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Physichochemical Properties of Kefir Drink Using Modified Porang Flour (*Amorphophallus oncophyllus*) During Storage Period

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Abstract

The purpose of this research was to compare physicochemical characteristics of goat milk kefir drink produced by using porang flour and modified porang flour throughout 21 days storage period. Goat milk kefir drink weretaken at 1, 7, 15 and 21 day of storage period, analysed for microstructure, particle size and ethanol content. Analyses of microstructure using confocal laser scaning microscopy (CLSM), particle size using particle size analyser,ethanol content usinggas chromatography (GC).There were significant differences considering the microstructure, particle size and ethanol content of goat milk kefir drink using modified porang flour during storage period.Lower ethanol content was observed in goat milk kefir drink with porang flour and modified porang flour as compared to control sample (no porang flour added) addition, lower ethanol in sample resulted from lower metabolism activity of microbial under lower water activity condition effect of glucomannan in porang flour. Goat milk kefir drink with modified porang flour addition produced lower particle diameter than control and porang flour treatment, it may due to the disruption of milk protein aggregate by modified porang flour. Confocal microscopy showed at modified porang flour treatment shows more homogenous protein milk aggregate.It can be concluded that modified porang flour produced lower ethanol content and particle diameter of goat milk kefir drink and more homogenous protein milk aggregate.

Article History

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Keywords

goat milk kefir drink,
modified porang flour,
microstucture,
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ethanol,
storage period.

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Introduction

Kefir is a fermented milk resulting from the activity of microorganisms that present in kefir grains[1].Kefir could be prepared from cow or goat milk[2], goat milkis an excellent raw material for dairy product production[3] aswell as goat milk kefir. Goat milk kefir drink is a functional drink conferring beneficial health attributes, however it need a development to improving the microstructure, particle size of goat milk kefir drink was stabilizers addition[4,5] as well as porang flour.

Porang (*Amorphophallus oncophyllus*) grown in Indonesian forest, glucomannan was major component of tuber that may benefits as food ingredient[6,7]. Glucomannan were copolymer of β –(1-4) D-glucose and D-mannose binding,molar ratio (1 : 1.6) and acetyl group (1 in each 17 residue)[8,9]. The problem related to porang flour and goat milkwere they could not formed colloid system.

Milk proteins such as casein are thermodynamically incompatible with neutral polysaccharides[10], which is characterised by two phases formation, one phase protein enriched and one phase polysaccharidesenriched[11].The methods to disperse porang flour had been developed using heat treatment using microwave combined with lactic acid, it had been successfully to modify the physicochemical of glucomannan to increased binding energy between glucomannan and κ-case in[12].The microwave advantage was efficient intime and energy heating due to electromagnetic environment and chemical components interaction,that induce an internal heating in nucleus and fasten the reaction[13].

However, the physicochemical properties of goat milk kefir drink prepared using modified porang flour are not yet studied.Therefore, the purpose of this research was to find out the physicochemical properties of goat milk kefir drink using porang flour, modified porang flour and no porang flour added that stored at different storage period.

Material and Methods

Modified porang flour (PT. Perhutani), lactic acid (Merck), ettawa goat crossbreed milk from local goat farm, kefir grainfrom laboratory of animal product technology University of Brawijaya.

Preparation of Modified Porang Flou

To prepare the modified porang flour, porang flour (3 g) was added with 8% (v/v) lactic acid solution (100mL) was stirred using magnetic stirrer for 15 min before radiated in a microwave at high power for 10 min. The porang flour solution centrifuged at 5000 rpm for 10 min[14].

Kefir Drink Preparation

Goat milk was heated using double wall pasteuriser (85 °C, 15 min) then cooled (25 °C). Kefir grains (3 % (w/v)) were inoculated into goat milk and incubated (25 °C, 24h) untill pH fell to ~4.6, kefir grains were separated from kefir and maintained at 4 °C. Goat milk kefir drink were prepared in the laboratory by mixing goat milk kefir with modified porang flour or porang flour 4.50 g 100 g^{-1} and sucrose 4.70 g 100 g^{-1} at pH 5.05, with water at 50 g 100 g^{-1}. Goat milk kefir drink were stored at 4 °C using refrigerator[15].

Methods

Confocal Laser Scanning Microscopy

The casein were labelled using rhodamine B solution, the image were made using Confocal laser scanning microscopy (CLSM) (Leica TCS-SP2, Germany) using an objective lens HCx PL APO 63x NA ¼ 1.2., excited using a heliumeneon laser at 543 nm and detected using a photomultiplier[16].

Gas Chromatography

Ethanol content of kefir drink determined by Gas Chromatography (GC)HP 5890 equipped MS 5A column and temperature conductivity detector (TCD). Helium as carrier gas programmed at 20 ml/min, the column programmed at 125-250 °C and detector at 275 °C[17].

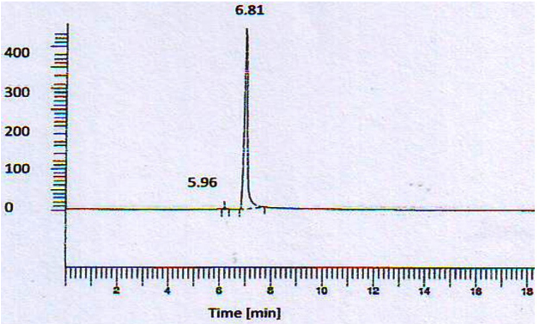
Particle Size

The size of kefir drink particle was observed using particle size analyser (CILAS 1090D)[18].

Result and Discussion

Ethanol Content

Kefir product have specific flavour typical originating from acetic acid, lactic acid, ethanol and CO_2, which produced during fermentation processes by yeasts, lactic acid and acetic acid bacteria and other microorganisms contained in kefir starter culture[19]. Ethanol were detected in almost all samples, higher ethanol contents were detected in goat milk kefir drink control samples than goat milk kefir drink containing porang flour or modified during storage period. During storage period the ethanol content increased in goat milk kefir drink control samples and goat milk kefir drink containing porang flour. Ethanol only found 0.06 g/L at 15 days storage periode of goat milk kefir drink containing modified porang flour (Table 1 and Fig 1).



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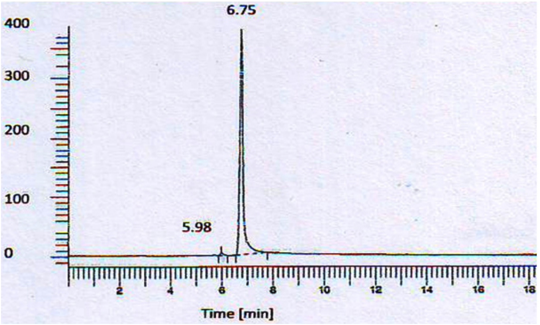

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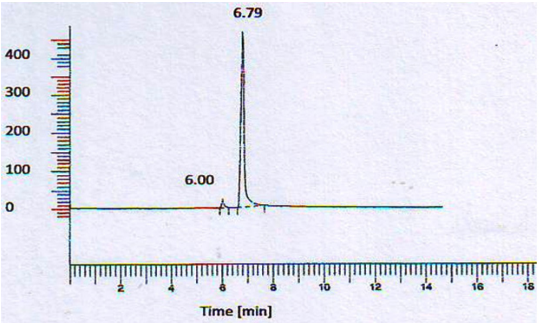

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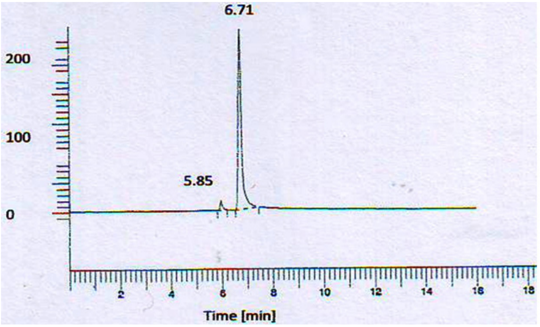

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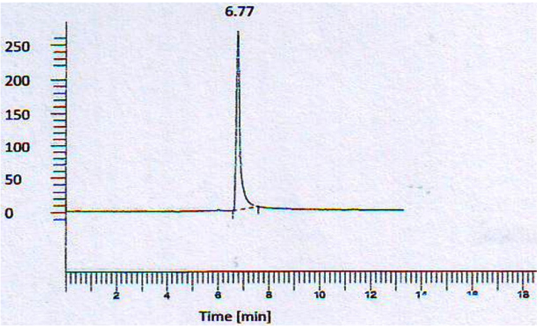

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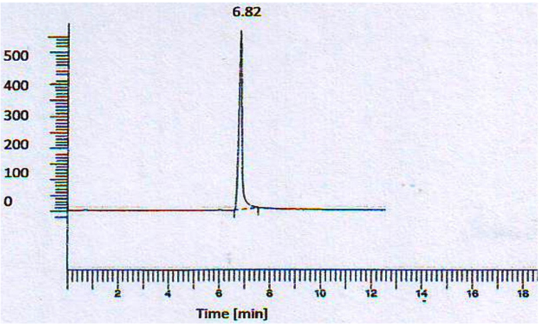

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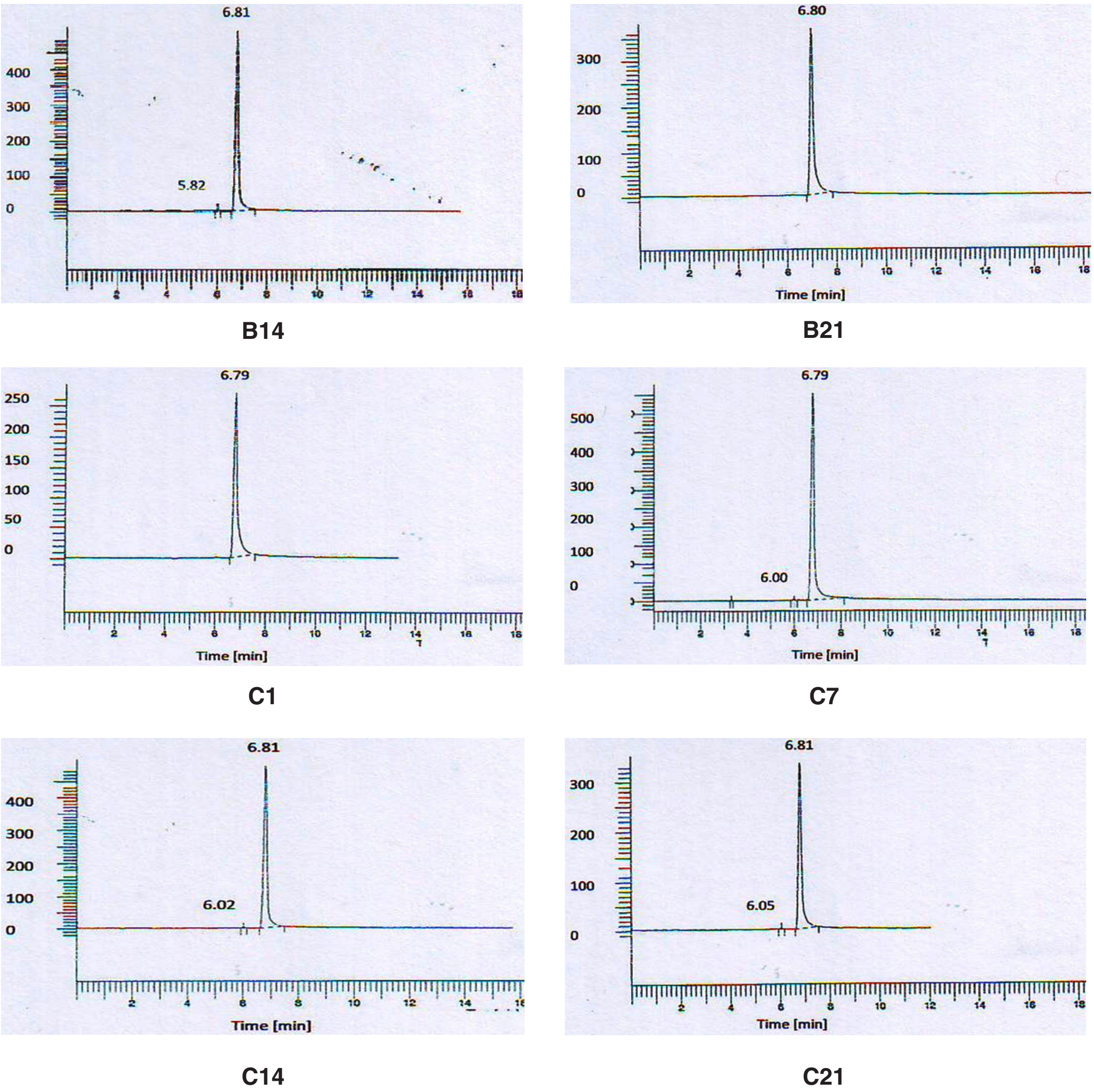


Fig.1: Ethanol content of goat milk kefir drink

The ethanol content were determined at 1, 7, 14 and 21 day storage period (from left to right). A= control; B= modified porang flour; C= porang flour.

Table 1: Ethanol content of goat milk kefir drink (%)

Treatment	Storage period (days)			
	1	**7**	**15**	**21**
Control (A)	0.18	0.26	0.8	0.94
modified porang flour (B)	-	-	0.06	-
porang flour (C)	0.04	-	0.20	0.32

The highest ethanol content wereobserved in goat milk kefir drink control samples after 21 days storage period. During the storage period, ethanol content increased in goat milk kefir drink control samples during storage period of goat milk kefir drink.The ethanol content changes is important according to the storage period. Ethanol is produced primarily by yeasts such as *Kluyveromyces* sp. and some heterofermentative bacteria that capable to produce ethanol[20],which were present in kefir grains[21].The ethanol content varies (0.0-0.94 %)depending on storage period of goat milk kefir drink.Microflora of the kefir grains ensures lactose metabolism through alcohol fermentation and the typical yeasty flavor formation[22].

The major component in porang flour and modified porang flour were glucomannan, glucomannan have ability to bind water, high moisture retention and lower water activity in food systemdue to their inherent hydrophilic nature[23]. Lower ethanol content in goat milk kefir drink produced using porang flour and modified porang flour maybe attributed with lower water activity (a_w) effect of glucomannan from porang flour and modified porang flour. The water activity (a_w) were 0.95, 0.94 and 0.93 for goat milk kefir drink produced no porang flour added, using porang flour and modified porang flour, respectively. Lower water activity will decrease lactose metabolism through alcohol fermentation.

Particle Size

The results of experiments on particle diameter are illustrated in Fig. 2 and Table 2. The longer the storage period, the lower span value or particle size distribution was observed in goat milk kefir drink for control and and modified porang flour treatment, however, the span value was stable for porang flour treatment.

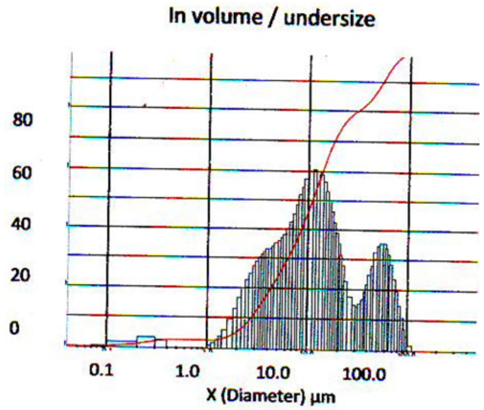
The particle size of goat milk kefir drink of control samples decreased during 15 days storage period, however, the particle size increase at 21 days storage period. The particle size of goat milk kefir drink decreased during 21 days storage period at modified porang flour treatment. The goat milk kefir drink particle size of porang flour treatment decreased slightly from 1 days to 15 days storage period, and then increased at 21 days storage period. They were almost constant during storage. This indicates that milk protein undergo aggegration at 21 days period at porang flour treatment, however, modified porang flour treatment indicating disruption of milk protein aggregate. Disruption of milk protein aggregate by modified porang flour resulted in decrease in particle size, indicating an interaction between modified porang flour and milk protein, however, porang flour had not adsorbed onto the casein micelle[24]. This results indicates that increased homogeneity of milk protein particle due to interaction between modified porang flour with milk protein.

During storage period of goat milk krfir drink no poang flour added treatment decreased the distribution of droplet size or span value, indicating that goat milk kefir drink formed narrower aggregate. The span value of of porang flour treatment was constant during storage, however the particle size increased at 21 days storage of goat milk kefir drink, indicating porang flour not enough to prevent aggregate formation anymore. Modified porang flour treatment decreased the span value and droplet size indicating that it have ability to prevent aggregate formation.

During storage period, porang flour treatment increased the droplet size indicating that goat milk kefir drink formed bigger aggregate and porang flour not enough to prevent aggregate formation anymore. However, modified porang flour treatment decreased the droplet size indicating that it have ability to prevent aggregate formation.

The micelles in goat milk kefir drink can be destabilized by acidification to about pH 4.625. The layer of κ-case in will collapse due to charge reduction of casein micelle surface at pH approaches 4.6 due to aggregation-coalescence[26].

The hydrophobicity of the milk proteins decreased when the casein micelles disassociate in the presence of ethanol. The ethanol reduces the functional hydrophobicity of the casein micelles. The presence of ethanol, a nonpolar solvent, promotes the solubility of the otherwise insoluble proteins during acidification through hydrophobic interactions of methyl groups with hydrophobic amino acids and inducing aggregation-dissociation of the micelles[27]. It is clear that modified porang flour resulted in a lower particle diameter of goat milk kefir drink than control and porang flour treatment.



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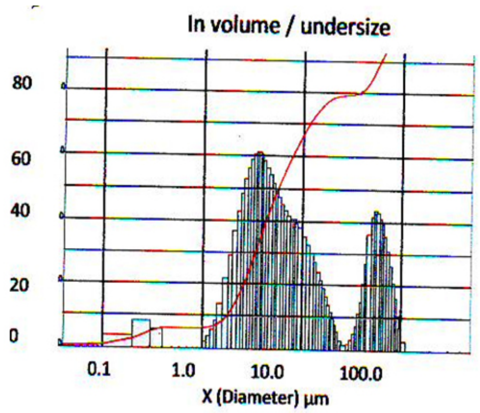

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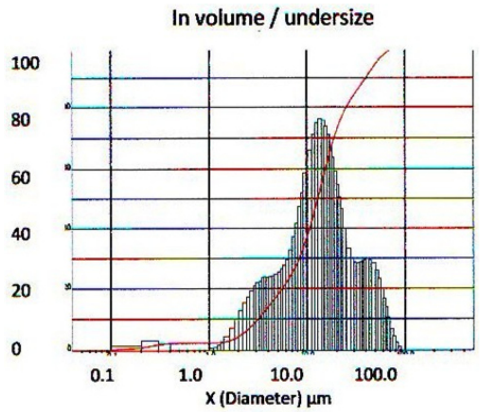

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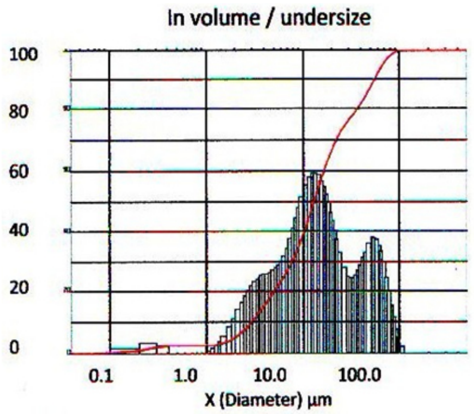

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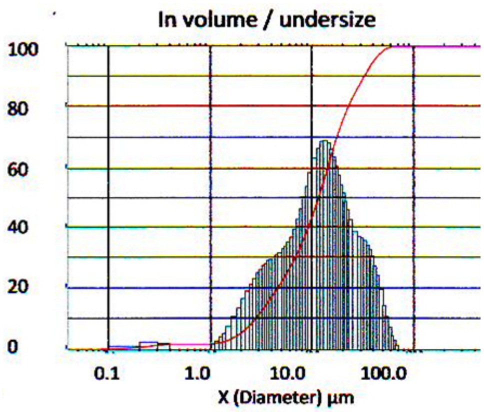

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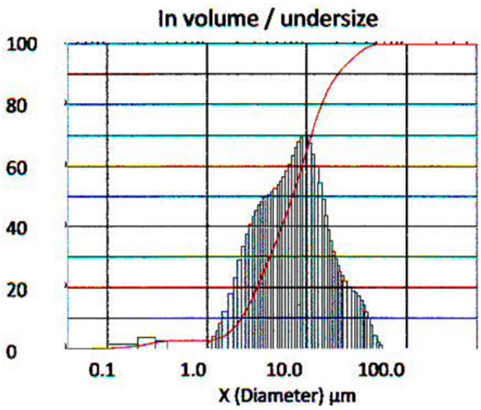

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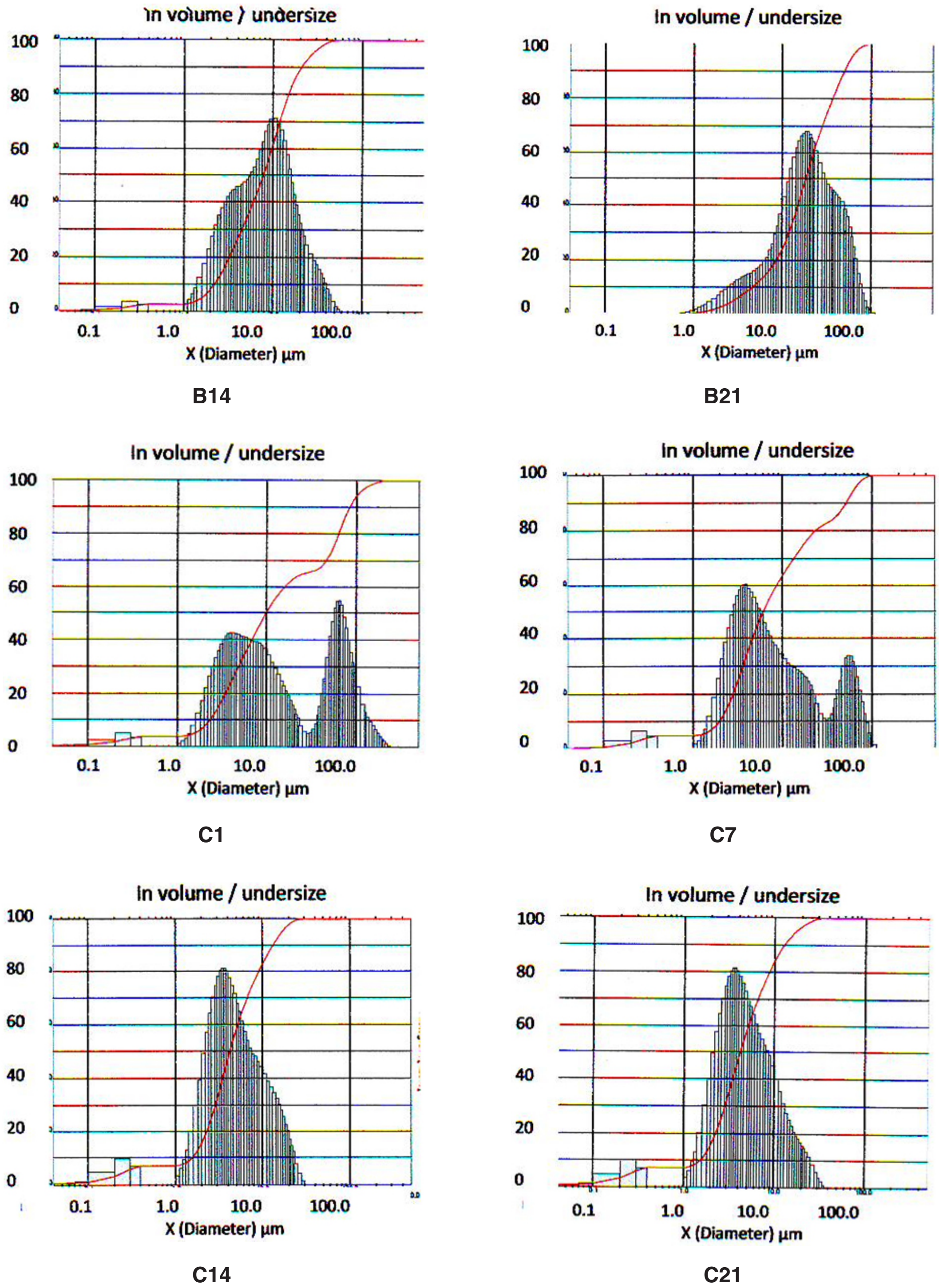


Fig.2: Particle size of goat milk kefir drink

The particle size were determined at 1, 7, 14 and 21 day storage period (from left to right). A= control; B= modified porang flour; C= porang flour.

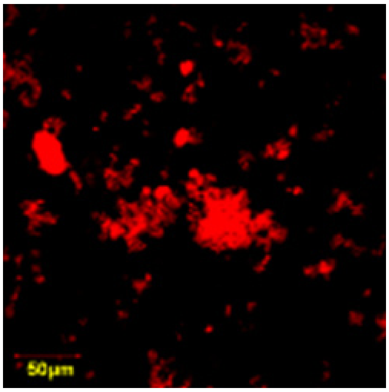
Table 2: Particle size of goat milk kefir drink (µm)

	Particle size of goat milk kefir drink (µm)											
	Control (A)				**Modified porang flour (B)**				**Porang flour (C)**			
	1	**7**	**15**	**21**	**1**	**7**	**15**	**21**	**1**	**7**	**15**	**21**
10%	1.75	2.70	3.09	2.90	2.30	1.93	1.53	1.51	2.77	2.16	2.19	4.49
50%	5.61	10.84	12.82	13.03	9.73	6.02	4.20	4.18	11.51	7.14	7.75	18.29
90%	58.23	53.80	39.77	55.01	83.58	51.41	13.11	12.62	32.74	20.54	20.69	49.92
Span	10.07	4.71	2.86	4.00	8.35	8.22	2.76	2.66	2.60	2,57	2.39	2.48

Microstructure

Micrographs (CSLM) of goat milk kefir drink of control samples show gel network protein phase (red areas) (scale 50 µm) and a distinct continous phase which appears as unevenly distributed grey inclusions. The control samples were found have only slight differences in CLSM images of goat milk kefir drink during 1, 7 and 15 days (Fig A1, A7, A14, A21) however, at 21 days storage period shows bigger aggregate of milk protein (red area). Some serum phase separation was observed at goat milk kefir drink control samples may be attributed with the higher ethanol content and the excess of casein aggregation that the lower water binding capacity of curd.

Modified porang flour treatment shows bigger milk protein (red area) aggregate at 1 day (Fig 3 B1) storage period, however, it have only slight differences and smaller in CLSM images of goat milk kefir drink during 7, 15 and 21 days (Fig 3 B7, B14, B21) storage period. Micrographs of goat milk kefir drink containing modified porang flour, shows gel network protein phase (red areas) (scale 50 µm) and a distinct modified porang flour phase which appears as unevenly distributed grey inclusions (scale 50 µm). The longer the storage period, decreased the size of the milk protein (red area) (scale 50 µm).



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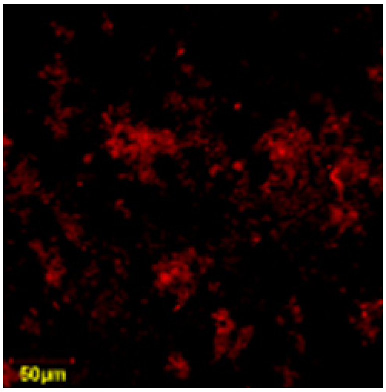

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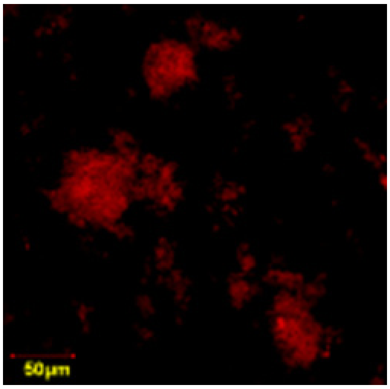

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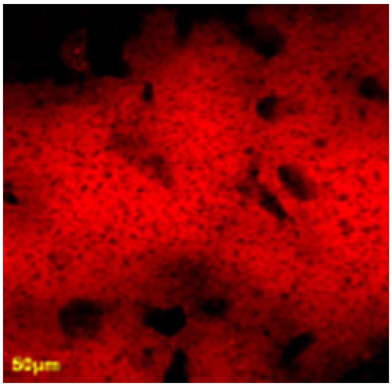

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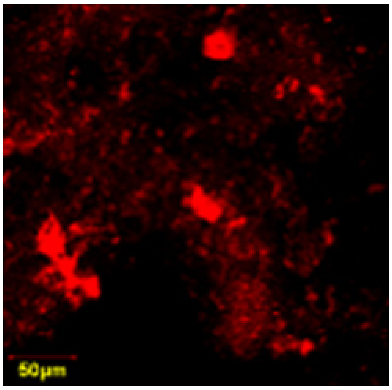

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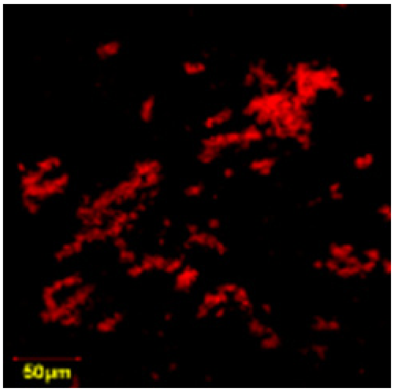

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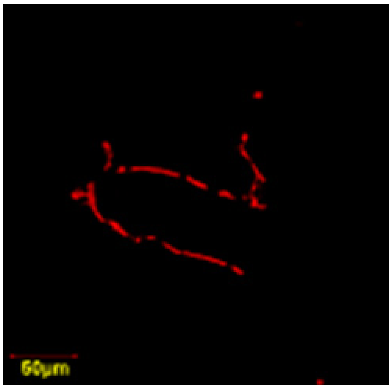

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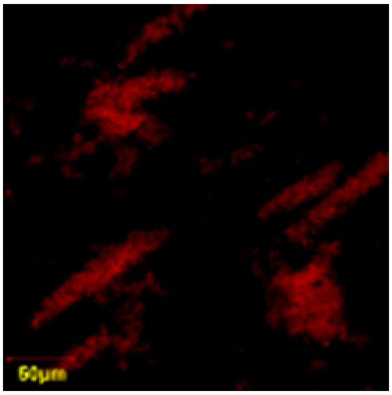

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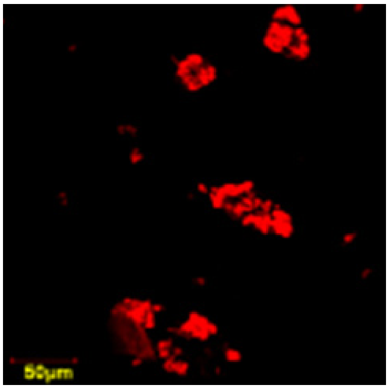

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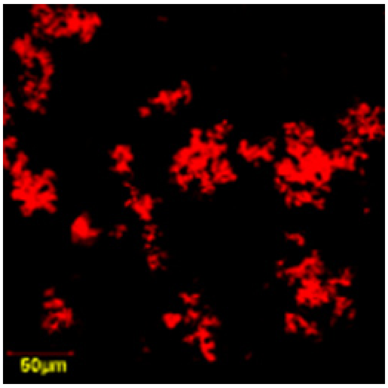

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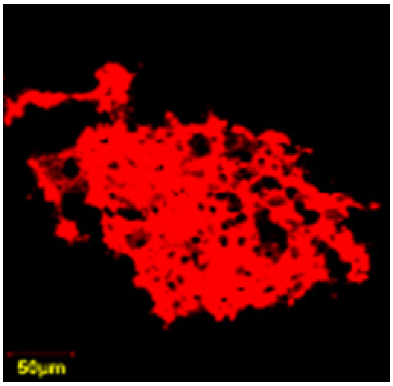

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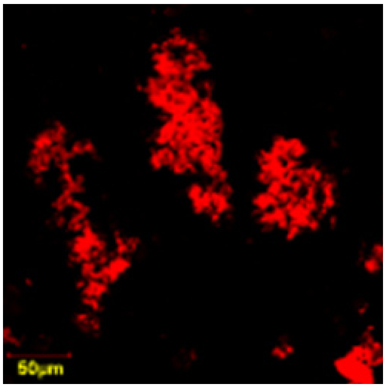

C21

Fig. 3: CLSM images of goat milk kefir drink

The microstructure were determined at 1, 7, 14 and 21 day storage period (from left to right). A= control; B= modified porang flour; C= porang flour.

Porang flour treatment shows only slight differences in CLSM images of goat milk kefir drink during1, 7, 15 and 21 days (Fig 3 C1, C7, C15, C21). The micrograph of goat milk kefir drink containing porang flour shows network protein phase (red areas) (scale 50 µm) and a distinct porang flour phase which appears as unevenly distributed grey inclusions (scale 50 µm). The longer the storage period, increased the size of the milk protein (red area) (scale 50 µm). Some serum phase separation was observed at goat milk kefir drink control samples and porang flour treatment, presumably corresponding to depletion destabilisation due to excess of unadsorbed porang flour to casein.

CLSM images confirmed the significant change in milk protein aggregate of goat milk kefir drink, in general milk protein aggregate were control>porang flour treatment> modified porang flour. Milk protein aggregate were significantly smaller in 15 and 21 days than in 1 and 7 days of storage period of modified porang flour treatment.

The milk protein aggregate of control samples decreased during 15 days storage period, however, the particle size increase at 21 days storage period of goat milk kefir drink. The milk protein aggregate of goat milk kefir decreased during 21 days storage period at modified porang flour treatment, however, porang flour treatment increase milk protein aggregate of goat milk kefir during 15 and 21 days storage period.

This indicates that milk protein undergo aggegration during storage period at control samples and porang flour treatment, however, modified porang flour treatment indicating disruption of milk protein aggregate. Disruption of milk protein aggregate by modified porang flour resulted in decrease in particle size was greater at 21 days, indicating an interaction between modified porang flour and milk protein. This results indicates that increased homogeneity of milk protein particle due to interaction between modified porang flour with milk protein.

Thermodynamic incompatibility can occur between protein and polysaccharides[26] such as glucomannan in porang flour may be attributed with the voluminous of porang flour that induces casein attractions and phase separation of casein-porang flour-water systems. This phase separation takes place where by casein - porang flour complexation is inhibited[27] known as depletion destabilization of aggregated casein- porang flour mixtures,the depletion of porang flour segments around a casein particle; these segments are excluded from the area between casein particles, leads to two phases formation, one concentrated in and one depleted in porang flour.

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Conclusion
Lower ethanol content in goat milk kefir drink maybe attributed with lower water activity (a_w) effect of glucomannan from porang flour and modified porang flour, which decrease alcohol production. Lower particle diameter of goat milk kefir drink in modified porang flour than control and porang flour treatment may due to the disruption of milk protein aggregate by modified porang flour. Confocal microscopy showed at modified porang flour treatment shows more homogenous protein milk aggregate was formed, without causing instability of milk protein aggregat in goat milk kefir drink.

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