Original Research Article

Influence of Maltodextrin Concentration onthe Proximate, Chemical, and Microbiological Properties of Powdered Bovine Colostrum Kefir

ABSTRACT

Incorporating filler during bovine colostrum kefir dehydration is necessary to protect microbial viability and produce desirable powder properties. In this study, the effect of different concentrations of maltodextrin (0%, 2.5%, 5%, 7.5%, 10%) on proximate, chemical, and microbiological properties of powdered bovine colostrum kefir were investigated. Increasing maltodextrin concentration significantly affected (P<.05) proximate, chemical, and microbiological properties, except for alcohol content, of powdered bovine colostrum kefir. Higher maltodextrin concentration increased carbohydrate content, yield, total dissolved solids, solubility, titratable acidity, alcohol, lactic acid bacteria (LAB), yeast, and total microbes, but decreased water content, protein content, fat content, ash content, $a_{w,}$ andpH.The highest concentration of maltodextrin provided the highest count of LAB and yeast to 7.39 log CFU/g and 7.07 log CFU/grespectively, while maintaining alcohol content 0.042%, still under HALAL regulations. However, the highest yield and solubility, 38.60% and 46.12% respectively, were still relatively low due to bovine colostrum characteristics. Addition of 10% (w/v)maltodextrin concentration was the best treatment which preserved LAB viability that complies with CODEX STAN243-2003 [45] and had desirable powder properties.

Keywords: Bovine, colostrum, kefir, maltodextrin, spray dry.

1. INTRODUCTION

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Bovine colostrum is the earliest secretion of postpartum cow characterized with viscous yellow reddish liquid with less lactose compared to mature milk [1]. The excess bovine colostrum is rich with immune factors, growth factors, and bioactive peptides necessary for the newborns' growth and immunity [2]. Although recently bovine colostrum products have risen on the market, the product development and commercialization are still limited to preserving colostrum as it contains high protein that easily aggregates at processing temperature. Fermenting bovine colostrum with kefir grain, a symbiotic culture of bacteria and yeasts, could expand colostrum functionality via increment of antimicrobial capacity [3], bioactive peptides [4], and probiotic properties of kefir microbiota[5]. Several researches have confirmed the potency of bovine colostrum to be fermented with kefir grain [6,7].

As liquid kefir contains high nutrition and water content that supports microbial activity, it has low shelf life indicated by undesirable shifts in physical, chemical, and sensory properties. In order to increase shelf life, also easing distribution and storage, dehydration of kefir is considered[8]. Spray drying is an economic and efficient method of drying that is utilized in many researches to preserve bacteria [9,10]. As a way of retaining microbial viability from

temperature stress [11] and achieving desirable physicochemical and morphology characteristics of powder [12], utilizing maltodextrin is considered. Several researches have been done on the efficacy of using maltodextrin as filler for dried products with bacteria [13,14]. To evaluate the efficacy of bovine colostrum kefir as powdered products, the powder's properties and microbial viability are necessary to be studied.

2. MATERIAL AND METHODS

Comment [MF2]: abbreviations

2.1 Preparation of Samples

2.1.1 Bovine Colostrum Kefir Production

Bovine colostrum kefir was produced according to Nurhasanah et al. [15] with modifications. Bovine colostrum, procured from Ungaran, Semarang, was pasteurized at 60°C for 30 minutes
[16]. Colostrum was cooled down to room temperature (±28°C) and filtered inside the laminar air flow (1300 Series A2, Thermo Fisher Scientific, America). In a sterile environment, 10% (w/v) kefir grain concentration, procured from Omah Kefir, Ungaran, was added into the colostrum, stirred slowly, and then wrapped with plastic. Colostrum was fermented at room temperature for 24 hours, then in a sterile environment, colostrum kefir filtrate was separated from the grains. Colostrum kefir was preserved at 4°C until spray drying.

2.1.2Bovine Colostrum Kefir Drying

Maltodextrin DE 10-12 (Lihua, China) was added to bovine colostrum kefir with different concentrations, specifically 0%, 2.5%, 5%, 7.5%, and 10% (w/v).The drying of bovine colostrum kefir followed KhalilianMovahhed and Mohebbi[16] with modifications. Bovine colostrum kefir samples with different concentration of maltodextrin were homogenized with Ultra Turrax (T-25, IKA, German) at 4000 rpm for 4 minutes, then heated and stirred with the magnetic stirrer (Cimarec, IKA, German) at 45°C and stirring level at 2 while being spray dried. The spray drier (B-290, Buchi, Swiss) used had Q flow 60 mm, inlet temperature 120°C, outlet temperature 62-84°C, aspirator 100%, pressure -60 mbar, nozzle cleaner 2, and feed 1. The powder output was collected in pouch bags with silica gel and preserved at -18°C.

2.2Analysis

2.2.1 Proximate Analysis

Powder samples were used for the proximate analysis. The determination ofcarbohydrate was proceeded with by difference method [18]. Water content was determined with gravimetric methodaccording to Legowo et al.[19]. Protein contentwas determined withkjeldahl method[20] with modifications. Protein content was determined with BuchiKjel Line (Buchi, Swiss) through destruction, destilation, and titration. During destruction, 0.5 g of sample, wrapped in filter paper, was put inside the digest tube and added with ¼ kjeldahl tablet and 10 ml of concentrated H_2SO_4 by turns, then digested at 400°C for 90 minutes.HCl 0.3 N was utilized for the titration.

Fat contentwas determined withsoxhlet method according to Kim et al. [21] with modifications.Briefly, sample weighed approximately 1 g (A) was wrapped in filter paper and dehydrated in oven at 70°C for 15 hours (B), then extracted inBuchi fat extractor (E-500, Buchi, Swiss) with benzene as solvent for 20 extraction cycles. Following the extraction, wrapped samples were laid out in desiccator to evaporate the solvent, then dehydrated in oven at 100°C for 1 hour before weighing (C). Fat contents were counted using the formula as follows.

Fat content (%)= ((B-C)/A)*100%

Ash content of samples were determined with ashingmethod by Legowo et al. [19] with modifications. Crucibles were dehydrated in oven at 105°C overnight. Crucibles were stored in a dessicator and weighed (X), then sample weighed approximately 1 g (Y) was put inside each crucible. Samples were charred in hotplate until it dissipated no smoke and turned white, then heated inmuffle furnace (Thermolyne, Thermo Fisher Scientific, America) at 550°C for 5 hours (Z) before weighing. Ash contents were counted as follows.

Ash content (%)= (Z-X)/Y*100%

2.2.2Chemical Analysis

Powder samples were used for the determination of yield [18]. Total dissolved solids (TDS)was determined according to Rizqiati et al.[18] with digital refractometer (PAL-1, Atago, Japan). Water activity (a_w)was determined with water activity meter (LabSwift-aw, Novasina, COUNTRY) by prepping the powder samples in a cylindrical plastic container for measurement. Solubilitywas determinedaccording to Rizqiati et al. [18] with modifications in drying time of wrapped samples after filtrating, which was 3 hours followed by another 1 hour in the oven at 105°C. According to Atalar and Dervisoglu [9]with modifications, pH was determined by pH meter (PC 700, Eutech Instruments, Singapore)by dissolving 1 g of sample in 10 ml aquadest before inserting the probe to the sample solution. Titratable acidity was determined by titration [18] with 0.1 N NaOH.

Alcohol content was determinedwith microdiffusion method according to Nahak et al. [22] with modifications.Preceding alcohol content determination, a standard curve was constructed. Two reagents of microdiffusion assay were reproduced according to Noriega-Medrano et al. [23] with modifications. Dichromate acid solution was made by dissolving 0.852 g of $K_2Cr_2O_7$ with 20 ml of aquadest, then added with 80 ml of concentrated H_2SO_4 slowly to avoid rapid exothermal reaction, whereas sodium carbonate was made by dissolving 20 g Na_2CO_3 of in 100 ml of aquadest.Standard solutions of 0%, 0.025%, 0.05%, 0.075%, and 0.1% (v/v) concentration of ethanol were prepared by diluting 0.5% ethanol stock solution in aquabidest. Ethanol and sodium carbonate were pipetted each 1 ml in the sides of the conways while dichromate acid was pipetted 1 ml in the middle, then the conways were incubated at 37˚C for 2 hours. Afterwards, dichromate acid solutions were pipetted and diluted in a ten-fold serial, then the absorbances were read using UV-Vis spectrophotometer (Cary 60, Agilent, America) with maximum wavelength of 480 nm. The regression line equation of Y=-4.2128x+0.8107 was obtained from plotting the absorbances with the respective standard solution concentrations. Alcohol content determination of powdered bovine colostrum kefir samples were carried out by dilution of powder samples with aquabidest (1:4), then repeating the procedure by replacing standard solutions with the diluted samples. Alcohol contents were calculated by plotting the acquired absorbances to the regression and reversing the dilutions.

2.2.3 Microbiology Analysis

Lactic acid bacteria (LAB), yeast, and total microbes were evaluated by total plate count method according to Sholichah et al.[24] with modifications. Powder samples were diluted in ten-fold dilutions with 0.85% NaCl. Samples at 10^{-4},10^{-5}, and 10^{-6}dilutions were pipetted 1 ml, each duplo, into petri dishes, then MRSA, SDA, and PCA media were respectively poured into the petri dishes for determination of total LAB, yeast, and total microbes. Samples for total LAB and total microbes were incubated anaerobically at 37˚C for 48 hours[25], while samples for yeast were incubated aerobically at 30˚C for 48 hours. Colonies of 30-300 were counted using the formula as follows.

CFU/ml= total colony x1/dilution factor

2.2.4 Statistical Analysis

Data obtained was analyzed using Statistical Product and Service Solutions (SPSS) 26.0 for Windows. One-way analysis of variance (ANOVA) procedure was used to determine

difference in treatment means with a significance level of 0.05. Duncan's Multiple Range Test (DMRT) was used in the further analysis for mean separation.

3. RESULTS

3.1 PROXIMATE COMPOSITION OF POWDERED BOVINE COLOSTRUM KEFIR SAMPLES

The addition of maltodextrin with different concentrations has a significant effect ($p<.05$) on the carbohydrate content, water content, protein content, fat content, and ash content of powdered bovine colostrum kefir samples (table 1). Carbohydrate content of the samples ranged 10.78-27.64%, water content of the samples ranged 2.47-4.25%, protein content of the samples ranged 43.06-54.37%, fat content of the samples ranged 24.07-27.02%, and ash content of the samples ranged 2.77-3.57%. The highest content of carbohydrate was in 10% (w/v) maltodextrin treatment samples, whereas the highest content of water, protein, fat and ash was in control samples. For the lowest content, it was vice versa.

3.2CHEMICAL PROPERTIES OF POWDERED BOVINE COLOSTRUM KEFIR SAMPLES

The addition of maltodextrin with different concentrations has a significant effect ($p<.05$) on the yield, TDS, a_w, solubility, pH, andTA, but insignificant on alcohol content (table 2). Yield of the samples ranged 29.21-38.60%, TDS of the samples ranged 50.00-70.00%Brix, a_w of the samples ranged 0.334-0.391, solubility of the samples ranged 34.63-46.12%, pH values of the samples ranged 4.13-4.82, TA of the samples ranged 2.01-2.82%, and the alcohol content of the samples ranged 0.025-0.042%. Yield, TDS, solubility, TA, and alcohol content had the highest value in 10% (w/v) maltodextrin treatment samples, whereas a_w and pH had the highest value in control samples. For the lowest value, it was vice versa.

3.3MICROBIOLOGICAL PROPERTIES OF POWDERED BOVINE COLOSTRUM KEFIR SAMPLES

The addition of maltodextrin with different concentrations has a significant effect ($p<.05$) on the viability of LAB, yeast, and total microbes (table 3).The LAB of the samples ranged 6.17-7.39 log CFU/g, yeast of the samples ranged 6.24-7.07 log CFU/g, and total microbes of the samples ranged 5.97-7.10 log CFU/g. The highest count of LAB, yeast, and total microbes was in 10% (w/v) maltodextrin treatment samples, whereas the lowest was in the control samples.

Table 1. Proximate properties of powdered bovine colostrum kefir samples

Properties	Maltodextrin concentration (%w/v)					Method
	0	2.5	5	7.5	10	
Carbohydrate content (%)	10.78^{a}±0.71	15.23^{b}±0.69	16.05^{b}±1.03	20.80^{c}±0.44	27.64^{d}±1.03	By difference
Water content (%)	4.25^{a}±0.25	3.79^{b}±0.17	3.29^{c}±0.10	2.95^{d}±0.14	2.47^{e}±0.10	Gravimetry method
Protein content (%)	54.37^{a}±1.39	53.03^{a}±1.33	50.27^{b}±0.46	48.61^{c}±0.10	43.06^{d}±0.23	Kjeldahl method
Fat content (%)	27.02^{a}±1.13	26.34^{a}±0.77	25.14^{ab}±0.88	24.67^{bc}±0.43	24.07^{c}±0.82	Soxhlet method
Ash content (%)	3.57^{a}±0.16	3.54^{b}±0.06	3.32^{c}±0.31	2.96^{d}±0.13	2.77^{d}±0.08	Ashing with muffle furnace

**Values are means of four replicate readings with a standard deviation*
**Mean values having different superscript letters on the same column differ significantly at 5% significant level (p<.05)*

Table 2. Chemical properties of powdered bovine colostrum kefir samples

Properties	Maltodextrin concentration (%w/v)					Method
	0	2.5	5	7.5	10	
Yield (%)	29.21^{a} ±0.65	30.65^{b}±0.41	31.32^{b}±0.46	33.50^{c}±0.42	38.60^{d}±0.51	Yield test
Total Dissolved Solids (%Brix)	50.00^{a}±0.00	50.00^{a}±0.00	60.00^{b}±0.00	68.50^{c}±3.00	70.00^{c}±0.00	Refractometer
Water activity (a_w)	0.391^{a}±0.01	0.354^{b}±0.00	0.353^{b}±0.01	0.352^{b}±0.00	0.334^{c}±0.01	Water activity meter
Solubility (%)	34.63^{a}±0.99	38.00^{b}±0.82	40.35^{c}±0.46	44.61^{d}±0.90	46.12^{e}±0.67	Solubility test
pH	4.82^{a}±0.01	4.77^{b}±0.04	4.63^{c}±0.03	4.26^{d}±0.04	4.13^{e}±0.03	
Titratable acidity (%)	2.01^{a}±0.04	2.07^{a}±0.06	2.22^{b}±0.04	2.26^{b}±0.02	2.82^{c}±0.01	Titration
Alcohol(%)	0.025±0.01	0.0299±0.01	0.031±0.01	0.035±0.01	0.042±0.02	Microdiffusion method [19]

**Values are means of four replicate readings with a standard deviation*
**Mean values having different superscript letters on the same column differ significantly at 5% significant level (p<.05)*
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Table 3. Microbiological properties of powdered bovine colostrum kefir samples

Properties	Maltodextrin concentration (%w/v)					Method
	0	2.5	5	7.5	10	
Lactic acid bacteria (log CFU/g)	6.17^{a}	6.55^{a}	6.55^{a}	6.50^{a}	7.39^{b}	Total plate count method
Yeast (log CFU/g)	6.24^{a}	6.25^{a}	6.32^{a}	6.79^{ab}	7.07^{b}	
Total microbes (log CFU/g)	5.97^{a}	6.36^{a}	6.63^{ab}	6.69^{ab}	7.10^{b}	

**Values are means of four replicate readings*
**Mean values having different superscript letters on the same column differ significantly at 5% significant level (p<.05)*

4. DISCUSSION

4.1 Proximate Analysis Of Powdered Bovine Colostrum Kefir Samples

The increasing concentration of maltodextrinincreased the carbohydrate content of samples. Maltodextrin is a starch hydrolysis product that consists of D-glucose units connected by (1-4) glucosidic linkages, thus its addition will increase carbohydrate content. High degree of hydrolysis causes higher maltodextrin's dextrose equivalent (DE) value that increases the structure's hydroxyl groups [26], and thereforeaffecting water absorption capacity. In this study, increasing maltodextrin concentration decreasedwater content of samples due to the increasing hydrophilic groups. Maltodextrin DE 12 water absorption mechanism follows a type II isotherm with multilayer formation [27]. The water content in this study ranged from 2.47-4.25%, which complies to the standard water content in powdered milk. The reduction of water content will increase the drying process stability,diminish hygroscopicity during processing and storage [28], and reduces rehydration time due to increasing surface area.

The increasing concentration of maltodextrindecreasedthe protein content of samples due to increasing proportion of carbohydrate content. The protein content in this study ranged from 43.06-54.37%, which is higher than that of powdered milk kefir with dextrin [29], due to bovine colostrum which generally has 15.0% protein, whereas mature milk 3.0%, mainly because of higher casein and immunoglobulin [30]. Protein quantity is also affected by kefir proteolytic activity during fermentation. Lactobacillus have extracellular proteolytic capabilitiesand peptide transport system, which allow it to hydrolyze protein, then either release more peptides or absorb them [31].

Increasing maltodextrin concentration decreased the fat content of samples due to the shifting proportion. Generally, bovine colostrum has higher fat content than mature milk with higher composition in palmitic, palmitoleic, and myristic acids [32]. During fermentation, LAB'slipolytic activity[6] may affect colostrum fat content. Likewise, increasingmaltodextrin concentration also decreased the ash content of samples due to the shift in proportion. Ash is also utilized in the metabolism of carbohydrates, fats, and proteins for cell growth, maintenance, and energy [33].Additionally to the higher mineral content of bovine colostrum than mature milk [1], vitamin B1, B12, Ca, folic acid, and vitamin K levels increase during kefir fermentation [34].

4.2 Chemical Analysis Of Powdered Bovine Colostrum Kefir Samples

Yield indicates production efficiency that compares the resulted products to raw materials. The increasing concentration of maltodextrin increased sampels' yields. The range of yield found in this study is higher than that of powdered goat milk kefir with dextrin [29]. The increasing yield is due to maltodextrin acting as solid enhancer, thereby adding volume and mass. The reduced water content due to maltodextrin addition is paramount to increase glass transition temperature, thereby enabling the formation of the glassy matrix that retains sensitive materials and reduces transfer of oygen[11]. Inversely, below the glass transition temperature, maltodextrin forms a rubbery viscous matrix that supports material adherence to the wall of the drying chamber [12], hence lowering the yield.

As maltodextrin is characterized with high solubility [27], the increasing concentration of maltodextrin increased samples' total dissolved solids (TDS) values as well. High TDS increases encapsulant viscosity for better protection [35], also encapsulation efficiency via increasing the resistance against collapse [11]. Furthermore, TDS increases production efficiency byreducing water content that will otherwise evaporated, however a superfluous amount may hinder the feed and spraying process [36]. Thus, increase in maltodextrin concentration lowered water activity in this study. The aw found in this study ranged 0.33-0.39, which is still under 0.61, the limit for microorganism growth [37], to maintain product stability.

Solubility refers to the ability of powder to release encapsulated materials in solvent, which indicates convenience.The increasing concentration of maltodextrin increased the samples' solubility values due to its hydrophilic groups. Similar result was found in noni leaf powder drink study, where 5-15% maltodextrin concentration resulted in 93.14-97.13% solubility [38].The solubility found in this study ranged 34.63-46.12%, which according toMoghbeli et al. [39] still inefficient. The low solubility of powdered bovine colostrum kefir may be due to the high fat content of bovine colostrum [40], which consists more of the relatively insoluble long chain fatty acids [41]. Solubility is also affected by the tendency of small particles to dissolve easily due to the increasing solvent diffusion, which is supported by the process of kefir fermentation and spray dry atomizer.

The samples' pH values decreased with increasing maltodextrin concentration. Similar results were observed in other studies regarding yoghurt powder with maltodextrin addition [14, 42]. The decreasing pH value is due to the preserved LAB viability with higher maltodextrin concentration. LAB utilizes lactose into lactic acid during anaerobic fermentation [5],hence the accumulating acid will lower pH.The pH value ranged measured was higher than that of the colostrum kefir liquid reported by Windayani et al. [3] due to the lesser lactose in bovine colostrum. Bovine colostrum contains minimum lactose to 1.2% that can increase during postpartum [43]. Furthermore, the varying pH value may be affected by the characteristics of colostrum and microbial composition of kefir grain [44]. The pH value is inversely proportional to titratable acidity (TA), which is measured through NaOH titration. TA ranges 2.01-2.82%, which still complies with CODEX-STAN 243-2003 [45] standard on 0.6% as the least acid content. Aside from affecting flavor, lactic acid contributes towards pathogen inhibition via inducing acidic condition.

The increasing concentration of maltodextrin didn't affect the samples' alcohol contents significantly. Ethanol, one of the main products and kefir's distinguishing characteristic [5], is produced via yeast anaerobic activity in converting pyruvic acid from glycolysis in anaerobic condition [46], hence it is of paramount importance for halal certification. The alcohol content found in this study was lower than Windayani et al. [3] who reported 0,39% alcohol content of bovine colostrum kefir fermented for 24 hours with 10% grain. Kefir alcohol content is affected positively by kefir grain concentration and fermentation duration [47].

4.3 Microbiological Analysis of Powdered Bovine Colostrum Kefir Samples

The addition of maltodextrinmaintained the viability of microorganisms as indicated by the increase of total LAB, yeast, and total microbes along with the rising concentration. The linear increase of LAB and yeast due to filler addition is similar with the result reported by Rizqiati et al. [29]. The linear relationship of LAB and yeast could be explained by their symbiotic associations. Metabolism products of LAB provide energy for yeast, whereas yeast produce essential growth factors for bacteria, including vitamins and amino acids [5], also raising the environment pH suitable for LAB [48]. LAB of powdered bovine colostrum kefir with 10% (w/v) maltodextrin complies with CODEX STAN 243-2003 [45], which stated that the minimum microorganisms in kefir is 10^7 CFU/g. Total microbes also counted the viability of acetic acid bacteria (AAB), which have the capacity to oxidize ethanol to acetic acid aerobically [49].

The several first maltodextrin concentration treatments towards microbial viability in this study didn't have much statistical differences with the control samples due to the drying process.The process of spray drying generates heat and mechanical stress that impact cellular injuries that reduce viability, such as denaturation of intracellular proteins, dehydration, and destabilization of cellular structure due to cytoplasmwater content elimination [50]. The addition of maltodextrinprotects microorganism viability as maltodextrin quickly forms a glassy matrix at the beginning of drying to increase cell stability [11] and reduce the surface mechanical stress [51]. The higher concentration of maltodextrin provides higher viscosity to encapsulate and protect sensitive materials better [35]. Moreover, maltodextrin has thermoprotectant capability [12], thus lessening thermal degradation effect on viability of microorganisms.

4. CONCLUSION

Increasing concentration of maltodextrin significantly affected proximate, chemical, and microbiological properties, excluding alcohol content, of powdered bovine colostrum kefir. Addition of 10% maltodextrin concentration provided powdered bovine colostrum kefir with total LAB that complies with CODEX STAN 243-2003 [45] and desirable powder properties, thus being the best treatment.

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