Characteristics stability of cheese whey-tomato juice fermented beverages with pectin and CMC addition

N Saidah, E Nurhartadi, A Nursiwi, A M Sari and R Utami
Department of Food Science and Technology, Faculty of Agriculture, Sebelas Maret University, Indonesia
Email: edhi.nr@gmail.com

Abstract. The aim of this study was to investigate the chemical and microbiological stability of 12 days of storage (±4°C) of fermented beverages from cheese whey and tomato juice with addition of pectin:carboxymethyl cellulose combination (0%:0%; 0.15%:0.25%; 0.25%:0.15%; 0.2%:0.2%; 0.1%:0.3%) and to determine the best formula to find out it’s hypocholesterolemic effects. Fermented cheese whey beverages was prepared from cheese whey:tomato juice (95%:5%), sucrose (10% w/v), and 5 variation of pectin:CMC combination. This research used a Completely Random Design (CRD) with one factor, combination of pectin and CMC. The result was analyzed by applying one-way analysis of variance (ANOVA) followed by Duncan test with 5% significant level. The result showed that during 12 days of storage, the addition of pectin:CMC combination in fermented beverages from cheese whey reduce the rate of pH, antioxidant activity, total lactic acid bacteria, and the rate of lactic acid rise. The best formula of fermented beverage from cheese whey and tomato juice is fermented beverage with 0.1%:0.3% (w/v) of pectin:CMC addition which has 34.636% of hypocholesterolemic effect.

1. Introduction
Whey is a liquid separated from milk curd after coagulation of milk, cream, skim milk or butter of milk on cheese, casein or similar products especially with rennet type enzymes [1]. Whey consists of 96% water, a total milk solid of 50-55%, 70% lactose, 20% protein, 70-90% minerals, and almost all water-soluble vitamins such as vitamin B complex and vitamin C [2]. Conversion of whey into a fermented beverage is one of the best ways of utilizing whey for human consumption [3]. The addition of fruit juice can improve the physicochemical properties of fermented beverages [4].

Fermented cheese whey beverages using 1% Lactobacillus acidophilus and Lactobacillus plantarum with a ratio 1:1 gives a good taste. Lactobacillus acidophilus produced more lactic acid than other LAB, Lactobacillus plantarum could improved sensory properties and inhibited pathogenic microorganisms, so the combination of both starter improved physicochemical and sensory properties of fermented beverages [5]. Lactobacillus acidophilus and Lactobacillus plantarum are lactic acid bacteria that can lower cholesterol [6, 7, 8, 9]. Tomato juice addition on fermented beverage can increase nutrical values of fermented beverages. Tomato contains phytosterols, B vitamins, and potassium which can lower cholesterol, and contains high antioxidants [10, 11]. Tomato fruit is also a source of fructooligosaccharide (FOS). FOS serves as a prebiotic that can stimulate the growth of lactic acid bacteria [12].

Separation of fermented cheese whey beverage might take place during storage. This separation occurs because high methoxyl pectin can form gel in acidic environments [13]. So to apply pectin in
an acidic fermented beverage, pectin needs to be combined with a more acid-resistant stabilizer i.e. carboxy methyl cellulose.

Pectin is a polymer of D-galacturonic acid linked to α- (1,4)-glucose bonds having gelling, thickening, and stabilizing properties [14]. According to a study by Schonbrun (2002), the addition of pectin in yoghurt showed high viscosity and overall acceptability. Pectin has a large water holding capacity [15]. However, high pectin metoxyl can form gel in acidic environments. Carboxy methyl cellulose (CMC) is a polysaccharide hydrocolloid containing a α- (1,4)-D-glucose- polymer derived with carboxy methyl groups. Carboxy methyl cellulose can be used as a stabilizer in dairy products, because it can react with casein near the isoelectric point, producing a soluble complex [13].

This study was to examine the effect of pectin and carboxy methyl cellulose on whey fermented beverage characteristics with the addition of tomato juice (Solanum lycopersicum) using starter Lactobacillus acidophilus FNCC 0051 and Lactobacillus plantarum FNCC 0027, to find the stability of formula chemically and microbiologically during storage of low temperatures, as well as to find the cholesterol-lowering activity of selected formulas in-vitro.

2. Materials and Methods
2.1. Materials
The ingredients used for the manufacture of fermented beverages in this study are cheese whey mozzarella wastes collected from the cheese industry in Boyolali, fresh ripe tomatoes (tomato varieties of permata) bought from Pasar Gede Surakarta, sucrose (Gulaku), Lactobacillus acidophilus FNCC 0051 and Lactobacillus plantarum FNCC 0027 taken from Food and Nutrition Culture Collection (FNCC), Food and Nutrition Laboratory, Gadjah Mada University, Yogyakarta.

2.2. Methods
2.2.1. Mother Starter
Each pure culture of Lactobacillus acidophilus FNCC 0051 and Lactobacillus plantarum FNCC 0027 propagated by aseptically taking 1 ose bacterial culture then put in test tubes containing 5 ml sterile MRS broth and then incubated at 37°C for 24 hours. The results of bacterial culture in MRS broth was then centrifuged at 1000 rpm for 10 minutes. Natan which was obtained would then be used as many as 3 ose and inoculated in 5 ml of 10% skim milk solution which has passed the pasteurization process at a temperature of 85°C for 30 minutes. Furthermore, they were then to be incubated at 37°C for 24 hours [16, 17].

2.2.2. Culture Starter
Manufacture of culture starter was with 100 ml of 10% skim milk pasteurized at 85°C for 30 minutes and then cooled to 37°C. After that, inoculated with 2% mother starter, then incubated at 37°C for 24 hours [17].

2.2.3. Tomato Juice
Fresh red tomato fruit washed and cut into small pieces then put into juicer to separate the pulp and its juice. The tomato juice that has been obtained then filtered using a filter cloth.

2.2.4. Fermented Whey Beverage
The preparation of fermented beverages was done by mixing homogenous cheese whey (95%) with sucrose (10% w/v of total cheese whey and tomato juice), and pectin: CMC (0%: 0%; 0.15%: 0, 25%, 0.25%: 0.15%, 0.2%: 0.2% and 0.1%: 0.3% w/v of the total volume of cheese whey and tomato juice) which have been mixed dry. Tomato juice (5%) was then added into the cheese whey mixture, sucrose, and stabilizer (pectin: CMC). The ingredients are mixed, then homogenized and continued pasteurization at 75°C for 30 minutes. The heat was cooled and inoculated with 1% starter Lactobacillus acidophilus FNCC 0051 and Lactobacillus plantarum FNCC 0027 with 1: 1 ratio (v/v
of total cheese whey and tomato juice) with $10^8$ CFU / ml starter. Furthermore, it was incubated at 37°C for 18 hours [18, 19].

2.2.5. Sample Analysis
The characteristic stability of the five varieties of fermented cheese whey beverage samples were analyze (pH, lactic acid content, antioxidant activity, and total lactic acid bacteria) during 12 days of low temperature storage to obtain the preferred formula. The selected formula was then tested for in-vitro cholesterol-lowering activity. The packaging is done during storage using a sealed glass bottle.

2.2.6. Cholesterol Lowering Activity Analysis
10 μl of standard cholesterol was added to 50 ml of tomato juice inoculated 1% of probiotic bacteria *Lactobacillus acidophilus* FNCC 0051: *Lactobacillus plantarum* FNCC 0027 (1: 1) and not inoculated bacteria (control sample) which then incubated in incubator shaker 37°C for 24 hours. The samples were incubated for 24 hours, then taken 40 μl and added with 4000 μl of Cholesterol FS Diasys reagent. The mixture of sample and reagent then homogenized continued incubation at room temperature for 20 minutes. The sample absorbance was subjected to a spectrophotometer with $\lambda$ 500nm [20]. Cholesterol levels are then calculated using standard curve equations.

3. Results and Discussion
3.1. Antioxidant Activity
During 12 days of storage, all samples of fermented drinks decreased antioxidant activity. The table for decreasing antioxidant activity during storage can be seen in Figure 1. The activity of antioxidants during storage due to degradation of antioxidant compounds contained in fermented beverages [21]. Antioxidant compounds such as ascorbic acid would eliminate oxygen, control the activity of polyphenol oxidase enzymes, and increase the stability of lycopene, so that during storage the amount would decrease. During storage, the phenolic component in the fermented beverage would be combined with ascorbic acid to inhibit the occurrence of oxidation reactions, so the amount also decreased. An increase in antioxidant activity on day 3 might be caused by antioxidant activity produced by *Lactobacillus acidophilus* and *Lactobacillus plantarum*. 0.1% pectin and 0.3% CMC might be the best prebiotic combination that could stimulated bacterial growth to produced higher antioxidant compound. Probiotic lactic acid bacteria demonstrated antioxidant ability both in-vivo and in-vitro. Lactic acid bacteria might resist reactive oxygen species (ROS), peroxide radicals, superoxide anions, and hydroxyl radicals.

![Antioxidant Activity of Fermented Beverages from Cheese Whey and Tomato Juice with Variation of Pectin:CMC Combination During Cold Storage ± 4°C](image)

3.2. Total Lactic Acid Bacteria, Lactic Acid Level, and pH
The results in Figure 2 showed that the amount of lactic acid bacteria in the cheese whey fermented beverage sample decreased for 12 days of storage. The reducing viability of lactic acid bacteria during storage can be caused by the decreasing amount of nutrients available in fermented beverages from cheese whey and tomato juice. During storage, lactose and protein as a source of nutrients lactic
acid bacteria would reduce because it has been converted into lactic acid and other organic acids. This caused lactic acid bacteria could not survive due to lack of nutrients. During 12 days of storage, all samples of fermented beverages increased lactic acid levels. This was because during the storage fermented beverages still underwent a fermentation process that made lactic acid bacteria had more time to utilize nutrients especially lactose in its medium [22, 23]. The amount of *Lactobacillus acidophilus* and *Lactobacillus plantarum* during this reasonably high storage temperature of ± 4°C (Figure 2), caused lactic acid as *Lactobacillus acidophilus* and *Lactobacillus plantarum* metabolites to increase during storage. This increasing of lactic acid levels, caused the pH of fermented drinks to decrease during the storage of low temperatures.

![Figure 2](image)

**Figure 2.** (a) Total Lactic Acid Bacteria (b) Lactic Acid Level (c) pH Value Fermented Beverages from Cheese Whey and Tomato Juice with Variation of Pectin:CMC Combination During Cold Storage Storage ± 4°C
3.3. Cholesterol Lowering Activity of Fermented Beverage from Cheese Whey and Tomato Juice
Selected Formula
Formulas of fermented beverage from cheese whey and tomato juice with the addition of pectin:CMC stabilizers was selected using an effectiveness index test [24]. Based on the results of effectiveness index test, fermented cheese whey with 0.1% pectin: 0.3% CMC selected for continued cholesterol-lowering activity test in vitro. The results of cholesterol measurements of the sample can be seen in Table 1. Beverages of cheese whey fermentation with the addition of tomato juice and stabilizer can lower cholesterol levels through several mechanisms. There are three possible mechanisms of probiotics in lowering cholesterol, the first mechanism of fermented products by LAB inhibits synthetic cholesterol thereby lowering cholesterol production. The second mechanism is through the removal of bile salts through the feces, where the conjugated bile salts are not absorbed by the intestine, and are more easily removed from the gastrointestinal tract than the conjugated bile salts. This results in more cholesterol needed to synthesize bile salts again so that it will lower cholesterol levels. The third mechanism is the ability of LAB to bind cholesterol so as to lower cholesterol levels in the media [25].

Table 1. Results Measurement of Cholesterol Levels In-vitro

| Sample | Absorbance | Cholesterol Level (mg/dl) | Cholesterol Reduction (mg/dl) | Cholesterol Reduction (%) |
|--------|------------|---------------------------|-------------------------------|---------------------------|
| Control | 0.206      | 1.941                     | 0.059                         | 2.957                     |
| Sample  | 0.136      | 1.307                     | 0.693                         | 34.636                    |

4. Conclusion
The result showed that during 12 days of storage, the addition of pectin:CMC combination in fermented beverages from cheese whey improved the rate of viscosity rise, reduced the rate of dispersion index decline, pH, antioxidant activity, total lactic acid bacteria, and the rate of lactic acid rise. The best formula of fermented beverage from cheese whey and tomato juice was fermented beverage with 0.1%-0.3% (w/v) of pectin:CMC addition which has 34.636% of hypcholesterolemic effect.

References
[1] Badan Standarisasi Nasional 1996 SNI 01-4220:1996 Tentang Tajian Susu (Whey) Bubuk (Jakarta: BSN)
[2] Archer RH 2013 III-Dairy-G-Whey Products Protein and Powder Technology Section (New Zealand: Dairy Research Institute Press)
[3] Kumar PA and Bangaraiah P 2014 Int J Pharm Bio Sci 5(4) 1101-11
[4] Widagda S and Nisa FC 2015 Jurnal Pangan dan Agroindustri 3(1) 248-258
[5] Nursiwi A, Nurhartadi E, Utami R, Sari AM, Laksono PW and Aprilia EN 2016 IOP Conf. Series: Materials Science and Engineering 193 012009
[6] Gilliland SE, Nelson CR and Maxwell C 1985 Applied and Environmental Microbiology 49(2) 377-381
[7] Guo LD, Yang LJ and Huo GC 2011 Czech J. Food Sci 29(3) 219–225
[8] Vani M, Prakash MS and Devi PY 2012 Indian Journal of Clinical Practice 23(4) 224-230
[9] Fuentes MC, Lujo T, Carrio JM and Cune J 2013 British Journal of Nutrition 109 1866–72
[10] Bhowmik D, Kumar KPS, Paswan S and Srivastava S 2012 Journal of Pharmacognosy and Phytochemistry 1(1) 33-43
[11] Nasir MU, Hussain S and Jabbar S 2015 Science 3(1) 1-5
[12] Maltos DAF, Mussatto SI, Esquivel, Herrera RR, Teixeira JA and Aguilar CN 2014 Critical Reviews in Biotechnology 36(2) 259-267
[13] Schonbrun R 2002 Thesis University of Florida
[14] Winarno FG 2004 Kimia Pangan dan Gizi (Jakarta: PT. Gramedia Pustaka Utama)
[15] Arioui F, Saada DA and Cheriguene A 2016 *Food Science and Nutrition* (Algeria: Wiley Periodical, Inc)
[16] Setioningsih E, Setyaningsih R and Susilowati A 2004 *Bioteknologi* 1(1) 1-6
[17] Utami R, Andriani MAM and Putri ZA 2010 *Caraka Tani* 25(1) 50-55
[18] Maryana D 2014 *Thesis* Hasanuddin University Makassar
[19] Krasaekoopt W and Cabraal TL 2011 *AU J.T.* 14(4) 253-258
[20] Fadhilah AN, Hafsan and Nur F 2015 *Prosiding Seminar Nasional Mikrobiologi Kesehatan dan Lingkungan*
[21] Porto MRA, Okina VS, Pimentel TC and Prudencio SH 2017 *Beverages* 3(36) 1-12
[22] Gianti I and Evanuarini H 2011 *Jurnal Ilmu dan Teknologi Hasil Ternak* 6(1) 28-33
[23] Temesgen M 2015 *Food Science and Quality Management* 37 90-102
[24] De Garmo EP, Sullivan WG and Canada JR 1984 *Engineering Economy (7th ed)* (New York: Macmillan Publishing Company)
[25] Lee DK, Jang S, Baek EH, Kim MJ, Lee KS, Shin HS, Chung MJ, Kim JE, Lee KO and Ha NJ 2009 *Lipids in Health and Disease* 8(21) 1-8

**Aknowledgement**

This work was financially supported by research project by PNBP UNS No. 1073/UN27.21/PP/2017 from Sebelas Maret University, Indonesia.