Influence of milk pH on the chemical, physical and sensory properties of a milk-based alcoholic beverage

Rodrigo A. Ibáñez, Stefanie Vyhmeister, María F. Muñoz, Natalia Brossard, Fernando Osorio, Fernando N. Salazar, María Angélica Fellenberg and Einar Vargas-Bello-Pérez

SUPPLEMENTARY FILE

Fig. S1 Appearance of *Licor de Oro* made from milks acidified to different pH values.

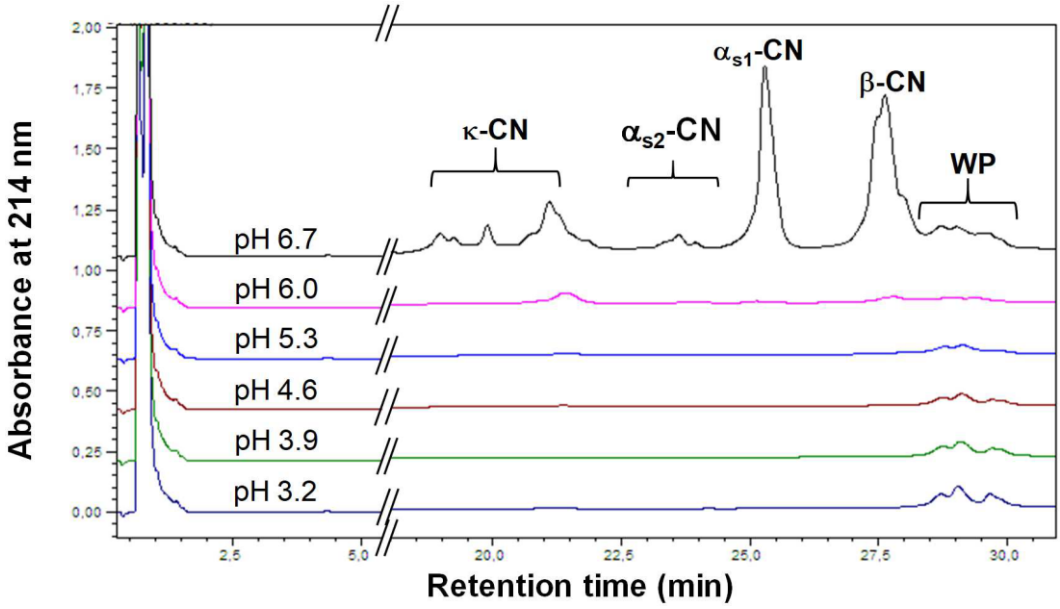

Fig. S2 Protein profile chromatograms obtained from *Licor de Oro* made with different pH values

Table S1 Description of methodology used for the analysis of *Licor de Oro*.

Analysis	Method description
Total Solids	Oven-drying method (AOAC 2007).
Total protein	Kjeldahl (%N × 6.38; IDF 1986).
Protein profile	Reversed-phase high performance liquid chromatography (Bonizzi *et al.* 2009) with some modifications using a Shimadzu Prominence system which consisted of a DGU-20A5R degassing unit, a LC-20AD quaternary pump, a SIL-20A autosampler, a CTO-20A column oven and a SPD-M20A diode array detector interfaced with LabSolutions software (Shimadzu Corporation, Tokyo, Japan). The column used for analyses was a Restek® Viva C4, 5 μm spherical particle size, 300 Å pore size, 2.1 × 150 mm. Elution was monitored at 214 nm and the mobile phase consisted of two solvents: A, 0.1% trifluoroacetic acid (TFA, Sigma-Aldrich, St. Louis, MO, USA) in liquid chromatography (LC) grade water (LiChrosolv®; EMD Millipore Corporation, Billerica, MA, USA); and B, 0.1% (v/v) TFA in LC grade acetonitrile (LiChrosolv®; EMD Millipore Corporation, Billerica, MA, USA). Aliquots of 0.4 mL of *LO* were mixed with 1.6 mL of an urea buffer, filtered through 0.45 mm polyethersulfone filter (Biocomma Limited, Shenzhen, P.R. China) and 30 μL of the filtrate was injected for LC analysis at an eluent flow rate of 0.75 mL/min, with column oven equilibrated at 40°C. The elution gradient was linear from 20% B (0 min) to 50% B (30 min), followed by isocratic gradient of 20% B from 30.1 to 35 min to maintain initial conditions of analysis for following samples. A blank consisted of LC grade water mixed with urea buffer at a ratio 1:5 was run before and after each *LO* sample to prevent accumulation excessive sugar in the system. Chromatogram peaks were identified by comparing peak intensity and retention times with casein (α_s-, β- and κ-) and whey protein (α-lactoalbumin and β-lactoglobulin) standards (Sigma-Aldrich, St. Louis, MO, USA).
Ash	Gravimetric method by heating samples at 550°C × 4 h (AOAC 2007).
pH	Direct measurement with pH electrode on samples at 20°C (INN 1979).
Titratable acidity	Addition of NaOH 0.1 N until phenolphthalein endpoint at pH 8.3 (INN 1998).

Continued

Table S1 (Continued)

Analysis	Method description
Ethanol content	Direct measurement with with an alcoholmetre at 20°C on distilled alcohol obtained from 250 mL of sample.
Density	Use of a 5 mL pycnometre.
Viscosity	Use of a controlled stress rheometre (Discovery HR-2; TA Instruments, Waters LLC, Leatherhead, Surrey, UK) equipped with a conical geometry (60 mm diameter, 1.0081° and 27 μm gap; H/A-AL ST, TA Instruments). Shear rate was increased from 0.1 to 300/s over 8 min at 25°C.
Turbidity	Direct measurement on a nephelometre (HI 83749, Hanna Instruments, USA) at 20°C.
Colour	Direct measurement with a colorimetre (Konika-Minolta CR-400, Konika-Minolta Optics Inc., Osaka, Japan) set to the CIELAB system (Hunterlab 2012), illuminant D65 and a visual angle of 2°, using a glass cuvette (CM-A96) contained in a sample holder (CR-A505) and a white calibration plate (CR-A43) as background.
Sensory analysis	The appearance, texture and flavour attributes of *LO* samples were measured by a combination of Spectrum and quantitative sensory analysis (Meilgaard *et al.* 1999). *LO* samples were evaluated in duplicate by 12 panellists with at least 15 h of training. Samples were identified with random 3-digit code. *LO* samples (45 mL) were served in 90 mL transparent cups at ~22°C. Sensory evaluation was performed using a numerical scale, ranging from 0 to 15. Description of evaluated attributes and their references are shown in Table S2.

Table S2 Definition of the attributes used by trained panelists to evaluate the sensory properties of *Licor de Oro* at 22°C[*].

Attribute	Definition and evaluation procedure	References used, preparation instructions and anchor points (0-15)
Turbidity	Degree of visual haze in beverage caused by suspended particles.	Deionized water = 0.0. Skim milk 0% fat (Colun) = 15.0.
Whiteness	Degree of white color developed in beverage.	Orange Crush soft drink (orange color; Dr. Pepper Snapple Group) = 0.0. Pap soft drink (intense yellow color; CCU) = 5.5. Skim milk 0% fat (white color; Colun) = 15.0.
Creaminess	Degree of thickness in beverage perceived by pressing the tongue with on the palate.	Deionized water = 0.0. Full-fat cultured milk (Soprole) = 5.0 Sweetened condensed milk (La Lechera Nestlé) = 14.0
Alcohol	Sensation perceived due to the presence of ethanol.	None to pronounced.
Sweet	Basic taste sensation elicited by sweet compounds.	None to pronounced.
Acid	Basic taste sensation elicited by acids.	None to pronounced.
Bitter	Basic taste sensation elicited by bitter compounds	None to pronounced.
Milkfat	Aromatics and flavor associated with milk or fresh cream.	None to pronounced.
Vanilla	Aromatics and flavor associated with vanillin.	None to pronounced.
Cloves	Aromatics and flavor associated with eugenol.	None to pronounced
Pungent	Chemical feeling factor associated with high concentrations of irritants to the mucous membranes of the oral cavity	None to pronounced.
Astringent	Harsh, drying, puckering sensation on the surfaces of the mouth.	None to pronounced.

[*]Attributes were evaluated using Spectrum and quantitative descriptive analysis (Meilgaard *et al.* 1999).

References

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