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Journal of Brewing and Distilling (JBD) provides rapid publication (monthly) of articles in all areas of the subject such as Fermentation Technology and Product Analysis, health effects of gin, Filtration and Packaging, Malt induced Premature Yeast Flocculation etc.

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Editors

Prof. Yujie Feng,

Huanghe Road,
Nangang District,
Harbin 150090,
Heilongjiang Province,
China.

Prof. Sunil Kumar,

I-8, Sector “c”,
East Kolkata,
Kolkata 700 107,
West Bengal,
India.

Dr. Marcus Vinicius Alves Finco,

University of Hohenheim,
Stuttgart-Germany Steckfeldstrasse,
170599,
Germany.

Dr. Noor El-Din Mahmoud,

Egyptian Petroleum Research Institute (EPRI),
1-Ahmad El Zomor st.,
Nasr City,
Cairo,
Egypt.

Prof. Edison Barbieri,

Instituto de Pesca,
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School Of Life Sciences Srtm university,

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Dr. Mahmoud Reyad Noor El-Din Mahmoud,

Egyptian Petroleum Research Institute (EPRI)
1-Ahmad El Zomor St., Nasr City, Cairo,
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Prof. Michael McAleer,

Erasmus University Rotterdam,
Rotterdam,
Netherlands.

Dr. Yogesh Chandra Sharma,

Institute of Technology, Banaras Hindu University,
Department of Applied Chemistry, Varanasi 221 005,
India.

Prof. Yujie Feng,

Harbin Institute of Technology,
No 73,Huanghe Road, Nangang District,
Harbin 150090,
Heilongjiang Province,
China.

Dr. Marina Bezhuaahvili

059, Tbilisi, Tsotne Dadiani av.34, 2, 11,
Georgia
USA.

Dr. Anup Maiti,

Pharmacy College Azamgarh, U.P.,
India.
Editorial Team

Dr. Juan Carlos González-Hernández,
Instituto Tecnológico de Morelia,
Avenida Tecnológico # 1500,
Colonia Lomas de Santiaguito,
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Dept. of Microbiology,
Osmania University,
Hyderabad,
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Dr. Kaustav Aikat,
Department of Biotechnology,
National Institute of Technology,
Mahatma Gandhi Avenue,
Durgapur-713209,
India.

Dr. Babu Joseph,
Acharya’s Bangalore B- School,
Off Magadi Main Road,
Andhra halli,
Bangalore 91,
India.

Dr. Amir Oron,
Kaplan Medical Center,
Rehovot,
Israel.

Dr. Augustine C. Ogbonna,
Department of Food Science & Technology,
University of Uyo,
Pmb 1017,
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Nigeria.

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Netherland.

Dr. Anup Maiti,
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U.p. India,
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Prof. Mohan Karuppayil,
School of Life Sciences,
S.r.t.m. University,
India.

Dr. Limpon Bora,
Dept of Biotechnology,
Dibrugarh University,
Dibrugarh-786004,
India.

Dr. Norbert Orbán,
Királyszék Pharmacy,
H-9012 Győr,
Királyszék Street,
Hungary.

Dr. Ogechukwu Akoma,
The Federal Polytechnic,
Pmb 55 Bida, 912001,
Nigeria.
Dr. Ponnan Arumugam,  
Prist University,  
Thanjavur – 403613,  
Tamil Nadu,  
India.

Dr. Abouzar Mirzaei Paiaman,  
National Iranian South Oil Company (NISOC),  
Department of Petroleum Engineering,  
Ahwaz,  
Iran.

Dr. Anup Maiti,  
Pharmacy College,  
Chandeswar,  
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Dr. Omoniyi Kehinde Israel,  
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Federal University of Technology,  
Owerri,  
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Dr. Raghunath Rashmi Krishna,  
Centre for post graduate studies,  
Jain University,  
Jayanagar,  
Bangalore-560011,  
India.

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Faculty of Bioscience Engineering,  
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Gent,  
Belgium.

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Louisiana State University,  
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New Orleans,  
U.S.A.

Dr. Shameul Alam,  
Kagawa University,  
Kagawa,  
Kitagun,  
Kagawa 761-0701,  
Japan.

Dr. Mihaela Begea,  
Institute of Food Research,  
3rd Gradistea Street,  
Bucharest,  
Romania.

Dr. Diogo Miguel Franco Dos Santos,  
Department of Chemical and Biological Engineering,  
Instituto Superior Técnico,  
Lisbon,  
Portugal.

Dr. R.A. Balikai,  
University of Agricultural Sciences,  
Dharwad-580 005,  
Karnataka,  
India.

Dr. Omoniyi Kehinde Israel,  
Ahmadu Bello University,  
School of Basic and Remedial,  
Nigeria.

Dr. Kamlendra Awasthi,  
Leibniz Institute of Polymer Research Dresden (IPF),  
Hohe Strasse 6, Dresden,  
Germany.

Dr. Kalpana Swain,  
College of Pharmaceutical Sciences,  
Mohuda,  
Berhampur,  
Orissa,  
India.

Dr. M. Abhilash,  
Dept. Of Biotechnology Engineering,  
The Oxford College of Engineering,  
Bangalore,  
India.
<table>
<thead>
<tr>
<th>Name</th>
<th>Institution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dr. Selvaraj Jagannathan</td>
<td>Pasteur Institute of India, India.</td>
</tr>
<tr>
<td>Dr. John C Abu-Kpawoh</td>
<td>Njala University, Private Mail Bag, Freetown, Sierra Leone.</td>
</tr>
<tr>
<td>Dr. S. Sandilyan</td>
<td>6a/18c 5th new street, Mayiladuthurai, Tamilnadu, India.</td>
</tr>
<tr>
<td>Dr. Ta Yeong Wu</td>
<td>School of Engineering, Monash University, Jalan Lagoon Selatan, Bandar Sunway, 46150, Selangor Darul Ehsan, Malaysia.</td>
</tr>
<tr>
<td>Dr. Shanmuga Priya</td>
<td>Manipal Institute of Technology, Dept of Chemical Engg, Mit, Manipal University-576 104, Karnataka, India.</td>
</tr>
<tr>
<td>Prof. Vasanthy Arasaratnam</td>
<td>Department of Biochemistry, Faculty of Medicine, University of Jaffna, Kokuvil, Sri Lanka.</td>
</tr>
<tr>
<td>Dr. Camelia Bonciu</td>
<td>Dunarea de Jos University, Food Science and Engineering Faculty, Domneasca Street no. 111, Galati, Romania.</td>
</tr>
<tr>
<td>Dr. Hassan Ali Zamani</td>
<td>Department of Applied Chemistry, Mashhad Branch, Islamic Azad University, Mashhad, Iran.</td>
</tr>
<tr>
<td>Dr. Manoj Kumar Mishra</td>
<td>Bhabha Pharmacy Research Institute, Bhopal c-5, Gagan Housing Society, Jatkhedi, Bhopal-403646, India.</td>
</tr>
<tr>
<td>Dr. Shah Ali Ul Qader</td>
<td>The Karachi Institute of Biotechnology and Genetic Engineering (KIBGE), University of Karachi, Karachi-75270, Pakistan.</td>
</tr>
<tr>
<td>Dr. M. Naeem</td>
<td>Botany Department, Aligarh Muslim University, Aligarh, India.</td>
</tr>
<tr>
<td>Dr. Riham Rashad Mohamed Ali</td>
<td>Faculty of Science, Department of Chemistry, Cairo University, Egypt.</td>
</tr>
<tr>
<td>Dr. Abiola Olusegun Peter</td>
<td>Dept of Science Laboratory Technology, The Polytechnic Ibadan, Nigeria.</td>
</tr>
<tr>
<td>Dr. Prakash Kumar Sarangi</td>
<td>Ravenshaw University, Qtr no-2rb/115, Road no-1 unit-9, Bhubaneswar, India-751022&quot;, India.</td>
</tr>
<tr>
<td>Dr. Xiao-Qing Hu</td>
<td>State Key Lab of Food Science &amp; Technology (China), Jiangnan University, China.</td>
</tr>
<tr>
<td>Dr. Melina Nicole Kyranides</td>
<td>Department of Psychology, University of Cyprus, P.o.box 20537, Nicosia, Cyprus.</td>
</tr>
</tbody>
</table>
Dr. Carolyn Ross,
Washington State University,
School of Food Science,
FSHN 122, PO Box 646376,
Pullman WA 99164-6376
Washington.

Professor S. Chandraju,
University of Mysore
Sir. M. V. PG center,
Tubinakere, Mandya-671402, Karnataka,
India.

Dr. Asif Tanveer,
Department of Agronomy,
University of Agriculture,
Faisalabad,
Pakistan.

Dr. Norbert Orbán,
Királyszék Pharmacy,
H-9012 Győr,
Királyszék street. 33,
Hungary.

Mr. Ogechukwu Akoma,
The Federal Polytechnic PMB 55 Bida,
912001, Nigeria.

Dr. Ponnan Arumugam,
PRIST University, Thanjavur – 403613,
Tamil Nadu,
India.

Mr. Abouzar Mirzaei Paiaman,
National Iranian South Oil Company (NISOC)
Ahwaz,
NISOC, Department of petroleum engineering,
Iran.

Dr. Anup Maiti,
Pharmacy College,
Itura,Chandeswar,
Uttar Pradesh,
India.

Mr. Omoniyi Kehinde Israel,
Ahmadu Bello University,
zaria,
Nigeria.

Dr. Ikenna Nwachukwu,
University Sains Malaysia;
Federal University of Technology,
Owerri,
Nigeria.

Prof. Yujie Feng,
No 73,Huanghe Road,
Nangang District, Harbin 150090,
Heilongjiang Province, P. R.,
China.

Mis. Raghunath Rashmi Krishna,
Centre for post graduate studies,
Jain University,18/3, 9th main, 3rd block,
Jayanagar, Bangalore-560011,
India.

Dr. Ana Valenzuela,
Postdoctoral researcher in Belgium,
Faculty of Bioscience Engineering Gent,
University Coupure Links 653 - 9000
Gent,
Belgium.

Dr. Gyanendra Singh,
Louisiana State University Health Sciences Center
(LSUHSC),
New Orleans, LA 70112,
USA.

Dr. G.Gnana kumar,
Chonbuk National University, Jeonju,
Republic of Korea.

Dr. Mahmoud Reyad Noor El-Din Mahmoud,
Egyptian Petroleum Research Institute (EPRI),
1-Ahmad El Zomor St., Nasr City, Cairo,
Egypt.

Dr. Shameul Alam,
Kagawa University, Kagawa, Seiun sou-101,
Ikenobe2444-2, Miki-cho, Kitagun,
kagawa 761-0701,
Japan.

Dr. Mihaela Begea,
Institute of food research,3rd gradistea street,
Bloc a9, sc. A, ap. 4, s4, Bucharest,
Romania.
Mr. Diogo Miguel Franco dos Santos,
Electrochemistry of Materials Group,
Institute of Materials and Surfaces Science and Engineering,
Department of Chemical and Biological Engineering,
Instituto Superior Técnico, TU Lisbon, Portugal.

Miss. Kalpana Swain,
College of Pharmaceutical Sciences,
Mohuda, Berhampur, Orissa, India.

Dr. Abhilash M,
Dept. of Biotechnology engineering,
The Oxford college of Engineering,
Bangalore, India.

Dr. Selvaraj Jagannathan,
Pasteur Institute of India
TCARV division, Coonoor-643 103,
The Nilgiris,
Tamil Nadu, India.

Dr. Jamshid Farmani,
Department of Food Science & Engineering,
Faculty of Agricultural Engineering & Technology,
University College of Agriculture & Natural Resources,
University of Tehran, Karaj-Iran.

Dr. John C Abu-Kpawoh,
Njala University Private Mail Bag,
Freetown,
Sierra Leone.

Prof. Edison Barbieri,
Instituto de Pesca
Av. Prof. Besnard s/n. Cananéia (SP),
Brazil.

Mr. S. Sandilyan,
6A/18C 5th New Street, Mayiladuthurai,
Tamilnadu, India.

Dr. Ta Yeong Wu,
School of Engineering,
Monash University,
Jalan Lagoon Selatan,
Bandar Sunway, 46150,
Selangor Darul Ehsan, Malaysia.

Miss. S. Shanmuga Priya,
Manipal Institute of Technology,
Dept of Chemical Engg, MIT,
Manipal University-576 104, Karnataka, India.

Prof. Vasanthy Arasaratnam,
University of Jaffna,
Department of Biochemistry,
Faculty of Medicine, Kokuvil.
Sri Lanka.

Dr. Sunil Kumar,
I-8, Sector “C”, East Kolkata,
New Township, E.M. Bypass,
Kolkata 700 107, West Bengal,
India.

Dr. Camelia Bonciu
Dunarea de Jos University,
Food Science and Engineering Faculty
Domneasca Street no. 111, Galati,
Romania.

Dr. Hassan Ali Zamani,
Department of Applied Chemistry,
Mashhad branch,
Islamic Azad University,
Mashhad, Iran.

Dr. Manoj Kumar Mishra,
Bhabha Pharmacy Research Institute, Bhopal
C-5, Gagan Housing Society,
Jatkhedi, Bhopal-403646,
India.

Dr. Shah Ali Ul Qader,
The Karachi Institute of Biotechnology and Genetic Engineering (KIBGE),
University of Karachi, Karachi-75270, Pakistan.
Dr. M. Naeem,  
Aligarh Muslim University, Aligarh,  
Botany Department, AMU, Aligarh, India.

Dr. Riham Rashad Mohamed Ali,  
Cairo university  
Faculty of science-Department of chemistry, Egypt.

Dr. Abiola Olusegun Peter,  
Ravenshaw University,  
Qtr no-2rb/115, road no-1, unit-9, bhubaneswar India.

Dr. Xiao-Qing Hu,  
State Key Lab of Food Science & Technology (China) Jiangnan University, China.

Dr. Melina Nicole Kyranides,  
Department of Psychology, University of Cyprus, P.O.Box 20537, CY 1678, Nicosia, Cyprus.

Dr. Carolyn Ross,  
Washington State University, School of Food Science FSHN 122, PO Box 646376, Pullman WA 99164-6376.

Dr. Jun Wei,  
Sanford-Burnham Medical Research Institute 10901 North Torrey Pine Road.

Dr. Harpreet Singh Grover,  
Dr. B R Ambedkar National Institute of Technology, Jalandhar H No 7, Room No 150, G T Road Bye Pass, NIT Jalandhar 144011.

Dr. Aline Augusti Boligon  
Universidade Federal de Santa Maria (RS, Brazil) Coronel Niederauer 1565, 209, Brazil.

Dr. Amir Oron,  
Kaplan Medical Center, Rehovot, Israel.

Dr. Camelia Bonciu,  
Dunarea de Jos University, Food Science and Engineering Faculty Domneasca Street no. 111, Galati, Romania.

Dr. Babu Joseph,  
Acharya’s Bangalore B- School Off Magadi Main Road, AndhraHalli, Bangalore 91

Dr. Augustine C. Ogbonna,  
University of uyo, Nigeria  
Department Of Food Science & Technology, University of uyo, pmb 1017, uyo, Nigeria.

Dr. Radha Mahendran,  
Bioinformatics Dept, VelsUniversity, Old Pallavaram, Chennai-117, India.

Dr. S. Chandraju,  
University of Mysore  
Sir. M. V. PG center, University of Mysore, Tubinakere, Mandya-671402, Karnataka, India.

Dr. Asif Tanveer,  
Department of Agronomy, University of Agriculture, Faisalabad, Pakistan.

Dr. Santiago Cuesta-Lopez,  
Polytechnic University of Madrid.  
ETSI Industriales, 2, Jose Gutierrez Abascal, 28006, Madrid.
Dr. Carlos Alberto Padrón Pereira,
Asociación Revista Venezolana de Ciencia y Tecnología de Alimentos
Avenida Andrés Bello Nº 101-79, entre Calles Independencia y Libertad,
Parroquia Urbana El Socorro, Municipio Valencia, Valencia, Estado Carabobo,
República Bolivariana de Venezuela.

Dr. Juan Carlos González-Hernández,
Instituto Tecnológico de Morelia,
Avenida Tecnológico # 1500,
Colonia Lomas de Santiaguito, Morelia, Michoacán, México.

Dr. Kaustav Aikat,
National Institute of Technology, Durgapur,
Department of Biotechnology, National Institute of Technology, Mahatma Gandhi Avenue, Durgapur-713209,
India.

Prof Dr Qadar Bakhsh,
Qurtuba University
Sector-K 1, Phase-3, Hayatabad, Peshawar,
Pakistan

Dr Gurvinder Singh Kocher,
Department of Microbiology,
Punjab Agricultural University,
Ludhiana,
India.

Dr. Selvaraj Jagannathan,
Pasteur Institute of India
TCARV division, Coonoor-643 103,
The Nilgiris, Tamil Nadu,
India.

Dr. Kamlendra Awasthi,
Leibniz Institute of Polymer Research Dresden (IPF)
Hohe Strasse 6, D-01069, Dresden,
Germany.

Dr. Prakash Kumar Sarangi,
Ravenshaw university
Qtr no-2rb/115, road no-1, unit-9, bhubaneswar,
India.

Dr. Xiao-Qing Hu,
State Key Lab of Food Science & Technology (China),
Jiangnan University, 1800 Li-Hu Ave., Wu-Xi 214122, P.R.C.,
China.

Prof. Layioyo Ola Oyekunle
University of Lagos
Department of Chemical Engineering,
Akoka-Yaba,
Lagos 101017, Nigeria.

Prof. Linga Venkateswar Rao,
Osmania university,
Head, dept. Of microbiology,
Osmania University,
Hyderabad.

Dr. M. Naeem,
Aligarh Muslim University,
Aligarh, India
Botany Department, AMU,
Aligarh,
India.

Dr. Radha Mahendran,
Vels University,
Bioinformatics Dept,
Old Pallavaram,
Chennai-117

Mr. Shameul Alam,
Kagawa University, Kagawa, Japan
Seiun sou-101, Ikenobe2444-2, Miki-cho,
Kitagun, kagawa 761-0701,
Japan.

Dr. Pradeep Parihar
Lovely Professional University
Room No. 406, Block-2, Division of Academic Affairs,
Lovely Professional University,
Near Chaheru Railway Bridge,
Phagwara-144002, Punjab,
India.

Dr. Priti Gupta,
Indian Agricultural Research institute,
Pusa road, New delhi -12,
India.
Ms. Rashmi Raghunath,  
Centre for post graduate studies, Jain University,  
18/3, 9th main, 3rd block, Jayanagar, Bangalore-560011,  
India.

Dr. Ngono Ngane Rosalie Annie,  
University of Douala,  
Po Box 24157 Douala,  
Cameroon,

Prof. S. Mohan Karuppayil,  
School Of Life Sciences  
Srtm university.

Dr. Saeed Zaker Bostanabad,  
IAU university, parand branch, Tehran,  
Parand new city, Tehran, Iran.

Dr. Taurai Mutanda,  
Durban University of Technology  
Dept of Biotech and Food Tech DUT,  
P.O. Box 1334 Durban,  
S. Africa

Dr. Hassan Ali Zamani,  
Mashhad branch,  
Islamic Azad University,  
Department of Applied Chemistry, Iran.

Dr. Rajarshi Pal,  
Stempeutics Research  
Lot 3-I-7, Enterprise 4,  
Technology Park Malaysia,  
Bukit Jalil,  
57000 Kuala Lumpur,  
Malaysia.

Dr. Giampiero La Rocca,  
University of Palermo,  
BIONEC Dept,  
Human Anatomy Section  
Via del Vespro 129,  
90127 Palermo,  
Italy.

Dr. Ranjeet Singh Mahla,  
CCMB, India  
LaCONES, CCMB, Habsigda,  
Uppal Road, Hyderabad, India.

Dr. Zhong-Dong Shi,  
Sloan-Kettering Institute and City College of New York,  
New York, NY USA.

Dr. Amin Akhavan Tabassi,  
University Science Malaysia,  
School of Housing, Building & Planning,  
Universiti Sains Malaysia,  
11800, Penang, Malaysia.

Dr. Hosni,  
Laboratoire des Substances Naturelles,  
Institut National de Recherche et d’Analyse Physico-chemique (INRAP),  
Biotechpôle, Sidi Thabet.

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Aligarh Muslim University,  
Department of Botany, Amu, Aligarh.

Dr. Siniša Srečec,  
Križevci College of Agriculture,  
M. Demerca 1, Križevci, HR-48260, Croatia.

Dr. Serkan Selli,  
Cukurova University, Agricultural Faculty,  
Food Engineering Department,  
Cukurova University, Agricultural Faculty, Turkey.

Mrs. Ruth Gomes de Figueiredo Gadelha,  
Universidade Federal da Paraíba,  
Av. Odon Bezerra, 279 apt 12 tambiá João Pessoa.

Dr. NSO Emmanuel Jong,  
National School of Agro - Industrial Sciences (ENSAI) of Ngaoundere,  
University of Ngaoundere, Cameroon.
Dr. Nandan Kumar Jana,
Heritage Institute of Technology,
Dept. of Biotechnology,
994, Chowbaga Road, Anandapur,
Kolkata, WB,
India.

Dr. Laleen Karunanayake,
University of Sri Jayewardenepura
Gangodawila, nuugeoda,
Sri Lanka.

Dr. Tapan Kumar Ghosh,
Heritage Institute of Technology
Anandapur, P.O – East Kolkata Township, Kolkata – 700107,
India.

Dr. Shashi Pandey Rai,
Botany Department, BHU, Varanasi,
India.

Dr. Brian Gibson,
VTT, Technical Research Centre of Finland,
Tietotie 2, Espoo, PO Box 1000, FI-02044 VTT,
Finland.

Prof. Asrhafl A M El-sayed,
Atomic energy authority,a
Cairo,
Egypt.

Dr. Abhishek Mathur,
Sai Institute of Paramedical & Allied Sciences,
26-A Rajpur road, Adjacent Hotel Meedo Grand,
Dehradun (U.K) - 248001,
India.

Mr. Steven Vendeland,
Ambassador’s Ink
2092 Edenhall Dr., Lyndhurst OH 44124

Dr. Huseyin Erten,
Cukurova University
Cukurova University, Faculty of Agriculture,
Department of Food Engineering,
Turkey.
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Application of high gravity fermentation worts to the brewing of industrial opaque beer
Misihairabgwi, Jane M., Kudita, Ivy and Zvauya, Remigio
Application of high gravity fermentation worts to the brewing of industrial opaque beer

Misihairabgwi, Jane M.1*, Kudita, Ivy2 and Zvauya, Remigio3

1Department of Biochemistry, School of Medicine, University of Namibia, Namibia.
2Chibuku Breweries, P. O. Box 3304, Southerton, Harare, Zimbabwe.
3Medical School, University of Birmingham, Edgbaston B15 2TT, Birmingham, England.

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Fermentation of worts with high concentrations of fermentable sugars has become common practice in large clear beer breweries that use high gravity worts containing 16-18% (w/v) dissolved solids to obtain high alcohol contents. Normal and high gravity worts, ranging from 12 to 20% (w/v) initial dissolved solid contents were used in industrial opaque beer brewing and the biochemical characteristics of the brews were determined. Characteristics of the beers varied according to wort gravity. Decreases in dissolved solids and maltose contents were recorded during fermentation for all the brews. Higher residual dissolved solids, ranging from 6.5 to 11% (w/v), were recorded for the high gravity brews as compared to the normal brews, which had 3.5% (w/v) residual dissolved solids. An increase in ethanol levels was recorded during alcoholic fermentation for all the brews and the maximum ethanol levels, recorded after 72 h of fermentation for all the brews, were 3.5 and 6.0% (v/v) for the normal and high gravity beers, respectively. The optimal wort dissolved solids content recorded with regard to ethanol productivity was 18% (w/v). Increases in lactic acid and acetic acid levels were recorded for the brews, reaching maximum levels of 2.25 and 0.48 g/L, respectively, after 96 h of fermentation. No propionic acid was detected in any of the brews. Considering the quality of the beer, there was no settling observed for all the beers at 48 h of aging. Viscosity values, ranging from 100 to 190 mPa.s were higher than the expected levels for the industrial opaque beer (60-80 mPa.s) and values recorded for head were within the expected range for the industrial opaque beer (3 to 4).

Key words: High gravity, opaque beer, dissolved solids, ethanol.

INTRODUCTION

Beer is a product of alcoholic fermentation of worts obtained from malted cereals, unmalted cereals and sugar sources (adjuncts) (Mathias et al., 2014). The production of opaque beer on an industrial scale in Southern African countries such as South Africa and Zimbabwe is well described and documented (Steinkraus, 1996; Togo et al., 2002). Chibuku is a popular industrialised opaque beer in Zimbabwe (Kutyauniso et al., 2009). The process of brewing of Zimbabwean industrial opaque beer begins with the cooking of the unmalted cereal adjunct, together
with commercial lactic acid for souring. Mashing is carried out by incubating the cooked adjunct with malt, then straining is carried out. The mixture is then heated for pasteurisation, cooled and more malt is added for the second conversion to produce the final brewing wort. The mixture is left to cool, after which active dried yeast is added to commence the alcoholic fermentation. The beer is left to cool to between 26 and 28°C and at this stage it is ready for consumption. The industrial process makes use of *Saccharomyces cerevisiae* yeast for alcoholic fermentation (Kutuayiripo et al., 2009).

Wort composition is extremely important in determining microbial activity and final beer quality (Mathias et al., 2014). Traditional brewing of clear beer results in the production of beers of 4-5% (v/v) ethanol from worts of 11 to 12% (w/v) (Casey et al., 1984; Puligundla et al., 2011). High gravity (HG) fermentation technology, which involves the fermentation of media containing normally 16 to 18% (w/v) initial dissolved solids, has been adopted in many clear beer breweries nationwide (Younis and Stewart, 1999; Patkova et al., 2000; Dragone et al., 2007; Mathias et al., 2014). Ethanol levels in the range of 9 to 11% (v/v) can be achieved when HG technology is applied to the brewing of clear beer. The high gravity beers are then diluted to equivalent ethanol levels to the normal beers (Patkova et al., 2000). The advantages of high gravity brewing are well-documented for clear beer production and include considerable saving of process water, efficient utilisation of fermenter space and production of more alcohol per given plant capacity and labour costs (Erten et al., 2007; Dragone et al., 2007; Puligundla et al., 2011). In the brewing of clear beer, high gravity fermentation technology has proved to be a technique that allows for high utilisation of raw materials and equipment while maintaining the quality of the beer. Disadvantages of HG technology reported for clear beer brewing include longer fermentation times to allow efficient fermentable substrate use, decreased foam stability and possible different flavour characteristics from normal gravity fermentations (Fernandez et al., 1985; Younis and Stewart, 1999; Erten et al., 2007; Dragone et al., 2007). While HG technology has many advantages, most research has mainly been directed to its application in clear beer. Our previous work reported the application of HG technology to opaque beer following the traditional procedure used in rural homes (Bvochora and Zvauya, 2001). Results of our previous studies showed that the application of HG fermentation technology to the brewing of traditional opaque beer resulted in the production of 4.74% (v/v) ethanol, higher than the 2.66% (v/v) recorded under normal fermentation conditions (Bvochora and Zvauya, 2001). To the best of our knowledge, there are no published reports on the application of HG technology to the brewing of industrial opaque beer. Potentially, the technique can be applied to industrial opaque beer brewing and improve the productivity of fermentation. The aim of this study therefore was to determine the biochemical characteristics of industrial opaque beer brewed using high gravity fermentation technology.

**MATERIALS AND METHODS**

The work was carried out at an opaque beer brewery in Harare, Zimbabwe. The yeast used in this study was *S. cerevisiae*, strain Y2/49, obtained from an opaque beer brewery in Harare, Zimbabwe. The sucrose used in the study was household sucrose obtained from a sugar refinery in Harare, Zimbabwe. The wort used in the study was obtained from an industrial fermentation process at an opaque beer factory.

**Preparation of high gravity wort**

Samples of wort (4 L) were collected from a 75 000 L brewing tank at 50°C, just before the addition of yeast. The wort samples were left to cool to 35°C. The wort samples were inoculated with dry *S. cerevisiae* yeast (1.2 g), and varying amounts of sucrose were added, with stirring, to obtain a range of initial dissolved solid contents of 12 (w/v) to 20% (w/v). An Agato N1 Brix sugar refractometer was used to determine the initial dissolved solids contents.

**Fermentation and opaque beer sample collection**

Fermentations were carried out in 5 L plastic buckets covered with aluminium foil at room temperature (approximately 27°C). After thorough mixing, samples (250 ml) were collected at intervals, filtered through Whatman 1 filter paper and kept at -20°C until required for analysis. All fermentations were carried out in duplicate.

**Analysis of opaque beer samples**

pH was measured immediately after sample collection, at room temperature (approximately 27°C), using a Mettler Delta pH meter. Dissolved solids contents were measured on the opaque beer supernatant using an Agato N1 Brix sugar refractometer. Opaque beer supernatant was used for analysis to determine sugar, ethanol and organic acid levels with fermentation time by HPLC analysis. HPLC analysis was carried out on a Shimadzu HPLC using an Aminex HPX 87H column, with column temperature 65°C, flow rate 0.6 ml/min and a sample injection volume of 5 μl. H2SO4 (4 mM) was used as the mobile phase. A refractive index detector was used at a wavelength of 215 nm. Samples were filtered through 0.45 μm syringe filters before HPLC analysis. A standard mixture was prepared from 0.1% v/v concentrations of formic acid, lactic acid, propionic acid and butyric acid and was run together with the beer samples to determine organic acid concentrations in the beers.

Beer quality determinants such as viscosity, head and settling were determined. A sample of opaque beer (250 ml) was placed in a plastic container and the viscosity of the beer was measured using a Brookfield Dial Viscometer at room temperature (approximately 27°C), according to the instructions of the manufacturer. The head of the beer was measured by placing 200 ml of opaque beer in a 500 ml glass measuring cylinder and recording the volume occupied by the head, which is the foam of the beer. Head was expressed as a percentage of the beer volume. To determine the settling of the solids in the beer, an opaque beer sample (200 ml) was placed in a 500 ml glass measuring cylinder and left to stand for 30 min. The amount of liquid above the settled solids of the opaque beer was noted and expressed as a percentage of the beer volume. The higher the amount of liquid recorded, the higher the settling value of the beer.
RESULTS AND DISCUSSION

The results presented in this section refer to biochemical changes occurring during the alcoholic fermentation stage of industrial opaque beer brewing. All results are means of duplicate determinations. Where no error bars are visible, standard deviation = 0.

Changes in pH

A general decrease in pH was recorded with fermentation time for the brews at all the initial dissolved solid contents used (Figure 1). pH values dropped from an initial maximum of 5.25 to a final minimum of 3.50. There was apparently no relationship between the rate of pH decrease and the initial dissolved solids contents of the wort for the range studied. Thomas and co-workers (2002) reported poor growth of S. cerevisiae as a result of a very rapid decrease of pH to values below 2.5 and that the inhibitory effect of low pH on yeast growth was compounded by the presence of organic acids such as acetic and lactic acid in the medium. Low pH values, below 4.5, may have had inhibitory effects on yeast growth as no buffering system was present in the beers. Raising the external pH places less stress on yeast cells (Thomas et al., 2002).

Changes in dissolved solid contents

A decrease in dissolved solid contents was recorded during fermentation for all the brews due to the use of sugars during fermentation (Figure 2). For all the fermentations, there were residual dissolved solids when fermentation was terminated. Fermentable sugars remained in the wort as a result of incomplete fermentation. Dragone and co-workers (2007) reported similar results when determining the effect of wort original gravity on fermentation performance. Residual dissolved solid contents were higher for the HG brews as compared to the normal brew where an initial dissolved solids content of 12% (w/v) was used. The residual dissolved solids content in the normal Zimbabwean industrial opaque beer is about 3.6% (w/v) (Bvochora and Zvauya, 2001), which is lower than levels obtained in the HG beers, ranging from 6.5 to 11% (w/v). There is therefore, a need to optimize fermentation conditions to allow for maximal use of dissolved solids during fermentation to result in a product of acceptable quality as high levels of residual dissolved solids may result in an unacceptably sweet beer.
Changes in ethanol

An increase in ethanol levels was recorded during alcoholic fermentation for all the brews and the maximum ethanol level recorded was 6% (v/v) after 96 h of fermentation when an initial dissolved solids content of 18% w/v was used (Figure 3). Under normal fermentation conditions (12% w/v dissolved solids), the maximum ethanol level obtained was 3.5% (v/v) after 72 h. A maximum ethanol level of 4.74% (w/v) was obtained in our previous studies where we applied HG technology to the brewing of traditional opaque beer, at an initial dissolved solids content of 16.89% (w/v) (Bvchora and Zvauya, 2001). Patkova and co-workers (2000) reported ethanol levels of about 9% (v/v) at an initial dissolved solids content of 16.89% (w/v) (Bvchora and Zvauya, 2001). Patkova and co-workers (2000) reported ethanol levels of about 9% (v/v) at an initial dissolved solids content of 20°Plato, after about 8 days of fermentation when HG technology was applied to clear beer brewing. There may be a need to increase yeast pitching levels proportionally to the increase in wort gravity and to optimize temperature conditions, as well as increase nutritional conditions if higher levels of ethanol are to be attained. Increased levels of new yeast cell mass synthesis have been reported when a nitrogen source is added to very high gravity worts (Casey et al., 1984; Betite et al., 2012; Mathias et al., 2014).

Changes in organic acid contents

An increase in lactic acid was recorded during the alcoholic fermentation for all the brews as proliferation of lactic acid bacteria occurred (Figure 4). Despite the addition of commercially prepared lactic acid during wort preparation, spontaneous lactic acid fermentation has been demonstrated to occur during the later stages of Chibuku brewing (Togo et al., 2002). There were no apparent differences in the rate of increase of the lactic acid for the brews at varying initial dissolved solid contents. An increase in acetic acid was also recorded, reaching a maximum level of 0.48 g/L after 96 h of fermentation at an initial dissolved solids content of 18% (w/v) (Figure 5). There was no acetic acid detected in the first 20 h for the brews with initial dissolved solid contents of 18 and 20% (w/v). Thomas and co-workers (2002) reported that acetic acid (167 mM) and lactic acid (548 mM) completely inhibited growth of S. cerevisiae both in minimal medium and nutritionally supplemented media. However, the yeast grew when the pH of the medium containing acetic acid or lactic acid was adjusted to 4.5. Based on the report by Thomas et al. (2002), acetic and lactic acid levels may have been inhibitory to yeast growth in the beers in this study. Lactic acid levels above 0.5% (v/v) have been reported to be undesirable in Chibuku (Kutyauripo et al., 2009). No propionic acid was detected by HPLC analysis. Propionic acid imparts off flavours in beers. Organic acid contents are useful as an indicator of spoilage and acceptability of opaque beer (Kutyauripo et al., 2009).

*S. cerevisiae* yeasts, under aerobic conditions, can use ethanol and short-chain organic acids, such as acetic...
Figure 3. Levels of ethanol during the alcoholic fermentation stage of industrial opaque beer at various dissolved solid contents. Initial dissolved solid contents: 12 % (w/v), 14 % (w/v), 16 % (w/v), 18 % (w/v), 20 % (w/v).

Figure 4. Levels of lactic acid during the alcoholic fermentation stage of industrial opaque beer at various dissolved solid contents. Initial dissolved solid contents: 12 % (w/v), 14 % (w/v), 16 % (w/v), 18 % (w/v), 20 % (w/v).
acid and lactic acid, as carbon sources, which may explain the slight decreases in ethanol and lactic acids in some beers after 72 h fermentation (Thomas et al., 2002).

Changes in maltose

A general decrease in maltose was recorded during the alcoholic fermentation for all the brews due to the use of the sugars by fermenting microbes (Figure 6). Constant levels of maltose were recorded during the first 24 and 48 h of fermentation for brews with initial dissolved solids contents of 18 (w/v) and 20% (w/v), respectively. This may have been due to either the high levels of sugars initially present inhibiting yeast fermentation or the need to induce maltase enzyme under the conditions. Maltose levels were negligible for brews with initial dissolved solid levels of 12 and 14 % (w/v) at 96 h of fermentation.

Determination of beer quality parameters

Considering the quality of the beer, there was no settling observed for all the beers. Settling is an undesirable characteristic in opaque beer brewing. Viscosity values were higher than the expected levels for the industrial beer and values recorded for head were within the expected range (Table 1). While our values for head were within the expected range, other workers have reported that HG fermentation results in a reduction in the formation and stability of head in beer due to greater losses of hydrophobic proteins in the beers (Cooper et al., 1998; Patkova et al., 2000). Different brews of opaque beer will vary in product quality depending on the ratio and quality of raw materials used and initial wort gravity. The need arises to employ a taste panel to determine the quality and acceptability of the beer. To reduce the adverse effects of HG fermentation on the behaviour of yeast, it may be necessary to modify the medium by adding various nutrients such as amino acids, unsaturated fatty acids and sterols (Fernandez et al., 1985).

Conclusion

The study showed that higher ethanol levels can be achieved when HG technology is applied to the brewing of industrial opaque beer. However, further research would be necessary to determine suitable substrates for attaining the desired gravities and suitable diluents to produce beers with acceptable qualities and similar ethanol levels with the normal opaque beers. The study
Figure 6. Levels of maltose during the alcoholic fermentation stage of industrial opaque beer at various dissolved solid contents. Initial dissolved solid contents: 12% (w/v), 14% (w/v), 16% (w/v), 18% (w/v), 20% (w/v).

Table 1. Viscosity and head values of beers at 48 h of beer aging.

<table>
<thead>
<tr>
<th>Initial dissolved solids (% w/v)</th>
<th>12</th>
<th>14</th>
<th>16</th>
<th>18</th>
<th>20</th>
<th>Expected values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Viscosity (mPa.s)</td>
<td>110</td>
<td>100</td>
<td>190</td>
<td>118</td>
<td>112</td>
<td>60-80</td>
</tr>
<tr>
<td>Head (%)</td>
<td>2.5</td>
<td>2.5</td>
<td>4</td>
<td>4</td>
<td>5</td>
<td>3-4</td>
</tr>
</tbody>
</table>

forms a basis for further investigation, which includes scaling up the process, carrying out a sensory evaluation of the beers and aiming to produce beers with acceptable properties.

Conflict of interests

The authors did not declare any conflict of interest.

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