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RESEARCH ARTICLE

UTILIZATION OF CHICKEN BONE WASTE BE CHICKEN BROTH AGAR (CBA) AS A SUBSTITUTE FOR NUTRIENT AGAR (NA)

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ABSTRACT

This study aims to create an alternative media in the form of Chicken Broth Agar (CBA) by utilizing chicken bones. This study succeeded in proving that CBA media could grow more bacteria than NA. This quantitative research is carried out by conducting practical and direct analysis related to the media-making process, and colony analysis in each media. The originality of the research lies in making CBA media in the utilization of chicken bone waste as an effort to increase the variety of bacterial culture media. The limitation of this study lies in the analysis sample which is not representative of all types of microbes. The sample should be adjusted to the composition of the chicken bones.

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INTRODUCTION

Chicken meat is one of the most widely used alternatives to meet the protein needs of the human body (Geiger, 1979). Apart from having high nutritional value, chicken meat is the easiest food to obtain and the price is relatively cheaper. Along with the amount of chicken consumed, the more chicken bones waste. Chicken bones are solid waste that has not been used optimally. When viewed from its chemical content, it consists of organic and inorganic compounds (Geiger, 1979; Rajesh *et al*., 2012). Utilization of chicken bones as raw material for making *chicken broth is* expected to provide a solution to reducing chicken bone waste. The chemical and physical composition of chicken bones is very suitable when used as a medium for bacterial growth. Mineral content can replace NA as a medium (Geiger, 1979; Nungester, 1929). Referring to table 1, it is possible to analyze and try its use by comparing NA which has a composition of meat, peptone and agar (North, 1902) This study utilizes chicken broth (CB) as raw material for nutrient agar (NA). Efforts to modify bacterial culture media continue to develop (Bridson & Brecker, 1970; Geiger, 1979; Herrold, 1931). This study has similarities in media engineering from a mixture of CB with beef (Ibrahim *et al*. 2009), fish extract (Jassim *et al*., 1988), eggs (Herrold, 1931).

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The use of chicken bone waste as raw material for making NA was the initial inspiration for this study. The use of CB as a substitute for NA in this study is called Chicken Broth Agar (CBA). In addition, NA modification is also carried out in an effort to obtain the ideal composition of NA so that the flexibility of the composition follows the characteristics of the bacteria (Vartoukian *et al*., 2010). This research offers the latest media as an alternative to NA by utilizing chicken bone waste to make CBA. This utilization is expected to reduce this waste and the use of chicken bone waste is inspired by the composition that is needed by microbes so that it can be used for making bacterial media (Herrold, 1931). The composition of the media must continue to change according to the characteristics of the desired bacteria (Bridson & Brecker, 1970). This triggers innovation to get the ideal composition of a media.

LITERATURE REVIEW

Bone is divided into hard / true bone (osteon) and cartilage (cartilage). True bone is bone that has undergone ossification (bone maturation) so that the structure is harder. The matrix contains very little collagen, while other inorganic materials such as calcium, phosphorus, bicarbonate, sirat, Mg, Na, and K. Cartilage is bone with a thick fibrous and elastic matrix. Cartilage has a high collagen content so that it is strong and flexible. Cartilage does not have nerves and blood vessels. Cartilage is found in the end of the hard bones, nose, ears and vertebrates (vertebrae).

Cartilage (cartilage) can be brittle when reacted with acids such as vinegar because acidic solutions have a tendency to dissolve elements such as calcium (Ca). Elemental calcium (Ca) is contained in bone (Spector & Glimcher, 1972). The process of dissolving with acid is the principle in the extraction of chicken bones into CB. Changes in the quality of CB are largely determined by PH (Pippen *et al*., 1965), so that from these properties the effect of PH in bacterial growth will be tested.

Microbes

Microbes require nutrients as a source of energy and cell growth. These basic elements include carbon, nitrogen, hydrogen, oxygen, sulfur, phosphorus, iron and a small number of other metals (Bushell, 1987). The main role of nutrients is as a source of energy, building materials for cells, and as electron acceptors in bioenergetic reactions. Therefore, the necessary food ingredients consist of water, energy sources, carbon sources, electron acceptor sources, mineral sources, growth factors, and nitrogen. In addition, in general, the nutrients in the hatchery media must contain all the elements that are important for biological synthesis (Bridson & Brecker, 1970). Growth of microorganisms requires water as a mineral solvent to form cell material and obtain energy (Bushell, 1987). The characteristics of different microorganisms require an engineering medium based on the composition of the water solvent. Basically, the solution must meet the requirements and provide all the elements in the formation of cellular components such as: C, H, N, P, which are constituents of membranes, proteins, nucleic acids and other cellular structures called macronutrients. Organic molecules that are unable to be synthesized by microbes such as vitamins and amino acids.

Media

Microorganism growth media is a material that consists of a mixture of nutrients (nutrients) that are needed by microorganisms for growth (Bridson & Brecker, 1970; Bushell, 1987; Newman, 1994). Microorganisms take advantage of the media's nutrients in the form of small molecules. Growth media can be isolated microorganisms into pure culture and also manipulate the components of the growth media (Bridson & Brecker, 1970). The microorganism medium must be in accordance with the requirements of the microorganisms concerned. Growing microorganisms must understand their basic needs and then formulate a medium or material that will be used (Bushell, 1987). Referring to previous literature (Bridson & Brecker, 1970; Bushell, 1987; Newman, 1994) the materials used for microbial media are water, agar, gelatin, and silicagel. The media must be given nutrients in the form of: C, H, O, N, P, micro elements such as Fe, Mg, vitamins and other elements. Media can be added with additional ingredients such as: peptone, meat extract, yeast extract and carbohydrates. The reference media used in this study is NA because it is a general medium (a medium that can be grown by various types of microorganisms). Agar nutrients are a common medium for testing water and other products. NA is also used for non-selective growth of microorganisms. This media is a simple medium made from extracts of beef, peptone, and agar (Bridson & Brecker, 1970). In making NA medium, peptone is added so that microbes grow quickly, because it contains a lot of N2 (Holmes *et al*., 1986), agar and meat extracts.

MATERIALS AND METHODS

The research method includes: making media, testing all media and comparing the results of bacterial growth in each medium. The stages of the research process can be seen in Table 2

RESULTS AND DISCUSSION

In this research, there are two methods in making CBA, namely without acid immersion and acid immersion. The difference in the treatment of the two methods is in the preparation stage of the material before extraction. If without acid soaking, the chicken bones to be extracted are cleaned and then heated. Meanwhile, if the acid reduction is done, before heating the chicken bones, immersion is carried out with vinegar as much as 1% for 12 hours. The addition of this acid aims to dissolve the mineral elements from chicken bones, especially calcium (Ca). After cooling CBA with acid immersion yields more yield (48.38%) than without acid immersion (15.14%). In addition, the difference from the CBA produced from the two methods is the color produced, CBA with acid immersion has a sharper color. This is an indication that the mineral acid immersion in chicken bones is more extracted than without acid suppression. The comparison can be seen in Table 3

Table 1.Chemical and physical composition of chicken bones

Component / Content	(%)
Gelatin	33.3
Calcium carbonat	3.85
Magnesium phosphate	2.05
Sodium carbonate	3.45
Water	1.8 - 44.3
Fat	1.2 - 26.9
Calcium phosphate	57.35
Collagen	15.8 - 32.8
Gelatin	33.3

Testing media in this study, using samples of bottled drinking water (AMDK), *Escherichia coli* and *Salmonella typimurium bacteria.* The three samples were treated the same by testing the optimal growth pH of the NA and the actual pH of the media. In table 4, shows two treatments of pH adjustment (pH adjusted to pH NA and actual pH). Testing media with samples of bottled water, NA media gave the largest calculation results compared to CBA1 and CBA2. This shows that the composition of NA is able to grow bacteria better. Inbacteria samples *Escherichia coli, the* growth of these bacteria was more on CBA media than NA. The same results occurred inbacteria samples *Salmonella typimurium*, CBA media had more growth than NA. In table 4, we can actually prove that the role of CBA can be used as an alternative to NA. Efforts to get optimal results with NA media engineering are the objectives of this study. NA media engineering in this study were: ***NA1,2,3*** - meat extract on NA was replaced by CB (0.3g, 0.5g, 0.7g); ***NA4*** - makes NA from (Pepton + agar) without CB; ***NA5*** -makes NA from CB + peptone + agar. The next process is to compare the results with NA media. The results of comparison of several media are shown in table 5 and Table 6 In Table 5, the largest growth of *Escherichia coli* on NA5 media and the lowest yield was NA1. NA medium is smaller than NA5.

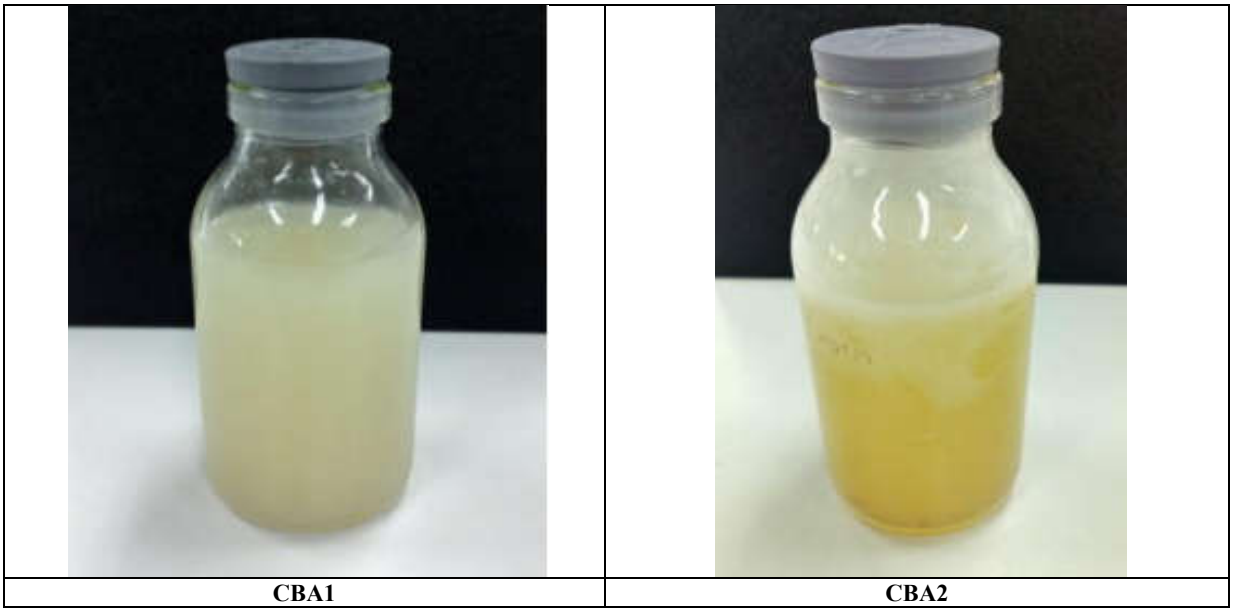
Table 2. Research Methodology

NO	STAGES	PROCEDURE
1	Making Media	
	CBA without acid (CBA1)	500g (extract CB) water + 1 lt, put it in a pressure cooker,cook for 2 hours, then strain CBA generated and refrigerate. Weigh CBA generated and stored in sterile packaging
	CBA + acid (CBA2)	500g CB + water 1 lt + CH_3COOH 1% soak for 12 hours then enter into the *pressure cooker*. cook during the 2 hours. strain CBA generated and refrigerate. Weigh CBA and store in sterile packaging
	NA	NA 0.2 g + *purified water* (PW)100 ml. Heat use *stirrer hot plate*up boiling. Adjust the pH of a media according to standard Sterilisasikan with autoclaving at 121°C for 15 minutes.
	NA (Modification) NA1, NA2, NA3, NA4, NA5	0.5 g peptone + 0.3 gCB+ 1.2 g agar, dissolve by *purified water* (PW) 100ml. Heat with *stirrer hot plate*up boiling. Adjust the pH according to standard. Sterilisasikan with using an autoclave at a temperature of 121°C for 15 minutes. Note: NA1 (CB=0.3 g) ; NA2 (CB=0.5 g); NA3 (CB=0.7 g) ; NA4 (peptone); NA5 (CB+Peptone)
2	Test Media (all media)	Enter sampel1 ml into a petri dish (duplo), pour the media already adjusted the ph in a petri dish. Incubation media + sample at 32.5°C, 48 hours. Count the number of microbes that grow.

Table 3. Comparison of CBA Yield CBA produced a physical apparition, seen in the picture 1

No	Treatment	Trial		Average
		1	2	
1	CBA + CH_3COOH	46.33%	50.42%	48.38%
2	CBA	15.30%	14.98%	15.14%

Physical sighting of the CBA that produced, seen in figure 1



CBA1 **CBA2**

Table 4. Media Test Results

No	Analysis	pH	Trial (cfu/ml)		Average (cfu/ml)
			1	2	
Growth bacteria test samples of drinking water in packaging (AMDK)					
1	CBA 1	6.37	6.70×10^3	7.00×10^3	6.85×10^3
2	CBA 2	6.37	7.40×10^3	8.30×10^3	7.85×10^3
3	NA	6.96	8.30×10^3	8.80×10^3	8.55×10^3
Growth the bacteria in the test sample bacteria *Escherichia coli*					
1	CBA 1	6.37	1.78×10^8	1.80×10^8	1.79×10^8
2	CBA 2	6.37	1.80×10^8	1.90×10^8	1.85×10^8
3	NA	6.96	1.48×10^8	1.56×10^8	1.52×10^8
Growth bacteria *Escherichia coli*(settings PH)					
1	CBA 1	6.97	1.92×10^8	1.72×10^8	1.82×10^8
2	CBA 2	6.95	1.76×10^8	1.86×10^8	1.81×10^8
3	NA	6.96	1.48×10^8	1.56×10^8	1.52×10^8
Growth bacteria *Salmonella typimurium*					
1	CBA 1	6.37	1.26×10^8	1.20×10^8	1.23×10^8
2	CBA 2	6.37	1.78×10^8	1.40×10^8	1.59×10^8
3	NA	6.96	1.38×10^8	1.44×10^8	1.41×10^8
Growth bacteria *Salmonella typimurium* (PH settings)					
1	CBA 1	6.97	1.38×10^8	1.32×10^8	1.35×10^8
2	CBA 2	6.95	1.44×10^8	1.46×10^8	1.45×10^8
3	NA	6.96	1.38×10^8	1.44×10^8	1.41×10^8

Table 5. Comparison of the growth of *Escherichia coli bacteria*

No	Analysis	Code	pH	Trial (cfu/ml)		Average (cfu/ml)
				1	2	
1	NA (CB 0.3 grams)	NA1	7.26	1.7×10^7	0.8×10^7	1.25×10^7
2	NA (CB 0.5 grams)	NA2	7.35	1.5×10^7	1.3×10^7	1.40×10^7
3	NA (CB 0.7 grams)	NA3	7.38	2.6×10^7	1.9×10^7	2.25×10^7
4	NA (Peptone)	NA4	6.33	1.1×10^7	2.8×10^7	1.95×10^7
5	NA (CB+ peptone)	NA5	6.37	1.7×10^8	1.8×10^8	1.79×10^8
6	NA	NA	6.96	1.4×10^8	1.5×10^8	1.45×10^8

Table 6. Comparison ofbacterial growth *Salmonella typimurium*

No	Analysis	Code	pH	Trial (cfu/ml)		Average (cfu/ml)
				1	2	
1	NA (CB 0.3 g)	NA1	7.26	1.9×10^7	0.5×10^7	1.20×10^7
2	NA (CB 0.5 g)	NA2	7.35	1.2×10^7	1.6×10^7	1.40×10^7
3	NA (CB 0.7 g)	NA3	7.38	0.8×10^7	1.3×10^7	1.05×10^7
4	NA (Peptone)	NA4	6.33	4.0×10^7	3.2×10^7	3.60×10^7
5	NA (CB+ peptone)	NA5	6.37	1.3×10^8	1.2×10^8	1.25×10^8
6	NA	NA	6.96	1.4×10^8	1.4×10^8	1.40×10^8

This condition opens a gap regarding the NA engineering process by replacing meat extract with CB. Table 6 shows the data that NA media has more growth inbacteria *Salmonella typimurium.* Referring to tables 5 and 6, it cannot be concluded that NA engineering is better than NA. This condition proves that NA5 can replace NA function as an alternative and opens the door for further research. This study offers new alternative media in the form of modified CBA and NA. This alternative media is an effort to reduce chicken bone waste. Concern for the environment is a strong impetus for this research. Based on the data, comparing CBA and NA modification, it turns out that CBA media is better than modified NA and NA media. So it is necessary to have a similar test related to NA modification in order to obtain an ideal and flexible NA composition in the use of its composition. The theoretical contribution of this research is the addition of variance of CBA, NA media (modification) as an alternative medium in an effort to flexibility the composition of NA. This research has a strong principle on the characteristics of bacteria that must be responded to by making special media, so that their growth can be optimal (Bushell, 1987). Contribution in practice is the process of making CBA and NA (modification) which need to improve the procedures and critical points of media creation.

Conclusion

CBA media can be used as an alternative to NA, the acid curing stage must be a critical point to obtain optimal results. NA5 is a modification of NA whose results are almost similar to NA media, so it is feasible to carry out further tests regarding the effectiveness and optimization of NA5.

Suggestion: It is necessary to carry out further tests in the form of CBA and NA stability tests (modification) related to the acid curing process.

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