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Visual Performance of Fermented Whey with the Addition of Pectin and Chitosan During 24 Hours Storage at Refrigerator Temperature

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Abstract

Fermentation of whey often faces problems with the product clarity due to the milk solids residue from previous cheese making process. The aim of this research was to determine the clarity and the performance of fermented whey precipitation process in the presence of pectin and chitosan as coagulant. Whey was initially pasteurized and added with pectin and chitosan prior to fermentation with mixed starter culture. Fermented whey was stored in refrigerator for 24 hours. Data were obtained using visual analysis by 15 semi-trained panelists. Qualitative scorings were given by (+) or (-) markings from the specified criteria. The data were compiled in a table, showcasing the observed characteristics at the initial and final states of fermentation. The results showed that the highest clarity (+++) was achieved by the fermented whey with the addition of pectin, as well as most stable sediment performance (+++) was also produced by pectin treatment. However, while fermented whey with the addition of pectin exhibited clarity, the level of clarity achieved by fermented whey with the addition of chitosan was superior.

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Introduction

Developments in the field of food science continue to progress rapidly, especially in the efforts to increase added value and quality of food products such as fermented whey. In cheese manufacture, whey is also produced as by-product (Al-Baarri et al., 2019, Scalone et al., 2019). As a by-product, the topic that is attracting researcher is the modification of the whey through a fermentation process. However, one problem is often found during the process, i.e., poor visual performance. This issue is due to the incomplete process of cheese making resulting in a small amount of casein and soluble protein remaining in the whey fraction (Gazi and Huppertz, 2015). The addition of pectin and chitosan was the exit strategy to solve this issue due to its capability as coagulant (Colombo et al., 2018, Yue et al., 2023).

Whey, as a by-product in the milk processing industry, has great potential to be further utilized to produce high-value products (Hernandez et al., 2022). The milk processing industry produces whey as a by-product in abundance. Whey is a by-product that is rich in nutrients, but if it is not processed before disposal, it can cause environmental problems (Lavelli and Beccalli, 2022). Therefore, research continues to be carried out to explore the potential for utilizing whey into value-added products. One interesting approach is through a fermentation process with the addition of pectin and chitosan, it is hoped that the physical performance can be enhanced resulting in the increase in its physical performance, such as the clarity of the product. Clarity enhances the visual appeal of the product. A clear and visually appealing liquid is often more attractive to consumers, creating a positive first impression. Clear liquids are often associated with purity and quality. Consumers may perceive a clear drink as being free from impurities, leading to a positive perception of the product's overall quality.

Fermentation is an effective biotechnology method in increasing the functionality of various food products. According to Zotta et al. (2020) the process of fermentation is a way to preserve food, increase nutritional value, and improve sensory properties. In this research, the addition of pectin and chitosan is an interesting aspect of attention since both have coagulant functionality. Furthermore, pectin and chitosan have unique characteristics that can affect the texture, stability, and nutritional value of fermented whey. Pectin, which is found in various types of fruit, can form gels and increase viscosity, meanwhile, chitosan which comes from chitin can provide stability and increase absorption capacity (Mohadi et al., 2022).

Based on our best knowledge, there was a limited number of documents regarding the utilization of pectin and chitosan to enhance the performance of fermented whey. This research was conducted to analyze the sensory properties through the visual performance of fermented whey with the addition of pectin and chitosan as coagulants. The benefits of this research are not only limited to dairy industry waste management but can also open up opportunities to produce new functional food products that meet consumer demands for high nutritional value and environmental sustainability. Whey is a byproduct of cheese-making, and utilizing whey helps reduce waste in the dairy industry. By finding valuable uses for whey, it minimizes the environmental impact of waste disposal. Through further understanding of the effect of the combination of pectin and chitosan on whey fermentation, it is hoped that this can make a positive contribution to the development of a sustainable, innovative, and environmentally friendly food industry. Thus, this research not only creates a potential solution to the dairy industry waste problem, but also opens up new avenues in the development of better and more sustainable food products.

Materials and Methods

This research was carried out in July – November 2023 at the Integrated Laboratory UPT, Diponegoro University, Semarang.

Materials

This research used whey that was produced from mozzarella cheese manufacture in Central Java and treated with pasteurization after received. The dry mixed culture of *Lactobacillus casei* and *Lactobacillus plantarum* were used to ferment the whey. High fructose syrup was also used to achieve the better taste in the final process, while pectin and chitosan were added. Measuring cups, glass beakers, induction cookers, pans, thermometers, magnetic stirrers, incubators, autoclaves, analytical scales, laminar air flow, micropipettes, micropipette tips, centrifuge tubes, bunsens, and showcases were also used to achieve the goal of research.

Methods

This research used twenty-five liters of whey produced from cheese manufacture in Central Java, Indonesia. After it was received, the whey was then pasteurized with the temperature of 85˚C for 10 second based on the treatment in factory. The research that was carried out using *L*. *casei* and *L*. *plantarum* bacteria as starter cultures and the pectin and chitosan were also added in the beginning of fermentation process. The parameters were whey clarity and sediment performance during 24 hours of storage at refrigerator.

Preparation of mixed culture starter. The mixed starter was used by mixing the commercial fermentation drink of Yakult and commercial dry mixed culture received from factory. Manufacture of starter from dry mixed culture was carried out by adding 3 grams of culture powder to 1 liter of liquid UHT milk, incubating for 6 hours at 37°C. After its production, Yakult and starter from dry mixed culture was then added to 885 ml of liquid UHT milk to produce 1 liter of working starter. This process was repeated until a working starter volume was obtained, with incubation for 6 hours at 37°C in aseptic control heated-laminar air flow. This method was done according to previous method by other researcher with slight modification (Pescuma et al., 2008).

Preparation of *L*. *casei* and *L. plantarum* starter cultures. Starter culture was made by transferring the *L. casei* FNCC 0090 culture into a centrifuge tube containing sterilized de Man Rogosa Sharpe Broth (MRSB) media. The tube was incubated at 37°C for 24 hours until an active culture was formed. A similar procedure was carried out for the bacteria *L. casei* FNCC 0090 and *L. plantarum* FNCC 0027. As much as 3% of the active culture was inoculated into a skim milk solution in an Erlenmeyer, which was then incubated at 37°C for 24 hours. This method was done according to previous study (Al-Baarri et al., 2018).

The preparation of fermented whey with a mixed starter culture. The process of making fermented whey was started with pasteurizing the whey at 72°C for 15 seconds and then adding pectin and chitosan. Subsequently, the mixture was allowed to cool until the temperature reaches 42°C. The whey was then supplemented with 5% (v/v) HFS and 5% (v/v) working starter, followed by homogenization for 15 minutes. Fermentation taken place at 37°C for 8 hours. After filling 35 ml into centrifugation tubes, the whey was stored in a showcase at a temperature of 4 – 6°C. This method was done as previous researcher (Solieri et al., 2022).

The preparation of fermented whey with *L. casei* and *L. plantarum* starter culture. The whey sample was pasteurized at 72°C for 15 seconds. Pectin and chitosan were added while homogenizing, and then the whey was allowed to cool until the temperature reached 42°C. After the whey temperature reached 42°C, high fructose syrup 4% (v/v), L. *casei* 2.5% (v/v), and L. *plantarum* 2.5% (v/v) (ratio 1:1) were added. The sample was put into a glass bottle, homogenized, and then incubated in an incubator at 42°C for 8 hours. This method was done as previous researcher (Solieri et al., 2022). The whey was stored in a showcase at a temperature of 4 – 6°C after filling 35 ml into centrifugation tubes.

Research variable. The variables observed in this research were whey clarity and fermented whey sediment performance that was done by 15 semi-trained panelists. Semi-trained panelists for whey evaluation underwent internal training to enhance their sensory evaluation skills. Panelists had a baseline level of sensory acuity, including the ability to perceive and distinguish various tastes, aromas, textures, and appearances in food as well as possess genuine interest in whey and a willingness to participate in sensory evaluations.

Fermented whey clarity testing procedure. The test was visually conducted by panelists who observed the sediment preparations in each fermented whey treatment. The test results were expected to be clear and free of impurities. Testing on fermented whey was carried out for 24 hours at cold temperatures to ensure that the fermented whey preparation did not contain any floating particles.

Testing procedures. A visual test for sediment in fermented whey was carried out to detect sediment that might have formed during or after the fermentation process. After the whey underwent the fermentation process and was stored in cold temperatures for 24 hours, it was directly observed that sediment could appear as solid particles settling at the bottom of the container or floating on the surface. Data were obtained through visual testing of fermented whey after inoculation and 24 hours after storage at temperatures of 4-6°C. Observations involved 15 semi-trained panelists with assessment criteria in the form of plus signs (+) and minus signs (-) in the sediment and suspension sections. The plus signs for sediment consisted of (+), (++), and (+++), each indicating settling a little, settling moderately, and settling a lot, respectively. For suspensions, the signs were (+), (++), and (+++), indicating slightly turbid, moderately turbid, and very turbid, respectively. A detailed explanation regarding these criteria can be found in Table 1.

Table 1. Criteria for Precipitation and Suspension

Symbol	Precipitate	Suspension
–	The precipitate is mixed with the suspension	The suspension appears very cloudy and impenetrable to light
+	The precipitates are discrete and very unstable	The suspension looks cloudy, and a lot of light is blocked. In the suspension, it is also very visible that there are floating casein granules
++	The precipitates are separate and quite stable.	The suspension seems less transparent and there are lots of fine grains floating around
+++	Separate and stable deposits	The suspension appears translucent and fluorescent. The suspension does not show any floating particles

Data analysis

Data were obtained by collecting criteria, counting the number of + or – marks. The number of + values was averaged and rounded to the nearest value. The data was presented in tabular form, comparing the conditions at the start of fermentation and the end of fermentation, specifically during 24 hours of storage. The data obtained was explained descriptively and compared with relevant comparative literature.

Results and Discussion

Based on the results of observations for 24 hours, data on the performance of fermented whey sediment with the addition of pectin and chitosan are presented in Table 2.

The precipitate performance of fermented whey is a crucial indicator for assessing the stability of the product (Zhang et al., 2022), particularly when exposed to changes in physical conditions, such as shaking. In this study, the results indicated that fermented whey with the addition of chitosan exhibited a tendency to be more susceptible to separation when shaken. In contrast, fermented whey with the addition of pectin demonstrated better stability, and the starter bacteria did not have a significant effect.

Table 2. Performance of Fermented Whey Precipitation with the Addition of Pectin and Chitosan

Coagulant	Starter	Beginning	End
Pectin	Mixed culture	–	+++
	L. casei and *L. plantarum*	–	+++
Chitosan	Mixed culture	–	++
	L. casei and *L. plantarum*	–	++

Chitosan, with its cleaning properties and ability to form gel, may result in deposits that are more susceptible to changes in physical conditions, such as shaking (Zhang and Rhim, 2019). The possibility of separation could be caused by the interaction of chitosan with particles in the whey which can settle when movement occurs. As a result, the sediment becomes more susceptible to disruption and separation when the whey is agitated.

Pectin can form a gel and increase viscosity, providing additional stability to fermented whey. Pectin can help prevent precipitation and dissolved particles in the whey from remaining homogeneously dispersed, so that when shaken, the whey tends to be more stable and does not experience significant separation (Bindereif, *et al.*, 2022).

Interestingly, the influence of starter bacteria in this context is not very significant on the performance of fermented whey sediment. This may be due to the dominant properties of pectin and chitosan in influencing the stability and clarity of whey, so that the influence of starter bacteria was not significantly visible under the test conditions carried out.

The stability of the precipitate in fermented whey can influence the organoleptic properties and quality of the final product. Whey, which tends to be stable when shaken, has better potential to maintain its homogeneity and quality in processing and storage (Patel, 2015).

Table 3. Clarity of Fermented Whey with the Addition of Pectin and Chitosan

Coagulant	Starter	Beginning	End
Pectin	Mixed culture	–	++
	L. casei and *L. plantarum*	–	+++
Chitosan	Mixed culture	–	++
	L. casei and *L. plantarum*	–	+++

The clarity of fermented whey is an important visual indicator that reflects the quality and fermentation process that has been carried out. In this research, the observation results showed that fermented whey with a combination of mixed culture starter and the addition of chitosan showed optimal clarity. The clarifying properties of chitosan and pectin which can precipitate particles in solution can be a key factor in increasing whey clarity since both agent helps to agglomerate and settle suspended particles, contributing to the clarification of the liquid.

Fermented whey with a combination of *L. casei* and *L. plantarum* starters and the addition of chitosan also showed the clearest results. The combination of these two starter bacteria may have synergy that supports the fermentation and whey clarification processes. The addition of chitosan in this context can make a positive contribution to the separation of liquid and solid phases, producing whey with a better level of clarity.

Whey fermented with a combination of mixed culture starter and added pectin showed a satisfactory level of clarity. Pectin, as a natural adhesive agent, can help settle particles into solution, leading to increased clarity (Owusu et al., 2021) and stable emulsion (Li et al., 2022) in drinking products. The clear and stable performance may increase the consumer preference (Han and Du, 2022). Although it may not be as optimal as whey with the addition of chitosan, the still good clarity shows the potential of pectin as a cleaning agent in fermented whey.

Fermented whey with a combination of *L. casei* and *L. plantarum* starters and the addition of pectin showed acceptable clarity results. Even though it may not be as clear as whey with the addition of chitosan, the clarity obtained still meets quality standards. The use of a combination of certain starter bacteria and pectin can have a positive effect on whey clarification, although with a slightly lower level of clarity compared to the use of chitosan.

Chitosan and pectin are two food additives that are often used in the food industry to improve the quality and physical properties of a product. Chitosan is a natural polymer derived from chitin, a compound found in the exoskeleton of crustaceans such as shrimp and crabs (Artiningsih, 2017). The unique properties of chitosan include the ability to form a gel, increase stability, and have cleansing properties, making it an attractive ingredient in improving the clarity of fermented whey.

On the other hand, pectin is a complex anionic polysaccharide found in primary cell walls and intercellular layers of plants (Cheng et al., 2023). Pectin can form a gel with water, giving a distinctive texture to various food products. In addition, pectin can also be used as a cleaning agent because of its ability to help settle particles and increase the clarity of a solution.

The use of chitosan and pectin in whey fermentation has different impacts on the level of clarity. Chitosan, with its cleansing properties, tends to precipitate particles and solids in the whey, thereby significantly improving clarity. Chitosan's ability to form gel can also provide additional stability to the whey solution, causing a clear and transparent result (Zhao and Xiao, 2016).

Meanwhile, pectin, with its ability to form gel and increase viscosity, can also make a positive contribution to the clarity of fermented whey. However, pectin may not have as great an effect as chitosan in terms of clarification, but it can still improve the physical properties and stability of whey.

The effect of chitosan and pectin on whey clarity depends not only on the type of additive, but also on the combination with specific starter bacteria. The complex interaction between starter bacteria, chitosan, and pectin can produce synergistic or antagonistic effects that influence the clarity of fermented whey. The level of turbidity can also be influenced by the presence of sediment that is filtered out during the whey filtration process which causes the color of the liquid to become cloudy, and apart from that by the presence of suspended solids that do not dissolve during fermentation due to polymerization occurring during storage resulting in clumping (Jaya et al., 2017).

Conclusion

The addition of chitosan to fermented whey tended to have a more positive impact on clarity compared to pectin. This result may contribute to the knowledge suggesting the use of chitosan to achieve better clarity than pectin, especially in whey fermentation.

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