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European Journal of Experimental Biology, 2016, 6(3):19-24

ISSN: 2248 –9215
CODEN (USA): EJEBAU

Formulation of microbial growth media using brewers' spent grains (BSG) and growth rate assessment with three bacterial species

***Archibong Etim J., Obuboegbunam Eberechukwu C., Ewelukwa Uju C., Onuora Veronica C., Ezemba Constance C., Okeke Chidi B. and Okafor Ugochukwu C.**

Dept. of Applied Microbiology & Brewing, Faculty of Biosciences, Nnamdi Azikiwe University, Awka, Nigeria

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ABSTRACT

Studies on the formulation of microbial growth media from brewers' spent grains and their gelling properties were carried out. Three bacterial species; Staphylococcus aureus, Streptococcus pyogenes and Escherichia coli were used to assess the ability of the formulated media to support microbial growth. Two formulations of brewers' spent grains obtained from 100% barley mash and a mixture of 80% barley and 20% sorghum mash were used. Proximate analysis of the dried spent grains from lautered 100% barley mash gave protein, fat, ash, moisture, fibre and carbohydrate content as 21.26%, 7.8%, 2.8%, 7.89%, 13.7% and 45.59% respectively. Whereas, analysis of the mixed dried spent grains gave 19.26%, 4.6%, 1.4%, 6.25%, 15.52% and 50.77% for protein, fat, ash, moisture, fibre and carbohydrate respectively. Total plate counts of test microorganisms on formulated media were compared to that of their standard growth media. At $P \leq 0.05$, the result showed a significant difference of total bacterial count between organisms grown on formulated media and those grown on standard media. In the formulations at different ratios with gelling agent, efficient gelling properties were obtained with active growth of tested microorganisms. Thus, brewers' spent grains can be harnessed into microbial growth media for cost reduction.

Keywords: Brewers' Spent Grain(BSG), media, microbial, mash, barley, sorghum.

INTRODUCTION

Microorganisms require nutrients to grow. These nutrients are supplied by either solid or liquid culture media. A growth medium or culture medium is a liquid or gel designed to support the growth of microorganisms or cells [5] or small plants like moss [3].

Industries generate a lot of wastes and residues whose accumulation in the environment results in pollution [10]. These industrial wastes contain vast amount of nutrient which can be harnessed for growth of microorganisms and subsequent production of useful primary and secondary metabolites like enzymes. [1].

Spent grains are the by-product of the mashing process, which is one of the initial operations in brewing. It is available at low or no cost throughout the year, and is produced in large quantities not only by large but also small breweries [9]. Despite its availability, the main application of brewers' spent grain has been limited to livestock feeding [7].

However recently, attempts have been made to use brewers' spent grains (BSG) in biotechnological processes such as in the cultivation of single cell proteins, source of value added products e.g ferulic and p-coumaric acids, xylitol and arabitol production [6]; [8]. This research was aimed at: Formulating a growth media from BSG and ascertaining the gelling properties of the formulated media, culturing some species of bacteria on the formulated media and comparing their growth rate with those grown on standard media.

MATERIALS AND METHODS

Collection and preparation of spent grains

Brewers' spent grains from lautered all-barley mash and a mixture of barley and sorghum malt were collected from a brewing company in Onitsha, South East Nigeria for five consecutive weeks. The wet spent grains were dried in an oven for 24hours at 60 ^{o}C and then ground into powder and filtered using a 0.06mm pore size filter and stored dry.

Collection of Test Microorganisms

The test organisms, *Staphylococcus aureus*, *Escherichia coli* and *Streptococcus pyogenes* were obtained from Glanson Medical Laboratory, Awka, South East Nigeria and maintained on agar slant at 22^{o}C in Applied Microbiology and Brewing Laboratory, Nnamdi Azikiwe University, Awka, Nigeria. Preliminary biochemical tests were also carried out on the microorganisms for confirmation.

Inoculation of test microorganisms

Serial dilutions of the test microorganisms were done in each case and 10^{-4} was used in the various inoculations.

Sample Analysis

Proximate nutrient analyses were carried out on the samples of brewers' spent grains according the recommended method of Association of Official Analytical Chemists, AOAC (1990) to determine their moisture, ash, fat, protein, carbohydrate and fibre contents.

Spent Grains Media Formulation and gelling capacity test

The powdered dry spent grains were mixed at different ratios with agar. The mixtures were stirred well to homogenize in measured de-ionized water and autoclaved for 15 minutes at 121^{0}C. The media were allowed to cool to a temperature of about 45^{0}C and dispensed into labeled plates. The gelling time was determined for different ratios of formulated media. The properly gelled media were selected and used for culturing.

Statistical Tool

A two way ANOVA was used to analyze the results.

RESULTS AND DISCUSSION

Table 1: Shows the proximate Analysis of spent grains (barley) collected for five weeks.
Table 2: Shows the proximate Analysis of spent grains (barley and sorghum mash), collected for five weeks
Table 3: Shows the gelling properties of powdered spent grains from all barley mash mixed with agar at different ratios.
Table 4: Shows the gelling properties of powdered spent grains (80% barley and 20% sorghum) mixed with agar at different ratios.
Table 5: Shows Total plate count of test microorganisms grown on formulated medium from all barley mash.
Table 6: Shows Total plate count of test organisms grown on formulated medium from a mixture of barley and sorghum mash.

Table 1: Proximate Analysis of Spent grains (all-barley mash), sampled for five weeks

Parameters (%)	Week 1	Week 2	Week 3	Week 4	Week 5	Mean value
Protein	21.28	21.24	21.26	21.25	21.27	21.26
Fat	8.00	7.60	7.80	7.70	7.90	7.80
Fibre	13.50	13.70	13.80	13.60	13.90	13.70
Ash	2.80	2.60	2.70	3.0	2.90	2.80
Carbohydrate	45.58	45.59	45.60	45.61	45.57	45.59
Moisture	7.91	7.88	7.90	7.87	7.89	7.89

Table 2: Proximate Analysis of Spent grains (Barley and Sorghum mash), sampled for five weeks

Parameters (%)	Week 1	Week 2	Week 3	Week 4	Week 5	Mean Value
Protein	19.91	19.87	19.89	19.93	19.95	19.91
Fat	4.80	4.70	4.50	4.60	4.40	4.80
Fibre	15.53	15.51	15.54	15.50	15.52	15.52
Ash	1.20	1.60	1.50	1.30	1.40	1.40
Carbohydrate	50.77	50.72	50.75	50.80	50.79	50.77
Moisture	6.51	6.54	6.50	6.53	6.52	6.52

Table 3: Gelling properties of powdered spent grains mixed with agar for Barley

Ratio of combination in g(spent grain: agar)	Gel formation	Time taken (in minutes)
06:00	No gelling	.....
5.5:0.5	No gelling	.....
05:1.0	Gelled	20
4.5:1.5	Gelled	17
04:2.0	Gelled	15
3.5:2.5	Gelled	13
03:3.0	Gelled	10
2.5:3.5	Gelled	9
02:4.0	Gelled	7
1.5:4.5	Gelled	5
01:5.0	Gelled	5
0.5:5.5	Gelled	5
00:6.0	Gelled	5

Table 4: Gelling properties of powdered spent grains mixed with agar for a mixture of Barley80% and Sorghum20%

Ratio of combination in g(spent grain: agar)	Gel formation	Time taken (in minutes)
06:00	No gelling	.....
5.5:0.5	No gelling	.....
05:1.0	No gelling	.....
4.5:1.5	No gelling	.. ..
04:2.0	Gelled	25
3.5:2.5	Gelled	22
03:3.0	Gelled	18
2.5:3.5	Gelled	15
02:4.0	Gelled	11
1.5:4.5	Gelled	10
01:5.0	Gelled	8
0.5:5.5	Gelled	5
0.0:6.0	Gelled	5

Table 5: Total Plate Count of test microorganisms (bacteria) grown on formulated medium (from all-barley spent grain)

Ratio of combination	Test microorganisms		
in g(agar: spent grain)	*Staphylococcus aureus*	*Streptococcus pyogenes*	*Escherichia coli*
01:01	15 x 10^5 cfu/ml	7 x 10^5 cfu/ml	12 x 10^5 cfu/ml
01:02	19 x 10^5 cfu/ml	12 x 10^5 cfu/ml	17 x 10^5 cfu/ml
01:03	27 x 10^5 cfu/ml	16 x 10^5 cfu/ml	25 x 10^5 cfu/ml
01:04	39 x 10^5 cfu/ml	22 x 10^5 cfu/ml	31 x 10^5 cfu/ml
Blood agar medium	57 x 10^5 cfu/ml	55 x 10^5 cfu/ml	.....
MacConkey agar	.....	.....	35 x 10^5 cfu/ml

Table 6: Total Plate Count of test microorganisms (bacteria) grown on formulated medium(from a mixture of Barley 80% and sorghum 20% mash)

Ratio of combination	Test microorganisms		
in g(agar: spent grain)	*Staphylococcus aureus*	*Streptococcus pyogenes*	*Escherichia coli*
02:01	6 x 10^5 cfu/ml	5 x 10^5 cfu/ml	7 x 10^5 cfu/ml
02:02	13 x 10^5 cfu/ml	7 x 10^5 cfu/ml	12 x 10^5 cfu/ml
02:03	17 x 10^5 cfu/ml	11 x 10^5 cfu/ml	19 x 10^5 cfu/ml
02:04	28 x 10^5 cfu/ml	15x 10^5 cfu/ml	25 x 10^5 cfu/ml
Blood agar medium	57 x 10^5 cfu/ml	55 x 10^5 cfu/ml	.....
MacConkey agar	.....	.....	35 x 10^5 cfu/ml

DISCUSSION

The results of the proximate nutrient analysis of brewers' spent grain collected for five weeks shows from its mean values that the BSG contained sufficient amount of nutrients to support the growth of the test organisms (*Staphylococcus aureus, Escherichia coli and Streptococcus pyogenes*). The mean values of the protein, ash, fat,

carbohydrate, moisture and fibre contents for both varieties of the sample brewers' spent grains agreed with the report of [12] who observed that brewers' spent grains contain vast amount of nutrients which can be harnessed for growth of microorganisms and biomass production.

The study of the gelling capacities of the formulated media showed that spent grains from barley of various concentrations gelled when mixed with 1g of agar unlike the spent grains from a mixture of barley and sorghum which gelled with 2g of agar. This could be attributed to the fact that spent grains from a mixture of barley and sorghum had lower protein and fat content than the spent grains from barley (Tables 3 and 4). Total plate count of test microorganisms showed that the formulated media adequately supported microbial growth. However the media from barley spent grains recorded higher growth rate than that of a mixture of barley and sorghum. Comparing microbial growth rates, results showed that each test organism gave higher colony count in their standard medium than in the formulated media (Tables 5 and 6). However, the formulated media supported the growth of the test microorganisms maximally, indicating that Brewers' Spent Grains can be used effectively for media formulation.

Acknowledgements
The authors appreciate Obuboegbunam, E. C., Ewelukwa, U. J. and Onuora, V. C whose efforts led to the realization of this research. We also appreciate the Department of Applied Microbiology and Brewing Science, Nnamdi Azikiwe University, Awka , Intafact Beverages Limited and the National Agency for Food and Drug Administration (NAFDAC) all in Nigeria for providing us with facilities and a conducive environment for this research work.

CONCLUSION

In conclusion, microbial growth media was formulated from brewers' spent grains with different gelling properties as the media from 100% barley mash gelled faster than that with a combination of sorghum. The formulated media supported the maximal growth of the test microorganisms, indicating that Brewers' Spent Grain can be used effectively for commercial media formulation.

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