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Interaction between phospholipase and transglutaminase in the production of semi-soft fresh cheese and its effect on the yield, composition, microstructure and textural properties

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ABSTRACT

Cheese is considered one of the most important products from milk production worldwide, and its world production has long been growing due to the high and constant demand for dairy products. However, cheese yield has always been a main problematic for cheesemakers from an economic viewpoint because of low values. This research aimed to determine the effect of the type of enzyme and its addition time on the composition, yields, texture, and microstructure of a semi-soft fresh cheese. A multi-factor categorical design was used. Type of enzyme: transglutaminase at 10 g/100L, phospholipase at 6.52 mL/100L, or the interaction between them. And, its time addition were: before, simultaneously or after the addition of rennet. The type of enzyme and its addition time affect statistically the chemical composition of the cheese, yield and texture. Whereas, the addition of transglutaminase together with phospholipase after rennet showed the highest moisture-adjusted yield cheese value (Yma:15.460 g/100L). It is worth noting that an increase of up to 3.89% in the yield was achieved when transglutaminase and phospholipase were used. The addition of transglutaminase together with phospholipase after rennet presented most of the best adjusted yields, suggesting an economical application of the enzyme in cheese making.

1. Introduction

World cheese production is approximately 19 million tons per year, obtained from 35% of total milk production (Fox, Guinee, Cogan, & McSweeney, 2017). In Colombia, its estimated production per year is 41 thousand tons, with a per capita consumption of 0.9 (kg per caput) (Fox, Guinee, Cogan, & McSweeney, 2016). The cheeses have been classified according to a series of characteristics, such as texture (depending on the type of humidity), method of coagulation coupled with other criteria and rates of maturation, thus finding a wide variety of these products on the market (McSweeney, Ottogalli, & Fox, 2017). For the case Colombian, one of the most popular and consumed cheeses is the farmer cheese, which is a typical Colombian dairy product prepared from fresh pasteurized cow's milk (López & Novoa, 2009) by enzymatic coagulation using rennet and is characterized by being a non-acid, non-ripe, fresh product, generally pressed, with a soft texture and classified as semi soft-and fatty.

One of the problems that most affects the cheese sector in the world is the yield (Cadavid et al., 2020; Lilbæk et al., 2006; Trancoso-Reyes, Gutiérrez-Méndez, Sepulveda, & Hernández-Ochoa, 2014), which depending on the type of cheese currently ranges from 9 to 20%, and for farmer fresh cheese this varies between 10 and 13%. Cheese yield is a very important parameter for cheesemakers from an economic viewpoint since small differences in yield translate into big differences in profits (Cadavid et al., 2020; Lilbæk et al., 2006; Trancoso-Reyes et al., 2014). Likewise, with the increase in cheese yield, the content of cheese whey produced in the manufacture of the cheese can be reduced, thus decreasing the rates of environmental contamination that this generates,

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and that despite the alternatives for industrial use, it is known that still it is discarded or used in low-cost methods such as feeding pigs or that generate environmental contamination such as dumping them into waterways (Fox et al., 2016). In this sense, different strategies for improving cheese yield have been employed, such as modifying the diet of goats with enzymatic extracts obtained from the spent substrate of fungi, with which the chemical composition of the milk is increased and consequently the yield of the cheese (Trejo-López, Ayala-Martínez, Zepeda-Bastida, Franco-Fernández, & Soto-Simental, 2021). Similarly, online real-time milk classification systems have been developed such as AfiMilk MCS technology, which selects the appropriate cheese milk according to its optical measured coagulation properties and composition, which consequently will favor the improvement of the cheese yield (Katz et al., 2016). Likewise, other strategies have been used, such as the incorporation of whey proteins by high heat treatment of milk and in situ denaturation, by addition of denatured whey proteins to cheesemilk or by high concentration factor ultrafiltration of cheesemilk (Fox et al., 2016). Enzymatic modification of milk proteins to produce cheese with optimized yield and desired properties has also been especially important for the dairy industry for a long time (Topcu, Bulat, & Özer, 2020), which is why in the dairy market there are two enzymes designed for this purpose, which refer to transglutaminase (mTG) and phospholipase, that due to their specificity for the hydrolysis site do not generate sensory defects (Gharibzahedi et al., 2018; Karahan & Akin, 2017).

However, one of the most emerging strategies in promoting the biofunctional properties of these products is the cross-linking of milk proteins with mTG (Gharibzahedi et al., 2018). mTG (EC 2.3.2.13) is an enzyme that, among others, catalyzes acyl transfer reactions between the γ-carboxamide group of a glutamine residue in one protein and the ε-amino group of a lysine residue of a different one, with the formation of ε-(γ-glutamyl)-lysine bond, resulting in the protein cross-linking (Cadavid et al., 2020), among them those of milk (αs_1, αs_2, β, κ-caseins and whey proteins) improving the rheological and physical properties of milk-based acid gels, as well as yield by strengthening functional properties such as emulsifying potential, water binding capacity and solubility (Gharibzahedi et al., 2018). Mainly 3 times have been reported in which mTG is added in the cheese-making process, which consists of (1) adding before curdling with rennet; (2) simultaneously with the rennet and; (3) after coagulating the milk and cutting the curd (Romeih & Walker, 2017). The time of addition of mTG has an effect on the quality properties of the cheese (De Sá & Bordignon-Luiz, 2010De Sá & Bordignon-Luiz, 2010; Mahmood & Sebo, 2009; Pierro et al., 2010).

Phospholipase (EC 3.1.1.32) is an enzyme that hydrolyzes the *sn*-1 ester bond of phospholipids releasing lysophospholipids, free fatty acids, diacylglycerols, among others, depending on the site of hydrolysis, and are highly specific and have little or no activity towards di or triglycerides (Karahan & Akin, 2017). The use of phospholipids increases the yield as a result of better emulsification and water-holding capacity as a consequence of the lysophospholipids that act as surface active agents in the cheese curd, helping in the emulsion of water and fat during processing and reducing syneresis (Karahan & Akin, 2017; Lilbæk et al., 2006).

Give above and that it is important for the cheese industry to improve yields to obtain benefits from the economic and environmental point of view, the objective of the present work was to determine the effect of the type and time of enzyme addition (mTG, phospholipase or the interaction between them) on the composition, yield, texture and microstructure of a semi-soft fresh cheese, taking into account that there are still no reports where their interaction or combined effect and time of addition of the set of enzymes are studied.

2. Materials and methods

2.1. Raw materials

Fresh cows' milk was supplied from a local dairy (Derivados Lácteos del Norte S.A., Medellín, Colombia) and the chemical composition of the standardized cheese milk was as follows (g/100 g): total solids, 11.06 ± 0.10; fat, 3.01 ± 0.01; protein, 2.74 ± 0.01; ash, 0.68 ± 0.02. Chymosin (EC 3.4.23.4, from *Aspergillus nigervar*) was used as a standard cheese-making rennet with strength 1000 international milk-clotting units (IMCU)/mL (CHY-MAX® M, Chr. Hansen's Laboratory Ltd., Medellín, Colombia). Microbial transglutaminase (protein-glutamin γ-glutamyl-transferase, E.C. 2.3.2.13) whose formulation includes milk proteins and lactose, and its enzymatic activity is of 125 U/g of protein, was provided by BDF Natural Ingredients (Probind CH, Girona, Spain). Phospholipase (phosphatidylcholine1-acylhydrolase; EC 3.1.1.32, from *A. Oryzae*) had the strength of 2300 Lecitase units (LEU)/mL and was added into the milk at a rate of 5 LEU per gram of milk fat, according to the specifications of the manufacturer (YieldMAX, Chr. Hansen's Laboratory Ltd., Medellín, Colombia). The calcium chloride was supplied by Tecnas S.A., Medellín, Colombia.

2.2. Cheese making

For the manufacture of semi-soft fresh cheese, the procedure described by Mahmood and Sebo (2009) was considered with some modifications. Standardized fresh cow milk (3% fat) was pasteurized in a pilot plant ultra-high temperature (UHT) plate heat exchanger (E&M, S.A.S, Medellín, Colombia) at 72 °C for 15 s in the milk and dairy derivatives laboratory of the University of Antioquia, cooled down to 36 °C then, calcium chloride (2.5 g/100 L) was added. The pasteurized milk was divided into batches of 2.5 L using a volumetric measuring cylinder, and the rennet was added (5.5 g/100 L) followed by incubation for 40–45 min for coagulation. Then, the curd was cut (size: 30 mm × 30 mm x 20 mm cuboid shape) and left to stand for 5 min at 36 °C. The curd was then heated for 5 min up to 39 °C in a 5 L cheese-vat (E&M, S.A.S, Medellín, Colombia). The separated whey was drained out and the salt was added (1.5% of curd weight) with manual mixing for 5 min. The curd was then transferred into molds and pressed (2 kg/cm^2) for 2 h at room temperature (20 ± 2 °C) in a cheese press (Jarinox®, Colombia). The produced cheese was then stored at 4 ± 1 °C.

2.2.1. Treatment with transglutaminase and phospholipase

mTG (10 g/100 L), phospholipase (6.52 mL/100 L), or mTG and phospholipase together, respectively, were added during the manufacturing process, (a) before (36 °C for 30 min), (b) simultaneously to, (c) after the addition (after 5 min of cutting the curd, incubation at 36 °C for 30 min) of the coagulating enzyme (chymosin) as shown in Table 1.

2.3. Compositional analysis

The moisture, protein and fat content of the cheeses were determined according to the methods established by the AOAC (AOAC, 2000). The moisture content was determined in an air oven (Thermo Scientific™, USA) (AOAC 930.15). Protein was determined by the Kjeldahl method (Velp scientifica, Italy) (AOAC 954.01). The fat was estimated using a Soxhlet extractor (Radleys, USA) (AOAC 920.39). Gross composition of cheese milk was determined according to Gharibi, Rashidi, Jahani-Azizabadi, and Mahmoudi (2020) using a Lactoscan (Milkotronic Ltd., Nova Zagora, Bulgaria). The total solids of the cheese whey were estimated by modified oven method (AOAC 92.523).

2.4. Cheese yield

The yield of each batch was expressed in different calculations, as

Table 1
Levels of independent variables established according to the full factorial design 3^2 with 2 replicates.

Code	Type of enzyme			Time of enzyme addition		
	T	P	TP	B	S	A
TB	+	−	−	+	−	−
TS	+	−	−	−	+	−
TA	+	−	−	−	−	+
PB	−	+	−	+	−	−
PS	−	+	−	−	+	−
PA	−	+	−	−	−	+
TPB	−	−	+	+	−	−
TPS	−	−	+	−	+	−
TPA	−	−	+	−	−	+

TB: transglutaminase before rennet; TS: transglutaminase simultaneously with rennet; TA: transglutaminase after rennet; PB: phospholipase before rennet; PS: phospholipase simultaneously with rennet; PA: phospholipase after rennet; TPB: transglutaminase + phospholipase before rennet; TPS: transglutaminase + phospholipase simultaneously with rennet; TPA: transglutaminase + phospholipase after rennet. -: no/+: yes; T: transglutaminase; P: phospholipase; TP: transglutaminase + phospholipase; B enzyme addition before rennet; S: simultaneous addition of enzyme and rennet; A: addition of the enzyme after coagulation and curd cutting.

can be seen in equations 1-5. Actual yield (Ya) (Equation. 1) and moisture-adjusted cheese yield (Yma) (Equation. 2) were calculated according to Topcu et al. (2020) and Fox et al. (2017). For Yma calculation, reference moisture content was taken as 58 g/100 g cheese considering resolution 1804/1989 of Colombian regulations (Ministerio de Salud, 1989).

$$Actual\ yield\left(Ya, \frac{g}{100g}\right) = \left[\frac{Cheese\ weight\ (g)}{Milk\ weight\ (g)}\right] \times 100 \quad (1)$$

$$Yma\left(\frac{g}{100g}\right) = \left[\left(\frac{100 - actual\ cheese\ moisture\ content)}{100 - reference\ cheese\ moisture\ content}\right)\right] Ya \quad (2)$$

In order to get more information about cheese quality, the yield was also expressed as a percentage of recovery of fat (FY), protein (PY), and total solids (TSY) respectively, according to Lilbæk et al. (2006) and Fox et al. (2017), as shown in the following equation (Equation (3)):

$$Y_{component}\left(\frac{g}{100g}\right) = \left[\frac{Component\ weight\ in\ cheese\ (g)}{Weight\ of\ the\ component\ in\ the\ starting\ milk(g)}\right] \times 100 \quad (3)$$

Likewise, the yield was expressed as the final use of solids in the cheese in relation to each liter of milk worked (GL coefficient) (Furtado, & Brasil Ltda, 2017). Yield was also expressed as yield adjusted to total solids cheese and cheese whey (R_2) (Verdalet, 1991), relative to a reference cheese, for which the control cheese (without mTG and phospholipase) was considered, as shown in the following equations (Equation. 4 and 5):

$$GL\left(\frac{g}{L}\right) = \left[\frac{\left(\frac{\%\ total\ solids\ of\ cheese}{100}\right) \times cheese\ weight\ (\mathrm{Kg}) \times 10}{Milk\ volume(\mathrm{L})}\right] \times 100 \quad (4)$$

2.5. Texture profile analysis

The texture profile analysis was carried out according to and García-Gómez, Vázquez-Odériz, Muñoz-Ferreiro, Romero-Rodríguez, and Vázquez (2019) and Monsalve-Atencio, Ospina-Millán, and Contreras-Calderón (2021), with some modifications. Fresh cheese was cut into small cubes (1.8 × 1.8 × 1.8 cm). Texture profile was determined to 25 ± 1 °C using a EZ Test/CE texturometer (Shimadzu, Japón) and software Trapecium X version 1.12. The compression test was performed using a cylindrical metal compression plate of 75 mm diameter. The compression speed was 50 mm/min with double compression cycles at constant displacement. Samples were compressed to 50% of the original height.

For the interpretation of the texture parameters, the report by Liu, Cao, and Liu (2019), was considered. It was measured hardness (N), adhesiveness (J), cohesiveness, gumminess (N), springiness, and chewiness (N). Hardness was measured as the maximum peak height during the first compression cycle. Adhesiveness was measured as the area under the x-axis on the curve of the compression graph. Cohesiveness was calculated as the maximum height peak in the second compression divided by the peak height in the first compression. Gumminess as the product of hardness and cohesiveness. Springiness was calculated as the ratio of the areas of the second and the first compression. Chewiness was calculated as the product of hardness, cohesiveness and springiness.

2.6. Microstructure by scanning electron microscopy (SEM)

To analyze the microstructure of cheese a JEOL JSM- 6490 L V (Tokyo, Japan) SEM was used according to a modified method described by Vega-Castro et al. (2021). Samples were lyophilized prior to analysis and were fixed on graphite tape, underwent a thin coating of gold (Au) (equipment Denton Vacuum Desk IV) and analyzed under high vacuum to obtain high-resolution SEM images, for this the samples were imaged at an acceleration voltage of 20 kV. Selected micrographs with a proper magnification (1000 ×) were obtained as representative of each sample.

2.7. Experimental design

The influence of the type and time of addition of enzyme (mTG and phospholipase) and its interaction on the yield, and physicochemical and textural properties of semi-soft fresh cheese was evaluated using a full factorial design type multi-factor categorical with duplicate runs, composed of 2 factors, which were set at 3-levels each (Table 1). All run was compared with a control. Statistical differences were determined using analysis of variance (ANOVA), followed by multiple range comparison tests, using the Fischer test (LSD) to determine if there is a minimal significant difference between treatments, and between the levels of each factor and their interactions, with a 95% confidence level. The correlations between the variables were evaluated using the Pearson correlation test with a 95% confidence level. The data were analyzed using STATGRAPHICS Centurion® 19 version 19.1.2 (64 bits), 2020; licensed for use by the Universidad de Antioquia.

3. Results and discussion

3.1. Compositional analysis

Table 2 shows the protein, fat, total solids and moisture content of the cheese, and total solids content of the whey, and shows significant differences between levels of factors enzyme type and time of addition. In general, the addition of phospholipase (P) generated the lowest levels ($p < 0.05$) of protein and fat content of the cheese, and whey total solids content, while the addition of mTG (T) presented a tendency to show the highest values ($p < 0.05$) protein and moisture in cheese, and whey total solids content, which could be related to the results obtained by Pierro et al. (2010), in whose investigation they found that the cheeses manufactured in the presence of mTG presented higher values of moisture and protein compared to the control, meanwhile, the together addition of mTG and phospholipase (TP) presented the highest values ($p < 0.05$) of fat and total solids content of the cheese. The time of enzyme addition presented a variable effect, however, the addition after rennet (A) presented the lowest values ($p < 0.05$) of protein, fat, and total solids content of the cheese, and total solids content of the whey.

On the other hand, the treatments that presented the lowest and highest values of protein content were PA and PS, with 10.840 ± 0.224 and 14.840 ± 0.409 g/100g, respectively (Table 2) ($p < 0.05$); on the

Table 2
Effect of the addition of mTG, phospholipase, or mTG together with phospholipse on the chemical composition of farmer cheese and cheese whey.

		Protein (g/100 g)	Fat (g/100 g)	Total solids (g/100 g)	Moisture (g/100 g)	Whey total solids(g/100g)
Samples						
TB		$14.419 \pm 0.254^{e,f}$	17.706 ± 1.043^{a}	37.843 ± 3.817^{a}	62.157 ± 3.817^{e}	3.516 ± 0.451^{a}
TS		$13.954 \pm 0.176^{d,e}$	$20.866 \pm 0.305^{c,d}$	40.426 ± 0.216^{b}	59.574 ± 0.216^{d}	5.001 ± 0.416^{b}
TA		$13.607 \pm 0.343^{c,d,}$	22.084 ± 0.389^{e}	44.250 ± 0.761^{d}	55.750 ± 0.761^{b}	5.571 ± 0.379^{b}
PB		12.530 ± 0.461^{b}	$20.260 \pm 0.539^{b,c}$	$41.756 \pm 0.581^{b,c}$	$58.244 \pm 0.581^{c,d}$	3.847 ± 0.813^{a}
PS		14.840 ± 0.409^{f}	$21.570 \pm 0.934^{d,e}$	46.950 ± 0.733^{e}	53.050 ± 0.733^{a}	3.261 ± 0.581^{a}
PA		10.840 ± 0.224^{a}	17.490 ± 0.125^{a}	37.549 ± 0.799^{a}	62.451 ± 0.799^{e}	3.201 ± 0.420^{a}
TPB		13.120 ± 0.099^{c}	$20.900 \pm 0.512^{c,d}$	$42.780 \pm 0.724^{b,c,d}$	$57.220 \pm 0.724^{b,c,d}$	3.305 ± 0.320^{a}
TPS		$13.490 \pm 0.375^{c,d}$	$21.377 \pm 0.300^{d,e}$	$43.988 \pm 0.572^{c,d}$	$56.012 \pm 0.572^{b,c}$	3.329 ± 0.484^{a}
TPA		14.180 ± 0.407^{e}	19.410 ± 0.277^{b}	$42.610 \pm 0.587^{b,c,d}$	$57.390 \pm 0.587^{b,c,d}$	3.090 ± 0.307^{a}
Factors						
Type of enzyme	T	c	a,b	A	b	b
	P	a	a	a,b	a,b	a
	TP	b	b	B	a	a
Time of enzyme addition	B	b	a	A	b	a
	S	c	b	B	a	a
	A	a	a	A	b	a

Values are means ± standard deviation (n = 3). Means in the same column having different superscript letters are significantly different ($p < 0.05$). For sample codes refer to section 2.2.

other hand, the treatments that presented the lowest and highest ($p < 0.05$) values of fat content were PA and TA, with 17.490 ± 0.125 and 22.084 ± 0.389 g/100g, respectively (Table 2).

Pierro et al. (2010) found that the addition of mTG simultaneously with the rennet presented a higher protein content in the cheese, concerning the protein content of the cheese obtained with the addition of mTG after cutting the coagulum, while De Sá and Bordignon-Luiz (2010) De Sá and Bordignon-Luiz (2010) reported that the addition of mTG 7 min after rennet presented higher protein content in the cheese compared to cheeses made with the addition of mTG 7 min before and at the same time as the rennet. Mahmood and Sebo (2009) reported that the addition of the enzyme before rennet prevented milk coagulation. These results are contradictory with those obtained in the present investigation since it was the addition of the mTG before rennet presented the highest protein contents, however, other authors have shown that the addition of mTG before the addition of the rennet improved the protein content in the cheese (Darnay, Králik, Oros, Koncz, & Firtha, 2017). Several studies about the use of phospholipase for the manufacture of cheeses have shown its application with addition before rennet (Fatum, Hoeier, Lyne, & Broe, 2008; Lilbæk et al., 2006; Trancoso-Reyes et al., 2014). However, in the present investigation, it was found that the addition of phospholipase simultaneously with rennet had the highest protein and fat contents, ($p < 0.05$) followed by the addition before and after rennet (Table 2). Unlike, other variables as total solids, moisture and whey total solids did, the addition of mTG and phospholipase together, not present significant ($p > 0.05$) differences between the enzyme addition times.

The moisture and total solids content of the cheese, and whey total solids for the samples PS-PA, PA-PS and TPA-TA ranged from 53.050 ± 0.733–62.451 ± 0.799, 37.549 ± 0.799–46.950 ± 0.733 and 3.090 ± 0.307–5.571 ± 0.379 g/100g, respectively (Table 2). Moisture content presented a significant ($p < 0.05$) negative correlation with protein (−0.46) and fat (−0.69) content, a behavior similar to that reported by Topcu et al. (2020) in a Kashar cheese in which mTG was added for its manufacture.

3.2. Cheese yield

TA and TB samples presented the lowest and highest ($p < 0.05$) protein yield (PY) values, with 61.256 ± 6.272 and 82.932 ± 3.924 g/100g, respectively, and it was observed that when mTG and phospholipase were added together, the highest ($p < 0.05$) PY was obtained when the enzymes were added after rennet (Fig. 1 a and Fig. 2a), while the fat recovery (FY) values showed a range of 92.686 ± 9.501–99.017

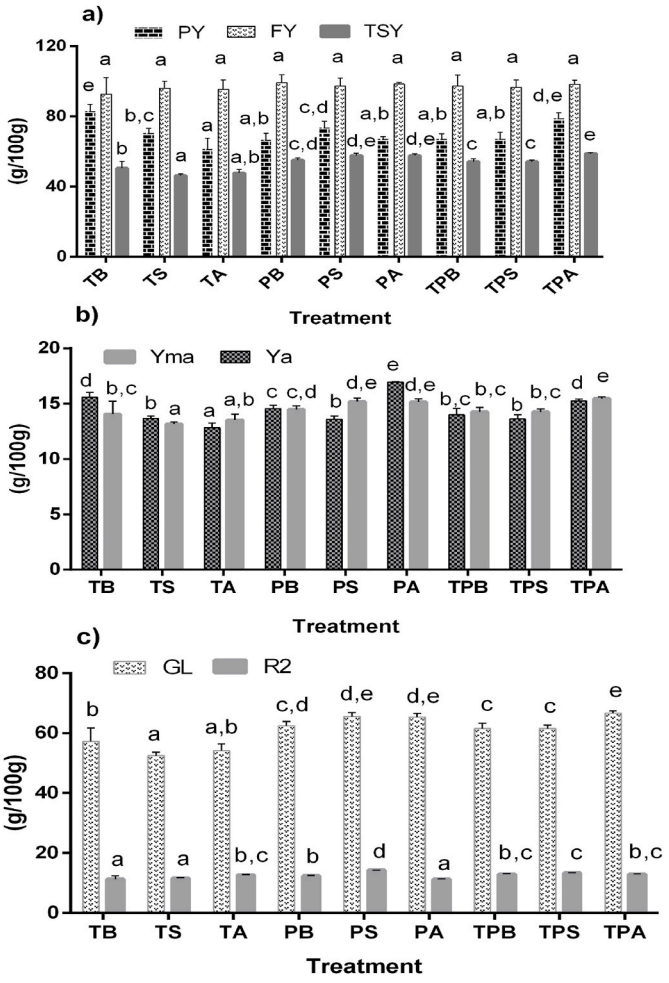

Fig. 1. Mean values and standard deviation for cheese yield. Different letters show significative statistical differences between the treatments (a) Protein (PY), fat (FY), and total solids (TSY) yield; (b) Actual (Ya) and moisture-adjusted (Yma) cheese yield; (c) solids recovery ratio (GL), total solids adjusted cheese yield (R_2). For sample codes refer to section 2.2.

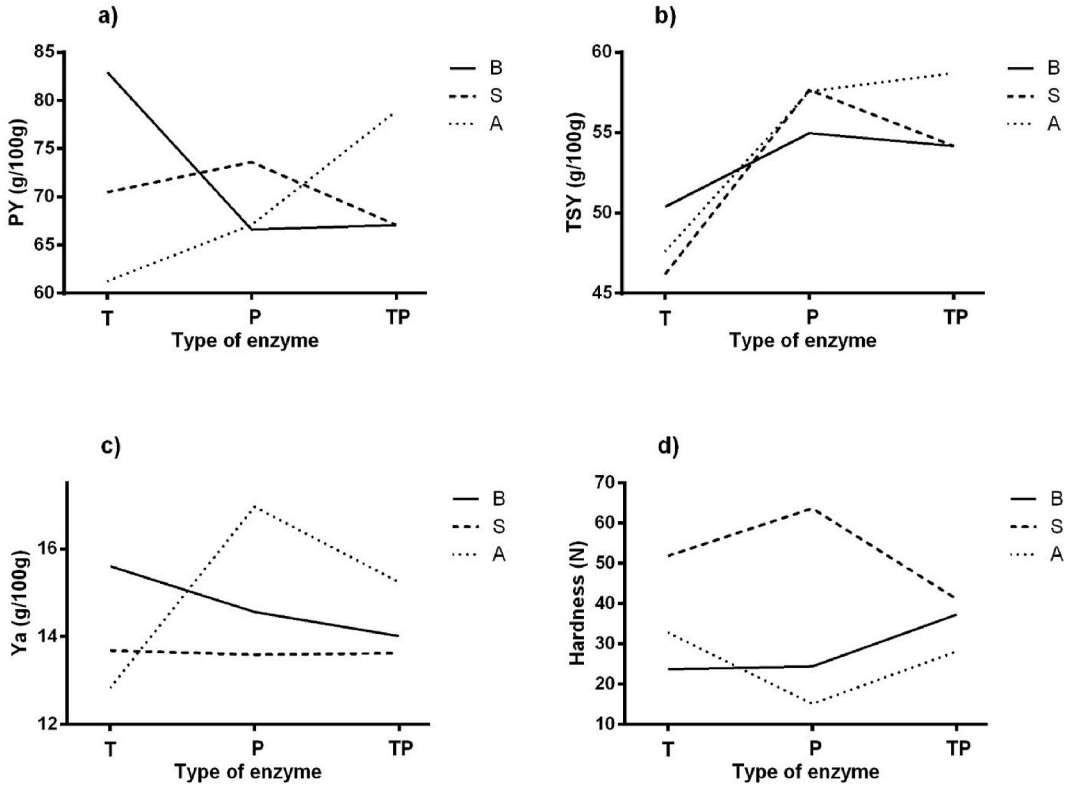

Fig. 2. Interaction effects, (a) Protein yield (PY); (b) Total solids yield (TSY); (c) Actual yield (Ya); (d) Hardness. For sample codes refer to section 2.2.

± 4.584 g/100g ($p > 0.05$), corresponding to samples TB and PB, respectively (Fig. 1a). However, the type of enzyme (combined or separately) and the time of enzyme addition did not show significant differences in protein recovery (PY) and fat recovery (FY) ($p > 0.05$) (Table 3), which could be because both mTG and phospholipase have been shown to increase protein content in cheese or fat recovery, as reported by different authors, which found that the addition of mTG generated an increase in the protein content of the cheese (Cadavid et al., 2020; Darnay, Králik, Oros, & KonczFirtha, 2017; De Sá & Bordignon-Luiz, 2010De Sá & Bordignon-Luiz, 2010; Mazuknaite, Guyot, Leskauskaite, & Kulozik, 2013; Pierro et al., 2010; Topcu et al., 2020), due to the catalytic function of mTG that leads to the formation of additional intra- and inter-molecular covalent bonds between proteins (i.e., αs-, β-, κ-caseins) (Aaltonen, Huumonen, & Myllärinen, 2014), and smaller peptides may also be bound to proteins by mTG (Topcu et al., 2020). Other authors also found that the addition of phospholipase into the cheese milk for the manufacture of cheese significantly increased protein content, that could be explained by the formation of lysophospholipid-protein complexes (Trancoso-Reyes et al., 2014), since lysophospholipids and fatty acid derived from phospholipase action interact with milk proteins, including whey proteins and caseins, that prevent protein-protein interactions, improving protein recovery (Lilbæk, Hanna, Fatum, Ipsen, & Sørensen, 2007). Likewise, a slight increase in fat yield with the use of mTG (Sayadi, Madadlou, & Khosrowshahi, 2013), as well as an increase in fat retention has been reported with the use of phospholipase (Fatum et al., 2008; Lilbæk et al.,

Table 3
Effect of the type and time of enzyme addition on the yield and texture of the farmer cheese, observed through a full factorial desing type multi-factor categorical.

		Ya (g/100 g)	PY (g/100 g)	FY (g/100 g)	TSY (g/100 g)	GL (g/L)	R2 (g/100 g)	Yma (g/100 g)
Factors								
Type of enzyme	T	A	a	a	A	a	a	a
	P	B	a	a	b	b	b	b
	TP	A	a	a	b	b	b	b
Time of enzyme addition	B	B	a	a	a,b	a,b	a	a,b
	S	A	a	a	a	a	b	a
	A	B	a	a	b	b	a	b

		Hardness (N)	**Adhesiveness (J)**	**Cohesiveness**	**Gumminess (N)**	**Springness**	**Chewiness (N)**
Factors							
Type of enzyme	T	A	b	a	a	c	a
	P	A	a	b	a	a	a
	TP	A	a,b	a	a	b	a
Time of enzyme addition	B	B	b	a	b	a	b
	S	C	a	a	c	a	c
	A	A	a	a	a	b	a

Different letters in the same column indicate significant difference ($p < 0.05$). For sample codes refer to section 2.2.

2006), which may be due to the lysophospholipids are released from the fat globule membranes on hydrolysis which interact with milk protein, the incorporation of lysophospholipid-protein complexes into the cheese matrix favors the retention of lysophospholipid (Lilbæk et al., 2006).

TSY of the cheese for the samples TS-TPA ranged from 46.196 ± 1.035–58.170 ± 0.642 g/100g (Fig. 1b.). The treatments that were not added with phospholipase presented the lowest TSY ($p < 0.05$) (Fig. 1 a and Fig. 2b), which clearly suggests that the addition of phospholipase improves TSY, in which the moment of addition of the enzyme present significant ($p < 0.05$) differences (Table 3), which is related to the reports presented by Lilbæk et al. (2006) who found that the addition of phospholipase improved TSY of a mozzarella cheese, while Aaltonen et al. (2014) reported that the addition of mTG in the manufacture of an Edam cheese had no significant effect on TSY. Also, it is noteworthy that the results of the whey total solids content were consistent with the TSY, where the treatments that were not added with phospholipase presented highest ($p < 0.05$) whey total solids content and lowest ($p < 0.05$) TSY, as confirmed a significant ($p < 0.05$) and negative correlation strong between whey total solids and TSY (−0.74), which also presented a significant ($p < 0.05$) and negative correlation with PY (−0.47). The behavior of the time of mTG addition for the samples to which only mTG was added, was the same as that reported by De Sá and Bordignon-Luiz (2010)De Sá and Bordignon-Luiz (2010) concerning the moisture content and total solids of the cheese.

The Ya ranged between 12.835 ± 0.402–16.008 ± 0.562 g/100g, for the TA and PA samples, respectively (Fig. 1b). The addition of phospholipase (P) presented the highest ($p < 0.05$) Ya (Fig. 2c), while the addition of mTG (T), as well as mTG together with phospholipase (TP), did not show significant ($p > 0.05$) differences between them (Table 3); the time of addition of the enzyme also showed a significant effect where the addition of enzyme simultaneously with the rennet showed the lowest ($p < 0.05$) Ya, while the addition of the enzyme before and after rennet did not show significant ($p > 0.05$) differences between them (Table 3), behavior similar to that of moisture (Table 2), which could suggest that the lowest Ya at the time of enzyme addition simultaneously with rennet, with respect to the addition of the enzyme before or after rennet, is associated with the low moisture content of the cheese, as reported by some studies (Ahmed, El-Nimer, Mostafa, & Omar, 2015; Darwish & Taher, 2017; Fatum et al., 2008; Lilbæk et al., 2006; Metwally, El-Zeini, & Gazar, 2018; Pierro et al., 2010; Topcu et al., 2020). Ya showed significant positive correlations ($p < 0.05$) strong with moisture (0.74), and with TSY (0.47) and PY (0.42), which provides indications that the Ya could be explained by the entrapping of water, and recovery of total solids and protein, this could be due to the release of lysophospholipids from the fat globule surface on hydrolysis by action of phospholipase, because surface-active material present in the serum phase can help to emulsify water and fat (Lilbæk et al., 2006), and also the retention of protein (Lilbæk et al., 2007), which could explain the higher Ya values obtained with the addition of phospholipase, with respect to the addition of mTG. Besides, the effect of mTG on casein leads the formation of additional covalent bonds in which more free water is entrapped (Aaltonen et al., 2014). The Ya also presented a significant negative correlation ($p < 0.05$) with the whey total solids (−0.54), which confirms that the cheeses with higher Ya presented cheese whey less loaded with solids.

However, when adjusting Ya to moisture (Yma), to cheese total solids (GL and TSY), and to cheese and whey total solids (R_2), it is notable that for all cases, the treatments to which phospholipase (P and PT) were added presented the highest ($p < 0.05$) adjusted yields (Table 3); and the time of addition of the enzyme simultaneously with the rennet presented the lowest ($p < 0.05$) Yma, GL and TSY, while the addition of the enzyme after rennet showed the highest values ($p < 0.05$) for Yma, GL and TSY (Table 3).

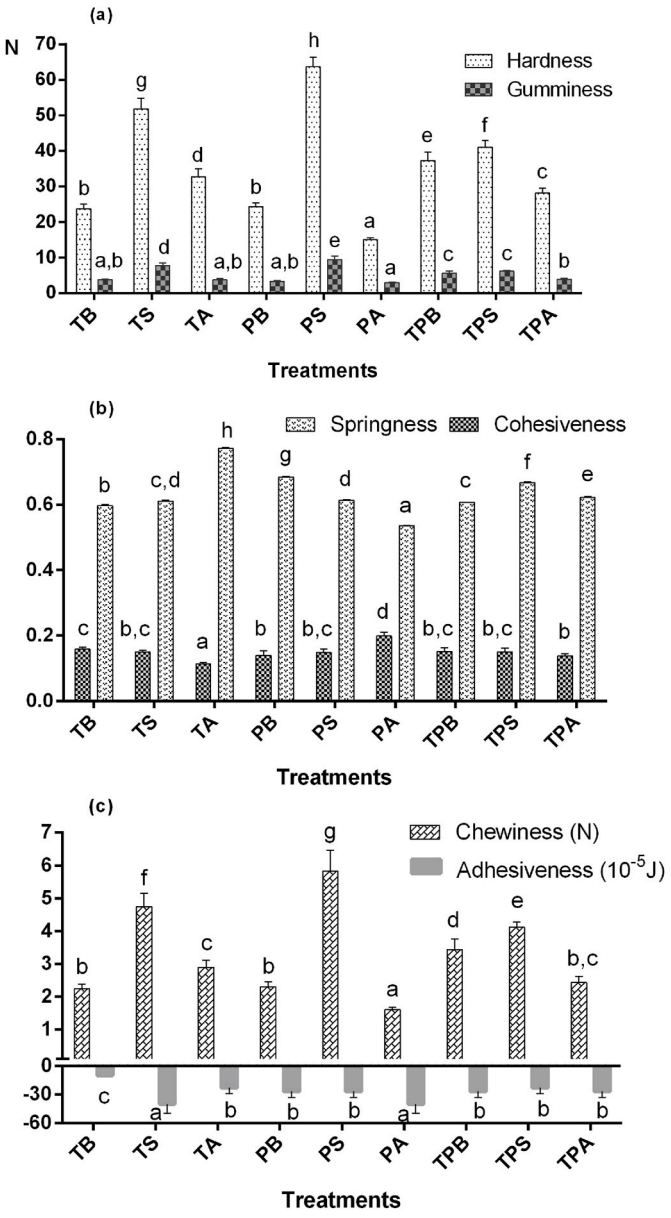

Fig. 3. Mean values and standard deviation for the texture profile analysis. Different letters show significative statistical differences between the treatments (a) Hardness (N), gumminess (N); (b) Cohesiveness, springness; (c) Chewiness (N), adhesiveness. For sample codes refer to section 2.2.

3.3. Texture profile analysis

The results of the texture profile analysis are shown in Fig. 3. It can be seen that the type of enzyme did not present a significant ($p > 0.05$) effect on the parameters of hardness, gumminess and chewiness; on the contrary, the addition of mTG (T) showed the highest ($p < 0.05$) adhesiveness and springness, and the addition of phospholipase (P) showed the lowest ($p < 0.05$) adhesiveness and springness, and higher ($p < 0.05$) for cohesiveness (Table 3); however, the time of addition of the enzyme did not show a significant ($p > 0.05$) effect on the cohesiveness, while the addition of the enzyme simultaneously with the rennet (S) presented the highest ($p < 0.05$) values of hardness, gumminess and chewiness; finally the addition of the enzyme after (A) rennet showed the lowest ($p < 0.05$) values of hardness, gumminess and chewiness.

The addition of enzyme before (B) and after (A) rennet presented the highest values of adhesiveness and springness, respectively. The effect of the interaction between the type and moment of enzyme addition on the

hardness can be observed in Fig. 2 d. Hardness presented a negative significant ($p < 0.05$) correlation strong with Ya (−0.70) and with moisture (−0.66), indicating that the cheeses that had higher Ya and moisture were softer, as mentioned by Darwish, El-Awady, and Mostafa (2019) and García-Gómez et al. (2019), who argues that the high content of moisture leads to weak the protein network resulting soft resulted cheese.

Hardness also showed significant ($p < 0.05$) positive correlations with the content of protein (0.61), as explained by Özer, Robinson, and Grandison (2003) who claims a harder body in a cheese network is a result of more protein level and/or more protein junction points in the network, and a harder body in cheeses is related with the formation of additional inter and/or intra-micellar cross-links induced by mTG (Topcu et al., 2020); in addition, the hardness also showed significant ($p < 0.05$) positive correlations with the content of fat (0.66), total solids (0.66) and R_2, results similar to those reported by Gemici and OneK (2017). Cohesiveness presented negative significant ($p < 0.05$) correlation with total solids (−0.53) and whey total solids (−0.51), and a strong positive significant ($p < 0.05$) correlation with Ya (0.73) and with moisture (0.53), result similar to that reported by García-Gómez et al. (2019), who reported that those samples with a greater cheese yield have been those that have shown the highest water content and cohesiveness.

Guminess showed very strong positive significant ($p < 0.05$) correlations with hardness (0.97), and with total solids (0.54), protein (0.53), fat (0.53) and R_2 (0.53), and negative with Ya (−0.55) and moisture (−0.54). Springness presented strong negative significant ($p < 0.05$) correlations with Ya (−0.71), and positive with fat (0.66), and total solids (0.49); the addition of only mTG showed the highest ($p < 0.05$) values of springness, which could be due to the cross-linking ability of TG which led to a compact protein network and increased the association between casein micelles (Gauche, Tomazi, Barreto, Ogliari, & Bordignon-Luiz, 2009). Chewiness presented negative significant ($p < 0.05$) correlations with Ya (−0.67) and moisture (−0.63), and very strong positive correlations with hardness (0.98), gumminess (0.98), and with fat (0.65), total solids (0.63), R_2 (0.59) and protein (0.56). The best treatments for each variable analyzed concerning the control can be seen in Table 4 in which a variable behavior stands out, therefore, the best treatment will correspond to the privileged variable based on the desired characteristics of the cheese producer.

3.4. Cheese microstructure

Fig. 4 shows the SEM microstructure of cheese made with and without the addition of enzyme (control (C), mTG (TB), phospholipase (PA) and mTG + phospholipase (TPA)). The microstructure of C cheese was clearly different from other samples, where also the PA, TB and TPA samples, which presented higher Ya ($p < 0.05$) compared to the control (see section 3.2), respectively, showed whey channels between cross-linked protein fibers containing fat globules, while the control cheese had a continuous protein matrix, this explains the higher hardness and lower moisture values of the control cheese compared to the other treatments (Danesh, Goudarzi, & Jooyandeh, 2018; Trancoso-Reyes et al., 2014); according to Hennelly, Dunne, O'Sullivan, and O'Riordan (2006), these channels between protein fibers might have resulted from microscopic pools of free water within the sample due to the increased hydration capacity of casein matrix.

The PA cheese was the softest, this could be due to the abundant presence of fat globules on the surface of the protein fiber, which were also in less quantity for the PA sample (Table 2), also giving a narrower structure (Metwally et al., 2018) compared to those who were treated with mTG (TB and TPA). Thinking that the PA cheese showed the highest moisture values, it is notable that the whey channels of less size, which could suggest that the water is emulsified as a consequence of the lysophospholipids resulting from the action of phospholipase (Lilbæk et al., 2006).

4. Conclusions

In the present study, the effect of the type of enzyme (mTG, phospholipase, or mTG + phospholipase) in the cheese making process was investigated, and it was found that it has a significant effect on the chemical composition of the cheese, yield and texture, where the addition of mTG together with phospholipase, generated the highest values of fat and total solids. The addition of mTG and phospholipase improve Ya which was correlated with moisture and recovery of protein and total solids, which shows the effect of enzymes alone or in combination on the ability to emulsify and retain water as corroborated by the microstructure analysis. The addition of mTG together with phospholipase after rennet showed most of the best adjusted yields, suggesting an economical application of the enzyme in cheese making. The results highlight the potential of using enzymes in cheese-making process to improve yield, however, future studies should be undertaken to investigate the time stability, shelf life and organoleptic properties of those cheeses to generate acceptance in the cheese market.

Declaration of competing interest

The authors declared that there is no conflict of interest.

Table 4
Effects of the addition of mTG, phospholipase or mTG together with phospholipse on the chemical composition of farmer cheese and cheese whey, yield and texture compared to the control.

Protein (g/100 g)	Fat (g/100 g)	Total solids (g/100 g)	Moisture (g/100 g)	Whey total solids(g/100 g)	Ya (g/100 g)
C	C	C	C	C	C
14.263 ± 0.358	21.174 ± 0.893	44.590 ± 3.081	55.410 ± 3.081	4.622 ± 0.687	13.065 ± 0.286
PS	TA	PS	PA	TPA	PA
14.840 ± 0.409	22.084 ± 0.389	46.950 ± 0.733*	62.451 ± 0.799	3.090 ± 0.307	16.958 ± 0.057
PY (g/100 g)	**FY (g/100 g)**	**TSY (g/100 g)**	**GL (g/L)**	**R2 (g/100 g)**	**Yma (g/100 g)**
C	C	C	C	C	C
68.614 ± 2.205	92.646 ± 6.746	50.518 ± 3.666	57.381 ± 4.164	13.065 ± 0.912	13.873 ± 1.045
TB	PB	TPA	TPA	PS	TPA
82.932 ± 3.924	99.017 ± 4.584*	58.710 ± 0.642	66.686 ± 0.730	14.282 ± 0.050	15.460 ± 0.169
Hardness (N)	**Adhesiveness (J)**	**Cohesiveness**	**Gumminess (N)**	**Springness**	**Chewiness (N)**
C	C	C	C	C	C
46.888 ± 0.640	−0.0002 ± 0.0001	0.170 ± 0.010	7.951 ± 0.397	0.600 ± 0.003	4.766 ± 0.217
PS	TB	PA	PS	TA	PS
63.657 ± 2.728	−0.0001 ± 0.000*	0.199 ± 0.011	9.504 ± 0.995	0.772 ± 0.003	5.826 ± 0.641

No significant difference with the control at $p < 0.05$. For sample codes refer to section 2.2.

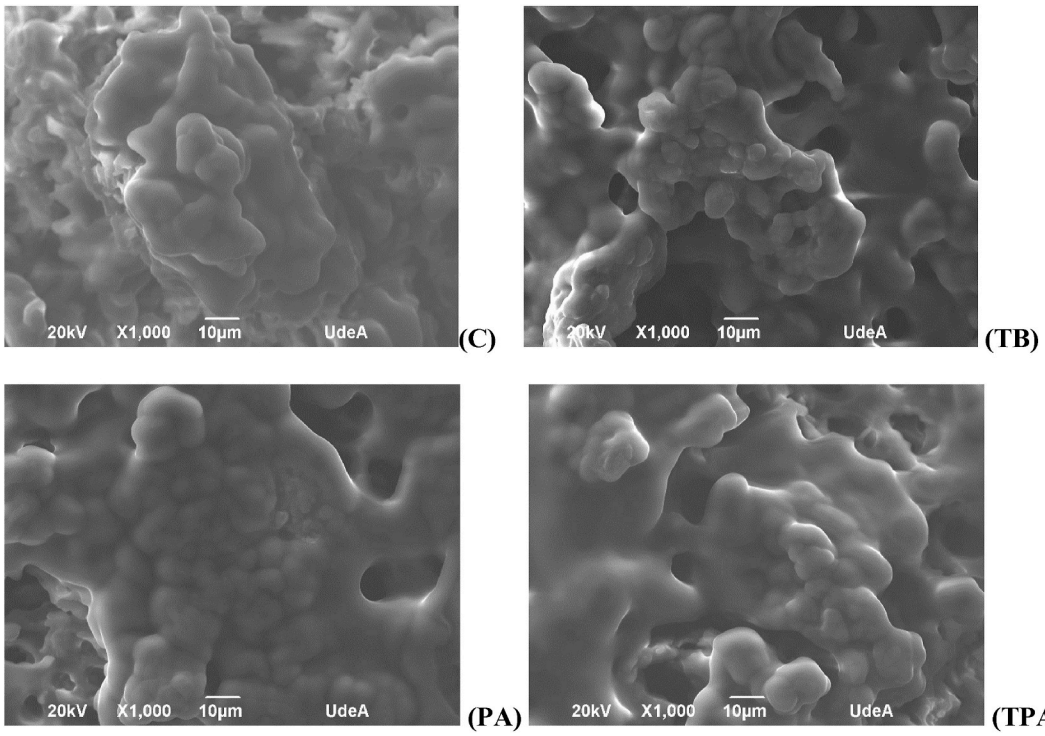

Fig. 4. Scanning electron microscopy of farmer's cheeses. C = control farmer cheese without mTG or phospholipase; TB: Farmer cheese with only mTG added before rennet; PA: Farmer cheese with only phospholipase added after rennet; TPA: Farmer cheese with addition of mTG together with phospholipase after rennet.

CRediT authorship contribution statement

Robinson Monsalve-Atencio: Conceptualization, Formal analysis, Investigation, Methodology, Project administration, Funding acquisition, Writing – original draft, Writing – review & editing. **Karolay Sanchez-Soto:** Conceptualization, Formal analysis, Investigation, Methodology, Funding acquisition, Writing – original draft, Writing – review & editing. **Juan Chica:** Investigation, Methodology, Funding acquisition, Writing – review & editing. **Jairo Andres Camaño Echavarría:** Investigation, Methodology, Funding acquisition, Writing – review & editing. **Oscar Vega-Castro:** Investigation, Methodology, Supervision, Writing – review & editing.

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