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Phospholipid classes and fatty acid composition of ewe's and goat's milk

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RESUMEN

Contenido de ácidos grasos de las diferentes clases de fosfolípidos de la leche de oveja y de cabra

Se ha analizado el contenido, distribución de las especies individuales y la composición en ácidos grasos de los fosfolípidos (FL) de la leche de oveja y de cabra. Se ha estudiado también la unión de cepas enterotoxigénicas y uropatogénicas de *Escherichia coli* a estos compuestos y el efecto de los FL sobre la hemaglutinación provocada por estas bacterias mediante inmunodetección en placa y ensayos en placas multipocillo, respectivamente. La leche de oveja y de cabra contiene más FL que la de vaca, pero menos que la leche humana. El perfil de FL individuales es similar en la leche de oveja y de cabra e incluye esfingomielina, fosfatidilcolina, fosfatidiletanolamina, fosfatidilserina y fosfatidilinositol. En cuanto a la composición en ácidos grasos, los FL de ambos tipos de leche presentan un contenido elevado de ácidos grasos de cadena larga (más de 16 carbonos) y no saturados, siendo el más abundante C18:1. La leche de oveja contiene ácidos grasos más largos y menos saturados, mientras que los de la leche de cabra son más cortos y más saturados. No se ha encontrado adhesión de ninguna de las cepas bacterianas estudiadas a los FL individuales de la leche de oveja o de cabra .Tampoco se ha observado inhibición de la hemaglutinación producida por las bacterias por los FL. Estos compuestos son constituyentes fundamentales de la membrana de los glóbulos de grasa de la leche, pero a la vista de los resultados obtenidos, no parece que participen en la defensa del recién nacido frente a infecciones bacterianas.

PALABRAS CLAVE: Ácidos grasos – Adhesión bacteriana – Fosfolípidos – Leche de cabra – Leche de oveja.

SUMMARY

Phospholipid classes and fatty acid composition of ewe's and goat's milks

The content, distribution of individual species, and the fatty acid composition of phospholipids (PL) from ewe's and goat's milk were analyzed. The binding of enterotoxigenic and uropathogenic *Escherichia coli* strains to PL and the inhibition of bacterial hemagglutination by PL were addressed using high performance thin-layer chromatography-overlay assays and microtiter plates, respectively. Ovine and caprine milk contained more PL than bovine milk but less than human milk. The profile of individual PL was similar, including sphingomyelin, phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine and phosphatidylinositol in both ovine and caprine milk. Regarding the fatty acid composition, a high content of long-chain fatty acids (more than C16) and unsaturated fatty acids, with C18:1 as the most abundant was found in ovine and caprine milk PL. Ovine milk has longer and less saturated fatty acids while caprine milk has shorter and more saturated ones. Neither the adhesion of any bacterial strains assayed to the individual PL from ovine or caprine milk nor the inhibition of bacterial hemagglutination by PL were observed. These are important constituents of the milk fat globule membrane, but it seems that they do not play a role in the defence of new-borns against bacteria if the results obtained are taken into account.

KEY-WORDS: Bacterial adhesion – Fatty acid – Goat milk – Ovine milk – Phospholipid.

1. INTRODUCTION

The gross composition of ewe's and goat's milk has been studied in depth and it has been found to present fat contents of over 7.9 and 3.8%, respectively, of which 0.8% are phospholipids (PL) (Park *et al.*, 2007). Phospholipids are structural components of the membrane that surrounds milk fat globules, the so-called milk fat globule membrane. This membrane is a natural milk emulsifier since milk is an emulsion of lipid droplets in an aqueous phase, with PL playing a very important role in this process (Dewettinck *et al.*, 2008). However, in recent years, bovine milk PL, like other complex lipids such as glycosphingolipids, have gained special relevance as bioactive factors or functional components that may bring health-related benefits, probably stemming from to their fatty acid profile (Lock and Bauman, 2004; Duan and Nilsson, 2009; Küllenberg *et al.*, 2012). They have been used in many food products and in pharmaceutical and

cosmetic applications as an alternative source to egg yolk and soybean PL (Rabasco and González, 2000, Thompson and Singh, 2006). Additionally, some PL, such as sphingomyelin (SM) and phosphatidylcholine (PC), are mainly located in the outer leaflet of the plasma membrane of cells, including the epithelial cells of mammary tissue, and they appear at the surface of the milk fat globule membrane (Morin *et al.,* 2007) and could be involved in binding processes and participate in the protection of new-borns against enteropathogens. In this sense, the binding of *Helicobacter* subspecies to phosphatidylethanolamine (PE) has been reported (Sprong *et al*., 2002)

Milk from ewes and goats is currently used in the dairy industry for the manufacturing of cheese because of its fat and protein contents (Boyazoglu and Morand-Fehr, 2001; Raynal-Ljutovac *et al.,* 2008), and it also affords a high amount of by-products such as buttermilk, whey, and whey cream, which are increasingly appreciated by consumers (Michaelidou, 2008). The PL concentration of such products (buttermilk, etc) is low, and several technological procedures have been developed to increase this content and reduce non-polar lipids at the same time. In later studies, the PL profile and fatty acid composition have been shown to be important in regards to future applications of these ingredients (Miura *et al*., 2004; Rombaut *et al.,* 2006; Spence *et al.,* 2009). Bovine milk has usually been used as a source of PL for many industrial and nutritional applications, with egg and soybean lecithin as alternatives. Egg lecithin is mainly composed of PC (70%), PE (24%), SM (4%) and trace amounts of lyso-PC (LPC), lyso-PE, phosphatidylserine (PS) and phosphatidylinositol (PI) (Kuksis, 1992), while soybean lecithin contains PC (28%), PE (26%), PS/PI (30%), and LPC (16%), with no SM (Boyd *et al*., 1999). In both lecithins, the absence or the very low content of SM is remarkable. Regarding the fatty acid content of these lecithin products, soybean contains a very high proportion of unsaturated fatty acids (77%, with 66% of polyunsaturated) (Boyd *et al.,* 1999), while egg lecithin has 54% of unsaturated fatty acids (21% polyunsaturated) (Kuksis, 1992). The high unsaturated fatty acid content of these products contrasts with the low content of bovine milk PL (31%) (Sánchez-Juanes *et al*., 2009a). Moreover, there are few and somewhat outdated data about the PL content of ovine and caprine milk, including the fatty acid profile of total and individual PL (Morrison and Smith, 1967; Morrison, 1968; Cerbulis *et al.,* 1983).

Taking the above into consideration, the present study was designed to characterize the PL content of ewe's and goat's milk, including the determination of its fatty acid composition. Since PL are located at the surface of the milk fat globule membrane, their possible role in the defence of new-borns against pathogens was also addressed by studying the binding of enterotoxigenic and uropathogenic *Escherichia coli* strains to these compounds.

2. MATERIAL AND METHODS

2.1. Milk samples

Bulk-tank fresh raw ewes' and goats' milk samples from three consecutive days were obtained from the Sociedad Cooperativa Ganadera de Lácteos y Derivados (COGALAD, Valderrodrigo, Salamanca) and the Cooperativa CAPRISAN (Benavente, Zamora), respectively, in the month of November. The ewes and goats were mainly of the Spanish Assaf and Saanen breeds, respectively, that were managed in an intensive type system. They were fed a controlled diet based on alfalfa hay and concentrate (forage:concentrate ratio 50:50). The milk was cooled by the supplier and transported in an insulated container to the laboratory. Samples were immediately frozen at –20 °C, lyophilized, and exhaustively mixed to provide a homogeneous concentration of the different milk components in the sample. The milk corresponding to each day was considered as a different sample. From each sample, several independent PL extraction and determination experiments were carried out.

2.2. Extraction and purification of phospholipids

Phospholipids were essentially obtained from lyophilized milk as previously described for bovine milk (Sánchez-Juanes *et al*., 2009a), with some modifications. Briefly, neutral lipids were eliminated with cold acetone. Polar lipids were extracted with chloroform/methanol mixtures and subjected to a Folch partitioning procedure as modified by Vanier *et al*. (1971) to obtain a lower lipophilic phase and an upper aqueous phase.

Phospholipids were obtained from the lower phase of the Folch system with the Bligh and Dyer procedure (Sánchez-Yagüe and Llanillo, 1986). PL were further purified by TLC. Samples were spotted onto a glass-backed thin-layer chromatography (TLC) plate (Merck, Darmstadt, Germany) and developed in petroleum ether/ethyl ether/acetic acid (85:15:2, v/v/v) as the solvent system to remove non-polar lipids such as acylglycerides, free fatty acids and cholesterol (Astaire *et al.,* 2003). In that system, PL did not move from the origin of the application and the spot was scraped off and the PL were extracted from the silica with chloroform/methanol 2:1 (v/v). Samples were kept at –20 °C until further analysis.

2.3. Analytical procedures

Total lipids were determined with the Röse-Gottlieb method (International Dairy Federation Provisional Standard 1D 1996), as previously described (Zancada *et al*., 2010). Lipid contents were measured gravimetrically.

Phospholipid contents were determined in the lower and upper phases by measuring lipid phosphorus after hydrolysis of the samples with

$HClO_4$ (Rouser *et al.,* 1970). Lipid phosphorous contents were multiplied by a factor of 25 to obtain the PL content (Sánchez-Yagüe and Llanillo, 1986). Individual PL were separated by two-dimensional TLC, identified by co-migration with authentic standards from Sigma (St. Louis, MO, USA), and quantified as described by Sánchez-Juanes *et al.* (2009a). Since PS and PI were poorly separated with the two-dimensional system, they were separated by one-dimensional TLC with chloroform/methanol/acetic acid-water (50: 37.5: 3.5: 2, v/v/v/v.) as the solvent system. Spots of PS and PI were processed and quantified as described for other individual PL.

Because phospholipids were determined as lipid-bound phosphorus after hydrolysis, and because milk has been reported to contain a high amount of inorganic phosphate, the free inorganic phosphate present before hydrolysis was also quantified as described above.

PL purification was checked by high performance thin-layer chromatography (HPTLC). Replicate plates were developed with Phospray (Supelco, Bellefonte, PA, USA) (for PL), anisaldehyde (Sigma, St. Louis, MO, USA) (for glycolipids, glycerophospholipids and sphingomyelin), and orcinol (Sigma, St. Louis, MO, USA) (for glycolipids).

2.4. Determination of the fatty acid content of phospholipids

Phospholipid fatty acid contents were studied by gas chromatography-mass spectrometry at the Mass Spectrometry Service of the University of Salamanca. PL were transmethylated using a boron trifluoride (BF_3)-methanol reagent (Sigma, St. Louis, MO, USA). Individual PL were separated by two-dimensional TLC, except for PS and PI, which were separated by one-dimensional TLC, as described above: each spot was scraped off and its fatty acid content was determined as described for total PL. Since SM has an amide-bound acyl chain, fatty acid analysis of this PL was carried out as reported for glycosphingolipid fatty acids (Zancada *et al*., 2010).

Fatty acid methyl esters from PL were taken up in isooctane, injected into a gas chromatograph apparatus (Shimadzu GC 17A) coupled to a Shimadzu MS QP 5000 mass spectrometer (Kyoto, Japan) and analyzed in the conditions reported previously (Sánchez-Juanes *et al*., 2009a).

2.5. Bacterial strains and media

Four enterotoxigenic *E. coli* strains (ETEC) and two uropathogenic *E. coli* strains (UPEC) isolated from diarrheic calves and humans, respectively, were kindly provided by Dr. Jorge Blanco from the Reference Laboratory of *E. coli* (Lugo, Spain). The characteristics of each ETEC strain were: K99-12 (serogroup O8; adhesin K99), F41-15 (serogroup O20; adhesin F41), K99-4 (serogroup O101; adhesin K99 + F41) and CCB1 (serogroup O101; adhesin F17/Att 25), and for the UPEC strains: FVL3 (serogroup O6:H1; adhesin P), FVL25 (serogroup O2: H1; adhesin P-like). The bacteria were grown in Mueller-Hinton broth (Difco, Detroit, MI, USA) for 3-5 days at 37 °C. When grown in this medium, the bacteria lacked fimbriae. For fimbria expression, the bacteria were grown on Minca agar containing Isovitale X (Becton Dickinson, Cockeysville, MD, USA) at 37 °C for 16 h for the ETEC strains, (Guineé *et al*., 1977) and on CFA-agar plates at 37 °C overnight for the UPEC strains, (Evans and Evans, 1978). Bacteria were also grown at 18 °C for negative controls of fimbria expression.

2.6. Adhesion of bacteria to phospholipids on HPTLC plates

The adhesion of bacteria to PL in HPTLC was examined by immunodetection (Karlsson and Stromberg, 1987). Briefly, PL (4 μg in each lane) were separated in duplicate using one-dimensional HPTLC with an appropriate solvent system: chloroform-methanol-water (60:35:8, v/v/v). One half of the plate was developed with a Phospray reagent and the other was processed for adhesion as previously described for bovine milk PL (Sánchez-Juanes *et al*., 2009a). A bacterial solution of 10 mL (1×10^8 colony forming units (CFU)/mL) was used.

2.7. Hemagglutination assays

Ovine blood samples were provided by Matosa S.A. (Salamanca, Spain). A 5% erythrocyte suspension in 3% mannose containing 150 mM NaCl, 5 mM PBS, pH 7.5, was used. Bacterial growth corresponding to one Petri dish was recovered with 1 mL of PBS and the CFU present in this solution were calculated. From this original bacterial suspension, several dilutions were made to find the minimum number of CFU required to agglutinate erythrocytes. Hemagglutination tests were carried out at 4 °C on sterile V-shaped 96-well plates (Nalge Nunc International, Roskilde, Denmark), as previously reported (Sánchez-Juanes *et al*., 2009b). We assayed individual and total PL (25 and 50 μg).

3. RESULTS

The PL content, the distribution of individual PL species, and the fatty acid content of total and individual PL were analyzed in ovine and caprine milk. Bulk-tank milk was used to obtain a representative milk PL profile, regardless of the stage of lactation, parity, etc. Ovine milk was also used in a previous work by our group (Zancada *et al*., 2010), and some general parameters reported in that work are included here (total solids, fat and protein contents were 19.1%, 7.5% and 5.5% (w/v)), respectively. Caprine milk had values of 12.6% (total solids), 3.9% (fat) and 3.6% (protein).

Ovine milk had a PL content of 297.5 ± 12.8 mg kg^{-1} of fresh milk, corresponding to 308.1 mg

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L^{-1}; whereas caprine milk contained 276.2 ± 19.9 mg kg^{-1} fresh milk, corresponding to 281.6 mg L^{-1}. These values are the sum of the PL recovered from the upper and lower phases of the Folch partitioning procedure, the upper phase containing only 5 to 10% of the total PL.

Regarding the distribution of individual PL, ovine milk contained PE (26.1 ± 2.3); PC (27.0 ± 0.9); PS (10.7 ± 0.5); PI (6.4 ± 0.7), and SM (29.7 ± 2.1) (data expressed as percentages of total PL content: n=4). The values for caprine milk were 26.9 ± 1.1 (PE); 31.9 ± 1.2 (PC); 13.9 ± 0.7 (PS+PI), and 27.3 ± 0.4 (SM), also expressed as percentages of the total PL content (n = 4). PS and PI failed to be well separated.

The fatty acid contents of total and individual PL from ovine and caprine milk were analyzed by gas chromatography-mass spectrometry. The results are shown in Tables 1 and 2. Both milks had C18:1, C16:0 and C18:0 as the most abundant fatty acids in the total PL fraction. However, ovine milk contained more very long-chain fatty acids (C22-C24) (9.5% vs. 2.2%) and less medium-chain fatty acids (C10-C15) (3.4% vs. 19.6%) than caprine milk. Regarding the saturation level of these fatty acids, the amounts of monounsaturated and polyunsaturated fatty acids were slightly different in both milks. The fatty acid contents of individual PL are also shown in Tables 1 and 2. SM was the PL with the most saturated and longest chain fatty acids in both milks. On the other hand, PE had the lowest content of saturated fatty acids and the highest content of monounsaturated and polyunsaturated fatty acids. PC, PS and PI had intermediate values of fatty acid contents, with no very long-chain fatty acids (more than C22).

Since our group is involved in the study of bacterial adhesion to milk compounds, ovine and caprine milk PL were used in the experiments assaying ETEC and UPEC adhesion on HPTLC plates. However, we failed to observe the adhesion of any of the bacterial strains assayed

Table 1

Fatty acid contents of phospholipids from ovine milk (% wt)

FA	Total PL	PC	PS	PI	PE	SM
C10:0	0.2 ± 0.0	0.7 ± 0.0	1.1 ± 0.6	1.4 ± 0.0	–	0.1 ± 0.0
C12:0	0.7 ± 0.4	0.8 ± 0.5	4.3 ± 0.9	3.1 ± 0.3	0.2 ± 0.0	0.4 ± 0.3
C14:0	2.2 ± 0.1	2.8 ± 0.5	9.2 ± 0.6	9.1 ± 1.0	1.4 ± 0.7	1.6 ± 1.0
C15:0	0.3 ± 0.1	1.0 ± 0.5	0.9 ± 0.2	2.0 ± 0.0	1.3 ± 0.4	0.3 ± 0.1
C16:0	19.5 ± 1.6	34.8 ± 3.0	28.8 ± 1.7	25.5 ± 3.5	12.0 ± 1.4	25.0 ± 2.4
C16:1	0.7 ±0 .4	0.6 ± 0.3	–	–	0.5 ± 0.1	–
iso/anteiso C17:0	0.5 ± 0.2	0.9 ± 0.5	–	–	–	–
C17:0	0.5 ± 0.0	0.6 ± 0.2	0.9 ± 0.3	2.2 ± 0.0	0.5 ± 0.1	0.5 ± 0.1
C18:0	18.0 ± 1.0	18.9 ± 2.2	33.9 ± 1.9	28.6 ± 4.2	17.5 ± 0.9	6.3 ± 1.2
C18:1	38.0 ± 0.6	32.3 ± 5.8	16.7 ± 1.9	25.2 ± 3.3	53.6 ± 2.7	0.6 ± 0.2
C18:2	9.6 ± 0.3	5.7 ± 1.3	4.3 ± 0.9	5.6 ± 0.6	13.4 ± 1.0	–
C20:0	0.3 ± 0.0	0.6 ± 0.0	–	–	–	0.7 ± 0.2
C22:0	1.6 ± 0.6	–	–	–	–	10.6 ± 1.0
C23:0	5.1 ±0.6	–	–	–	–	32.1 ± 4.3
C24:0	2.8 ± 0.9	–	–	–	–	21.0 ± 0.1
C24:1	–	–	–	–	–	1.8 ± 0.9
SFA	51.7	61.1	79.1	71.9	32.9	97.6
MUFA	38.7	32.9	16.7	25.2	54.1	2.4
PUFA	9.6	5.7	4.3	5.6	13.4	–
MCFA	3.4	5.3	15.5	15.6	2.9	2.4
LCFA	87.1	94.4	84.6	87.1	97.5	33.1
VLCFA	9.5	–	–	–	–	65.5

FA, fatty acid; PL, phospholipids; PC, phosphatidylcholine; PS, phosphatidylserine; PI, phosphatidylinositol; PE, phosphatidylethanolamine; SM, sphingomyelin; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; MCFA, medium-chain fatty acids (C10-C15); LCFA, long-chain fatty acids (C16-C20); VLCFA, very long-chain fatty acids (C22-C24).
Values are means ± SD; n = 4.

Table 2
Fatty acid contents of phospholipids from goat's milk (% wt)

FA	Total PL	PC	PS/PI	PE	SM
C10:0	2.8 ± 0.0	1.5 ± 0.0	–	0.5 ± 0.0	0.1 ± 0.0
C12:0	4.8 ± 0.4	0.6 ± 0.5	0.1 ± 0.0	1.1 ± 0.2	0.1 ± 0.0
C14:0	10.4 ± 0.1	3.8 ± 0.5	1.3 ± 0.1	2.3 ± 1.4	2.5 ± 0.1
C15:0	1.6 ± 0.1	0.9 ± 0.5	0.5 ± 0.1	0.9 ± 0.0	0.3 ± 0.0
C16:0	22.6 ± 1.6	40.0 ± 3.0	13.3 ± 2.8	21.2 ± 5.1	20.7 ± 1.6
C16:1	1.5 ± 0.4	0.4 ± 0.3	0.2 ± 0.0	0.7 ± 0.3	0.2 ± 0.1
iso/anteiso C17:0	2.4 ± 0.2	1.4 ± 0.5	0.5 ± 0.3	0.4 ± 0.1	0.2 ± 0.0
C17:0	1.3 ± 0.0	0.7 ± 0.2	1.0 ± 0.2	0.5 ± 0.1	0.9 ± 0.1
C18:0	16.1 ± 1.0	8.0 ± 2.2	47.9 ± 8.3	12.4 ± 2.2	8.0 ± 0.1
C18:1	26.1 ± 0.6	34.4 ± 5.8	29.7 ± 10.7	50.6 ± 6.1	0.9 ± 0.2
C18:2	7.5 ± 0.3	5.2 ± 1.3	4.4 ± 2.4	8.9 ± 2.3	–
C20:0	0.8 ± 0.0	0.5 ± 0.0	–	0.5 ± 0.0	2.5 ± 0.2
C22:0	0.7 ± 0.6	0.9 ± 0.0	1.0 ± 1.4	–	18.3 ± 1.4
C23:0	0.8 ± 0.6	1.1 ± 0.0	–	–	25.8 ± 0.65
C24:0	0.7 ± 0.9	0.4 ± 0.0	–	–	16.6 ± 0.8
C24:1	–	–	–	–	2.8 ± 0.3
SFA	65.0	59.8	65.6	39.8	96.0
MUFA	27.6	34.8	29.9	51.3	3.9
PUFA	7.5	5.2	4.4	8.9	–
MCFA	19.6	6.8	1.9	4.8	3.0
LCFA	78.3	90.6	97.0	95.2	33.4
VLCFA	2.2	2.4	1.0	–	63.5

FA, fatty acid; PL, phospholipids; PC, phosphatidylcholine; PS, phosphatidylserine; PI, phosphatidylinositol; PE, phosphatidylethanolamine; SM, sphingomyelin; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; MCFA, medium-chain fatty acids (C10-C15); LCFA, long-chain fatty acids (C16-C20); VLCFA, very-long-chain fatty acids (C22-C24).
Values are means ± SD; n = 4.

to the individual PL in our samples. Because most fimbriae agglutinate erythrocytes, the inhibition of this hemagglutination by milk PL can be used as an indirect measurement of their binding capacity. We have assayed two different dilutions from the original bacterial suspensions. One was the minimum amount of CFU required to agglutinate erythrocytes. The procedure was completed with a further dilution from this reported above (1/2). With these two different amounts of CFU we ensured that erythrocytes would be agglutinated. This agglutination could be reversed and such reversal could be observed macroscopically. The hemagglutination elicited by all six strains assayed was not inhibited by total or individual PL obtained either from ovine or caprine milk.

4. DISCUSSION

Milk has been used for the manufacturing of cheese and other dairy products since the beginning of time. There are many by-products from these industrial treatments, such as buttermilk, whey derivatives, etc. that were formerly considered waste but that are now being taken into consideration for use in nutritional and pharmaceutical applications. Bovine milk has commonly been the origin of those products and this is why the composition of this milk and its derivatives has been extensively studied. For that reason, it has been found that they contain glycosphingolipids and PL, which could be considered bioactive components (Rombaut and Dewettinck, 2006). Ovine and caprine milk is mainly used for cheese production and hence the compositional study carried out with bovine milk also seems to be necessary for such milks.

The PL content recorded here was quite different from that previously reported for ewe's and goat's milk (Morrisey and Smith, 1967). The discrepancies with previous data concerning ovine and caprine milk could be attributed to breed, feeding, fat content, etc, although variations due to

methodological procedures cannot be discarded. Ovine and caprine milk has more PL than cow's milk (Bitman and Wood, 1990; Sánchez-Juanes *et al*., 2009a) but less than human milk (Morrison and Smith 1967).

The percentages of some individual PL such as PC and SM recorded here were similar to those reported elsewhere for ovine milk (Morrison, 1968); however, we detected more PS and PI and less PE. Regarding caprine milk, we found more PC and PS/PI and less PE than other authors (Cerbulis *et al*., 1983). The profile of individual PL found by us in both ewes and goats is also similar to that reported for other ruminants, such as bovines (Morrison, 1968). It appears that all milks have a similar individual PL profile, PC, PE and SM contributing to approximately one third.

There are no data in the literature concerning the fatty acid content of total PL from ovine or caprine milk. Therefore, the fatty acid content of ovine and caprine milks individual PL has been reported (Morrison and Smith, 1967, Cerbulis *et al*., 1983). Ovine and caprine milk total PL showed differences in their fatty acid profiles. Ovine milk had a lower content of saturated fatty acids (52% vs. 65%) and a higher content of long-chain (C16-C20) and very long-chain fatty acids (C22-C24) (96.6% vs. 80.5%) than caprine milk. It seems that the fatty acid composition of the PL from both sources are balanced: Ovine milk has longer and less saturated fatty acids while caprine milk has shorter and more saturated ones, probably giving rise to similar physicochemical characteristics in the MFGM from both sources. The fatty acid profile of total PL from ovine milk is different from that of bovine milk PL (Sánchez-Juanes *et al*., 2009a), including less medium-chain fatty acids and more long-chain or very long-chain fatty acids. The levels of C18:0 were quite similar (18% in ovine milk vs. 19.3% in bovine milk), but the levels of C18:1 and C18:2 were higher in ovine milk. The content of unsaturated fatty acids was higher than that found in bovine milk (26.3% of monounsaturated and 4.9% of polyunsaturated fatty acids; Sánchez-Juanes *et al*., 2009a). Ovine milk PL were less saturated than the PL from bovine milk. By contrast, goat's milk showed a fatty acid profile of PL similar to that of cow's milk.

Regarding the fatty acid content of individual PL from ovine milk, very similar profiles in PC and PE, but differences in the profiles of SM and PS/PI in comparison with previous data were found (Morrison and Smith, 1967). Although in that study the values of PS and PI were given together (Morrison and Smith, 1967), we found more C16:0 and C18:0 and fewer C18:1 and C18:2 fatty acids in our individual PS and PI. Regarding SM, we observed more very long-chain fatty acids (65.5% vs. 54.9%) and saturated fatty acids (97.6% vs. 88.4%) than the above mentioned authors. Ovine milk individual PL also had a fatty acid content similar to the PL from bovine milk. However, PE from ewes had a higher content of unsaturated fatty acids (67.5% vs. 33.5%) and a lower content of C16:0 (12.0% vs. 35.5%) than bovine milk PE (Sánchez-Juanes *et al*., 2009a). The fatty acid profile of goat individual PL was very similar upon considering PC and SM and slightly different for PE and PS/PI from that previously reported by Cerbulis et al. (1983) for goat's milk PL. We found a PE with more C16:0 and less C18:0 and a PS/PI with more C18:0 and less C18:1 than Cerbulis *et al*. (1983). As previously mentioned (Miura *et al*., 2004; Rombaut *et al*., 2006; Spence *et al*., 2009), bovine milk PL and egg and soybean lecithins have proven to be important in many nutritional and industrial applications. These lecithins have very reduced SM contents and high amounts of unsaturated fatty acids (Kuksis, 1992; Boyd *et al.,* 1999). By contrast, bovine milk contains 23% of SM but lower amounts of unsaturated fatty acids (Sánchez-Juanes *et al.,* 2009a). From our results it can be inferred that ovine or caprine milk PL lie in an intermediate relative position among bovine, egg and soyben PL since they have 27-30% of SM and their unsaturated fatty acid contents range between 35-48%. Ovine or caprine milks could be used in processes in which bovine, egg or soybean PL would not be suitable.

Fat globule membranes containing ovine and caprine milk by-products could be used in calf and infant nutrition. These membranes contain PL and could participate in the defence of new-borns against infections (Sprong *et al*., 2002). Accordingly, we assayed the binding of ETEC and UPEC to ovine and caprine milk PL that had been separated by HPTLC. Nevertheless, we failed to observe the adhesion of any of the bacterial strains assayed to the individual PL from our samples. These results are consistent with those obtained in a previous work by our group addressing bovine milk PL (Sánchez-Juanes *et al*., 2009a). Regarding the inhibition of bacterial hemagglutination by PL, our present results confirm those obtained in the HPTLC-overlay assays. The hemagglutination produced by all six ETEC and UPEC strains was not inhibited by total or individual PL from ovine or caprine milks. These results are also in agreement with those previously reported for bovine milk PL (Sánchez-Juanes *et al*., 2009a).

It seems that milk PL do not participate as putative receptors for bacteria in direct binding to cell membranes, although they are probably important in the binding of bacteria to membrane glycoconjugates through a modification of the packaging and presentation of sugar ligands.

5. CONCLUSIONS

In light of these and other previous results, a direct interaction or the adhesion of bacteria to PL, and hence a role of these compounds in new-borns' defence against bacteria, can be discarded. Despite this, since ovine and caprine milk derivatives or industrial dairy products are currently produced in large amounts, and since they have no acknowledged

usefulness, they could be an interesting and economically valuable alternative source of PL for use instead of bovine milk, egg, or soybean lecithin PL in infant formulas, food additives, and ingredients in therapeutic and cosmetic products.

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