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Whey utilization as milk substitution in the making of Caspian Sea Yogurt.

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ABSTRACT

This study aims to improve the efficiency of the production process of the Caspian Sea yogurt by utilizing cheese whey waste. Caspian Sea yogurt is yogurt that has a lower acidity and greater viscosity than regular yogurt. Bacteria commonly used for the production of the Caspian Sea Yogurt are *Lactobacillus lactis* ssp cremoris and *Acetobacter orientalis*. This yogurt is fermented at room temperature after adding 1/10 or 1/20 of the volume of the bacteria culture in the milk yogurt, because the Caspian Sea yogurt fermented at lower temperatures (around 25 to 30 ° C) than regular yogurt. The experiment was designed as randomized block (RBD) with two factors, namely: the proportion of milk: whey (100: 0 as the control; 75:25; 50:50; 25:75 as the treatment) and starter concentration (1.5%, 2% and 2.5% (v / v)) with three replications. It was found that the proportion of cow's milk:whey of 75:25 and 2.0% starter concentration was well received in organoleptic test with the value close to control (milk: whey ratio of 100:0 and starter concentration of 2.5%). This treatment has an average protein content of 3.58%, a total Lactic Acid Bacteria (LAB) of 6.8 x 109 cfu/ml, 4.156 pH, total solids 4.89 dPas and viscosity of 0.26.

Keywords: *Acetobacter orientalis*, Caspian Sea Yoghurt, starter concentration, *Lactobacillus cremoris*, whey.

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INTRODUCTION

Yogurt is a fermented product produced by the process involving aerobic and anaerobic microbial activity [1]. Yogurt has been recognized and appreciated by the public because it has a positive impact on health, can improve digestion of proteins and fats, stimulates the secretion of fluid necessary for digestion process such as saliva, gastric fluid, bile and pancreatic and reduce the incidence of allergic reactions to lactose [2]. Yogurt has a viscosity and distinctive taste that can be used as an alternative to dairy products for consumers [3].

Caspian Sea yogurt has a lower acidity and greater viscosity than regular yogurt [4]. The bacteria involved in the production of Caspian Sea yogurt is L. *lactis* ssp *cremoris* and *Acetobacter orientalis*. This type of yogurt is fermented by adding 1/10 or 1/20 the volume of the bacterial culture in the milk yogurt at room temperature. Caspian sea yogurt fermentation effective at lower temperatures (around 25 to 30 ° C) than regular yogurt [5]. Making yogurt in general, use mixed bacterial isolates starter culture of *Lactobacillus acidophilus* and *Streptococcus thermophilus*. However, for the making Caspian Sea yogurt used different isolates namely *Lactobacillus cremoris* and *Acetobacter orientalis*. Yogurt produced was relatively different, because the yogurt in general has a high acidity and relatively low viscosity.

Milk in yogurt production is to provide the lactose which will be hydrolyzed into glucose and galactose which are subsequently through glycolysis and fermentation will be converted into lactic acid and acetaldehyde. Besides that the casein (milk protein) will coagulate and make yogurt becomes thick. Whey is a by-product of the cheese-making industry which is a greenish-yellow clear liquid obtained from the filtration and pressing the curd during the cheese making process. The abundant of availability of whey, which is not optimally utilized and and to prevent environmental pollution are the reason of whey used as a medium to produce lactic acid [6]. Whey is a waste of cheese which still contains 5% lactose, 1% protein, 0.4% fat and some minerals [7] which are needed by bacteria to produce lactic acid [8]. In order to utilize the by-product of the cheese-making, accordingly, the potential utilization of waste whey for the production of Caspian sea yogurt was examined in this study.

MATERIAL AND METHODS

Raw materials and bacterial culture

Fresh milk and whey obtained from Jabung village, guided village of the Faculty of Agricultural Technology, Brawijaya University, Malang, Indonesia. Pure bacterial culture (*Lactobacillus cremoris* and *Acetobacter orientalis*) were obtained from the Laboratory of Food Microbiology Department of Agricultural Technology, Faculty of Agricultural Technology Brawijaya University, Malang, Indonesia.

Experimental design

Research was conducted using Randomized Block Design (RBD) with two factors, namely: the proportion of milk: whey (P) (100: 0 as control; 75:25; 50; 50; 25:75), and the concentration of starter (S) (1.5 %, 2% and 2.5% (v / v)) with three replications.

Research Methods

The experiment was conducted in two stages of the making a starter culture, and the stage the making yogurt.

Starter Culture Making Process

Slant culture of *Lactobacillus cremoris* and *Acetobacter orientalis* from the Laboratory of Food Microbiology Department of Agricultural Technology, Faculty of Agricultural Technology Brawijaya University, grown in 5 mL sterile MRS broth medium, and incubated at temperature of 30 ^{o}C, 24 hours.

Ready to use liquid starter culture

Both stock culture agar slant incised, and the culture was transferred into 5 ml of sterile MRS medium broth, incubated at 30°C, 24 hours. The culture was transferred into 45 ml of fresh milk and then incubated at room temperature for 18 hours.

Making caspian sea yogurt with the addition of whey

Fresh milk and whey (as specified proportion) pasteurized at 85-87°C for 15 minutes, and then cooled to 71°C. Subsequently inoculated with yogurt starter (corresponding concentration set, w / v), then fermented at room temperature (26 ± 1 ° C) for 24 hours.

Parameters and Data Analysis

Physical and chemicals property measured was total dissolved solids and total sugar [9]. Total LAB measuerd with the methode of Lay [10]. Organoleptic observations include aroma, texture, colour and taste was conducted. Data were analyzed by analysis of variants (ANOVA) with a 5% level of confidence. Then followed by Least Significant Difference test and Duncan Multiple Range Test at 5% confidence interval. For organoleptic test, the data were performed by Hedonic Scale Scoring.

RESULT AND DISCUSSION

Analysis of the content of Whey and Caspian Sea Yogurt produced

Whey used in this study contains a protein of 1.124%, 0.618 for fat, and lactose at 3.958% (Table 1)

Table 1: Protein, Fat and Lactose content of Whey

Parameter	Content (%)
Protein	0,924
Fat	0,618
Lactose	3,958

Meanwhile, whey nutrient content is 0.9% protein, 0.3% fat, 4.5% lactose, 0.2% lactic acid, 0.6-0.8% minerals and water [11]. Differences in levels of protein, fat, and lactose levels may be caused by the differences in the processes and methods used in the manufacture of cheese. The better the milk processed into cheese, the lower the nutritional value of whey produced.

Protein levels of the Caspian Sea yogurt in various proportions of milk and whey, as well as the concentration of starter

The protein content of yogurt is largely determined by the quality of the base ingredients of the milk used. The higher milk protein content the better the quality of the resulting yogurt. The protein content of the Caspian Sea yogurt with whey utilization varies from 0.93% to 3.60% (Table 2).

Table 2: Protein levels of the Caspian Sea yogurt in various proportions of milk and whey, as well as the concentration of starter

Proportion of milk : *whey* (%)	Starter Culture Concentration		
	1,5%	2%	2,5%
100:0	$3,5267^{a}$	$3,5967^{b}$	$3,5833^{a}$
75:25	$2,1267^{d}$	$2,2067^{cd}$	$2,3600^{c}$
50:50	$1,2167^{e}$	$1,1267^{ef}$	$1,1500^{ef}$
25:75	$0,7533^{g}$	$0,7133^{g}$	$0,9300^{fg}$

Protein content tended to decrease with the increase of whey added, where the higher the concentration of whey used, then the protein content Caspian Sea yogurt is also declining. The decreasing levels of this protein due to the protein content of whey lower than the protein content of milk. In addition,

milk is a suitable medium for the growth of some microorganisms, because there are a variety of substrates contained in it, such as lactose, fat, protein, vitamins and minerals [12]. The greater the proportion of cow's milk added, then microorganisms will grow optimally, because most of the microbes that exist in the Caspian Sea yogurt are lactic acid bacteria which will utilize lactose in the milk for growth, resulting in the conversion of the substrate that is in foodstuffs and causes the protein content to high.

The pH value of the Caspian Sea Yogurt in various proportions of milk and whey, as well as the concentration of starter

With the increasing amount of whey used, the pH of the Caspian Sea Yogurt tends to increase (Table 3). The tendency of increasing the pH is allegedly associated with the metabolic activity of the starter diminishing. Suspected proportion addition of cow's milk will increase microbial activity in yogurt. Increased microbial activity will cause the amount of acid produced also increased, so that the pH products will also be lower.

Table 3: The pH value of the Caspian Sea Yogurt in various proportions of milk and whey, as well as the concentration of starter

Proportion of milk : *whey* (%)	Starter Culture Concentration		
	1,5%	2%	2,5%
100:0	4,276^{g}	4,253^{g}	4,156^{h}
75:25	4,456^{e}	4,356^{f}	4,250^{g}
50:50	4,690^{ab}	4,616^{c}	4,526^{d}
25:75	4,733^{a}	4,670^{b}	4,573^{cd}

The decline of PH is due to the activity of lactic acid bacteria (LAB) to produce energy through a fermentation process by breaking the substrate into simpler components. In addition, lactic acid was form which then accumulates cause a decrease in the pH value. The lactic acid and acetaldehyde generated causes a decrease in the pH of the fermentation medium or increase the acidity and give rise to a distinctive aroma[13].

The more the concentration of the starter added, the more amount of LAB fermentation and will produce more lactic acid. The total amount of the acid associated with pH values, where the higher the lactic acid, the pH value will decrease. Lactose (milk sugar) will be hydrolyzed by lactic acid bacteria with the results in the form of pyruvate and is converted into lactic acid by the enzyme lactate dehydrogenase produced by the bacteria [14]. In addition, glucose hydrolysis by lactic acid bacteria will also be used by the lactic acid bacteria to form acid.

Fermentation by LAB is characterized by an increasing number of organic acids, wherein the amount and type of acid that is produced depends on the species, the composition of the fermentation medium and conditions change LAB [15] .As long fermentation, LAB will produce lactic acid. This is consistent with the statement which states that during fermentation, LAB will remodel the lactose into lactic acid which will give a sour taste and increase the stability of fermented dairy products, such as cheese, yogurt and kefir [16].

Viscosity Caspian Sea Yogurt in various proportions of milk and whey, as well as the concentration of starter

The viscosity of Caspian Sea yogurt produced ranges from 0.1 to 0.4 d.Pas (Table 4). The higher the concentration of whey added the viscosity of Caspian Sea yogurt decreases. Caspian Sea yogurt lowest viscosity was obtained from the treatment of milk: whey proportion of 25:75, while the highest viscosity was obtained from the control with a concentration of 2.5% starter. This is due to the whey added reducing the total solid in the milk. The lower total dissolved solid in the yogurt will produce yogurt with low viscosity and affect the viscosity value.

The lower the dissolved solids content in the yogurt will produce yogurt with lower viscosity [17]. A decrease in the total amount of milk solids will reduce the viscosity of yoghurt and give significant effect on the formation of gel in yogurt fermentation process [18]. Formation of lactic acid by lactic acid bacteria that cause an increase in total acid casein coagulation gelling experience [19]. The formation of the gel causes the texture

becomes semi in that its viscosity rises. Each type of whey proteins have functional properties that are associated with the texture, viscosity and gel forming ability of a product [20]. The formation of gel during the making of dairy products is basically occurs due to unstable complex of casein which is easily to coagulate to form gel in the present of acid substances [19]. The present of acid substances will affect the pH. At pH of 4.6 casein is in the isoelectric state where the activity of water particles decreased that will caused the coagulation of the protein and increase the viscosity.

Table 4: Viscosity Caspian Sea Yogurt in various proportions of milk and whey, as well as the concentration of starter

Proportion of milk : *whey* (%)	Starter Culture Concentration		
	1,5%	2%	2,5%
100:0	0,40^{a}	0,40^{a}	0,43^{a}
75:25	0,30^{b}	0,26^{b}	0,20^{c}
50:50	0,10^{d}	0,20^{c}	0,20^{c}
25:75	0,10^{d}	0,10^{d}	0,10^{d}

Total Solids Caspian Sea Yogurt in various proportions of milk and whey, as well as the concentration of starter

The total solids of Caspian Sea yoghurt produced ranged from 3.85 to 4.33% starter (Table 5). The total solids decreased with the increase of whey concentration. The lowest total solids concentration showed by the proportion of milk: whey at 25: 75 with starter concentration of 1.5%, while the highest total solids concentration was showed by the control with the starter concentration of 2.5%. This is due to the addition of whey caused the decrease of solids in yogurt.

Table 5: Total Solids Caspian Sea Yogurt in various proportions of milk and whey, as well as the concentration of starter

Proportion of milk : *whey* (%)	Starter Culture Concentration		
	1,5%	2%	2,5%
100:0	4,89^{a}	4,88^{a}	4,89^{a}
75:25	4,55^{b}	4,55^{b}	4,51^{b}
50:50	4,37^{c}	4,34^{cd}	4,28^{d}
25:75	3,98^{e}	3,87^{f}	3,85^{f}

Different from the effect of theproportion of milk:whey the addition of starter concentrations were not significantly affect the total solid. This is because the addition of microorganisms didn't affect the efectiveness of hydrolysis which cause no differences of total solids resulted.

Total LAB on the Caspian Sea Yogurt in various proportions of milk and whey, as well as the concentration of starter

The higher proportion of cow's milk added, the total LAB also increased (Table 6). Milk is a suitable medium for the growth of some microorganisms due to the avilability of substrates contained therein, such as lactose, fat, and some protein, it is also available in addition to growth stimulants, namely vitamins and minerals [12]. In addition to nutritional factors, the increase in the number of LAB is also attributed to the concentration of starter added to support growth. During the growth LAB will break down proteins into amino acids and peptides which are used as a source of nitrogen for the growth and multiplication of cells [21].

Table 6: Total LAB on the Caspian Sea Yogurt in various proportions of milk and whey, as well as the concentration of starter

Proportion of susu : *whey* (%)	Starter Culture Concentration		
	1,5%	**2%**	**2,5%**
100:0	1,79 x10^{9b}	2,23 x10^{9a}	2,27 x10^{9a}
75:25	1,06 x10^{9d}	1,41 x10^{9c}	1,74 x10^{9b}
50:50	6,23 x10^{8g}	7,73 x10^{8f}	9,00 x10^{8e}
25:75	4,51 x10^{8i}	5,50 x10^{8h}	6,28 x10^{8g}

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Organoleptic Test to the Caspian Sea Yogurt Produced

For the organoleptic test, only treatments comply with the standard of edible yogurt were tested. Therefore, there are only 3 treatments were tested, namely P1 is milk: whey 100: 0 as control, P2 (milk: whey 75: 25), and P3 (milk: whey 50:50).

Colour

The results of organoleptic test showed an increasing trend towards colour preference level with the increasing proportion of cow's milk and starter concentration (Figure 1). This is because the more addition of whey and yoghurt starter and the stronger colour of Caspian Sea yogurt resulted. The yogurts were bright, creamy and have a sour taste.

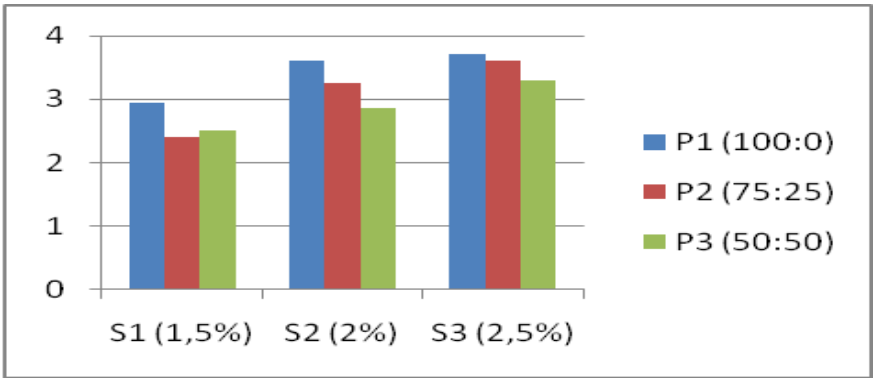

Figure 1: Respondents' preference level based on the colour of Caspian Sea yogurt with the addition of varying amount of whey and starter concentrations

The increased brightness of yogurt with increased concentration of solids starter supposedly because the existing food component will be hydrolized by the microbes and used for metabolism. Such as glucose was hydrolyzed to be used for the growth and the protein will also be broken down into amino acids for growth, so that the concentrations of the substrate is reduced and caused the increase of brightness of yogurt. The acid and alcohol produced by the hydrolisis of glucose will cause the sour and distinctive taste of yogurt.

Flavour

The results of organoleptic test showed a tendency towards increase in the level of preference for the taste by increasing the proportion of cow's milk and starter concentration (Figure 2). The more the concentration of the starter is added, the microbes will be more active to produce acid.

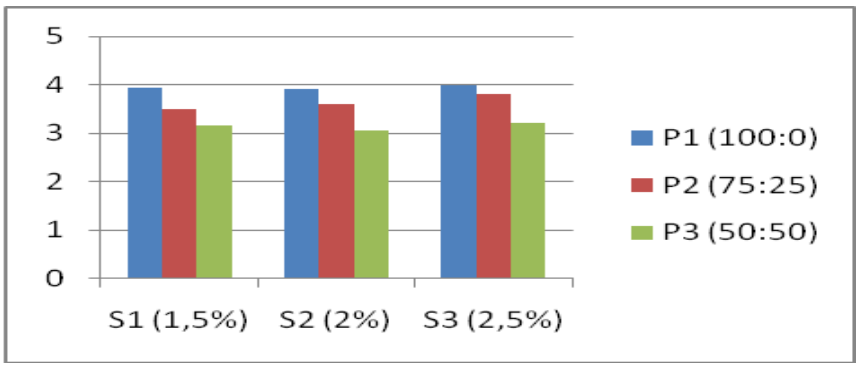

Figure 2:Respondents' preference level based on the flavour of Caspian Sea yogurt with the addition of varying amount of whey and starter concentrations

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In general, all of the samples have a sour taste caused by a decrease in pH during fermentation. Yogurt has a distinctive flavour, which is a blend of sour taste and alcohol [22]. The addition of 25% whey changes the flavour to a little bland and a bit of a bitter taste but it still acceptable.

Aroma

The results of organoleptic test showed a trend towards increase in the level of preference for the scent with the increasing proportion of cow's milk and starter concentration (Figure 3). The addition of 25% whey did not change the scent of sea Caspian Sea yogurt and still approaching yogurt scent made from cow's milk alone without the addition of whey. With the increasing concentration of the added starter until 2 %, the aroma of components that form the more that alcohol or diacetyl which can affect the flavor of Caspian Sea yogurt resulted was still preffered.

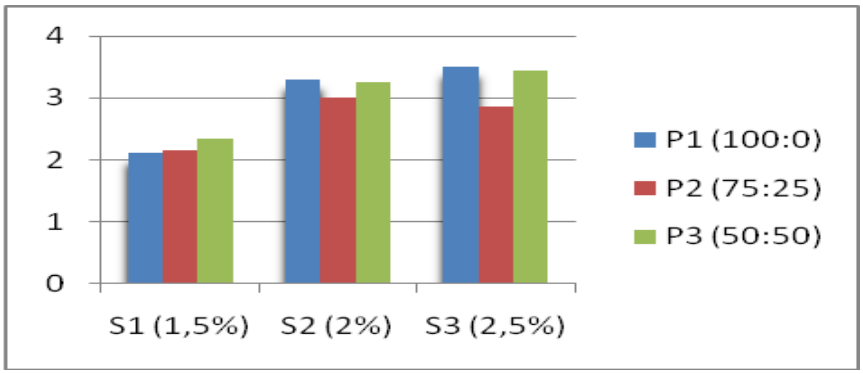

Figure 3: Respondents' preference level based on the aroma of Caspian Sea yogurt with the addition of varying amount of whey and starter concentrations

Best treatment

The best treatment is determined based on the testing of a modified calculation methods De Garmo [23]. The selection of the best treatment based on the calculation and weighting of the physical parameters, chemical and microbiological determined by the panellists.

The analysis shows that the highest value of the parameters measured shown by the control that the proportion of cow's milk: whey 100: 0 with a concentration of 2.5% starter. Controls containing 3.38% protein, total BAL 6.8 x 109 cfu / ml, pH 4.17, 4.89 DPAs total solids, and viscosity of 0.4. While the best treatment based on chemical and organoleptic analysis showed that the ratio of milk:whey of 75:25 and 2.0% starter concentration is the closest combination to control and can be accepted by the respondent. This treatment produced Caspian Sea yogurt with protein content of 2.21%, total BAL x109c 1.41 cfu / ml, pH 4.36, 4.55 DPAs total solids, and viscosity of 0.26.

CONCLUSION

The results showed that the proportion of milk: whey and concentration starter significant effect on total acid, pH, total BAL and total sugars. Interaction of the two treatments was not significant effect on protein content, total solids and viscosity. Caspian Sea yoghurt best treatment on physical parameters, chemistry and microbiology, with the most likely value of the control value is treated milk ratio: 75:25 whey starter and concentration of 2.0%. This treatment resulted in caspian sea yogurt with protein content of 2.21%, total BAL x109c 1.41 cfu / ml, pH 4.36, 4.55 DPAs total solids, and viscosity 0:26 most can be accepted by the respondent.

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