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Milk Clot from Rabbit Stomach (Oryctolagus Cuniculus)

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Abstract

The article examines the presence of milk clot from rabbit stomach (oryctolagus cuniculus). The cheese industry uses a wide variety of coagulants to produce cheese, including natural yeast (cow grass), enzymes from genetically modified microorganisms (Mucor miehei, M. pusillus and Endothia parasitica), plant enzymes from Cirsium spp. (milk thistle) and enzymes of animal origin (pork and chicken). Stomachs of edible animals, such as rabbits, can be used to produce milk coagulants, mainly in traditional cheese dairies producing fresh and semi-cured cheeses. The proteolytic activity and coagulation potential of rabbit (Oryctolagus cuniculus) stomach extract with the addition of NaCl and ethanol to milk was evaluated. The investigation was carried out in two phases. First, the effects of salt on gastric desiccation, coagulation by the method of Chazarra et al. and proteolytic activity were investigated using freshly slaughtered rabbit stomachs. Treatments include: 1) brine (NaCl), 2) surface saturation and 3) NaCl-free. The stomach is then dried by inhalation. In the second step, determine the effect of NaCl concentration combined with ethanol on binding strength and proteolytic activity; six levels of ethanol addition and six levels of NaCl were used for this purpose. In the first stage the scheme is completely randomized and in the second stage completely randomized with a 664 factorial arrangement, with six levels of NaCl, six levels of ethanol and four salvages. Mean values were compared with Tukey's test ($p<0.05$). In the first stage, NaCl-free stomach showed higher enzymatic activity and when saturated with saline or surface saturation, higher binding strength was observed. In the second stage, ethanol concentration did not affect fruit set. The best results were obtained with 1-5% NaCl and differed ($p<0.05$) from those obtained without NaCl. Rabbit stomach supplemented with NaCl and ethanol was found to have the effect of coagulating milk. As a result, these properly processed internal components can be used to produce cheese. Rabbit stomachs contain coagulating enzymes that can be extracted with solutions containing 1 to 5% NaCl and pH 4. The addition of NaCl and ethanol to rabbit stomachs did not affect coagulant activity and fruit set strength when tested in cow's milk.

Keywords: *Concentration, Superficial saturation, Gastric desiccation.*

Introduction

The coagulant is a chymosin or renin, which is an aspartic protease produced in the stomachs of child calves and lacquered sheep, Salazar (2015) This enzyme is used as a milk coagulant, because it hydrolyzes ncasein and coagulates unstable casein micelles to form a gel matrix that retains fat water and some soluble components of milk this process is gelation or coagulation (Calvo, 2015). In addition to chymosin, the stomach of lactating ruminants contains lipolytic enzymes that help hydrolyze milk fat this gives the cheeses the characteristic flavor (Arteaga, Mendoza, Barre, & Vargas, 2019).

The quality of the coagulant is mainly related to the coagulant activity of milk during a certain period of time, this capacity is reduced during storage because enzymes, mainly chymosin, are autolytic and affect activity. After three months the binding capacity decreases by 16%, and after six months of storage it could be 26% (Rivadeneira & Mendoza, 2019).

According to (Gema López Guzmán1, 2014), the coagulation force decreased proportionally with the increase in milk temperature, the maximum coagulation activity occurred at the milk temperature of 30 °C and at 65 °C this activity was completely inhibited chymosin exhibited a maximum activity when the

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pH of milk from 5.5 to 5.8 and inactivity declines to 8.0 (Serpas, 2022).

Natural rennet is produced industrially or processed manually and sold in liquid form ((ALAIS, 2016)) or paste (Ramón-Canul, 2019). The disadvantage of Manual rennet is the limited microbiological control during production which affects its safety, therefore, additives that do not affect coagulation are used such as boric acid Ramos & Quispe (2019), NaCl or Castillo ethanol (2020), otherwise they will lower the pH (Ramos & Gretel, 2019).

To develop a milk coagulant similar to that of calves, coagulating milk proteases of microbial, vegetable and non-ruminant origin are being investigated (Carvajal, 2020). Although different proteases coagulate milk, most of them are specific to other their treatments resulting in cheeses with low yields or undesirable flavors (Alarcon, 2020).

Microbial coagulants contain only one type of chymosin (A or B) and the others are absent in natural rennet. Vegetable cheeses have a low coagulation capacity, contain mainly enzymes with high proteolytic activity resulting in a poor paste and low in

cheese consistency (Faya, 2019). Non-ruminant coagulants include gastric enzymes from chicken (Alva, 2019) and pork that have low coagulation capacities compared to those of pigs so commercial products allowed on the market must be introduced a type of animal whose stomach contains coagulation enzymes is the rabbit

Oryctolagus cuniculus: Rabbit's milk contains 12.3% protein, of which 70% is casein. Rabbits only fed their cubs once a day showing high protease production In this case the intensity of fruit set may be the same as ruminants. Therefore, the objective of the study was to evaluate the coagulation and proteolytic activity of the rabbit stomach with the addition of NaCl and ethanol to cow's milk.

METHODS

Biomaterials

To develop a milk coagulant similar to that of calves, coagulating milk proteases of microbial, vegetable and non-ruminant origin are investigated (Carvajal, 2020). Although different proteases coagulate milk, most of them are specific for other treatments resulting in cheeses with low yields or undesirable flavors (Alarcon, 2020).

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evaluate coagulation and proteolytic activity of rabbit stomach with the addition of NaCl and ethanol to cow's milk.

Peritoneal fat, remains of esophageal spleen and the pylorus is dried along with the rest of the duodenum and the contents of the stomach have been removed the stomach is put back to the exposed mucosa

gastric is washed with drinking water for 30 minutes the stomach is divided into three batches 1) 20 stomachs are immersed in saturated NaCl solution (37 g in 100 of water at 20 °C) for 5 minutes 2)20 the surface of the stomach is covered with a solution of NaCl stomach r1 3)60 immersed in saturated NaCl solution (37 g in 100 g of water at 20 °C) for 5 minutes 2)20 the surface of the stomach is covered with a solution of NaCL 2 g of NaCl stomach r1 3)60 stomachs remain free of NaCl.

The stomach is inflated to dry in a forced-air furnace and 45% relative humidity at 35°C is considered dry when they have reached a stable weight of approximately 48 hours and mill in 25 µm granules.

Enzymatic Substances

From 1 g of stomach were prepared 100 ml of the extract in acidified water (acetic acid, pH 4.0). Coagulation capacity was assessed in two stages. First, the effect of NaCl in brine, with and without surface saturation, was evaluated, and after 2 and 10 days, the effect of storage on fruit strength and protein degradation activity was identified. In the second stage, the complexing effect of NaCl (0, 1, 2, 3, 4 and 5%) and ethanol (0, 1, 2, 3, 4 and 5%), depending on the binding power and proteolytic activity, the extracts were evaluated at 4, 8, 12 and 16 days from the date of initiation of storage. All treatments in both phases were performed in triplicate. The strength indicates the amount of rennet that we must add to curdle the milk in the conditions of our manufacture (Rodriguez, 2018).

F = 2,400 x V

T(C)

Where: F rennet strength; T time in seconds; V volume of milk C volume of rennet.

Between enzyme extract applications and how long the clot can last upright with a straw (Chazarra et al., 2007). Extract has no effect when milk does not coagulate after 40 minutes. For these tests, pasteurized milk was used at 63 ° C.30 min, acidity 20 ° Dornic with 50% (CaCl) (2 ml 10 l1Milk).

Definition of Protein

Protein concentrations were determined by Bradford's (1976) colorimetric method to 595 nm on a spectrophotometer (Genesis 10 UV-VIS, THERMO), ovalbumin albumin calibration curve (2 mg ml-1 in 0.9% NaCl) and concentrations from 0 to 30 are expressed as µg of gastric extract ml-1 protein.

Proteolytic Action

It was determined according to the method of Corzo et al. (2012): 500 L of substrate (1% casein in monobasic and dibasic sodium phosphate buffer).0.1 M at pH 6) Shake 50 L of enzyme extract for 30 s, maintain at 35 ° C for 30 min, and after 20 min, 1 mL of 5% trichloroacetic acid was added to stop the reaction. The proteolytic activity was determined spectrophotometrically at 280 nm in the

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supernatant obtained after centrifugation (30 min at 3000 rpm at 4 ° C). Activity was expressed in enzyme activity units (UAE) (absorbance increased by 0.001 mg protein per minute). This analysis was performed in triplicate.

The stomach is inflated to dry in a forced air oven and 45% relative humidity at 35 ° C is considered dry when they have reached a stable weight of about 48 hours and are ground in granules of 25 µm.

Statistical Analysis and Design

From 1 g of stomach were prepared 100 ml of the extract in acidified water (acetic acid, pH 4.0). Coagulation capacity was assessed in two stages. First, the effect of NaCl in brine, with and without surface saturation, was evaluated, and after 2 and 10 days, the effect of storage on fruit strength and protein degradation activity was identified. In the second stage, the complexing effect of NaCl (0, 1, 2, 3, 4 and 5%) and ethanol (0, 1, 2, 3, 4 and 5%), depending on the binding power and proteolytic activity, the extracts were evaluated at 4, 8, 12 and 16 days from the date of initiation of storage. All treatments in both phases were performed in triplicate. The strength indicates the amount of rennet that we must add to curdle the milk in the conditions of our manufacture (Rodriguez, 2018).

RESULTS

The binding strength between gastric extracts with and without NaCl. The extract without NaCl did not coagulate in any of the storage periods. The gastric extract immersed in saline coagulated in the same way as the gastric extract saturated with NaCl (Table 1). This result suggests that NaCl affects coagulation activity, as Whitaker (1994) has shown that NaCl concentrations above 6% can affect enzyme activity, giving

resulting in partial or complete inhibition in several cases. found that NaCl concentrations below 2% increased the solubility and degradation of proteins, and increased enzymatic activity The variable cheese yield (%), in the production of fresh cheese did not present statistical differences (P>0.05), when evaluating with the different rennets, numerically highlighting the best treatment with adult rabbit rennet with 19.23 %, followed by means of 18.32 and 18.23 % in the treatments with commercial rennet and young rabbit rennet respectively, with a standard error of ± 0.78 %.

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Table 1: Effect of NaCl concentration on mycelial growth and inhibition of P. chlamydosporia sporulation in PDA medium.

Ttos. (mmoLL of NaCl	IAC (%)	IAC*	CPI(%)	CPI*
0	0	0,00 e	0	0.00 d

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40	6,55	0.52 d	21,57	0.96 c
80	10,26	0.65 c	71,19	2.01 b
120	42,7	1.42 b	77,69	2.17 ba
160	51,35	1.60 to	85,17	2.37 to
CV		**11,66**		**10,05**

Note; This table was taken from Rodriguez (2018)

DISCUSSION

The effectiveness of rennet by the use of rabbit stomach is a function of temperature, substrate concentration (milk), calcium concentration, and acidity. The usual coagulation temperatures can vary between 28 ° C and 41 ° C, although the most usual is one of 35 ° C, depending on the type of cheese can be mixed with milk with an acidity that can vary between 0.18% titratable acidity to 0.46% (Moreno, Villegas, & Flores, 2018). The

The cheese industry uses various coagulants for the production of cheeses, and natural rennets (calf abomasos), enzymes of genetically modified microorganisms (Mucor miehei, M. pusillus and Endothia parasítica), plant enzymes of Cirsium spp. (thistle) and enzymes of animal origin (pigs and chickens) stand out. The stomachs of food animals, such as rabbits, could be used for the production of milk coagulants, mainly in traditional cheese factories that make fresh and semi-matured cheeses (Dobler, Espinosa, Hernández, López, & Márquez, 2016). The advantage of rabbit breeding lies in the genetic plasticity of the species and the speed of its biological cycle. This plasticity is a function of genetic variability that has its origin in recent domestication and in an absence of intense artificial selection for a specialized target (Levas, Coudert, Rochambeau, & Thebault, 1996).

CONCLUSION

The domestic rabbit is more limited and less used than other domestic mammal species traditionally exploited to meet meat, milk, wool and fur needs. However, its genetic plasticity is great and, therefore, it seems that it can be adapted with sufficient zootechnical productivity to a very varied range of breeding media. Research on the zootechnical behavior of the rabbit and on the development of its breeding is recent (it began less than 40 Aryans ago), even though

Genetic research is older. In the field of breeding and selection, this can be both an advantage and a disadvantage. This advantage is because the tendency to import recipes without studying the country's own problems will be less, and because genetic variability makes it possible to adapt the farm to local conditions. Disadvantageous, although relative, because it will be necessary to establish the guideline of genetic improvement adapted to the needs of the country. The essential limitation is dependence on the breeding environment, which must be studied and then mastered.

Rabbit stomachs contain coagulating enzymes that can be extracted with solutions containing 1 to 5% NaCl and pH 4. The addition of NaCl and ethanol to rabbit stomachs did not affect coagulant activity and fruit set strength when tested in cow's milk. The use of rabbit stomachs as a source of coagulating enzymes is an alternative to conventional rennet, with potential for artisanal cheesemaking.

REFERENCES

1. ALAIS, C. (2016). Milk science, principles of dairy technique. Spain: Continental.

2. Alarcon, K. (2020). Effect of sheep rennet (Ovis aries) pretreatment and coagulation time on the sensory and physicochemical characteristics of fresh cheese.
3. Alva, G. (2019). Milk coagulation coincided by flame rennet enzymes (Lama glama). Peru: Journals - Universidad Nacional Jorge Basadre Grohmann.
4. Arteaga, R., Mendoza, F., Barre, R., & Vargas, P. (2019). Determination of the type of fruit set. SCIENCEMATRIA.
5. Calvo, M. (2015). Food biochemistry, coagulant enzymes.
6. Carvajal, G. (2020). Technical feasibility analysis for obtaining vegetable rennet from raw material produced in Ecuador. Quito: UCE.
7. Castillo, T. (2020). Evaluation of the quality and performance of fresh cheese made from cow's milk using two types of rennet: natural and artificial.
8. Dobler, J., Espinosa, E., Hernández, P., López, L., & Márquez, O. (2016). Coagulant extract of milk from the stomach of rabbits (Oryctolagus cuniculus sp.). Revista Agrociencia, 50(5), 583-593. Retrieved from https://dialnet.unirioja.es/servlet/articulo?codigo=6417850
9. Faya, E. (2019). The physicochemical and sensory characteristics of fresh cheese made with different concentrations of guinea pig rennet (Cavia porcellus).
10. Gema López Guzmán1, E. P. (2014). Types of rennets Venezuelan Journal of Food Science and Technology.
11. Levas, F., Coudert, P., Rochambeau, H., & Thebault, R. (1996). Rabbit breeding and pathology Food and Agriculture Organization of the United Nations, Rome. Retrieved from https://www.fao.org/3/t1690s/t1690s.pdf
12. Moreno, G., Villegas, C., & Flores, A. (2018). Evaluation of the coagulant action of enzymes of animal origin in the production of dairy products. Caribbean Journal of Social Sciences. Retrieved from https://www.eumed.net/rev/caribe/2018/07/elaboracion-derivados-dairying.html
13. Ramón-Canul, L. G. (2019). Efficiency of sensory techniques for the evaluation of artisanal cheeses made with different commercial rennets. Oaxaca.
14. Ramos, & Quispe. (2019). Effect of natural rennet on yield, consistency and color.
15. Ramos, C., & Gretel, M. (2019). Determination of parameters for obtaining and preserving bovine rennet. National University of Cajamarca.
16. Rivadeneira, F., & Mendoza, A. (2019). Characterization of raw material acceptability of four artisanal rennets.
17. Rodriguez, D. J. (2018). Online prediction of the elastic modulus of the curd. Autonomous University of Barcelona.
18. Salazar, V. M. (2015). Extraction and physicochemical characterization of chymosin. Guatemala: UNIVERSIDAD DE SAN CARLOS.
19. Serpas, S. M. (2022). Evaluation of the strength and performance of 4 commercial rennets used for the production of curd from cow's milk. San Salvador.