, contradictory results are observed, since some investigations report that there is no influence of propolis, however, In other cases, it is stated that if there are changes in the organoleptic properties when propolis extracts are added, for which microencapsulation must be applied, this is especially observed in fish products, so it can be considered that the effect of propolis in the sensory properties will depend on the type of meat to which it is added and in this particular case no significant changes are observed on alpaca meat, which is why it is considered an ideal natural preservative product as a substitute for artificial ones. Because propolis can affect the color of the hamburguer, it should be added in moderate amounts. Studies such as the one by Prakatur *et al.*[32] showed that propolis can affect the color of chicken meat when it is added to their feed, which shows that a color change in alpaca meat is consistent when propolis is applied. On the other hand, Vargas-Sánchez *et al.*[33] when applying propolis as a preservative in beef and pork hamburgers, they reported that it better preserves their color during refrigeration compared to hamburgers that did not contain it. This shows that, although propolis can cause color changes in meat, it can also be important for its preservation, so it should not be considered as a negative aspect of it.

Conclusion

The artisanal alpaca meat hamburger presented a nutritional content, according to the proximal analysis, which in some cases, such as protein, exceeds the average values of a commercial hamburger, due to the incorporation of propolis and soy flour that they add a protein content. This makes the alpaca meat burger a healthy and nutritious alternative for consumption. The antimicrobial effect of propolis was corroborated by the microbiological test with which an inhibition of the growth of the Staphylococcus aureus bacteria was achieved, achieving acceptable count values of the same at four weeks, within the permissible ranges according to the current regulations that govern the stuff. Regarding the sensory properties of the alpaca meat burger, it was found that the addition of propolis as a preservative does not cause negative changes in appearance, smell, taste and texture compared to the alpaca meat burger with synthetic preservative,

based on in the Kruskal-Wallis test ($p > 0.05$). However, an effect on the color ($p < 0.05$) product of the addition of propolis in proportions greater than 0.5 ml in 100 g of meat mixture was corroborated, so this proportion is considered as the limit of addition of propolis for that the hamburger with propolis as a preservative has the same sensory properties as the hamburger with a synthetic preservative and therefore a similar acceptance.

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Conflict of Interest
All the authors declared no conflict of interest.

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