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Research Article
Cost-Effective Media for Production of Lactic Acid Bacteria Isolated from Poultry in Kuwait

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Abstract

Background and Objective: Poultry is a primary source of meat in Kuwait. Abuse of antibiotics in the poultry industry can lead to unfavorable effects on food safety. However safer approaches, such as probiotic use, can be expensive. The present study screened cost-effective media formulations which support growth of lactic acid bacteria. **Materials and Methods:** Shake flask experiments were conducted using three bacterial strains isolated from Kuwaiti poultry farms (*Lactobacillus brevis*, *Lactobacillus parabuchneri* and *Pediococcus pentosaceus*). Ten media formulations containing different concentrations of tomato serum, molasses and yeast extract were inoculated with bacteria and incubated at 30, 35, or 37°C. Samples were taken periodically (0, 24 and 48 h), plated on selective agar and incubated overnight; growth was measured the next day. Statistical analysis revealed the formulation that supports the highest growth when compared to de Man Rogosa and Sharpe medium. **Results:** The formulation containing 5 g L^{-1} yeast extract, 200 mL L^{-1} tomato serum and 10 g L^{-1} molasses resulted in the best overall growth and supported a longer exponential phase compared to the commercial medium. **Conclusion:** This new media could be used as a cost-effective alternative for large-scale production of certain poultry probiotics.

Key words: Poultry production, probiotics, tomato serum, yeast extract, molasses, bacterial growth media

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Food safety and security is crucial to maintain nutritional stability in any country. In developed countries, legislators have established firm industry regulations that benefit the economy, farmers and citizens. For a fast-developing country, such as Kuwait, food security and safety is important due to a high demand for limited local food supplies. According to the UN Food and Agriculture Organization, poultry consumption in Kuwait increased from 58,000 metric tons in 1993-135,000 metric tons in 2013. Moreover, their Statistics Division reported the consumption of poultry meat per capita tripled from 1993 (23.3 kg capita^{-1} year^{-1}) to 2011 (63.6 kg capita^{-1} year^{-1})[1]. Since poultry is the most consumed meat in Kuwait, it is essential to find the most effective and economical way to ensure the safety of this industry and its products.

The poultry industry is highly affected by environmental factors (e.g., weather) and susceptible to infection by microbes from various sources, such as water. Microbes that affect animal health and mortality have a direct effect on a country's food security. Furthermore, transfer of some microbes (e.g., *Salmonella*) to humans can lead to sickness and e ven death. Various drugs and antibiotics, such as chlortetracycline, penicillin and diclazuril, used to control microbial infections have been approved by the US Food and Drug Administration[2-5]. However, several countries in the European Union have banned and/or limited the use of antibiotics in favor of natural biological approaches to protect and improve the quality and safety of poultry meat[4]. For example, successful application of probiotics to control foodborne pathogens is well-documented. Probiotics are defined as "live microorganisms that, when administrated in adequate amounts, confer a health benefit on the host[6]. Probiotics are an indigestible food ingredient that selectively stimulate the growth and activity of one or a limited number of bacterial populations in the host digestive system[7].

Specifically, lactic acid bacteria (LAB), such as *Lactobacillus* and *Bifidobacterium*, have been intensively used as human and livestock probiotics[8]. Considering the market for animal feed probiotics is expected to reach $7.0 billion by 2025[9], LAB use in the livestock industry will largely depend on the development of more economical media which support its mass production. Therefore, the present study examined various media formulations to determine a more cost-effective way to produce LAB for use with poultry in Kuwait.

MATERIALS AND METHODS

Bacterial Isolates: Bacterial isolates used were taken from local poultry farms in Kuwait in a previous study[10]. The selected strains used (*Lactobacillus brevis*, *L. parabuchneri* and *Pediococcus pentosaceus*) are considered to have probiotic characteristics helpful to poultry health. The selected strains were stored in cryogenic vials at -80°C, carefully thawed and then subcultured in de Man Rogosa and Sharpe (MRS)medium (Oxoid, UK) before experimental testing. Analysis of experimental media formations commenced once bacterial growth and purity were up to laboratory standards.

Media testing: Shake flask cultures were used to examine bacterial growth characteristics in 10 different experimental media formulations containing various concentrations of yeast extract, tomato serum and molasses (Table 1) compared to commercial MRS medium. For each experimental test, the media was sterilized by autoclave (121°C, 15 min), aliquoted into sterile tubes (30 mL tube^{-1}) and then inoculated with fresh bacterial cultures equal to 10% of the final volume. Tubes were incubated at three different temperatures (30, 35 and 37°C), with a fixed mixing speed of 100 RPM on a rotatory shaker. Bacterial samples (100 µL) were taken from each tube at different time intervals (0, 24 and 48 h), plated on selective MRS agar (Oxoid, UK), incubated at 37°C for 24 h[11] and then growth measured as the number of colony forming units; sampling after initial inoculation (0 h) served as a baseline

Table 1: Experimental media content

	Media formulation									
Ingredient concentration	1	2	3	4	5	6	7	8	9	10
Yeast extract (3 g L^{-1})	X	X			X		X		X	
Yeast extract (5 g L^{-1})			X	X		X		X		X
Tomato serum (200 mL L^{-1})	X		X				X			X
Tomato serum (400 mL L^{-1})		X		X				X	X	
Molasses (5 g L^{-1})							X		X	
Molasses (10 g L^{-1})					X	X		X		X

control. Growth of samples maintained in commercial MRS medium served as a positive control. Each formulation and temperature was tested in triplicate.

Statistical analysis: Data were presented as the averages for each formulation and temperature. Differences were determined by 2-way analysis of the variance and Duncan's multiple range testing using Statistical Analysis Software (SAS ©). Differences were considered significant at $p<0.05$.

RESULTS

***Lactobacillus parabuchneri* growth:** The results showed that media formulations 8, 9 and 10 yielded the highest *L. parabuchneri* growth at all three incubation temperatures (Fig. 1a, b) compared to other formulations at all temperatures. While medium 8 appears to support the most growth at 35°C, this difference was insignificant. Followed by medium 9 for 48 h at 30°C and medium 8 for 48 h at 30°C. Cultivation of *L. parabuchneri* in MRS medium resulted in higher growth at 24 h regardless of temperature, with highest growth at 37°C. Based on these results, medium 8 should be used for large-scale cultivation of *L. parabuchneri* for 24 h at 35°C as it promotes the most growth. Complete data for all other media formulations can be found in Fig. 2 (a-c).

***Lactobacillus brevis* growth:** Figure 3 (a, b) shows that *L . brevis* grew the best in media 9 and 10 at 24 and 48 h.

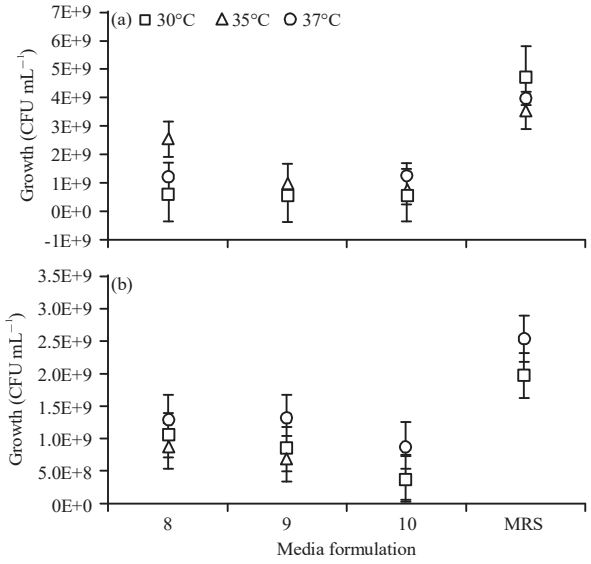

Fig. 1(a-b): Average growth of *Lactobacillus parabuchneri* after (a) 24 h and (b) 48 h

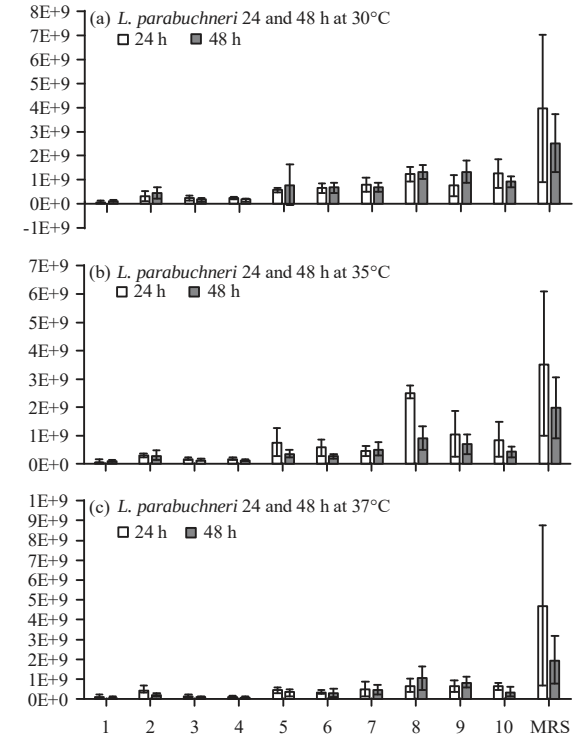

Fig. 2(a-c): Average growth of *Lactobacillus parabuchneri* at 30, 35 and 37°C after 24 and 48 h

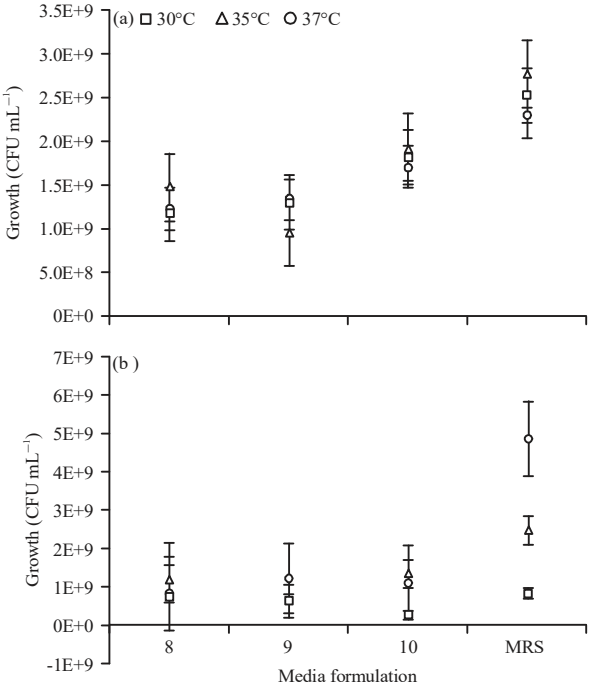

Fig. 3(a-b): Average growth of *Lactobacillus brevis* after (a) 24 h (b) and 48 h

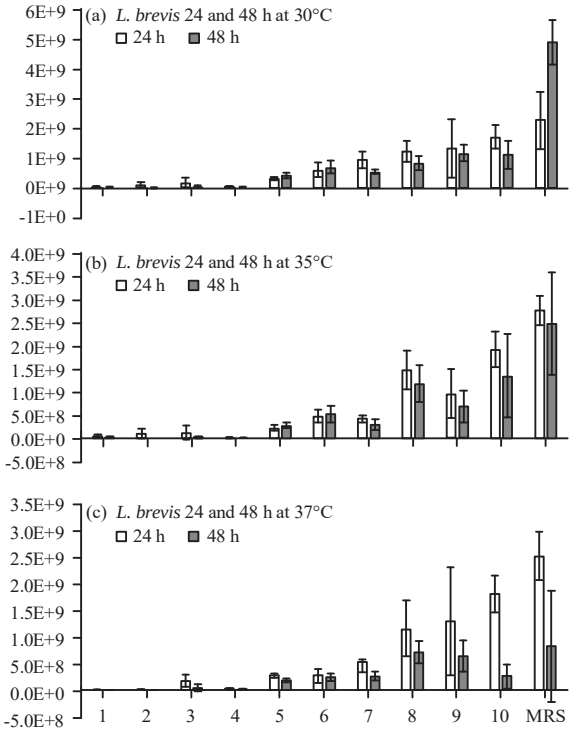

Fig. 4(a-c): Average growth of *Lactobacillus brevis* at 30, 35 and 37°C after 24 and 48 h

Cultivation in medium 10 for 24 h at 35°C resulted in the highest amount of growth ($\alpha = 0.05$) compared to all other media, temperatures and cultivation times. While, the best *L. brevis* cultivation time and temperature using MRS medium was at 30°C for 48 h. Moreover, 24 h growth at 35°C was higher than that at 48 h. These results demonstrate that cultivation of *L. brevis* in medium 10 at 35°C for up to 24 h produces the greatest amount of growth compared to other formulations. Complete data for all other media formulations is shown in Fig. 4 (a-c).

***Pediococcus pentosaceus* growth:** Interestingly, *Pediococcus pentosaceus* showed very unstable and inconsistent growth in all media formulations, especially when cultivated at 37°C (Fig. 5 a, b). Nonetheless, medium 10 supported the highest amount of growth at 37°C, followed by 30 and 35°C, relative to all other formulations. Optimal *P. pentosaceus* growth using MRS medium was at 30°C for both 24 and 48 h. Similar to the other bacterial strains, medium 10 supported the best *P. pentosaceus* grow that 30°C at 24 h. Fig. 6 (a-c) shows complete data for all other media formulations.

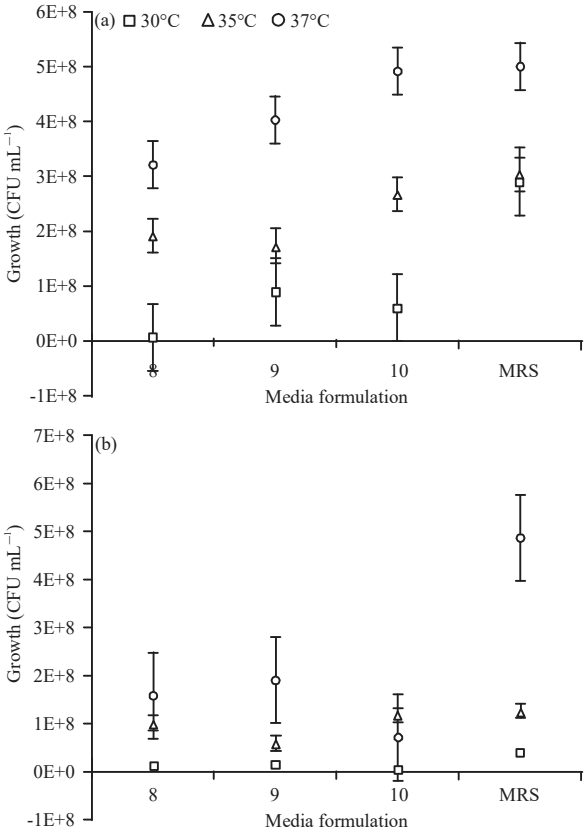

Fig. 5(a-b): Average growth of *Pediococcus pentosaceus* after (a) 24 h and (b) 48 h

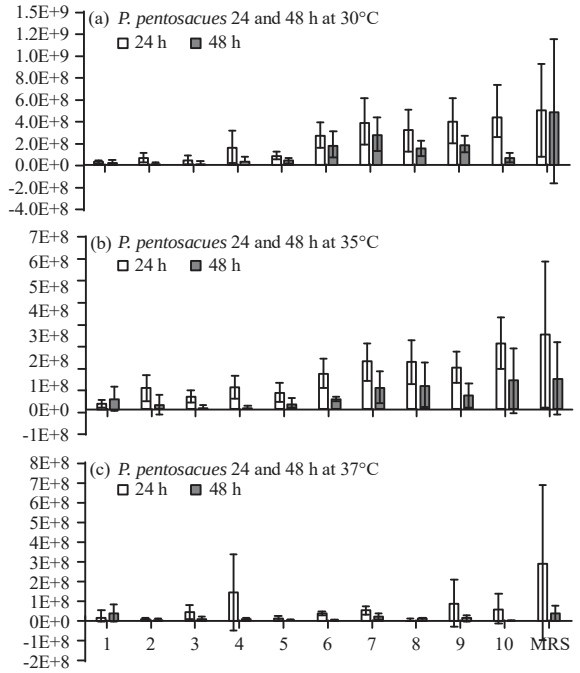

Fig. 6(a-c): Average growth of *Pediococcus pentosaceus* at 30, 35 and 37°C after 24 and 48 h

Table 2: Lactic acid bacteria cultivation summary

Strain	Media formulation	Cultivation Time (h)	Incubation temperature (°C)
Lactobacillus parabuchneri	8	24	35
Lactobacillus brevis	10	24	35
Pediococcus pentosaceus	10	24	30
Lactobacillus parabuchneri	MRS	24	37
Lactobacillus brevis	MRS	24	35
Pediococcus pentosaceus	MRS	24	30

MRS, de Man Rogosa and Sharpe media

Table 3: Media composition price in USD

	Medium 10 (USD)	MRS (USD)
Tomato 200 mL or180 g*	0.5.000	-
Yeast extract 5 g*	1.3.000	-
Molasses 10 g	0.0016	-
MRS 52 g L^{-1}	-	10.34
Cost USD L^{-1}	1.8000	10.34 (excludes shipping and handling)

*Based on higher cost, MRS, de Man Rogosa and Sharpe media

DISCUSSION

To support the cost-effective mass production of LAB as probiotics for poultry in Kuwait, the present study revealed that cultivation in media containing 5 g L^{-1} yeast extract, 200 mL L^{-1} tomato serum and 10 g L^{-1} molasses (medium 10) resulted in the best overall growth and supported a longer exponential phase of all three LAB tested. Specifically, growth of all strains was optimal after 24 h of cultivation, though each preferred a different incubation temperature (Table 2). Selection of these three relatively cheap media components was based on LAB growth characteristics and the content of different commercially available formulations. The experimental media assessed herein were compared to growth in MRS medium, which is commonly used to cultivate LAB and has replaced previous formulations by Briggs (tomato juice-based) and deMan (meat extract tomato juice-based)[12].

LAB lack various biosynthetic pathways, making them fastidious microbes that rely heavily on external nutrient sources. Yeast extract provides a rich source of nitrogen and vitamin B, which are known to enhance lactic acid production[13]. Tomato juice provides necessary carbon, protein, acidity and is considered especially important for the growth of tomato juice factor bacteria[14]. Moreover, 4'-o-(β-D-glucopyranosyl)-D-pantothenic acid is reportedly a growth factor present in tomato juice that stimulates *P. cerevisiae* growth[15] Molasses is an important agro-industrial byproduct that is high in sugar (48-50%) and other nutrients, making it a cheap carbon source. It also has a buffering capacity that is advantageous for maintaining the pH of the medium above 5.3 during fermentation[16]. Importantly, the nutrients required for growth in media containing yeast extract, tomato juice and molasses are still available after autoclaving[15]. The present study demonstrated media containing these three ingredients supports greater bacterial growth than MRS medium. Specifically, the addition of tomato juice and molasses apparently helped prolong the exponential phase of bacterial growth, which will be useful for continuous culture of LAB strains[17].

The three key media ingredients used herein are known to be relatively cost-effective in Kuwait. The price of tomatoes ranges from about 0.1-5 USD[18] kg^{-1}, depending on the season, with an annual average price of 1.3 USD[19] kg^{-1}. During the high-yield season (March–April), the average price is approximately 0.15 USD kg^{-1} and most of the harvested produce not sold ends up discarded as waste. During the low-yield season (October-January), the price of tomatoes increases to about 2.5 USD kg^{-1}. According to Informa UK Limited[20] molasses is a byproduct of the cane and beet sugar refining process, averaging approximately 160 USD t^{-1}. The price of yeast extract, on the other hand, averages from 100-260 USD kg^{-1} (e.g., from Hi Media and Sigma Aldrich).Though yeast extract is the most expensive component, its cost is offset by use of relatively larger amounts of the substantially cheaper tomato juice and molasses. Furthermore, media formulated with these three ingredients is much more cost-effective than MRS medium, which is priced at around 200 USD kg^{-1} (e.g., from Oxoid). In fact, 1 L of commercially available MRS media is 5 times more expensive than media formulation 10 (Table 3).

CONCLUSION

The results of the current study showed that media formulation 10 should serve as a beneficial substitute for producing large-scale quantities of probiotic LAB in a relatively short time period (24 h) and at a very reasonable price. Additionally, this media may also be suitable for a wider range of LAB strains, though this notion requires further investigation.

SIGNIFICANCE STATEMENT

This study reports formulation of a new medium for cultivation of probiotics using relatively cost-effective sources readily available in Kuwait. The composition of the formulated medium (tomato serum, molasses and yeast extract) supported optimal growth of three LAB strains. The results indicate that large-scale production of these bacterial strains for use as a possible feed additive in the poultry industry is economical and potentially profitable.

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REFERENCES

1. FAO., 2014. FAO Statistatical Year Book 2014. Food and Agriculture Organization, Rome, Italy.
2. Goncalves-Tenorio, A., B.N. Silva, V. Rodrigues, V. Cadavez and U. Gonzales-Barron, 2018. Prevalence of pathogens in poultry meat: A meta-analysis of European published surveys. Foods, Vol. 7, No. 5. 10.3390/foods7050069
3. Stromberg, Z.R., J.R. Johnson, J.M. Fairbrother, J. Kilbourne, A. van Goor 3rd, R. Curtiss and M. Mellata, 2017. Evaluation of *Escherichia coli* isolates from healthy chickens to determine their potential risk to poultry and human health PLoS One, Vol. 12, No. 7. 10.1371/journal.pone.0180599
4. Peralta-Sánchez, J.M., A.M. Martín-Platero, J.J. Ariza-Romero, M. Rabelo-Ruiz and M.J. Zurita-González *et al*., 2019. Egg production in poultry farming is improved by probiotic bacteria Front. Microbiol., Vol. 10. 10.3389/fmicb.2019.01042
5. Phan, T.T., L.T. Khai, N. Ogasawara, N.T. Tam, A.T. Okatani, M. Akiba and H. Hayashidani, 2005. Contamination of *Salmonella* in retail meats and shrimps in the Mekong Delta, Vietnam. J. Food Protect., 65: 1077-1080.
6. Hill, C., F. Guarner, G. Reid, G.R. Gibson and D.J. Merenstein *et al*., 2014. Expert consensus document: The International Scientific Association for probiotics and prebiotics consensus statement on the scope and appropriate use of the term probiotic. Nat. Rev. Gastroenterol. Hepatol., 11: 506-514.
7. Gibson, G.R. and M.B. Roberfroid, 1995. Dietary modulation of the human colonic microbiota: Introducing the concept of prebiotics. J. Nutr., 125: 1401-1412.
8. Vlasova, A.N., S. Kandasamy, K.S. Chattha, G. Rajashekara and L.J. Saif, 2016. Comparison of probiotic lactobacilli and bifidobacteria effects, immune responses and rotavirus vaccines and infection in different host species. Vet. Immunol. Immunopathol., 172: 72-84.
9. Research and Markets, 2019. Probiotics in animal feed market by livestock (Poultry, ruminants, swine, aquaculture, pets), source (Bacteria [*Lactobacilli*, *Streptococcus* Thermophilus, Bifidobacteria] and Yeast & Fungi), form (Dry and liquid) and region-Global forecast to 2025. https://www.researchandmarkets.com/reports/4763770/probiotics-in-animal-feed-market-by-livestock.
10. Balba, M., A. Yateem, S. Al-Zenki, T. Al-Surrayai, R. Al-Daher and Y. Al-Shayji, 2012. Isolation, characterization and evaluation of lactic acid bacteria for the development of poultry probiotics. Kuwait Institute for Scientific Research, Report No. KISR 11219.
11. Sanders, E.R., 2012. Aseptic laboratory techniques: Plating methods. J. Vis. Exp., Vol. 63. 10.3791/3064
12. De Man, J.C., M. Rogosa and M.E. Sharpe, 1960. A medium for the cultivation of *Lactobacilli*. J. Applied Bacteriol., 23: 130-135.
13. Juturu, V. and J.C. Wu, 2016. Microbial production of lactic acid: the latest development. Crit. Rev. Biotechnol., 36: 967-977.
14. Fugelsang, K.C. and C.G. Edwards, 2006. Wine Microbiology: Practical Applications and Procedures. 2nd Edn., Springer Berlin, Germany, Pages: 394.
15. Eto, M. and A. Nakagawa, 1975. Identification of a growth factor in tomato juice for a newly isolated strain of *Pediococcus cerevisiae*. J. Inst. Brew., 81: 232-236.
16. Quan, Z.X., Y.S. Jin, C.R. Yin, J.J. Lee and S.T. Lee, 2005. Hydrolyzed molasses as an external carbon source in biological nitrogen removal. Bioresour. Technol., 96: 1690-1695.
17. Tamizharasi, V., J. Srinkanth and G. Santhalakshmi, 2005. Molasses-based medium requires no nitrogen supplement for culturing three entomopathogenic fungi. J. Biol. Control, 19: 135-140.
18. Numbeo, 2019. Cost of living. https://www.numbeo.com/cost-of-living/
19. Abdal, M., M. Suleiman, N.R. Bhat and S. Jacob, 2009. Cost of cultivation of tomato in Kuwait in uncooled plastic tunnel system-A case study. World Applied Sci. J., 6: 1625-1628.
20. Informa UK Limited, 2019. World molasses & feed ingredients report. https://www.agra-net.com/agra/world-molasses-and-feed-ingredients-report/.