

## Bacterial Growth Enhancement Value and Nutritional Quality of Compounded Milk Media from Commercial Milk Brands

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### Abstract

**Background and Objective:** Milk agar is recommended for enumeration of microorganisms in dairy products. Most of the commonly used media are imported to Nigeria and hence available *in-house* substitutes are necessary. In this study, microbial growth enhancement values of compounded milk agar were compared to imported milk agar using two milk brands. Dawadawa (fermented locust beans), yeast powder and peptone in the proportions of 1: 3: 5, respectively, were used as nutrient sources.

**Material and Methods:** Growth enhancement values of the compounded media were assessed on three bacterial species of *Staphylococcus aureus*, *Escherichia coli* and *Lactobacillus plantarum*. Furthermore, imported milk agar served as the control.

**Results and Conclusion:** Significant differences were seen in nutritional contents of the compounded milk agar and imported milk agar as well as the elemental compositions of compounded milk agar and imported milk agar. No significant differences were seen between the total viable counts of *Staphylococcus aureus* and *Lactobacillus plantarum* on compounded milk agar and imported milk agar. However, significant differences were reported in the total viable counts of *Escherichia coli* on compounded milk agar and imported milk agar. Media containing Cowbell milk powder recorded the highest total viable counts ( $1.3 \times 10^5$ ,  $1.8 \times 10^6$  and  $2.0 \times 10^6$  CFU ml<sup>-1</sup>) for *Staphylococcus aureus*, *Escherichia coli* and *Lactobacillus plantarum*, respectively. In conclusion, the Cowbell milk powder in combination with yeast extract and peptone is the best milk of choice for compounding milk agar when using local resources.

**Conflict of interest:** The authors declare no conflict of interest.

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## 1. Introduction

Milk is a rich source of macro and micronutrients [1]. Milk can be classified based on the types of treatment they undergo, which include raw milk (unpasteurized), market milk (pasteurized fluid milk), dried milk powder and concentrated milk such as unsweetened condensed and sweetened condensed milks [2]. Milk provides a suitable source of energy and high quality protein including a number of essential micronutrients like zinc, potassium, magnesium, calcium and phosphorus [3]. Thus, it can serve as a suitable growth medium for microorganisms.

Milk composition and microbial load are essential factors that must be considered in assessing quality of milks because these factors affect processing of milks and their

nutritional quality and safety [4]. Powdered milk supports growth of bacteria, including *Staphylococcus (S.) aureus*; *Enterococcus*, *Micrococcus*, *Streptococcus*, *Bacillus* and *Clostridium* spp. as well as several other microorganisms [5]. Other common contaminants in milk and milk products include *Escherichia (E.) coli* and *Lactobacillus* spp. [6]. Bacterial growth in milk media is enhanced under conditions such as adequate water activity, favorable pH and adequate temperature [7].

The major bacteriological medium used in routine assays for the quality assessment of dairy products is milk agar. Various types of milk media include peptonized milk, litmus milk media, skim milk powder and milk agar. As

stated in the Product Catalog of the Becton Dickinson Diagnostics Systems, International Dairy Federation and British Standards Institute have published recommendations for the use of milk agar in microbial analysis of dried milk, liquid milk, whey and ice cream and whey. For Oxoid milk agar, constituents include yeast extract (3 g), peptone (5 g), milk solids (1 g) and agar (15 g) as specified in Oxoid Manual. Yeast extract and peptone provide essential nutrients while agar is a solidifying agent. Culture media include various compositions to provide growth requirements of certain culture species [8]. Complex media usually include components such as yeast extract, meat extract and peptone. Most of the media currently used in laboratories are imported and thus there is a need to use local substitutes that are abundant and available for the development of appropriate economic media in developing countries [9]. Presently, imported milk media such as milk agar (500 g) costs nearly \$ 200 on the average with no transportation/shipment costs. Thus, it is necessary to substitute compounds with similar results since milk media are expensive and not easily available. Therefore, this study assessed growth of common contaminants of dairy products, including *S. aureus*, *E. coli* and *Lactobacillus* spp. on compounded milk agar (CMA) using popular milk brands and readily available cheap protein substitutes independently and wholly, compared to nutritional and microbial growth enhancement qualities of imported milk agar (IMA).

## 2. Materials and Methods

### 2.1 Samples

Two commonly consumed brands of powdered milk in Nigeria were chosen for the study based on the consumer preference using questionnaires by a group of randomly selected panelists. Milk brands included 'Peak' and 'Cowbell.' Fermented locust beans of dawadawa were purchased from the open market. In contrast, edible yeast powder was purchased from a pharmaceutical store (Westgate Lifecare Pharmacy, Nigeria).

### 2.2 Preparation of various substitutes used in formulation of milk agar

Fermented locust bean condiment was dried at 40 °C for 24 h and blended into fine powder. The Oxoid LP0037 Bacteriological Peptone (Oxoid, Hampshire, UK) and Oxoid LP0021 Yeast Extract (Oxoid, Hampshire, UK) were used for the formulation of milk agar.

### 2.3 Preparation of imported milk agar and compounded milk agar

The IMA was prepared as positive control based on the manufacturer's instructions. Milk media were compounded using the two brands of milk as follows: 1 g of each milk brand was added separately to 3 g of yeast extract, 5 g of peptone and 15 g of agar and dissolved in 1 litre of distilled

water (DW). The solution was boiled to dissolve fully and sterilized by autoclaving at 121 °C for 15 min. It was then cooled to 45 °C before pouring into sterile Petri dishes. Other formulations were made following a similar procedure, using 3 g of edible yeast powder and 5 g of dawadawa as a substitute for yeast extract and peptone, respectively.

### 2.4 Collection of microorganisms

Briefly, *S. aureus* (ATCC 6538), *E. coli* (ATCC 25922) and *Lactobacillus* (*L.*) *plantarum* locally isolated and characterized with no typing, were provided by the Microbiology Laboratory, Abubakar Tafawa Balewa University, Bauchi, Nigeria, and maintained on agar slants at 35 °C. Isolates were characterized using biochemical tests and API 20E test systems.

### 2.5 Proximate analyses of the imported milk agar and compounded milk agar

Proximate analyses of IMA and CMA were carried out to assess moisture content, protein, lactose and fat based on the methods by Association of Official Analytical Chemists (AOAC) [10].

#### 2.5.1 Assessment of crude protein [10]

Briefly, 2 g of each milk agar was weighed and transferred to Kjeldahl digestion flasks. Then, 8 g of catalyst mixture (96% anhydrous sodium sulphate, 3.5% copper sulphate and 0.5% selenium dioxide) was dissolved in 25 ml of concentrated sulphuric acid. Flasks were heated gently in fume cupboard with occasional agitation until the solution turned clear. Digests were cooled and transferred into 250-ml volumetric flasks and made up to fixed volumes using DW. Each diluted digest was purified using distillation apparatus. The apparatus was washed using steam for nearly 15 min. Then, 5 ml of the boric acid indicator was transferred into a 100-ml conical flask with the tip of the condenser dipped into the boric acid indicator. Then, 5 ml of the digest was pipetted into the distillation tube via small funnels and mixed with 5 ml of NaOH washed down with DW. Steam was passed through the apparatus for 5-7 min and the distillate trapped in boric acid indicator was collected in conical flasks and titrated using 0.1 ml of hydrochloric acid. Crude protein in the sample was calculated by multiplying the total nitrogen by the empirical factor according to AOAC [10] as Eq. 1:

$$\text{Crude protein} = \frac{0.007 \times 6.25 \times 100 \times \text{titre}}{\text{Weight of sample used}} \quad \text{Eq. 1}$$

$$1 \text{ ml of } 0.1\text{M} = 0.0014 \text{ g}$$

#### 2.5.2 Assessment of crude fat [10]

A quantity of 2 g of each sample was extracted using Soxhlet apparatus and dried with anhydrous ether (bp 40-60). A timple with porosity permitting rapid passage of ether was used. Extraction period was nearly 4 h at a condensation rate of 5-6 drops per second. Extract was dried at 100 °C for 30 min and then cooled and weighed.

### 2.5.3 Assessment of lactose [10]

Briefly, 10 ml of dilute hydrochloric acid was added to 20 ml of the sample in conical flasks. The solution was transferred into a 100-ml volumetric flask, made up to a fixed volume using DW, and then titrated against Benedict's reagent.

### 2.5.4 Assessment of moisture [10]

Metal dishes and their covers were heated at 105 °C for 15 min using an oven and cooled using a desiccator. Then, dishes and covers were weighed. Briefly, 5 g of the sample was transferred to a dish and weight of the sample, dish and cover was recorded. Sample with the dish and cover were heated at 105 °C for 1.5 h using an oven. A cover was placed on the dish, cooled in desiccator and weighed at room temperature. Then, dish and sample were reheated for 30 min and weighed after cooling. This process was repeated continuously until a constant weight was achieved.

### 2.6 Elemental analyses of the imported milk agar and compounded milk agar

Elemental analyses were carried out based on the method by the Association of Official Analytical Chemists (AOAC) [10] to assess essential minerals such as Na, K, Mg and Ca as well as trace minerals such as S, P, Mn and Fe.

### 2.7 Assessment of pH

The pH values of IMA and CMA were assessed using pH meter (Eutech Instrument, Singapore).

### 2.8 Enumeration of bacteria

Viable colonies of *S. aureus*, *E. coli* and *L. plantarum* isolates were homogenized separately in normal saline. Serial dilutions were carried out; after which, 1 ml of 10<sup>-4</sup> dilution of each bacterium was inoculated separately in IMA and CMA in duplicates. The plates were incubated at 35 °C for 18-24 h, aerobically. At the end of a 24-h incubation, bacterial growth from various milk media was recorded as colony-forming units (CFU ml<sup>-1</sup>). For *L. plantarum*, plates were incubated anaerobically at 35 °C for 24 h.

### 2.9 Statistical analysis

Independent T-test was used to compare the mean count data for the microbial isolates on IMA and CMA as well as proximate and elemental analyses of the media carried out in duplicates. Differences were significant at  $p \leq 0.05$ .

## 3. Results and Discussion

Independent T-test showed no significant differences at 0.05%  $\alpha$ -level in moisture content, protein, fat and lactose contents of CMA and IMA (Table 1). Hence, protein, fat, moisture and lactose contents were approximately similar in CMA and IMA. Therefore, nutritional compositions of the compounded media were sufficient for the microbial growth. Generally, media containing Cowbell milk seemed to perform better than those with Peak milk powder. The percentage dry weight of each bacterial cell is 48% carbon, 12.5% nitrogen, 55% proteins, 9% carbohydrates, 7% lipids and 6% ash [11]. Nduka [11] also reported that nutrient is calculated based on the dry weight. For carbohydrates, 1 g of glucose is required per 0.5 g of dry cells while nitrogen requires 125 mg per gram of dry cells.

Elemental composition revealed no significant differences at 0.05% between the levels of ash, sulphur, phosphorus, sodium, potassium, iron, magnesium, calcium and manganese for CMA and IMA (Table 2). Hence, elemental contents were approximately similar for all media and sufficient for the microbial growth (Table 2). Nduka [11] reported that minerals and growth factors are usually added in very small amounts. Microorganisms generally need ten elements, which must be available in large quantities for growth (carbon, oxygen, hydrogen, nitrogen, sulphur, phosphorus, potassium, calcium, magnesium and iron) [12]. Carbohydrates, proteins, lipids and nucleic acids are synthesized by the first six elements while the last four elements serve as cations in cells. In addition to macroelements, several microelements such as Mn, Zn, Co, Mo, Ni and Cu as well as growth factors, which comprise of organic compounds, are important requirements of all microorganisms for growth [13].

**Table 1.** Proximate analyses of the imported milk agar and compounded milk agar with various protein sources

	Moisture Content (%)	Protein Content (%)	Ether Extract (%)	Ash (%)	Crude fiber (%)	Carbohydrate (%)
PD(1:5)	11.80	13.60	19.20	4.70	2.40	48.30
PDEY(1:5:3)	13.40	15.10	19.50	4.80	2.60	44.80
PYEP(1:3:5)	14.00	16.40	13.60	3.50	1.70	50.80
PEY(1:3)	12.00	12.90	13.10	3.10	1.50	57.40
CD(1:5)	12.10	13.80	18.40	4.70	2.50	48.50
CDEY(1:5:3)	13.20	15.70	19.00	4.70	2.60	44.80
CYEP(1:3:5)	14.30	16.50	13.10	3.30	1.60	51.20
CEY(1:3)	12.20	13.20	12.30	3.00	1.50	52.40
IMA	10.80	11.10	15.70	2.90	0.90	58.60
S.D	1.1447	1.8022	3.0333	0.8428	0.6160	4.8816

CYEP='Cowbell' milk + Yeast Extract + Peptone; PYEP='Peak' milk+ Yeast Extract + Peptone; CDEY='Cowbell' milk + 'Dawadawa' + Edible Yeast; PDEY='Peak' milk + 'Dawadawa' + Edible Yeast; CEY='Cowbell' milk + Edible Yeast; PEY='Peak' milk + Edible Yeast; CD='Cowbell' milk + 'Dawadawa'; PD='Peak' milk + 'Dawadawa'; IMA=Imported milk agar; S.D = Standard deviation

**Table 2.** Elemental analyses of the imported milk agar and compounded milk agar (mg/100g)

Sample	Ash	Sulphur (S)	Phosphorus (P)	Sodium (Na <sup>+</sup> )	Potassium (K <sup>+</sup> )	Iron (Fe <sup>2+</sup> )	Magnesium (Mg <sup>2+</sup> )	Calcium (Ca <sup>2+</sup> )	Manganese (Mn <sup>2+</sup> )
PD	4.7	56	38	105	42	18	48	307	0.01
PDEY	4.8	66	42	115	44	18	52	285	0.01
PYEP	3.5	48	35	95	35	15	44	262	0.00
PEY	3.1	52	38	102	30	16	52	280	0.00
CD	4.7	52	40	102	45	19	50	298	0.01
CDEY	4.7	55	42	105	50	18	52	287	0.01
CYEP	3.3	45	33	101	32	16	45	258	0.00
CEY	3.0	48	36	105	28	15	49	267	0.00
IMA	2.9	42	45	125	36	19	45	255	0.00
S.D	0.85	7.07	3.83	8.824	7.566	1.62	3.25	18.30	0.0053

CYEP= 'Cowbell' milk + Yeast Extract + Peptone; PYEP='Peak' milk + Yeast Extract + Peptone; CDEY='Cowbell' milk + 'Dawadawa' + Edible Yeast; PDEY='Peak' milk + 'Dawadawa' + Edible Yeast; CEY='Cowbell' milk + Edible Yeast; PEY='Peak' milk + Edible Yeast; PD='Peak' milk + 'Dawadawa'; IMA= Imported milk agar; S.D=Standard deviation

**Table 3.** PH of the various milk media

	With peptone and yeast extract	With 'Dawadawa'	With yeast powder	With 'Dawadawa' and Edible Yeast
CMA	6.3	6.9	4.8	6.5
PMA	6.2	6.6	5.0	6.3

IMA = 6.6; CBMA=Cowbell Milk agar; PMA=Peak Milk agar; IMA=Imported milk agar

Milk agar containing yeast and peptone recorded higher pH values, while milk agar with milk solids recorded lower pH values (Table 3). The pH of milk media compounded with dawadawa alone ranged within 6.6-6.9 while pH of the milk agar formulated with yeast powder alone ranged within 4.8-5.0 (Table 3). The pH of various formulations was moderately acidic and near alkaline. The mild acidic pH was favorable for the growth of the bacterial species. Naturally, most bacteria thrive under mild alkaline conditions. Most bacteria grow optimally at 37 °C within a narrow pH range of 6.7-7.5. However, there are exceptions with growth under acidic and alkaline conditions [14].

Generally, the highest total viable counts (TVC) ( $7.0 \times 10^3$  to  $1.3 \times 10^5$  CFU ml<sup>-1</sup>) were reported in milk agar containing Cowbell milk powder. Milk agar containing Peak milk powder and yeast powder recorded the lowest viable count for *S. aureus*, compared to IMA (Table 4). No significant differences were seen in TVC of *S. aureus* on IMA and CMA with yeast powder only and CMA with dawadawa only (Table 4). Generally, a lower TVC was achieved in these two combinations, compared to CMA containing yeast extract and peptone and CMA containing yeast powder and dawadawa. Therefore, *S. aureus* demands a high protein requirement for proper growth. In fact, *S. aureus* can grow on complex media in the absence of carbohydrates [15]. Small colony variants (SCV) characterized by slow growth rate, decreased toxin production and altered pattern of utilization are formed by the mutation of *S. aureus* [16]. In this study, TVC of *S. aureus* on IMA was not significantly different at 0.05%  $\alpha$ -level, compared to that on CMA with combined yeast extract and peptone.

However, TVC of *S. aureus* on IMA was significantly different at 0.05%  $\alpha$ -level from that on milk agar with a combination of yeast powder and dawadawa as well as dawadawa alone. Thus, edible yeast powder and dawadawa might not adequately substitute yeast extract and peptone in growth requirement of *S. aureus*.

Similarly, milk agar containing Cowbell milk powder recorded the highest TVC ( $1.0 \times 10^6$  to  $1.8 \times 10^6$  CFU ml<sup>-1</sup>) while agar containing Peak milk powder recorded the lowest TVC for *E. coli*. Milk agar formulations containing Cowbell milk powder recorded a higher TVC (Table 4). Significant differences were seen at 0.05%  $\alpha$ -level in TVC of *E. coli* on IMA and CMA containing Cowbell, yeast extract and peptone (Table 4). Certainly, *E. coli* grows rapidly and requires simple nutrients, especially carbon, which is supplied by glucose in growth media [17]. Furthermore, glucose is metabolized to smaller molecules (e.g. carbon dioxide, acetic acid or ethanol), which generates ATPs needed for energy-requiring activities of the cells [17]. Media containing Cowbell, yeast extract and peptone could be metabolized faster than the other media, making carbon dioxide, acetic acid and ethanol readily available to generate ATPs needed by *E. coli*. Previous reports have verified that *E. coli* survives with no growth factors and can synthesize amino acids, pyrimidines, purines and vitamins, where carbon serves as the starting point for its intermediary metabolism [14]. A similar trend was seen for cultures with *L. plantarum*; where, milk agar containing Cowbell milk powder recorded the highest TVC ( $2.0 \times 10^5$  to  $2.0 \times 10^6$  CFU ml<sup>-1</sup>), compared to those containing Peak milk powder (Table 4).



**Table 4.** Total viable counts of *Staphylococcus aureus*, *Escherichia coli* and *Lactobacillus plantarum* in various milk media with various protein constituents after 24-h incubation at 35 °C (CFU ml<sup>-1</sup>)

Milk agar with different protein constituents	Bacteria					
	<i>Staphylococcus aureus</i>		<i>Escherichia coli</i>		<i>Lactobacillus plantarum</i>	
	CMA	PMA	CMA	PMA	CMA	PMA
With yeast extract and peptone	1.3 × 10 <sup>5</sup>	6.4 × 10 <sup>4</sup>	1.8 × 10 <sup>6</sup>	1.2 × 10 <sup>6</sup>	2.0 × 10 <sup>6</sup>	4.0 × 10 <sup>5</sup>
With yeast extract and dawadawa	8.3 × 10 <sup>4</sup>	4.0 × 10 <sup>4</sup>	1.3 × 10 <sup>6</sup>	1.2 × 10 <sup>6</sup>	2.0 × 10 <sup>5</sup>	1.0 × 10 <sup>5</sup>
With yeast powder only	7.0 × 10 <sup>3</sup>	1.0 × 10 <sup>4</sup>	1.2 × 10 <sup>6</sup>	1.1 × 10 <sup>6</sup>	5.0 × 10 <sup>5</sup>	3.0 × 10 <sup>5</sup>
With Dawadawa only	4.4 × 10 <sup>4</sup>	3.1 × 10 <sup>4</sup>	1.0 × 10 <sup>6</sup>	1.1 × 10 <sup>6</sup>	3.0 × 10 <sup>5</sup>	1.0 × 10 <sup>5</sup>
	IMA = 1.0 × 10 <sup>5</sup> CFU ml <sup>-1</sup>		IMA = 1.2 × 10 <sup>6</sup> CFU ml <sup>-1</sup>		IMA = 2.3 × 10 <sup>6</sup> CFU ml <sup>-1</sup>	

CBMA=Cowbell Milk agar; PMA=Peak Milk agar; IMA=Imported milk agar

The TVC of *L. plantarum* on IMA was not significantly different at 0.05%  $\alpha$ -level from that on CMA with a combination of Cowbell milk powder, yeast extract and peptone (CYEP). However, TVC of *L. plantarum* on IMA was significantly different at 0.05%  $\alpha$ -level from that on milk agar with all remaining combinations. The higher TVC of *L. plantarum* on IMA showed that yeast extract and peptone were needed for the maximum growth of *L. plantarum*. The IMA supported growth of *L. plantarum* after 24 h as well as other locally compounded formulations when incubated anaerobically. The *L. plantarum* needs a high quantity of protein for adequate growth. This protein was available in Cowbell milk powder, yeast extract and peptone used for compounding the milk agar. *Lactobacillus* spp. needs amino acids, pyrimidines, purines and vitamins as part of the requirements for growth [18]. Bacteria may need specific nutrients and chemical requirements for their growth [19].

Bacteria showed irregular-shaped colonies on CMA while regular colonies with smooth edges were achieved on IMA. Furthermore, *E. coli* showed large irregular-shaped colonies on CMA while small regular-shaped colonies with smooth edges were achieved on IMA. Moreover, *L. plantarum* included similar characteristics on all CMA as well as IMA (Table 5). Morphological characteristics of *S. aureus*, *E. coli* and *L. plantarum* on various CMA and IMA were quite similar. Thus, cultural characteristics of various bacteria did not alter on CMA and IMA. Despite similar morphological characteristics of various bacteria on compounded and imported media, milk agar prepared with Cowbell milk powder showed better bacterial growth, compared to that prepared with Peak milk powder. Enhanced bacterial growth on media compounded with Cowbell milk could occur as a result of the presence of soy lecithin, which was present in Cowbell milk powder as reflected on the milk sachet. A recent study has verified that lecithin in the oil phase is responsible for the stability of milk powder [20].

**Table 5.** Morphological characteristics of *Staphylococcus aureus*, *Escherichia coli* and *Lactobacillus plantarum* in all compounded milk media of Cowbell and Peak

Features	Bacteria		
	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>	<i>Lactobacillus plantarum</i>
Shape	Irregular	Irregular	Circular
Size	Big	Big	Small
Color	Milky	Cream	Cream
Elevation	Flat	Raised	Raised
Edges	Serrated	Rough	Smooth
Surface	Dry/Rough	Wet	Shiny
Pigmentation	-	-	-
Margin	Entire	Not entire	Entire

#### 4. Conclusion

This study has shown that compounded media formulated with Cowbell better support microbial growth, compared to those formulated with Peak milk. It is generally believed that Cowbell milk contains soy fats (lecithin), which serves as a rich source of nutrient for the microbial growth and improves stability of the Cowbell milk powder. Overall, nutritional facts of the two milk brands have been satisfactory. Elemental and proximate analyses have revealed that composition of CMA is similar to that of IMA; thus, serving as a veritable means of maximizing IMA costs. Dairy products can routinely be assessed for their microbiological quality using locally CMA made from commercial brands of milk and locally available cost-effective resources.

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#### 6. Conflict of Interest

The authors report no conflicts of interest.

#### References

1. Jalili-Nik M, Shahri ZS, Hashemy SI, Mashkani B. Effects of major ingredients in cattle milk on enzyme kinetics of recombinant  $\beta$ -galactosidase (BGalP) expressed in *Pichia pastoris*. *Appl Food Biotechnol*. 2018; 5(4): 205-212. doi:10.22037/afb.v5i4.22107.

2. Roberts TA, Cordier JL, Gram L, Tompkin RB, Pitt JI, Gorris LGM, Swanson KMJ. Milk and Dairy Products. In: Roberts TA, Cordier JL, Gram L, Tompkin RB, Pitt JI, Gorris LGM, Swanson KMJ (eds). *Microorganisms in Foods 6*. Springer, Boston, MA, 2005; pp. 643-715
3. Gorska-Warsewicz H, Rejman K, Laskowski W, Czebotko M. Milk and dairy products and their nutritional contribution to the average polish diet. *Nutrients* 2019; 11(8):1771. doi: 10.3390/nu11081771
4. Flint S, Bremer P, Brooks J, Palmer J, Sadiq FA, Seale B, Teh KH, Wu S, Md Zain SN. Bacterial fouling in dairy processing. *Int Dairy J.* 2020; 101: 104593. doi: 10.1016/j.idairyj.2019.104593.
5. Pal M, Alemu J, Mulu S, Karanfil O, Parmar BC, Nayak JB. Microbial and Hygienic aspects of Dry Milk Powder. *Beverage Food World.* 2016; 43(7): 28-31.
6. Kivanc M, Evrim YE. Survival of *Escherichia coli* O157:H7 and *Staphylococcus aureus* during the fermentation and storage of kefir. *Food Sci Technol.* 2019; 39: 225-230. doi: 10.1590/fst.39517.
7. Sadhu SP. Effect of cold chain interruptions on the shelf-life of fluid pasteurised skim milk at the consumer stage. *Brazilian J Food Technol.* 2018; 21: e2017064. doi: 0.1590/1981-6723.06417.
8. Ryan K, Ray G. *Medical Microbiology*. 4th Edition, McGraw Hill York, 2004; pp. 120-123.
9. Esan EB, Muyiwa AA, Lawal JO. Performance of tea (*Camellia sinensis* L.) on culture media modified with locally sourced substitutes. *J Anim Plant Sci.* 2009; 4(1): 298-303.
10. AOAC. *Official Methods of Analysis*. 15<sup>th</sup> Edition. Association of Official Analytical Chemists, Washington DC, USA. 1990; 807-928.
11. Okafor N. *Industrial Media and the Nutrition of Industrial Organisms In: Modern Microbiology and Biotechnology*. Science Publishers, Enfield, New Hampshire, USA. 2007; pp. 54-55.
12. Merchant SS, Helmann JD. Elemental economy: Microbial strategies for optimizing growth in the face of nutrient limitation. *Adv Microb Physiol.* 2012; 60: 91-210. doi: 10.1016/B978-0-12-398264-3.00002-4
13. Basu S, Bose C, Ojha N, Das N, Das J, Pal M. Khurana S. Evolution of bacterial and fungal growth media. *Bioinformatics.* 2015; 11(4): 182-184. doi: 10.6026/97320630011182
14. Yuan Y, Zhao B, Zhou S, Zhong S, Zhuang L. Electrocatalytic activity of anodic biofilm responses to pH changes in microbial fuel cells. *Bioresour Technol.* 2011; 102(13): 6887- 6891. doi: 10.1016/j.biortech.2011.04.008.
15. Stainer RY, Ingraham JL, Wheelis ML, Painter PR. *The Microbial World*. 5th Edition, Prentice Hall Press, Englewood Cliffs, New Jersey, 1986; 689 pp.
16. McNamara PJ, Proctor RA. *Staphylococcus aureus* small colony variants, electron transport and persistent infections. *Int J Antimicrob Agents*, 2000; 2: 117-122. doi: 10.1016/S0924-8579(99)00170-3.
17. Lodish H, Berk A, Zipursky SL, Matsudaira P, Baltimore D, Darnell J. *Molecular Cell Biology*. 4th Edition. Freeman WH; New York, 2000; 1084 pp. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK21475/>
18. Hebert EM, Raya RR, De Giori GS. Nutritional requirements of *Lactobacillus delbrueckii* subsp. *lactis* in a chemically defined medium. *Curr Microbiol.* 2004; 49(5): 341-345. doi: 10.1007/s00284-004-4357-9
19. Tripp HJ, Kitner JB, Schwabach MS, Dacey JW, Wilhelm LJ, Giovannoni SJ. SAR11 marine bacteria require exogenous reduced sulphur for growth. *Nature* 2008; 452: 741-744. doi: 10.1038/nature06776
20. Toikkanen O, Outinen M, Malafrente L, Rojas OJ. Formation and structure of insoluble particles in reconstituted model infant formula powders. *Int Dairy J.* 2018; 82: 19-27. doi: 10.1016/j.idairyj.2018.03.001

## میزان افزایش رشد باکتریایی و کیفیت تغذیه‌ای محیط‌های کشت ترکیبی شیر حاصل از چند نام تجاری شیر

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### چکیده

**سابقه و هدف:** محیط کشت میلک-آگار برای شمارش ریزاندامگان‌ها در فرآورده‌های شیری توصیه می‌شود. اغلب محیط‌های کشت معمول مورد استفاده در نیجریه وارداتی بوده و لذا جایگزین‌های در دسترس داخلی<sup>۱</sup> ضروری می‌باشند. در این مطالعه، میزان افزایش رشد میکروبی میلک-آگار ترکیبی با میلک-آگار دو نام تجاری شیر وارداتی مقایسه شدند. داواوا (دانه‌های تخمیر شده خرنوب)، پودر مخمر و پپتون با نسبت به ترتیب ۵:۳:۱ به عنوان منابع مواد مغذی مورد استفاده قرار گرفتند.

**یافته‌ها و نتیجه‌گیری:** تفاوت‌های معنی‌داری در مقادیر کیفیت تغذیه‌ای میلک-آگار ترکیبی و میلک-آگار وارداتی همچنین ترکیبات تشکیل دهنده آنها ملاحظه شد. تفاوت معنی‌داری بین میزان کل زنده‌مانی استافیلوکوکوس اورئوس و لاکتوباسیلوس پلانتاروم در میلک-آگار ترکیبی و میلک-آگار وارداتی دیده نشد. اگرچه، تفاوت‌های معنی‌داری در میزان کل زنده‌مانی/اشرشیا کلی در میلک-آگار ترکیبی و میلک-آگار وارداتی گزارش شد. بالاترین میزان کل زنده‌مانی برای استافیلوکوکوس اورئوس، اشرشیا کلی و لاکتوباسیلوس پلانتاروم در محیط‌های کشت حاوی شیر گاو به ترتیب  $1.3 \times 10^5$ ،  $1.8 \times 10^6$  و  $2.0 \times 10^6$  CFU ml<sup>-1</sup> گزارش شد. در نتیجه، پودر شیر گاو در ترکیب با عصاره مخمر و پپتون بهترین انتخاب شیر برای میلک-آگار ترکیبی با استفاده از منابع داخلی می‌باشد.

**تعارض منافع:** نویسندگان اعلام می‌کنند که هیچ نوع تعارض منافی مرتبط با انتشار این مقاله ندارند.

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### واژگان کلیدی

- رشد باکتریایی
- محیط کشت ترکیبی
- میلک-آگار وارداتی
- نام های تجاری شیر

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