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Short Communication

Factors Affecting Foaming Properties of Milk in Cappuccino Coffee

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JW presented the concept. XL, JW, TW and WW planned methodology. JW perfomred formal analysis, data curation and wrote the manuscript. XL validated the results. WW, TW and XZ arranged resources. YS acquired funds, wrote and reviewed the manuscript.

Key words
Milk protein, Fatty acid profiles, Amino acid, Cappuccino coffee

ABSTRACT

The objective of this study was to compare milk protein, amino acid and free fatty acid content among two kinds of milk with different foaming properties, used in coffee. For the milk protein, a significant difference between milk types was found in β-lactoglobulin (β-LG) and α-lactalbumin (α-LA), but not in casein content. The content of β-LG and α-LA in milk with the high foaming property of 70-96% (milk C) was significantly higher than that of milk with the low foaming property 30-48% (milk E). In terms of essential amino acids (EAA), the leucine (Leu) and lysine (Lys) were present in the highest quantities, and of the nonessential amino acids (NEAA), glutamic acid (Glu) had the highest content. Of the free fatty acids (FFA), the saturated fatty acids(SFA) content in milk C was lower, and unsaturated fatty acid (UFA) content was higher, than in milk E. The highest FFA contents were observed for C14:0, C16:0 and C18:1, and the C16:0 content in milk E was significantly higher than in milk C. In brief, although whey protein improves foaming, but the presence of FFAs strongly impairs foaming.

Over the past century, the foaming properties of milk have been studied for a variety of purposes. Milk is a product that people consume daily, and its foaming properties are important both in the production of processed food and dairy-based foams such as whipped cream, ice-cream, or cappuccino-style beverages. Various molecules such as proteins, emulsifiers, and solid crystals of fat that are present in a liquid phase or in a semisolid to solid matrix, can act as surfactants in foams (Campbell and Mougeot, 1999).

Scientific interest in the foaming properties of milk began at the start of the last century, and since then considerable research has been carried out. Studies have examined how temperature alters the foaming properties of milk by influencing the conformation of milk proteins. Temperature appears to be the most important parameter influencing the foaming behavior of milk containing lipids. Kamath (2008) observed a pronounced minimum of foaming at 25°C, supporting earlier observations that the partially crystalline state of the milk fat globules at 10–30°C has detrimental effects on the foam-generating process. A frequently encountered problem is the failure of milks of similar fat content to foam. The interaction effects of fat and E/S levels were highly significant. Milk samples with higher fat levels had higher overrun, whereas those with higher E/S levels had smaller, more stable foams. In particular, milk proteins have been investigated as model systems (Tamma *et al*., 2013). Proteins are often used for the stabilization of liquid food foams, because of their strong adsorption to the gas/liquid interface as well as good steric and electrostatic stabilization (Murray, 2007). A recent review (Huppertz, 2010) indicates that a comprehensive overview of the scientific work done on the foaming properties of milk: next to the proteins, lipids have the largest influence on the foaming properties of milk. Skim milk produces very stable foams, and the presence of milk fat can negatively affect the stability of milk foams.

Our study examines the biochemical aspects of milk that affect its foamability. Milk foam is widely used in food production, and factors that determine foamability are not yet well understood. To explore the particular roles of the milk protein, amino acids and fatty acids in the foam ability of the milk, we performed systematic measurements of the content and composition of the protein, amino acid and fatty acid in milk. Our aim was to outline how these factors govern foaming and to

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clarify their specific roles.

Materials and methods

Milk samples were obtained from Bright Farming Co., Ltd., in Shanghai. A total of 18 milk samples were collected from two types of foaming properties. Two treatments were tested:1) milk C (milk with the high foaming property of 70-96%), 2) milk E (milk with the low foaming property of 30~48%). Milk samples were collected at 8:00h ,12:00h and 16:00h, and approximately 500 mL was taken for each type from the milk container into sterile glass bottles. Milk samples were collected into two parts; one part was used for testing of milk protein and fat content using the Milko Scan FT (Foss Electric, Denmark), and the second part was stored at 4 ^{0}C until further used for AA analysis.

For milk protein profiles the milk samples were prepared according to the Bobe (1998) method. The concentration of milk protein in the final diluted solution was approximately 4 mg/mL, detected by RP-HPLC. The gradient elution was performed in accordance with the method of Bonfatti (2008). The flow rate was 0.5 ml/min, the column temperature was kept at 45 °C and the detection was made at a wavelength of 214 nm.

For milk amino acid profiles the AA profiles of two milk samples were determined according to the Chinese standard method GB/T 5009.124-2003. Thawed milk samples were thoroughly mixed and hydrolyzed using 6 mol/L hydrochloric acid in sealed glass ampules for 24 h at 110°C. The hydrolyzate was centrifuged, and the supernatant was mixed with hydrochloric acid. The mixture was used for AA analysis filtering through a 0.22 µm syringe filter by using an amino acid analyzer (Beckman 6300 amino acid analyzer, Beckman Instruments, Palo Alto, California). Amino acids include free amino acids and amino acids in proteins were determined.

For milk fatty acid profiles, the total lipids of the two milk samples were extracted according to the Folch method (Folch *et al.*, 1957). Transmethylation was performed using sodium methylate and methanolic boron trifluoride (BF3) for 20 minutes at 95^{0}C and dissolved in hexane (Morrison *et al*., 1964). Fatty acids were analyzed and quantified using a gas chromatograph (Agilent 6890) equipped with a flame-ionization detector and a 5% phenyl methyl polysiloxane column (DB-23; 30 m × 0.32 mm internal diameter, a film diameter of 0.25µm; Santa Clara, CA, USA). Fatty acid quantification was achieved by utilizing the internal standard method, with those of a standard mixture of erucic acid (C22:1, F-45629, Sigma-Aldrich, USA) as standard. Data acquisition and processing were performed with Agilent-Chemstation software for gas chromatographic systems.

All data were presented as means and standard error means (SEM). Data were analyzed by One-way ANOVA using SPSS (version 17.0). Differences between means were considered significant at P<0.05.

Results

The protein composition of milk in coffee is presented in Table I. For the milk protein, a significant difference between milk types was found in β-lactoglobulin (β-LG) and α-lactalbumin (α-LA) (P<0.05), but not in casein content. The content of β-LG and α-LA in milk C was significantly higher than in milk E. The top content of protein in milk C was α-LA, κ-CN X2 and β-LG, about 2.97%, 2.96% and 2.94% respectively, the lowest protein was κ-CN X1 and β-CN, about 0.57% and 1.09% respectively. The top content of protein in milk E was κ-CN X2, about 2.25%, the lowest protein was α-LA, about 0.57%.

Table I. Protein profiles (%) of milk from the foaming properties in cappuccino coffee.

Item	Treatment		SEM	P-value
	C	E		
κ-CN X2	2.96	2.25	0.6548	0.294
αs2-CN	2.10	1.71	0.3794	0.317
κ-CN X1	0.57	1.19	0.4122	0.151
αs1-CNB	2.29	1.84	0.5550	0.436
αs1-CAN	1.53	1.32	0.3924	0.602
β-CN	1.09	1.30	0.2032	0.334
α-LA	2.97a	0.57b	0.5759	0.001
β-LG	2.94a	1.81b	0.3224	0.003

Note: C, E: milk with the low foaming property of 30-48% and milk with the high foaming property of 70~96%; SEM: Standard error of mean; ^{ab} Means bearing different superscripts in the same column differ significantly ($P < 0.05$).

Amino acid composition of two types milk from the foaming properties in coffee is showed in Table II. For the amino acids, no significant difference was found in the two groups. The top content of essential amino acids (EAA) was leucine (Leu) (4.38~4.49%) and lysine (Lys) (3.16~3.27%), the lowest EAA is phenylalanine (Phe) and methionine (Met), about 1.68% and 1.08% respectively. For the ten kinds of nonessential amino acids (NEAA), the Glu content was highest, nearly 10%, then is proline (Pro), about 5%, the glycine (Gly) is lowest, less than 0.5%.

Table III shows the fatty acid compositions in the two types of foaming properties in coffee. Fatty acids were grouped into the saturated fatty acids (SFA), unsaturated fatty acid (UFA), monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA). Cappuccino coffee milk contains at least ten fatty acids in amounts over 1%: C6:0, C10:0, C12:0, C14:0, C15:0, C16:0, C16:1, C18:0, C18:1 and C18:2. The top content of fatty acid was

C14:0 (9.71~10.39%), C16:0 (32.6~34.5%) and C18:1 (21.16~21.76%), the content of C16:0 in milk E was significant higher than milk C (P<0.05).

Table II. Amino acid profiles (%) of milk from the foaming properties in cappuccino coffee.

Item	Treatment		SEM	P-value
	C	E		
EAA				
THR	2.09	2.02	0.0585	0.226
VAL	3.09	3.00	0.0795	0.280
MET	1.08	1.08	0.0281	0.958
ILE	2.29	2.23	0.0615	0.319
LEU	4.49	4.38	0.1215	0.379
PHE	1.69	1.66	0.0441	0.433
LYS	3.27	3.16	0.0892	0.263
NEAA				
ASP	3.37	3.26	0.0938	0.239
SER	2.94	2.86	0.0816	0.383
GLU	8.44	8.22	0.2327	0.351
GLY	1.47	1.45	0.0387	0.713
ALA	2.17	2.12	0.0578	0.363
CYS	0.19	0.18	0.0086	0.586
TYR	1.44	1.42	0.0399	0.670
HIS	1.03	1.00	0.0282	0.399
ARG	1.17	1.12	0.0320	0.195
PRO	4.75	4.65	0.1347	0.475

Note: C, E: milk with the low foaming property of 30-48% and milk with the high foaming property of 70~96%; -SEM: Standard error of mean; ^{ab} Means bearing different superscripts in the same column differ significantly ($P < 0.05$).

Discussion

To a large extent, the protein composition determines the foamability and foam stability. The protein in milk was composed mainly of casein and whey protein, with a ratio of about 4:1 (Table 1). Whey protein in milk is mainly composed of β-lactoglobulin and α-lactalbumin, which will denature to a certain extent when heated. Some study found that whey proteins form viscoelastic layers at the air/water interface when they are adsorbed, which can lead to a resistant and cohesive interfacial network under certain conditions, improving foam stability. Other authors have claimed that the increase in surface hydrophobicity could be considered as a decisive factor for the formation and stabilization of protein foams due to the rapid formation of viscoelastic films (Dombrowski *et al*., 2016). After slight denaturation of whey protein, the intermolecular disulfide bonds and hydrogen bonds in the denatured whey protein enhance the interactions between proteins and improve foamability (Marinova *et al*., 2009; Lazidis *et al*., 2016; Dombrowskia *et al*., 2017). Other results showed that the membrane produced by whey protein showed high elasticity at the air-water interface, while the membrane produced by casein protein did not.

Table III. Fatty acid profiles (%) of milk from the foaming properties in cappuccino coffee.

Item	Treatment		SEM	P-value
	C	E		
C6:0	1.06^{b}	0.77^{a}	0.0471	0.000
C8:0	0.88^{b}	0.77^{a}	0.0355	0.006
C10:0	2.52^{b}	2.20^{a}	0.0906	0.002
C11:0	0.09^{B}	0.06^{A}	0.0144	0.047
C12:0	3.18^{b}	2.79^{a}	0.1024	0.001
C13:0	0.13^{b}	0.09^{a}	0.0031	0.000
C14:0	10.39^{b}	9.71^{a}	0.1226	0.000
C14:1	0.95	0.93	0.0334	0.454
C15:0	1.08	0.96	0.0922	0.223
C15:1	0.07	0.07	0.0094	0.413
C16:0	32.60^{B}	34.50^{A}	0.8587	0.037
C16:1	1.42	1.48	0.0549	0.250
C17:0	0.55^{B}	0.53^{A}	0.0092	0.039
C17:1	0.20	0.19	0.0073	0.076
C18:0	10.22	10.50	0.3693	0.448
C18:1	21.16	21.76	0.3371	0.087
C18:1T	0.64^{B}	0.21^{A}	0.1909	0.036
C18:2	3.00	2.73	0.1509	0.087
C18:2T	0.12	0.12	0.0086	0.958
C18:3	0.37	0.35	0.0232	0.341
C20:0	0.15	0.15	0.0049	0.270
C20:1	0.18	0.18	0.0215	0.907
C20:2	0.24	0.23	0.0237	0.616
C20:3	0.15^{b}	0.13^{a}	0.0047	0.001
C20:4n-6	0.20^{b}	0.17^{a}	0.0069	0.002
SFA,% total FA	62.84	63.02	0.87	0.097
UFA,% total FA	28.69	28.52	0.91	0.949
MUFA,% total FA	24.62	24.81	0.60	0.165
PUFA, % total FA	4.07b	3.70a	0.17	0.002

Note: C, E: milk with the low foaming property of 30-48% and milk with the high foaming property of 70~96%; -SEM: Standard error of mean; ^{ab} Means bearing different superscripts in the same column differ significantly ($P < 0.05$).

The content of EAA and NEAA was found not to differ among the C and E milk groups (Table II). The amino acids present in the highest quantities were Glu, Pro, Leu and Asp, consistent with other results (Singh *et al*., 1987). Research has shown that too much FFA reduces the quality and flavor of milk products. Cao *et al*. (2008) analyzed the free fatty acids in the used processing of ham. They found that during the long processing of dry-cured ham, the fat is strongly hydrolyzed by endogenous lipase and phospholipase, this produces large amounts of FFAs, which are further oxidized to form volatile flavors, causing ham to have its distinctive odor. Zhang *et al*.

(1999) studied the dynamics of free fatty acids in Perilla seed oil. They showed levels of free fatty acids in Perilla oil increased with the storage time, which greatly affecting the flavor and quality of Perilla oil. Studies have shown that C16:0, C18:0 and C14:0 account for 50~90% of the total FFAs (Farnworth *et al*., 2007). Our results show that FFA content was ranked in this order: C14:0, C16:0 then C18:1, the content of C16:0 in milk E was significantly higher than in milk C. The FFA content of milk increases through the action of the enzymes inherent in milk, and because of the decomposition of fat by enzymes produced by microbial growth; however, FFAs may also be by-products of oxidation reactions. There are many factors that determine the composition and content of FFAs in milk are mainly related to feed ingredients and structure, the ratio of concentrate to roughage in the diet, and to the composition and quality of roughage; these factors affect the physical and chemical environment in the rumen, which in return affects milk fatty acid composition.

Conclusion

This study compared the milk protein, amino acid and fatty acid content of two types of milk that have different foaming properties. We found that whey protein improved foaming, but the presence of free fatty acids strongly impaired foaming. We have focused on the impact of raw milk quality on the foaming. This work provides a theoretical basis for the scientific adjustment of pasture conditions, and the production and screening of high-quality milk to improve foamability.

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Statement of conflict of interset

Authors have declared no conflict of interset.

References

Anneke, H.M., Katja, G., Martin, A.B., Martien, A., Cohen, S. and Ton van, V., 2002. *J. Colloid Interf. Sci.,* **254**:175-183. https://doi.org/10.1006/jcis.2002.8592

Bobe, G., Beitz, D.C., Freeman, A.E. and Lindberg, G.L., 1998. *J. Agric. Fd. Chem*., **46**: 458-463. https://doi.org/10.1021/jf970499p

Bonfatti, V., Grigoletto, L., Cecchinato, A., Gallo, L. and Carnier, P., 2008. *J. Chromatogr. A*., **1195**:101-106. https://doi.org/10.1016/j.chroma.2008.04.075

Campbell, G.M. and Mougeot, E., 1999. *Trends Fd. Sci. Technol*., **10**: 283e296.

Cao, Y.Z. and Lu, Q.H., 2008. *Acta Agric. Jiangxi*, **20**:72-73. https://doi.org/10.1111/j.1601-5215.2008.auindex_1.x

Dombrowski, J., Mattejat, C. and Kulozik, U., 2016. *Int. Dairy J*., **61**: 166-175. https://doi.org/10.1016/j.idairyj.2016.05.006

Dombrowskia, J., Gschwendtnera, M. and Kulozika, U., 2017. *Coll. Surf. A: Physicochem. Eng. Asp*., **516**: 286-295.

Fains, A., Bertrand, D., Baniel, A. and Popineau, Y., 1997. *Fd. Hydrocoll*., **11**: 63e69. https://doi.org/10.1016/S0268-005X(97)80012-4

Farnworth, E.R., Chouinard, P.Y., Jacques, H., Venkatramanan, S., Mafu, A.A., Defnoun, S. and Jones, P., 2007. *Nutr. J*., **6**: 1–15. https://doi.org/10.1186/1475-2891-6-15

Folch, J., Lees, M. and Stanley, G.H., 1957. *J. biol. Chem*., **226**: 497–509.

Geraldine, V. and Gamboa1, L.B., 2012. *Philipp. Agric. Scient*., **4**: 416-421.

Huppertz, T., 2010. *Int. J. Dairy Technol*., **63**: 477–487. https://doi.org/10.1111/j.1471-0307.2010.00629.x

Jacob, E., Wolfgang, C., Claudia, G., Benjamin, Z., Jochen, W., Jorg, H., Timo, S. and Lutz, F., 2016. *Int. Dairy J*., **62**: 1-9.

Kamath, S., Huppertz, T., Houlihan, A.V. and Deeth, H., 2008. *Int. Dairy J*., **18**: 994–1002. https://doi.org/10.1016/j.idairyj.2008.05.001

Katrin, O., Christine, B.K., Mania, M. and Thomas, W., 2014. *Coll. Surf. A: Physicochem. Eng. Asp*., **460**: 280–285.

Kechun, Z., Guozhong, X. and Xiangzhen, S., 2010. *Dairy Sci. Technol*., **1**:37-38.

Lazidis, A., Hancocks, R.D. and Spytopoulos, F., 2016. *Fd. Hydrocoli*., **53**: 209-217. https://doi.org/10.1016/j.foodhyd.2015.02.022

Ling, Z., Qingfeng, T., Muhammad W. I., Zhongsheng, X., Feng, H., Li, L., Mingzhen, L., Bo, L., Guangsheng, Q. and Caixia, Z., 2018. *Italian J. Anim. Sci*., **4**: 873-878.

Marinova, K.G., Basheva E.S. and Nenova, B., 2009. *Fd. Hydrocoli*., **23**: 1864-1876. https://doi.org/10.1016/j.foodhyd.2009.03.003

Morrison, W.R. and Smith, L.M., 1964. *J. Lipid Res*., **5**: 600–608.

Murray, B.S., 2007. *Curr. Opin. Coll. Interf. Sci*., **12**: 232e241.

Singh, H., 1987. *J. Dairy Res*., **56**: 235-248. https://doi.org/10.1017/S0022029900026455

Tamma, F., Sauer, G., Scampicchio, M. and Drusch, S., 2013. *Fd. Hydro-coll*., **27**: 371–377. https://doi.org/10.1016/j.foodhyd.2011.10.013

Zhang, H. and Zhang, Y.P., 1999. *Guangzhou Fd. Sci. Technol*., **16**: 49-53.