Buletin Peternakan 44 (1): 43-49, February 2020

ISSTAP
Indonesian Society for Sustainable Tropical Animal Production

Bulletin of Animal Science

ISSN-0126-4400/E-ISSN-2407-876X Accredited: 36a/E/KPT/2016
http://buletinpeternakan.fapet.ugm.ac.id/

Doi: 10.21059/buletinpeternak.v44i1.49130

Physical and Microstructural Characteristics of Kefir Made of Milk and Colostrum

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Article history
Submitted: 30 August 2019
Accepted: 11 February 2020

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ABSTRACT

This research set out to compare the physical and microstructural characteristics of kefir made of milk, colostrum, and milk-colostrum mixes at various proportions. Kefir was made by adding kefir grains to 100% milk (P0), 80% milk + 20% colostrum (P1), 60% milk + 40% colostrum (P3), 40% milk + 60% colostrum (P4), 80% milk + 20% colostrum (P5), and 100% colostrum (P6). Fermentation was allowed under room temperature for 24 hours. The characteristics observed were color values, viscosity, pH, water holding capacity (WHC), syneresis, and microstructure. The result showed that the color of kefir (L* value, lightness); (b* value, yellow-blue), (a*, red-green), and whiteness index (WI) was significantly affected by raw materials. The viscosity of kefir was also affected by the raw materials ($p<0.05$), in which the kefir made from a mix of 80% milk and 20% colostrum showed the highest viscosity (1524.20 m.Pa.S). However, other characteristics such as pH, WHC, and syneresis were not significantly affected by raw materials. The microstructure of kefir made from 20 to 40% colostrum showed a string and compact protein tissues, while that made from 80 to 100% colostrum showed a clumping gel and concentration dominated by protein and fat tissues. This study demonstrated that milk kefir produced from milk-colostrum mixes posses a yellowish color (b*), low whitenes index, negative a* value, low lightness, whereas kefir made from 100% colostrum showed slightly greenish with low lightness level. Kefir with highest viscosity was produced from combined 80% milk and 20% colostrum. The microstructure of kefir produced from mixes with 40% and 60% colostrum showed a strong, tight, and compact microstructure of protein tissues.

Keywords: Colostrum, Kefir, Microstructure, Milk, Sensory characteristics

Introduction

Physical and microstructural characteristics of fermented kefir product are crucial for the complete functional properties. Physical characteristic is greatly attributed to the composition of the main raw materials, total solid, the type and amount of starter, fermentation temperature, and storage time (Wang *et al*., 2017). Physical characteristics are derived from gel formation. Kefir physical characteristics are affected by pH decrease that would affect water holding capacity (WHC) and syneresis. Kefir physical characteristics are important for the optimization process, product design, and quality control (Yovanoudi *et al*., 2013; Dinkçi *et al*., 2015). Kefir is a fermentation product that exhibits acidic properties and contains alcohol derived from freeze-dried commercial kefir starter, or the traditional, reusable kefir grain. Kefir main raw materials include cow milk (Gul *et al*., 2018), goat milk (Setyawardani *et al*., 2014), and buffalo milk (Nguyen *et al*., 2014). The physical characteristics of kefir include color, viscosity, and WHC. (Corona *et al*., 2016) showed that kefir was made using a different percentage of kefir grain to test the viscosity (Delgadillo *et al*., 2017). Kefir added with ‘porang’ (*Amorphophallus muelleri*) was tested for physical characteristics that include viscosity and pH (Nurliyani, 2017) physical characteristics and microstructure (Bensmira and Jiang, 2012; Wang *et al*., 2017; Gul *et al*., 2018).

This study contributes to enhance the current knowledge of factors affecting the quality characteristics of milk-based kefir. Incorporating colostrum to milk as raw materials to produce kefir highlight the potential usefulness of colostrum for producing kefir. Colostrum is more viscous, yellowish, with lower pH than milk. More importantly, compared to milk, colostrum contains higher nutrition, such as protein vitamin, mineral, enzymes, antimicrobials peptide, and immunoglobulin (Górová *et al*., 2011). Colostrum color is related to 65% β-carotene content (Calderón *et al*., 2007). The casein micelle contributes to the particle aggregation and affects viscosity. Colostrum at the early lactation includes a large amount of 600-nm casein micelle (Walstra *et al*., 2006). The present study was designed to determine the effects of raw materials, which were fresh milk, colostrum, and milk-colostrum mixes at various percentages, on the pH, viscosity, color index, syneresis, WHC, and microstructure.

Materials and Methods

Materials

Kefir was made from cow's milk and colostrum comes from local farmer Baturaden Central Java Indonesia. Kefir grains collection of the Faculty of Animal Science laboratory, Jenderal Soedirman University. Colorimeter (Minolta Croma Meter, CR-400, Osaka Japan). Viscometer Brookfield RVT; SEM type JSM-5000 (JEOL, Tokyo, Japan) and the equipment were tools for kefir production.

Sensory analysis of milk and colostrum

Sensory charateristics of milk and colostrum as raw materials were determined by a panel of 10 trained panelists (Gomes *et al*., 2013b). All panelists were trained held for definitions, attributes development, and test procedures.

Kefir manufacture

Kefir was manufactured according to the procedure of Setyawardani and Sumarmono (2015). Cow milk and colostrum were pasteurized at 72^{o}C for 15 second, and cooled to 28^{o}C. Kefir grains were added and incubated at 28^{o}C for 24 hours to allow fermentation process. Milk kefir was separated from kefir grains by using a fine plastic stainer and ready for further treatment. Kefir gains were placed in a plastic container and ready for another batch of fermentation.

Color measurement

A total of 25 ml kefir sample was poured into a 25-ml Erlenmeyer tube and color analysis was carried out using Minolta Croma Meter (CR-400, Osaka Japan). Values of L* (lightness), a* (red-green) and b* (yellow-blue) were recorded. Whiteness index (WI) was calculated according Gul *et al*. (2018) using a formula: 100 - [(100-L*)2 + a*2 + b*2]1/2.

Viscosity measurement

Kefir viscosity was tested using a viscometer (Brookfield RVT) following a method by Gul *et al*. (2018) as much as 250ml sample was put into a beaker glass. Sample viscosity was using a spindle no. 2 at 125 rpm speed with readbility 5-85%. Viscosity test was performed for two minutes to reach stable condition, and then the sample was conditioned at room temperature. The result was recorded as kefir viscosity at m.Pa.s.

Degree of acidity (pH) measurement

Kefir pH was measured using a digital pH meter (AMT 16) according to Setyawardani *et al*. (2014), pH meter was calibrated to pH 4 and pH 7 before being used. As much as 20 ml kefir sample was put into a beaker glass, and pH meter was inserted for a while to obtain the result of kefir pH.

Water holding capacity measurement

Water holding capacity (WHC) was measured with a method by Harte *et al*. (2003) by modifying the amount of sample and centrifugal speed. As much as 10 ml kefir sample was put into a centrifuge tube and centrifuged at 3500 rpm speed for 15 minutes at room temperature. WHC calculation was as follows: WHC (%) = 1(W1 – W2)×100

W1 = sample weight after the whey is separated
W2 = kefir sample weight

Syneresis measurement

Syneresis was measured based on centrifugation according to Amatayakul *et al*. (2006) with a modified sample weight. As much as 10 ml of the sample was put into a centrifuge tube and rotated at 3500 rpm speed for 15 minutes at room temperature. Syneresis calculation was as follows: syneresis =$\frac{A-B}{C}$ x 100 %

A= tube + sample weight
B= whey weight after centrifugation
C= sample weight

Microstructure determination

Measuring kefir microstructure with Scanning Electron Microscopy (SEM) was following a method by (Goldstein *et al*., 1992) using an SEM type JSM-5000 (JEOL, Tokyo, Japan) with 15.000-time magnification and 20 kV ACCV. Kefir sample was centrifuged to discard the supernatant, then added with a 2% glutaraldehyde. The solution was centrifuged again, then added with 2% tannin solution, and let sit for several hours. The solution was recentrifuged, and the fixative fluid was discarded, then added with cacodylate buffer and let sit for 10 minutes. The fluid was removed and added with 50% alcohol then soaked for 10 minutes. The centrifuged solution was added with 1% osmium tetraoxide, and let sit for an hour. More alcohol was added gradually from 70%, 80%, 95% to absolute alcohol. The sample was recentrifuged, and removed the fluid, t-butanol was added and let sit for 10 minutes. The next step was discarding butanol liquid, and then the remaining was made into suspension in butanol. The pieces were solidified, made into suspension swab on a slipcover, then let dry.

Data analysis

The obtained data was subjected to data tabulation in Excel 2016 and analysis using SPSS 16, followed by a Duncan's Multiple Range Test (DMRT).

Result and Discussion

Milk for kefir main ingredients exhibits physical characteristics, namely specific gravity, pH, and freezing point according to Indonesian National Standard (SNI) 3141.1:*2011*. Colostrum for kefir ingredients contains a higher specific gravity, but lower pH and freezing point. Sensory properties of milk showed standard color, aroma, and viscosity, but colostrum was yellowish and rancid and with a normal viscosity. Both ingredients are deemed suitable for making kefir.

Kefir color

Table 2 showed that kefir with and without colostrum had a varied level of lightness (L*). The highest lightness (73.74±7.12) was showed in 40% colostrum, while the lowest (63.79±4.80) was in 20% colostrum. Additional colostrum in the ingredients significantly affected ($p<0.05$) lightness (L*) in kefir produced. Milk has a higher lightness than colostrum, namely 68.2 compared to 55.0, respectively (unpublished data). Kefir made with more milk, and additional 20% colostrum resulted in the highest L* and significantly different ($p<0.05$) than kefir made of 100% colostrum. Accordingly, colostrum contributes to kefir light color.

A higher percentage of colostrum in making kefir would increase b* value (yellowish) in kefir product. The yellow color is attributed to riboflavin in colostrum more than fat content (Dimitreli *et al*., 2014). b* value was identified from the yellow-blue color in the tested sample. Table 2 showed that the higher the colostrum percentage in kefir making, the more solid the yellow color. b* value keeps increasing and reaches its peak (29.44±4.49) at 100% colostrum for kefir making. It showed that colostrum addition for kefir significantly affected ($p<0.05$) b* value. Additional of 80% colostrum resulted in a relatively similar yellowish color. The b* value, which is yellowish color, was affected more by riboflavin than by fat content (Dimitreli *et al*., 2014). Color properties a* (red-blue) of kefir samples were negative; all kefir showed greenish color. The a* value was affected by a color intensity that was significantly ($p<0.05$) affected by the combined milk-colostrum for making kefir. Red color a* showed red-green coordinate of a sample. Kefir made of 100% colostrum showed the highest a* value. Kefir color tends to be yellowish white; therefore, a* value becomes negative.

Whiteness index value (WI) of kefir made of 100% colostrum was significantly lower ($p<0.05$) than kefir made of 100% milk. Overall, the higher colostrum proportion produced kefir with lower WI. WI value negatively correlated with b* value. The high b value was likely correlated to the high riboflavin content of colostrum (Dimitreli *et al*., 2014), which contributes to the formation of yellowish color. WI value in this study was lower than that reported by Gul *et al*. (2018). In addition to riboflavin, WI value was affected by the type of milk as kefir ingredients. Kefir made of buffalo milk contains lower riboflavin than that of cow milk (El-Salam and El-Shibiny, 2011).

Viscosity

The average viscosity ranged from 1072.23 to 1424.20 m.Pa.S. The combined ingredients showed a significantly different of viscosity ($p<0.05$). The highest viscosity was obtained in kefir with 20% colostrum. Additional 40 to 100% colostrum to kefir showed the same viscosity with kefir without colostrum. The viscosity of fermentation product was affected by milk fat. Fat globules in protein tissue would increase WHC value, which stimulated viscosity and a more stable gel. In fermentation product, it would decrease pH and stimulated the interaction between milk fat and casein that would increase viscosity. A decreased pH during fermentation of solid matter in the milk would form a matrix that increased viscosity in the fermentation product. A similar result was reported by (Delgadillo *et al*., 2017). Kefir concentration up to 20% in kefir ingredients would increase viscosity by 2.5 times from 1. 3770 (5%) to 2.4831 Pa*s.

The result of viscosity test on kefir made of combined milk-colostrum could increase viscosity up to 30% colostrum addition. It was different from a study by (Nurliyani, 2017) that kefir viscosity was not significantly different with additional glucomannan derived from porang (*Amorphophallus muelleri*). Viscosity, particularly

Table 1. Physical and sensory characteristics of milk and colostrum

Parameter	Fresh Milk*	Colostrum*	SNI, 2011
BJ	1.0289	1.0425	1.027-1033
pH	6.25	5.505	6.3-6.8
Freezing point (^{o}C)	-0.535	-0.775	-0.520 sd -0.560
Color	white	yellowish	white
Aroma	aromatic	fishy	normal
Viscosity	normal	normal	normal

* measurement data with four replication.

Table 2. Average kefir color with the addition of colostrum

Treatments	L*	a*	b*	WI
100% milk	68.95±5.14^{ab}	-2.20±0.367^{a}	8.31±0.69^{a}	67.72±5.55^{b}
80% milk + 20% colostrum	63.79±4.80^{a}	-2.25±0.549^{a}	16.26±2.41^{b}	60.06±4.22^{ab}
60% milk + 40% colostrum	73.74±7.12^{b}	-2.17±0.548^{a}	17.13±3.38^{b}	68.31±7.55^{b}
40% milk + 60% colostrum	72.23±5.72^{ab}	-1.45±0.749^{a}	20.45±4.95^{bc}	64.91±3.96^{b}
20 % milk + 80% colostrum	69.36±1.23^{ab}	-1.12±0.768^{ab}	23.86±5.20^{cd}	61.01±3.82^{ab}
100% colostrum	64.93±1.04^{a}	-0.15±1.454^{b}	29.44±4.49^{d}	54.13±3.79^{a}

^{a,b,c,d} Different superscripts in the same column show significantly different at the level of 5% ($P<0.05$).

Each treatment was repeated 4 times. L* (Lightness); a* (red-blue); b* (yellow-green); WI (Whiteness Index).

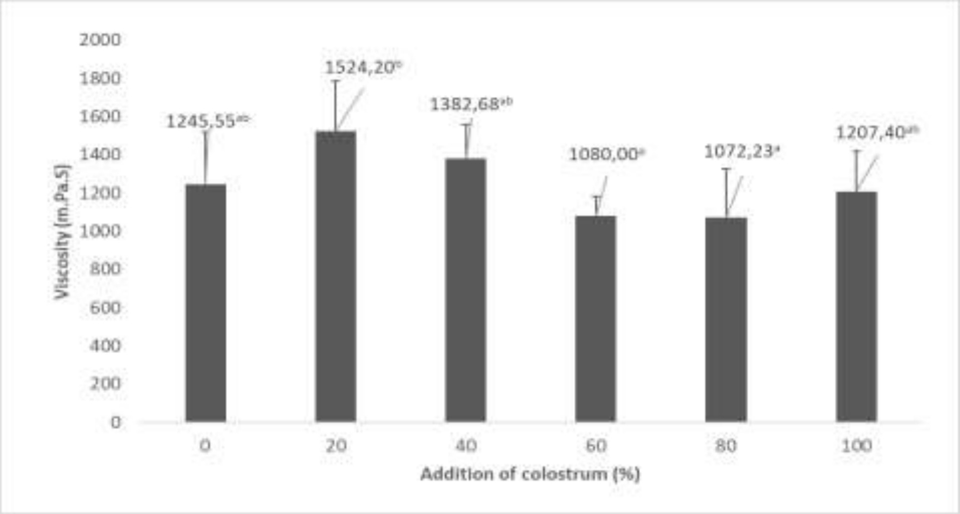

Figure 1. The viscosity of kefir made from fresh milk and combined milk-colostrum.

viscosity index, is attributed to the type of milk (Cais-Sokolińska *et al*., 2016). Kefir viscosity with a combined ingredient was lower than that with 17750 -1771 cPs incubation and the lowest viscosity was 1250 -1277 cPs. Viscosity would increase during incubation (Yoo *et al*., 2013).

pH, WHC and syneresis

Table 3 showed that kefir pH of the combined milk-colostrum was 3.91 to 3.98. pH value of kefir made of milk, combined milk-colostrum, and pure colostrum was relatively similar (p>0.05). Kefir grains generally contain different types of lactic acid bacteria that would affect the acidity of kefir pH (Kasenkas, 2011). The result of this study showed that WHC and syneresis of kefir made of 100 % milk, milk-colostrum combined, and 100% colostrum was not significantly different (p>0.05). The average of WHC and syneresis were 30.88 - 35.10% and 62.18 - 67.00%, respectively. WHC of fermentation product was resulted from an aggregated protein particle by gravitation (Kasenkas, 2011). A changing WHC would be detected during incubation, and it would affect the level of acceptance (Nguyen *et al*., 2014). The highest WHC value and the lowest syneresis were found in kefir made of 80% milk and 20% colostrum. Fat content and surface fat are other contributing factors of WHC value in fermentation product; protein could be absorbed by other globule fat surface to form tissues that affect the WHC value of fermentation product Nguyen *et al*. (2014).

Fat content in milk and colostrum was relatively similar, namely 4.87% and in colostrum was 4.57%; therefore, WHC value in kefir fermentation product was relatively similar. WHC and syneresis were affected by the pH value. According to Öztürk and Öner (1999), the decreased pH during fermentation would increase casein particle resistance against syneresis. The type of milk and the main ingredients for kefir would affect WHC value. Buffalo milk and goat milk contain higher casein micelle than cow milk because the protein tissue has smaller pores and higher solidity, which make WHC value higher (Gomes *et al*., 2013a). Syneresis value in this research was lower than in yogurt made with extra caseinate sodium and WPI at different concentration. Low syneresis reflects a better quality fermentation product (Supavititpatana *et al*., 2009). High syneresis shows that water holding capacity in the product was weak; therefore, more nutrition would be disconnected from the solid matter because the gel strength was low. Factors contributing to syneresis are total solid and milk composition (Vareltzis *et al*., 2016).

Microstructure

Kefir microstructure in figure A did not show a strong gel composed of protein tissues, which resulted in fragile gel and led to syneresis. Figure A showed many voids that reflected low viscosity. Kefir made of soy milk showed a gel structure with small and solid branches (Bensmira and Jiang, 2012). A compact structure is due to

Table 3. Average pH, WHC dan syneresis of kefir with addition of colostrum

Treatments	pH	WHC (%)	Syneresis (%)
100% milk	3.94±0.21	32.87±2.77	64.93±3.03
80% milk + 20% colostrum	3.98±0.20	34.60±5.14	62.18±4.90
60% milk + 40% colostrum	3.92±0.18	34.24±3.17	64.37±2.61
40% milk + 60% colostrum	3.91±0.22	33.31±3.13	64.45±3.27
20% milk + 80% colostrum	3.94±0.18	35.10±3.72	65.03±3.98
100% colostrum	3.94±0.23	30.88±3.35	67.00±4.13

No significant differences were observed among treatments (P>0.05).
The different letters in figure show the significant difference (P<0.05).

the high total solid (Pereira *et al*., 2003). In Figure B, the gel was stronger, protein tissue was more transparent, and the gel was more stable, the voids were small and tight; therefore, the viscosity was higher than other product. Figure C also showed protein tissues that formed a strong gel matrix, but many visible voids may cause syneresis because the gel was unstable. Figure D was similar to Figure B but more voids; therefore, the viscosity was lower. Additional colostrum as much as 80 to 100% to kefir showed gel clumping and solidity that was dominated by protein and fat tissue. Kefir made of a high-protein ingredient would showed a smaller but denser protein tissue, which made the gel stronger and more stable. Figure B showed a porous, spongy structure. Figure E and F formed a strong tissue and a cross tie during kefir fermentation. High protein milk before fermentation process would result in smaller and more compact protein tissue (Schkoda *et al*., 2001). Figure E and F used 80-100% colostrum, which yielded a cluster of large particles and showed a relation between aggregate and microstructure. It plays a role in forming viscosity and a fine, thick texture profile. Fermentation product would form a gel with a 3-dimensional structure where casein micelle is the main frame structure (Peng and Guo, 2015).

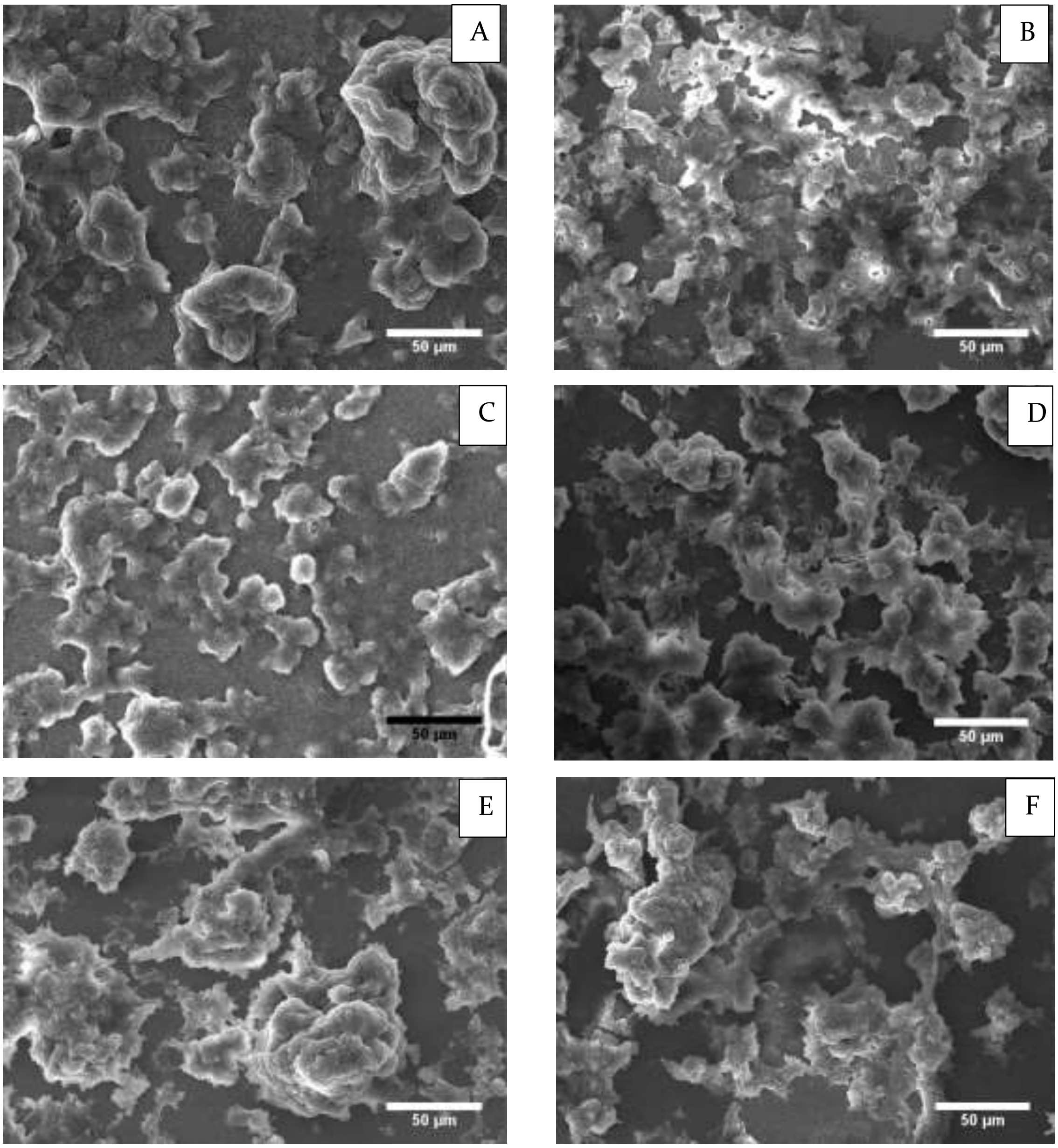

Figure 2. Microstructural characteristics of kefir made from milk and colostrum
A = 100 % cow's milk; B = cow's milk 80 % + colostrum 20 %; C = cow's milk 60 % + colostrum 40 %; D = cow's milk 40 % + colostrum 60 %; E= cow's milk 20 % + colostrum 80 % dan F = 100 % colostrum (magnification 500 X).

Conclusions

The combined milk-colostrum produced kefir with dominant yellowish color (b*), low WI, negative a* value, low lightness (L*), while kefir with 100% colostrum showed slightly greenish kefir with low lightness level. The highest viscosity was obtained from combined milk with 20% colostrum, but with similar pH, WHC, and syneresis. The microstructure of the combined milk with 40-60% colostrum resulted in a strong, tight, and compact microstructure of protein tissue.

Acknowledgments

The study was funded by the Ministry of Research, Technology and Higher Education of Indonesia through Fundamental Research Scheme (No. 111/UN/23.14/PN.01.00/2019).

References

Amatayakul, T., A. Halmos, F. Sherkat, and N. Shah. 2006. Physical characteristics of yoghurts made using exopolysaccharide-producing starter cultures and varying casein to whey protein ratios. Int. Dairy J. 16: 40-51.

Bensmira, M. and B. Jiang. 2012. Effect of some operating variables on the microstructure and physical properties of a novel Kefir formulation. J. Food. Eng. 108: 579-584.

Cais-Sokolińska, D., J. Wójtowski, and J. Pikul. 2016. Rheological, texture and sensory properties of kefir from mare’s milk and its mixtures with goat and sheep milk. Mljekarstvo: časopis za unaprjeđenje proizvodnje i prerade mlijeka 66: 272-281.

Calderón, F., B. Chauveau-Duriot, P. Pradel, B. Martin, B. Graulet, M. Doreau, and P. Nozière. 2007. Variations in carotenoids, vitamins A and E, and color in cow's plasma and milk following a shift from hay diet to diets containing increasing levels of carotenoids and vitamin E. J.Dairy Sci. 90: 5651-5664.

Corona, O., W. Randazzo, A. Miceli, R. Guarcello, N. Francesca, H. Erten, G. Moschetti, and L. Settanni. 2016. Characterization of kefir-like beverages produced from vegetable juices. LWT-Food Sci.Technol. 66: 572-581.

Delgadillo, J. O. V., M. d. J. L. Lara, C. K. L. Santillan, and C. Bulbarela. 2017. Physicochemical and Rheological Characterization of an Acidic Milk Product: Kefir Concentration Effect. J. Food Sci. Eng. 7: 86-92.

Dimitreli, G., D. Petridis, P. Akakiadou, and S. Chrysalidou. 2014. Effect of protein supplementation, fat globule size and storage time on the rheological and sensory properties of buffalo milk stirred yogurt. J. Food Res. 3: 31.

Dinkçi, N., H. Kesenkaş, F. Korel, and Ö. Kınık. 2015. An innovative approach: cow/oat milk based kefir.

El-Salam, M. H. A., and S. El-Shibiny. 2011. A comprehensive review on the composition and properties of buffalo milk. Dairy Sci.Technol. 91: 663.

Goldstein, I., D. E. Newbury, P. Echlin, D. C. Joy, C. Fiori, and E. Lifshin. 1992. Electron Microscopy and X-Ray Microanalysis. Text for Biologist, Material Scientists and Geologists. Plenum Press, New York.

Gomes, J. J. L., A. M. Duarte, A. S. M. Batista, R. M. F. de Figueiredo, E. P. de Sousa, E. L. de Souza, and R. d. C. R. do Egypto. 2013a. Physicochemical and sensory properties of fermented dairy beverages made with goat's milk, cow's milk and a mixture of the two milks. LWT-Food Sci.Technol. 54: 18-24.

Gomes, J. J. L., A. M. Duarte, A. S. M. Batista, R. M. F. de Figueiredo, E. P. de Sousa, E. L. de Souza, and R. d. C. R. d. E. Queiroga. 2013b. Physicochemical and sensory properties of fermented dairy beverages made with goat's milk, cow's milk and a mixture of the two milks. LWT - Food Science and Technology 54: 18-24.

Górová, R., E. Pavlíková, J. Blaško, B. Meľuchová, R. Kubinec, M. Margetín, and L. Soják. 2011. Temporal variations in fatty acid composition of individual ewes during first colostrum day. Small Rum. Res. 95: 104-112.

Gul, O., I. Atalar, M. Mortas, and M. Dervisoglu. 2018. Rheological, textural, colour and sensorial properties of kefir produced with buffalo milk using kefir grains and starter culture: A comparison with cows’ milk kefir. Int. J. Dairy Technol. 71: 73-80.

Harte, F., Luedecke, B. Sawanson, and G. V. Barbosa-Canovas. 2003. Low-Fat Yogurt Made from Milk Subjected to Combinations of High Hydrostatic Pressure and Thermal Processing. J. Dairy Sci. 66: 1074-1082.

Kasenkas, H. 2011. Antioxidant properties of kefir produced from different cow and soy milk mixtures. J. Agric. Sci. 17: 253-259.

Nguyen, H. T. H., L. Ong, C. Lefèvre, S. E. Kentish, and S. L. Gras. 2014. The microstructure and physicochemical properties of probiotic buffalo yoghurt during fermentation and storage: a comparison with bovine yoghurt. Food Bioprocess Technol. 7: 937-953.

Nurliyani, N. 2017. Properties of Goat Milk Kefir Supplemented with Glucomannan from Porang (*Amorphophallus oncophyllus*) Tuber. In: International Seminar on Tropical Animal Production (ISTAP). p 419-424.

Öztürk, B. and M. Öner. 1999. Production and evaluation of yogurt with concentrated grape juice. J. Food Sci. 64: 530-532.

Peng, X. and S. Guo. 2015. Texture characteristics of soymilk gels formed by lactic fermentation: A comparison of soymilk prepared by blanching soybeans under different temperatures. Food Hidrocoll. 43: 58-65.

Pereira, R., H. Singh, P. Munro, and M. Luckman. 2003. Sensory and instrumental textural characteristics of acid milk gels. Int. Dairy J. 13: 655-667.

Schkoda, P., A. Hechler, and J. Hinrichs. 2001. Improved texture of stirred fermented milk by integrating fat globules into the gel structure. Milchwissenschaft 56: 85-89.

Setyawardani, T., A. H. Rahardjo, M. Sulistyowati, and S. Wasito. 2014. Physiochemical and organoleptic features of goat milk kefir made of different kefir grain concentration on controlled fermentation. Anim. Prod. 16: 48-54.

Setyawardani, T. and J. Sumarmono. 2015. Chemical and microbiological characteristics of goat milk kefir during storage under different temperatures. J. Indonesian Trop. Anim. Agric. 40: 183-187.

Supavititpatana, P., T. I. Wirjantoro, and P. Raviyan. 2009. Effect of sodium caseinate and whey protein isolate fortification on the physical properties and microstructure of corn milk yogurt. Chiang Mai University (CMU) J. Natural Sci. 8: 247-265.

Vareltzis, P., K. Adamopoulos, E. Stavrakakis, A. Stefanakis, and A. M. Goula. 2016. Approaches to minimise yoghurt syneresis in simulated tzatziki sauce preparation. Int. J. Dairy Technol. 69: 191-199.

Walstra, P., J. T. M. Wouters, and T. J. Geurts. 2006. Dairy Science and Technology. Taylor and Francis Group, Florida, USA.

Wang, H., C. Wang, M. Wang, and M. Guo. 2017. Chemical, physiochemical, and microstructural properties, and probiotic survivability of fermented goat milk using polymerized whey protein and starter culture Kefir Mild 01. J. Food Sci. 82: 2650-2658.

Yoo, S.-H., K.-S. Seong, and S.-S. Yoon. 2013. Physicochemical properties of kefir manufactured by a two-step fermentation. Korean J. Food Sci. Anim. Res. 33: 744-751.

Yovanoudi, M., G. Dimitreli, S. Raphaelides, and K. Antoniou. 2013. Flow behavior studies of kefir type systems. J. Food. Eng. 118: 41-48.