FORMULATION OF SELECTIVE MEDIUM FOR THE GROWTH OF *STAPHYLOCOCCUS AUREUS* AND *ESCHERICHIA COLI* USING SPOILT VEGETABLES.

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ABSTRACT

A selective medium for the growth of Staphylococcus aureus and Escherichia coli was formulated from spoilt vegetables (cucumber, cabbage and carrot). Their growth on the formulated medium was compared with the growth on commercial medium. On the formulated medium, Staphylococcus aureus showed $2.3x10^4$cfu/ml while on Nutrient agar (NA) it showed $2.6x10^4$cfu/ml. E. coli showed $2.0x10^4$cfu/ml on the formulated medium and on NA it showed $2.5x10^4$cfu/ml. The moisture, ash,fat, crude fibre, protein and carbohydrate contents of cucumber were 89%, 14%, 9%, 5.0%, 5.42%, 5.5%, while for cabbage was 78%, 12%, 1%, 8%, 6%, 8% and carrot had 75%, 16%, 0.5%, 0.30%, 0.34%, 11% respectively. The moisture content of the vegetables was high: 89%, 78% and 75% respectively for cucumber, cabbage and carrot respectively. Cabbage and carrot had the lowest ash content of 1.0% and 0.5% respectively, while carrot had the lowest fat, crude fibre and protein contents of 0.5%, 0.30% and 0.34% respectively. The study revealed that spoilt vegetables (cucumber, cabbage and carrot) contain nutrients that were able to provide the nutritional requirement necessary for the growth of Staphylococcus aureus and Escherichia coli. Thus, spoilt vegetables can be used for the preparation of culture media for isolation and cultivation of these bacteria.

Introduction

A medium is solid or a liquid preparation containing materials for the culture and growth of microorganisms, animal cells or plant tissue cultures (Talaro and Talaro, 2004). Different microorganisms require different nutrient material. Thus, culture media vary in form and in composition depending on the species to be cultivated (Ryan and Ray, 2004).

A culture medium is simply defined as any material in which microorganisms find nourishment and can reproduce themselves (Pelezar, 1993). Therefore, for a microbiological medium to fulfill its specific purpose, it must contain all the substances and compounds necessary for the growth and reproduction of the organism. Various substances have been conbined into nutritive concoction media and have successfully been used to isolate important microorganisms from materials such as water, soil, food, clinical specimen and dairy products. (Pelezar, 1993).

A medium may be formulated as either permissive with the intent of allowing the growth of whatever organisms are present or restricted or selective with the intent of only selecting a particular subset of organisms (Ryan and Ray, 2004).Microorganisms need

nutrients, as a source of energy and certain environmental conditions in order to grow and reproduce. In the environment, microbes adapt to the habitats most suitable for their needs while in the laboratory, these requirements must be met by a culture medium (Simin, 2011). When a medium is being prepared for microbial growth, consideration must be given to the provision of carbon and energy sources and other growth factors that are essential for the organisms. (Laleye, 2007).

Media used in the laboratory for the cultivation of microorganisms supply the nutrients required for cellular growth and maintenance. A wide variety of culture media is employed by the microbiologist for the isolation, growth, maintenance of pure cultures and identification of bacteria according to their biochemical and properties (John, 2006).

Different media for the growth and isolation of bacteria have been reported from different substrates. Plant materials have been used to recover bacteria from different sample sources such as groundnut, sorghum, extracts local food stuff waste, cassava whey, yam, african oil bean, maize, beans and pigeon pea (Famurewa and David, 2008). According to Ritchiz (2002), bacteria prefer a neutral pH for growth on artificial media between 25-30°C. The nutritional content of vegetables varies considerably, though generally they contain little protein or fat and varying proportions of vitamins, provitamins, dietary minerals, fiber and carbohydrates. This quality makes the vegetables to be suitable environment for bacterial and other microbial growth and development (Woodruff, 1995; Whitaker, 2001).

Media for culturing bacteria is an important tool in microbiology laboratory. The high cost of the microbial culture media has paved a way for the production of alternative media using cheap local raw materials and also based on the value and the scarcity of culture media, screening of alternate media is found to be an important tasks. Thus, the need to formulate a media for the growth of bacteria using spoilt vegetables (cabbage, carrot and cucumber) as growth medium is necessary. The aim of the study is to formulate a selective mediumfor the growth of *Staphylococcus aureus* and *Escherichia coli* using spoilt vegetables waste.

Materials and Method

Sample Collection

Spoilt cucumber, carrot and cabbage were collected from Muda Lawal market within Bauchi metropolis in Bauchi state and transported to the microbiology laboratory of the Science Laboratory Technology department, Federal Polytechnic Bauchi in a clean container.

The *Escherichia coli* and *Staphylococcus aureus* were collected from the microbiology laboratory of Abubakar Tafawa Balewa University Teaching Hospital Bauchi, Bauchi state and authenticated.

Pre-Treatment of Samples

The spoilt vegetables were washed and sliced. They were cut manually into 3mm thickness. The sliced samples were subjected to dry heat in oven at 80 ºC until the samples were completely dried. The sliced samples were air dried at room temperature,

ground into powder using pestle and mortar, sieved and stored separately in sterile container until it was used (Umechuruba and Elenwo, 1999).

Proximate Analysis
The crude fibre, protein, ash, moisture and carbohydrate contents were determined according to the method described by AOAC (2005).

Formulation of medium
2g of cucumber, 2g of cabbage and 2g of carrot were weighed separately and mixed with 2g of agar, which is a solidifying agent and 0.1g sodium chloride in 100ml of distilled water. The pH was adjusted to 7±0.5. The medium was sterilized at 121°C for 15 minutes and approximately 20ml was dispensed into each of the sterilized petridishes after cooling to about 45-50°C.

Preparation of Commercial medium
Nutrient agar (NA) was prepared according to the manufacturer's instruction.

Confirmation of bacteria
Morphological and biochemical tests were carried out using the method described by Cheesbrough, (2004) to confirm the organisms (*Escherichia coli* and *Staphylococcus aureus).* The tests carried out include coagulase, catalase, citrate utilization, methyl red, oxidase, sugar fermentation, indole and motility tests.

Growth of bacteria on the Formulated and Commercial media
The pure culture was dissolved in distilled water and its turbidity was compared to that of the McFarland standard.
Serial dilution of the pure culture solution were in 10-1 fold from 10-1 to 10-7 and 1ml of the solution was poured in a sterile plate and molten agar medium of both formulated and commercial medium cooled at 47°C was poured and mixed thoroughly, which was allowed to solidify. The plates were inverted and incubated at 47°C for 24hours. The dilution was made in duplicate plate.

Viable Count of Discrete Colonies
The total viable count (TVC) of the discrete colonies formed was determined using a colony counter. After 5 to 7 days of incubation, the plates were taken to the bench and the colonies were counted using the colony counter device. The number of colonies counted was multiplied by the dilution factor and expressed in colony forming unit per ml (cfu/ml).

Results
The result of proximate analysis of spoilt vegetables (cucumber, cabbage and carrot), were as presented in table 1. As can be seen, the moisture content of the vegetables was high: 89%, 78% and 75% for cucumber, cabbage and carrot respectively. Cabbage and

carrot had the lowest as conten of 1.0% and 0.5% respectively while carrot had the lowest fat, crude fibre and protein contents of 0.5%, 0.30% and 0.34% respectively. The moisture, ash,fat, crude fibre, protein and carbohydrate contents for cucumber were 89%, 14%, 9%, 5.0%, 5.42%, 5.5%, while for cabbage was 78%, 12%, 1%, 8%, 6%, 8% and carrot had 75%, 16%, 0.5%, 0.30%, 0.34%, 11% respectively.

The viable counts for *Escherichia coli* and *S. aureus* on formulated media were $2.0x10^4$ cfu/ml and $2.3x10^4$ cfu/ml respectively while on commercial medium they were $2.5x10^4$ cfu/ml and $2.6x10^4$ cfu/ml respectively (Table 2).

Table 3 shows the cultural and biochemical characteristics of the bacteria *Escherichia coli* and *S. aureus* on Formulated Agar.

Table 1. Proximate composition of spoilt cucumber, cabbage and carrot (% dry matter, DM).

Constituents	Cucumber %	Cabbage %	Carrot %
Moisture content	89.0	78.0	75.0
Ash content	14.0	12.0	16.0
Fat content	9.0	1.0	0.5
Crude fibre	5.0	8.0	0.30
Protein	5.42	6.0	0.34
Carbohydrate	5.5	8.0	11.0

Table 2: Shows viable counts of *E. coli* and *S. aureus* on Formulated media (FM) and Nutrient agar (NA) media

Organisms	FM (cfu/ml)	NA(cfu/ml)
S. aureus	$2.3x10^4$	$2.6x10^4$
E. coli	$2.0x10^4$	$2.5x10^4$

Key: FM – Formulated Media, NA – Nutrient Agar, cfu – colony forming unit

Table 3: Cultural and Biochemical Characteristics of bacteria on Formulated Agar

Sample colony On FM	Cell shape	Gram reaction	Motility	Catalase	Coagulase	Glucose	Sucrose	Lactose	Maltose	Organism
Slightly Milky colony	Rod	-	-	+	-	+	A	A	A	*E. coli*

Yellow, raised round	Clusters	+	-	+	+	+	AG	-	+AG	*S. aureus*

Key: - = Negative, + = Positive, A = Acid, AG = Acid and Gas, FM = Formulated Media

Discussion

The result of this study shows that cucumber, cabbage and carrot had a high moisture content of 89%, 78%, 75% respectively. This agrees with work of Olutiola *et al*., (2000) who reported that moisture is required by organisms for their life process, as they require water for extracellular digestion of nutrients.

According to report by James (2000), the average moisture content of vegetables is about 88%, with an average content of 8.6% carbohydrates, 1.9% proteins, 0.3% fat, and 0.84% ash. From the standpoint of nutrient content, vegetables are capable of supporting the growth of bacteria. The higher water content of vegetables favour the growth of spoilage bacteria, and the relatively low carbohydrate and fat contents suggest that much of this water is in available form (James, 2000).

The crude fibre in cucumber and cabbage are 5% and 18% respectively, which indicates the presence of organic matter in the sample, according to Nwanze *et al.,* (2000) who reported that crude fibre gives an estimate of the proteins, lipids (fats), carbohydrate and the nucleic acid composition although there is a relationship between lipids content of the media and fungi growth.

Furthermore, the ability of the formulated selective medium to support the growth of *S. aureus* and *E. coli* is because they utilize the protein in the spoiled vegetable medium. This agrees with the work of Adesemoye and Adedire, (2005) who reported that protein is important for synthesis of cells substance.

Fat content was higher in spoilt cucumber (24%) than the other vegetables used for the study. Fats are vital to the structure and biological functions of cells and are used as alternative energy source. (Trease and Evans, 1989).

The growth of bacteria on the formulated media used in the study was slightly lower when compared to the growth on the commercial media (NA). This also agrees with the report of Ravathie (2012),who used edible leguminous seeds such as green gram, black gram, soya meat and cowpea as formulated medium and observed that *Staphyllococcus* sp. grows better on nutrient agar and grows comparatively slowly in all other protein formulations (as far as time duration for initial growth is concerned). Ravathie (2012) also reported that in comparison with the performance on conventional nutrient agar media, the prepared formulations were found to be cheap and good alternative culture media for bacteriological studies.

Conclusion

The relative performance of the bacterial growth on the formulated medium, when compared with the commercial medium illustrated a good growth of *Staphylococcus aureus* and *Escherichia coli* . Hence the alternative medium produced from raw vegetables can be used for the cultivation of microbes, which is found to be cost effective in the present scenario of getting commercial media.

Recommendation

It is therefore recommended that cheap spoiled vegetable waste agar medium can effectively be an efficient medium for cultivation of bacteria. Further research should be

carried out on the use of spoilt vegetable to produce broth agar for culturing both clinical and general microorganisms. Spoilt vegetable formulation is comparable to the commercial medium and should be taken as a substitute. The improvement of drying, grinding and filtering should be employed.

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