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Editor-in-Chief

Dr. VK Joshi

(FBRSI, FISHA)

Adjunct Professor, Shoolini University, Solan

Prof. and Head (Retd)

Department of Food Science and Technology,

Dr YS Parmar University of Horticulture and Forestry

Nauni, Solan, Himachal Pradesh, INDIA

Wine consultant CSIR (Palampur), Editor WJB, IJFN

Member FSSAI, AFST

vkjoshipt@rediffmail.com / editorijfft@gmail.com

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INTERNATIONAL JOURNAL OF FOOD AND FERMENTATION TECHNOLOGY

About the Journal

The Journal publishes research papers on all the subjects related to the Food and Fermentation Technology. All aspects of food and fermentation technology would be considered. Papers on bio-technology, bio-chemical engineering, toxicological aspects having direct bearing or are related with food would be welcomed. R & D work related to the fermentation covering microbiology, bio-chemical aspects, genetics, indigenous fermented foods, toxicology or nutritive value would also be included. Articles highlighting the food standards and safety issues will be given special emphasis. Preparation and evaluation of alcoholic beverages would be an important aspect of the articles published. The review on any aspect of food processing, composition, nutrition, and fermentation would be considered. Management of food processing industrial waste would be an integral component of the papers that could be submitted. The other aspects of food processing like low temperature preservation, dehydration, thermal processing, irradiation, emerging technologies viz., ohmic preservative, pulse electric field, high pressure preservation, enzymology, microbiological quality, food safety and standards, and food engineering, shall also be considered.

In brief, the International Journal of Food and Fermentation Technology, a half yearly journal, publishes original research papers, short communications and review papers on topics which include the following but is not limited to:

- Food microbiology • Bio-chemical aspects of food • Genetic and genetically modified foods • Enology • Indigenous fermented foods
- Toxicology, safety and quality • Food processing • Fermentation technology • Food engineering • Quality assurance • Food preservation
- Food additives • Food marketing • Marketing of processed products • Tourism in relation food and food processing.

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(FBRSI, FISHA)

Adjunct Professor, Shoolini University, Solan
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Department of Food Science and Technology,
Dr YS Parmar University of Horticulture and Forestry
Nauni, Solan, Himachal Pradesh, INDIA

Wine consultant, former Consultant CSIR (Palampur), Editor WJB, IJFN
Member FSSAI, AFST

vkjoshipt@rediffmail.com / editorijfft@gmail.com

Honorary Editor(s)

Dr (Mrs) Sunita Garg

National Institute of Science Communication and Information Resources,
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sunitag@niscair.res.in

Dr N S Thakur

Department of Food Science and Technology
Dr. YS.Parmar University of Horticulture and Forestry, Nauni, Solan,
Himachal Pradesh, INDIA
nsthakur1964@gmail.com

Executive Editorial Board

Prof. R M Sharma

Division of Fruits & Horticultural Technology
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New Delhi, INDIA
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drgargi.dey@gmail.com

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Department of Microbiology, Yogi Vemana University
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lvereddy@gmail.com/lvereddy@yahoo.com

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Department of Biotechnology and Bioinformatics
Padmashree Dr. D.Y. Patil University, INDIA
madhavirevankar@rediffmail.com

Dr Om Prakash Chauhan (Fruit and Vegetable Processing)

Fruits and Vegetables Technology Division
Defence Food Research Laboratory Siddharthanagar,
Mysore, INDIA
opchauhan@gmail.com

Dr. K. Jayaram (Indigenous Fermentation Technology)

Microbial Reserves Division
Institute of Bioresources and Sustainable Development
Imphal, Manipur, INDIA
saccheromyces@rediffmail.com

Dr S Kapoor (Mushroom Production)

Department of Microbiology, College of Basic Sciences and
Humanities, PAU, Ludhiana, INDIA
skapoorpau@pau.edu

Dr Satish Kumar Sharma (Fruit and Vegetable Processing)

GB Pant University of Agriculture & Technology, Hill Campus,
Ranichauri, Distt Tehri-Garhwal, Uttarakhand, INDIA
drsatis10@yahoo.com

Dr. Seema Garcha (Probiotic Foods)

Department of Microbiology
Punjab Agricultural University, Ludhiana, INDIA
seemagarcha@hotmail.com/seemagarcha@gmail.com

Dr Somesh Sharma (Fermentation Technology)

Department of Food Technology
School of Bio engineering and Food Technology
Shoolini University, Solan HP, INDIA
sharmawine@gmail.com

Dr Wamik Azmi (Food Biotechnology)

Department of Biotechnology, Himachal Pradesh University,
Summer Hill, Shimla, Himachal Pradesh, INDIA
wamikazmi@rediffmail.com

Dr Shashi Bhushan (Fermentation, Health Related Products)

Division of Biotechnology, Institute of Himalayan Bio-resource
Technology (CSIR), Palampur, Distt Kangra,
Himachal Pradesh, INDIA
sbhushan@ihbt.res.in

Dr S K Patyal

Department of Entomology and Apiculture,
DR. Y S Parmar University of Horticulture and Forestry,
Nauni Solan, INDIA
patyalsk@yahoo.co.in

Dr. R Kumar (Food Scientist)

Defence Food Research Laboratory
Scientific Panel Member
Food Safety Standards Authority of India
Mysore, INDIA
kumardfrl@gmail.com

Dr. Neetu Kumra Taneja (Molecular Microbiology)

Department of Basic and Applied Sciences
National Institute of Food Technology Entrepreneurship and
Management
Sonapat, Haryana, INDIA
neetu.niftem@gmail.com

Dr. Savitri (Biotechnology)

Department of Biotechnology
Himachal Pradesh University
Summer Hill, Shimla, HP, INDIA
savvy2000in@yahoo.com

Dr. Naveen Batra (Biotechnology)

Department of Biotechnology
GGSDS College
Chandigarh, INDIA
navneet05@gmail.com

Dr. G.S. Meena

Scientist
Indian Council of Agricultural Research- National Dairy Re-
search Institute, Karnal, Haryana, INDIA
gsiitkgrp@gmail.com

Dr. Prasad Jayprakash Rasane

Assistant Professor (Food Technology) and Entrepreneurship
Coordinator (Agriculture)
Lovely Professional University,
Jalandhar, Punjab, INDIA
rasaneprasad@gmail.com

Dr. Vikas Kumar

Assistant Food Technologist,
Department of Food Science and Technology,
Punjab Agricultural University
Ludhiana- -141004, Punjab, INDIA

Editorial Advisory Board

Dr Aline Lonvaud (Wine and Brandy)

Faculty of Enology
University Victor Segalen Bordeaux 2, FRANCE
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The Australian Wine Research Institute, AUSTRALIA
creina.stockley@awri.com.au

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Department of Food Science and Technology
Dr. YS. Parmar University of Horticulture and Forestry, Nauni
Solan, Himachal Pradesh, INDIA
devinavaidya@yahoo.com

Dr Eveline Bartowsky (Wine Microbiology)

The Australian Wine Research Institute
P.O. Box 197, Glen Osmond, AUSTRALIA
eveline.bartowsky@awri.com.au

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Departamento de Quimica Agricola, Geologia y Edafologia
Facultad de Quimica. Universidad de Murcia
Campus Universitario de Espinardo, Murcia, SPAIN
gnavarro@um.es

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Laboratorio de Microbiologia y Genetica. Facultad de Ciencias del Mar y Ambientales. Universidad de Cadiz. Poligono del no San, SPAIN
laureana.rebordinos@uca.es

Dr Luca Cocolin (Wine Microbiology)

Dipartimento di Scienze degli Alimenti, Universita degli studi di Udine, Facolta di Agraria, via Marangoni, Udine, ITALY
lucasimone.cocolin@unito.it

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remedios.marin@unavarra.es

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Department of Biology, McMaster University, Hamilton, Ontario, CANADA
elsheikha.aly@gmail.com

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Department of Freeze Drying and Animal Product Technology Defense Food Research Laboratory, Mysore, INDIA
dfrlmysore@sancharnet.in

Dr Neerja S. Rana (Biochemistry)

Department of Basic Sciences
Dr.YS. Parmar University of Horticulture and Forestry, Nauni Solan, Himachal Pradesh, INDIA
drneerjauh24@yahoo.co.in

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Laboratory of Enology and Applied Chemistry, Unite de Recherche sur la Vigne et le Vin de Champagne, Research Unit N°2069 University of Reims, Faculty of Science, FRANCE
philippe.jeandet@univ-reims.fr

Professor Pradeep Khanna (Mushroom Production)

College of Basic Science, PAU Ludhiana Punjab, INDIA
pkhanna_pau@rediffmail.com

Dr Pura Naik J (Plantation Crops)

Division of Plantation, Spices and Flavour Tech. Central Food Technological Research Institute Mysore, INDIA
puranaik@yahoo.com

Dr RC Ray (Food Fermentation)

Regional Centre of Central Tuber Crops Research Institute Dumuduma Housing Board, Bhubaneswar, Orissa, INDIA
rc_rayctcri@rediffmail.com

Dr. Rintu Banerjee (Microbial Technology)

Microbial Biotechnology and Downstream Processing Laboratory, Agricultural & Food Engineering Department Indian Institute of Technology, Kharagpur, West Bengal, INDIA
rin_tuin@yahoo.com

Dr R K Gupta (Statistics)

Department of Basic Sciences
Dr. Y S Parmar University of Horticulture and Forestry, Nauni Solan, Himachal Pradesh, INDIA
rkgupta9@rediffmail.com

Dr R S Singh (Food Fermentation and Enzyme Technology)

Department of Biotechnology,
Punjabi University, Patiala, Punjab, INDIA
rssingh11@lycos.com

Dr. Sanjeev Kumar Soni (Enology)

Department of Microbiology
Panjab University, Chandigarh, INDIA
sonisk@pu.ac.in

Dr. Vasudeo P. Zambare

Sequence Biotech. Pvt. Ltd.
Nashik, Maharashtra, INDIA
vasudeo.zambare@sdsmt.edu

Dr S S Kanwar (Food Fermentation)

Department of Microbiology, CSK HPKV, Palampur Himachal Pradesh, INDIA
sskanwar1956@gmail.com

Dr Sumit Arora (Dairy Science)

Dairy Chemistry Division, National Dairy Research Institute Karnal, Haryana, INDIA
sumitak123@yahoo.com

Prof Tek Chand Bhalla (Food Fermentation & Enzyme Tech.)

Department of Biotechnology,
Himachal Pradesh University, Shimla Himachal Pradesh, INDIA
bhallatc@rediffmail.com

Dr V M Pratape, (Grain Science and Technology)

Department of Grain Science and Technology, Central Food Technological Research Institute Mysore, INDIA
gst@cftri.res.in

Dr Y S Dhaliwal (Food and Nutrition)

Department of Food Science and Nutrition
College of Home Science
CSK HPKV, Palampur, Himachal Pradesh, INDIA
ysdhaliwal44@yahoo.co.in

Dr. S S Thorat (Food Science Technology)

Department of Food and Industrial Microbiology
Vasanthrao Naik Marathwada Agriculture University
Parbhani, INDIA
drssthorat@rediffmail.com

Dr. D N Gandhi (Dairy Microbiology)

Gandhi House, New Housing Board
Karnal, Haryana, INDIA
dngandhi@rediffmail.com

Dr. Raj Bala Grewal (Foods and Nutrition)

Centre of Food Science and Technology
CCS Haryana Agriculture University
Hisar, Haryana, INDIA
grewalrb@hau.ernet.in/grewalrb@gmail.com

Dr. Ome Kalu Achi (Microbiology)

Department of Microbiology
Michael Okpara University of Agriculture
Abia State, NIGERIA
omekachi@yahoo.com

From the Desk of Editor-in-Chief



I am happy to state that, we have successfully brought out Eight volumes of our journal “*International Journal of Food and Fermentation Technology*” and presenting volume 9(2) to you. It is a unique journal on the subject so earnestly request the scientific community to respond with their high quality papers. It has been rated by NAAS and Index Copernicus, which is though quite encouraging needs improvement to achieve the set standards by taking measures by all those involved, especially the Editorial Board members and the Publisher to increase the visibility of the Journal.

During the past eight years, we have published papers on various aspects of food and fermentation technology, which could be achieved only with the active cooperation of all. It is hoped that the readers must have relished the style, general get-up, cover page, coverage of the journal, scientific and technical contents, with an attractive look of A4 size format but would welcome suggestions from the readers, contributors and peers of the field in the form of letters to the Editor-in-Chief for its improvement. I would be happy even to publish such letters and place before the Editorial Board various suggestions for possible implementation. I would like to express my deep gratitude to all those who have extended their help, guidance and co-operation especially the help extended by the editorial members/referees involved in review process in bringing out this issue.

I welcome the new editorial board members of the Journal and hope that their cooperation would help achieve new heights. I earnestly believe that the Journal would develop by leap and bounds in future. I seize this opportunity to request all the members of editorial board to exert more so that the journal gets high recognition among the peers. I am sure that through your efforts we would get not only more number of papers but of better quality. They are welcome to contribute themselves papers of high quality and to encourage others to do so. They are expected to write at least once conceptual editorial, and review or a research paper when asked to do so.

From Volume 2, we had introduced conceptual editorial which is being continued. I have been inviting all the members of editorial board to contribute liberally to various issues. It is your journal and you should make efforts to make it excellent. Certainly, there would not be any page charges for this invited contribution. However, there is somewhat slower response to my request. I again request all of you to please send conceptual editorial with your photo. The scientists from abroad can definitely contribute to improve the quality of this journal presently in infancy by submitting conceptual editorial, state of art reviews, research papers and suggestions. Their contributions would be given due considerations.

We had started ‘Open Forum’ for the readers, peers, educations researchers and general public to express their views which would be published. However, no response to the same has been received. The topic for the same for this issue is appearing just after conceptual editors. There are several issues raised in the open forum where the ethics of paper publication.

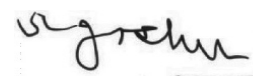
It is a very sensitive issue being discussed world over. I expect from the academicians to response with their comments and suggestions /views, large number of journals, papers, quality of papers, predatory journals, fees etc have been raised. There is also a provision of publishing of book reviews in the journal for which all readers, scientists, editors can review the book of their choice except their own books and

send their contributions to the Editor-In-Chief. Similarly, the publishers of the scientific books have opportunity to submit their books for review to the Editor-in-Chief, for which no page charges are levied. The publishers, however are a bit shy, perhaps they think sending out a complimentary book for review would be too costly for them through in reality they could earn many times more than one copy of the book. Needless, to say that book reviewed get overwhelming recognition and in many cases more sale orders. A review would shortly be made to assess the performance of all the members of the editorial board after this volume and would only be continued to be a member if at least once they have responded positively to my request in a befitting manner. From volume 10th the editorial board would be restructured to make more effective. I request all those with the background of editing /academics research and teaching to send their CV s for consideration

On behalf of the editorial board and the publisher, I assure the readers and the contributors that we would strive hard to ensure high level of scientific integrity, transparency, impartiality and accuracy in selection of articles and finally, printing of the journal. Comparatively, the number of reviews submitted are very less and definitely, more reviews are welcome. The contributors of review or mini-review can consult the editor-in-chief, any member of the editorial board for the topic of review to contribute. I must express very clearly that just because of some page charges we will accept and publish substandard articles, but would accept and publish only peer reviewed and accepted papers. All the contributors are requested to check their contributions for the copy right problems, as we have a software to check the same. Besides, rejecting the paper, where violation is detected, it may lead to black listing of such authors. Similarly, I must say the many contributors want to publish their papers very quickly. Since, it takes time in review process of the paper and getting it reviewed, time is always consumed for publishing a paper. We strive to select papers for publication to cover as much as possible the entire spectrum of food and fermentation technology and the papers with some novelty get preference. Not only this, some authors get their papers reviewed and after getting the same improved or reviewed then publish in other journals, which is highly improper and unethical, would lead to black listing and publishing their names on website of the journals. I think such scientists are slur on the scientific community.

I understand very well that the final scientific quality of any journal would be determined by the type of papers submitted to the journal and published, so the cooperation of all would be a pre-requisite in this endeavour and would welcome especially from all all the contributors to contribute liberally.

V.K. Joshi



Editor-in-chief
IJFFT

Conceptual Editorial

Anticancer properties of horticultural based food

Now-a-days, cancer is considered as one of the leading causes of mortality and morbidity, and has become the most serious life-threatening disease, affecting people of all ages worldwide. It is the second most common cause of death after cardiovascular diseases in developed countries. As per the study of Indian Council of Medical Research (ICMR), the incidence of cancer is likely to go up from 9.8 lakh cases in 2010 to 11 lakh in 2020. While some cancers can be cured if detected early, treatment of others remain a great challenge. In accordance with the recent report from the World Health Organization (WHO), the average number of new diagnosed cases of cancer in the World overcomes 14 million per year and, among these, more than 60% result in death (8.8 million in 2015). The term “cancer” can be described as a set of complex processes involving impaired cells death, unlimited cell proliferation and temporal–spatial changes in cell physiology that often leads to malignant tumor formation resulting in invasion of distant tissues to form metastasis. Multistage carcinogenesis (namely initiation, promotion and progression) is a widely accepted hypothesis in the development of cancers, resulting from extensive DNA damage. DNA damage as a term encapsulates both frank single and double-stranded DNA breaks, as well as stable modifications to nitrogen bases in DNA or its sugar-phosphate backbone, caused by external (e.g., IR) or internal sources (e.g., reactive oxygen species (ROS) generated during oxidative metabolism), which impact the cell by disrupting gene function and/or impairing transcription, DNA replication and cell proliferation. The oxidative hypothesis of carcinogenesis claims that many carcinogens can generate free radicals that damage cells, setting these cells to malignant changes. DNA contains reactive groups in its bases that are highly susceptible to free radical attack and oxidative DNA damage can lead to deleterious mutations. Most oxidative lesions are efficiently repaired by specific DNA glycosylases, but unrepaired lesions accumulate with age. When the cells divide, the lesions become fixated and mutations and cancer may result. Cancer cells are characterized by mutations and genetic instabilities which consequently lead to impaired regulation of cell cycle, uncontrolled proliferation and overcoming of apoptosis and similar checkpoint mechanisms. Anticancer treatments usually use compounds that target fast-dividing cells. This approach, regrettably, has a negative side effect because normal, fast-dividing cells such as hair follicles and epithelial cells in the digestive system are also affected. Furthermore, one of the aggravating circumstances is that many cancer cells gradually develop resistance to conventional forms of therapy.

Fruits and vegetables (F & V) provide a diversified low caloric, and protective, micro-nutrient rich diet. Low F & V intake is considered as the sixth main risk factor for mortality in the world. Overall it is estimated that low F & V intake is attributable to approximately 2.7 million (4.9%) annual deaths, and causes about 31% of ischaemic heart diseases (IHD), 11% of stroke and 19% of gastro-intestinal cancers and still significantly associated (protective) with lung/pharyngeal/laryngeal/oral cancer, type-2 diabetes mellitus, bone-health,



Prof. R.M. Sharma

*Division of Fruits & Horticultural
Technology
ICAR-Indian Agricultural Research
Technology,
New Delhi-110012*

vision/cataract and micronutrient deficiency state. A commonly accepted estimate among experts is that at least one third of cancer cases are related with diet. Based on the evidence from the accumulated studies indicates that there is a low risk of cancers in people with a high intake of fruits and vegetables. The potential number of cancer cases that might be prevented if half the U.S. population increased its fruit and vegetable consumption by one serving each per day. The cancer risks were estimated using U.S. Environmental Protection Agency (EPA) methods, cancer potency estimates from rodent bioassays, and pesticide residue sampling data from the U.S. Department of Agriculture (USDA). The resulting estimates are that approximately 20,000 cancer cases per year could be prevented by increasing fruit and vegetable consumption. Total vegetable intake is inversely related to breast cancer risk. Reduced risk is also related to high intake of allium vegetables and fresh legumes. High intake of citrus fruits and Rosaceae fruits are inversely associated with breast cancer risk. The high intake of total vegetables, certain fruits, milk, and eggs may reduce the risk of breast cancer, whereas high consumption of animal-source foods may increase the risk. Approximately 200 studies that examined the relationship between fruit and vegetable intake and cancers of the lung, colon, breast, cervix, esophagus, oral cavity, stomach, bladder, pancreas, and ovary are reviewed. A statistically significant protective effect of fruit and vegetable consumption was found in 128 of 156 dietary studies in which results were expressed in terms of relative risk. For most cancer sites, persons with low fruit and vegetable intake (at least the lower one-fourth of the population) experience about twice the risk of cancer compared with those with high intake, even after control for potentially confounding factors. It would appear that major public health benefits could be achieved by substantially increasing consumption of these foods.

Chemoprevention through diet modification, i.e., increased consumption of plant-based food, has emerged as a most promising and potentially cost-effective approach to reduce the risk of cancer. Research Fund (WCRF) report (Food, Nutrition, Physical Activity, and the Prevention of Cancer: a Global Perspective, World Cancer Research Fund/American Institute for Cancer Research, Washington DC, 1997) estimated that adequate consumption of fruit and vegetables could reduce by 23% the incidence of all cancer types, classifying as “convincing” the level of available scientific evidence. In this regard, natural compounds from plants are proving to be suitable candidates for such a therapy. Interfering with the process of carcinogenesis through diet or by the added digestion of natural compounds has been termed as “chemoprevention”. An increasing importance is being given today to alternative medicine and dietary approach in prevention and treatment of cancer.

There are many categories of plant bioactive compounds, such as alkaloids, glycosides, polyphenols, tannins, gums, resins and oils, and many of these phytochemicals have been shown to possess low intrinsic toxicity and exert prominent effects on cancerous *versus* normal cells. An encouraging fact is that, in the last few decades, nearly 70% of all anticancer drugs are originated from natural sources or are derivatives of natural products. Phytochemicals can be defined as substances found in edible fruits and vegetables that may be ingested daily by humans (in gram quantities), and that exhibit a potential for modulating human metabolism in a manner favourable for the prevention of cancer and other degenerative diseases. Any reduction in the risk of disease that is associated with a high antioxidant nutrient intake may result from consuming a mix of foods rich in antioxidants rather than consuming antioxidants as single nutrients. The important group of phytochemicals include carotenoids (α -Carotene, β -Carotene, β -Cryptoxanthin, Lutein, Zeaxanthin, Astaxanthin and Lycopene) and phenolics. ROS scavenging capacity of phenolic acids (Hydroxybenzoic acid -Gallic, Protocatechuic, Vanillic and Syringic; Hydroxycinnamic acid-p-Coumaric, Caffeic, Ferulic and Sinapic), Flavonoids (Flavonols-Rutin, Quercetin, kaempferol, Myricetin, Galangin, Isorhamnetin and Fisetin; Flavones-Apigenin, Chrysin, Luteolin, Orientin, Baicalein and Acacetin; Flavanols (Catechins)- Catechin, Epicatechin, Epigallocatechin,

Epicatechingallate and Epigallocatechingallate, Proanthocyanidins and Thearubigins; Flavanones-Eriodictyol, Hesperetin, Butin, Poncirin, Sakuranin and Naringenin; Anthocyanidins-Cyanidin, Capensinidin, Pelargonidin, Rosinidin, hirsutidin, Delphinidin, Peonidin and Malvidin; Isoflavonoids- Genestein, Daidzein, Glycitein, Biochanin A, barbigerone and Formononetin) has made them an inseparable part of anticancer diet for human.

Flavonoids are naturally occurring polyphenols that are ubiquitous in plant-based foods such as fruits, vegetables and teas as well as in most medicinal plants. Over 10,000 flavonoids have been characterized over the last few decades. Flavonoids comprise of several sub-classes including flavonols, flavan-3-ols, anthocyanins, flavanones, flavones, isoflavones and proanthocyanidins. Flavonoids have been shown to be able to act as antioxidants by scavenging free radicals, an activity related to their phenol rings containing hydroxyl groups. Flavonoids have the ability to act as reducing agents, making them capable of donating hydrogens to free radicals and causing their removal. Flavonoids can also act as singlet oxygen quenchers and as chelators of transition metals such as copper and iron, which are known pro-oxidants in foods. Certain flavonoids have anticarcinogenic activity due to their ability to induce the hepatic enzymes affecting the metabolism of carcinogens (e.g. benzo[a]pyrene), and their effect on the metabolic activation of benzo [a] pyrene. Citrus fruits are particularly high in a class of phytochemicals known as the limonoids (limonin and nomilin). It has been determined by animal studies that citrus limonoids and derivatives have certain biological activities that may be used as chemopreventive agents for cancer. Apart from the anticancer activity, flavonoid-mediated health benefits include antioxidant activity through the removal of free radicals, which are capable of damaging lipids, proteins and DNA, antiinflammatory, neuroprotective and antiproliferative activity, as well as an ability to modulate signalling pathways involved in central cell processes. Apigenin (amongst abundant flavonoid) is found in significant quantities in a variety of vegetables and fruits such as parsley, celery, chamomile, oranges, thyme, onions, honey and spices, as well as beverages derived from plants; tea, beer and wine. It is a secondary plant metabolite, usually found in nature in glycosylated form, more soluble than its pure form which is unstable and quite insoluble in water and organic solvents. The actions of apigenin in inhibiting the cell cycle, diminishing oxidative stress, improving the efficacy of detoxification enzymes, inducing apoptosis, and stimulating the immune system are also known. The beneficial effects of apigenin have been established well on a variety of cancers (head and neck, breast, prostate, colorectal, pancreatic, skin, liver and cervical and ovary).

Glutathione S-transferase (GST) is a major detoxifying enzyme system that catalyses the conjugation of glutathione with electrophiles that induce activated carcinogens. The glutathione conjugates are usually less reactive and more water soluble, and hence, facilitate excretion. Many chemicals that are GST enhancers have been found to inhibit chemically induced carcinogenesis. The phytochemical classes (flavonoids, glucarates, carotenoids, coumarins, mono-terpenes, tri-terpenes and phenolic acids) found in citrus have already been identified by the United States National Cancer Institute as warranting further research with regard to protection against cancer and cardiovascular disease. Limonin and nomilin follow the same trend as their ability to induce GST activity. Nomilin, being a much better inducer of GST, was more active as an inhibitor of carcinogenesis than the less effective limonin. The primary problem is that limonin is intensely bitter. A second problem is that this citrus chemical is only soluble in organic solvents. A third problem is that the concentration of limonin and nomilin in citrus juices is fairly low and, therefore, the overall consumption of these two specific limonoids is not high. Moreover, many other structure-related limonoids such as ichigan, isoobacunoic, obacunone, and others, act as good GST inducers.

Tumour promoters, such as epidermal growth factor (EGF), 12-O-tetradecanoylphorbol-13-acetate (TPA), and tumour necrosis factor- α , can induce neoplastic cell transformation by regulating MAPKs, PI3K/Akt

signalling, AP-1, and NF κ B. Cyanidin, an anthocyanidin found in fruits and vegetables, has been reported to possess anti-cancer effects. It has been found that cyanidin, but not cyanidin-3-glucoside, inhibits the EGF-induced Akt/p70S6K phosphorylation. Moreover, cyanidin directly suppressed the activity of PI3K by binding to PI3K directly in an ATP-competitive manner, which indicates that PI3K is one of the molecular targets of cyanidin.

The protective effects of β -carotene, at least from food, although not necessarily as an isolated molecule, are thought to occur through one or more of several modes. These modes include singlet oxygen quenching (photo protection), antioxidant protection and enhancement of the immune response. Singlet oxygen is a highly reactive form of oxygen that participates in reactions that can alter or destroy important cellular components such as membranes, enzymes and nucleic acids (e.g. DNA). β -Carotene may function as a redox reagent, an immunological regulator or by increasing cell-to-cell communications.

Carotenoids have been shown to enhance both specific and non-specific immune functions in addition to enhancing tumour immunity. It has been postulated that carotenoids may enhance activity by (i) quenching excessive reactive species formed by various immunoactive cells, (ii) quenching immunosuppressive peroxides and maintaining membrane fluidity, (iii) helping to maintain membrane receptors essential for immune functions, and (iv) acting in the release of immunomodulatory lipid molecules such as prostaglandins and leukotrienes.

Vitamin C may protect against cancer through several mechanisms, in addition to inhibiting DNA oxidation. One potential mechanism is chemoprotection against mutagenic compounds such as nitrosamines, which can be formed by reaction of nitrite or nitrate (common in food and cigarette smoke) with amines and amides. Vitamin C prevents the reaction of nitrites with amines and amides that form potent carcinogenic nitrosamines within the digestive tract, and prevents oxidation of specific chemicals to their active carcinogenic forms.

Dietary fibre is commonly defined as 'plant polysaccharides and lignin, which are resistant to hydrolysis by the digestive enzymes of man'. They are generally classified into two groups: soluble and insoluble dietary fibres. Soluble fibres are highly fermentable and are associated with carbohydrate and lipid metabolism, while insoluble fibres contribute to faecal bulk and reduce transit time. The mechanisms by which fibre can influence colon cancer include physical dilution of colon content, absorption of bile acids and carcinogens, decreased transit time, altered bile acid metabolism and the effects of fermentation, namely, the production of short-chain fatty acids, lowering of pH and stimulation of bacterial growth.

The possible anticarcinogenic mechanism mediated by substances supplied by Horticultural based diet includes antioxidant effects, effects on cell differentiation, increased activity of carcinogens detoxifying enzymes, blocked formation of nitrosamines, altered estrogen metabolism and altered colonic milieu (including bacterial flora, bile acid, composition, pH, fecal bulk), preserved integrity of intracellular matrixes, effect of DNA methylation, maintenance of normal DNA repair, increased apoptosis of cancer cells and decreased cellproliferation.

Open Forum

The academics, research papers and advancement of research

In one of the earlier issues of the journal we had introduced a specific feature entitled as OPEN FORUM with the hope that the teachers, researchers, students, publishers, and the research managers would respond but unfortunately no response at all has been received so far. Perhaps no body has time to focus on such issues. A few developments in academic sphere has taken place which affect all which I would like to share here with the earnest hope to get the response in the OPEN FORUM.

I share with you that these days, we have a large number of journals many dealing with very specific aspects so the results of research are very much focused and receive the right audience. At the same time, a question arises, is there any regulatory mechanism to control the planned and scientific growth. As a right approach, these journals should be scanned if the any journal with the same name in a particular country is existing or not? If already existing, with similar content or objectives etc., it should not be allowed to register.

The journals are name like Journal of biotechnology, biotechnology research journal, International journal of biotechnology, so on and so forth. Similarly, there are many journal like food and Nutrition, Nutrition and food Science., Journal of microbiology, International journal of Microbiological research, Microbiological research. It is agreed that there can be different journals with same, even can be on the similar subjects but their coverage should be different, style of paper presentation could be different, with different focus it can easily be achieved with some sort of registry at national and international level. Naturally, when the coverage is such that the findings have implication at international level it should be named international otherwise would remain national or simply the name of the journal without international or national only.

What type of papers would be published? Should there be any fees for publication of a paper? Now if they charge money, it is called predatory journal if not who will bear the cost of production, being discussed separately. In earlier times, the papers publication was free but many times they would charge the cost of reprint bur providing tear sheets free to the contributors. Now, some journal charge fees But the fees are charged after the papers are refereed and accepted. Such journals therefore cannot be called predatory. The others advertise that the paper would be evaluated in 2 or 3 weeks time and published in a period of say two weeks. I could not understand how the paper is evaluated in week, or got revised in two weeks and published. So some sort of regulations should be there which could differentiate the predatory journal from the non- predatory. It can easily be done from the call of the paper they give, promise to publish the same and seeing the quality of paper . Acceptance or rejection rate of the papers can easily reflect the same.

It is equally heartening to note that the publication of the papers have become very rapid compared to the earlier times when it used to take almost a year to publish a paper. The question arises if this has really

improved the quality of the research papers or for that matter the overall quality of research. Perhaps the answer is certainly not yes if not a blanket no. I could not imagine a prestigious and renowned referee who can evaluate the paper say in week and the same is revised in two weeks time and published. Are the referee and the editor are so competent or it is assumed that everything is ok.

These days publication of paper has become essential to get the degree like M.Phil or Ph.D., apparently to improve the quality of research for the award of degree. While it is a good idea it also compels the students to publish the paper using the means at his or her disposal including the publication in the paid journals. Where is the quality then? The idea of publications for the award of degree is more prevalent where it is completely research based. Where the Ph.D. degree is by partial fulfillment how one can do the research of high quality in one and half year and then, publish in journal of high impact journal. Many journals of high impact also charge very high fees which some students barring those coming on paid seats, can pay. Of course, the publication of a large numbers of papers has given a large numbers of papers to the publishers and has increased the numbers of journals and subsequently, the publishers. Some of the publishers only aim to get a large numbers of papers and even skip the editorial review process. Otherwise, how could a paper be published in just a week? A point to ponder upon? The regulatory agencies should consider such points, while evaluating the journals.

Interestingly, a large number of the journals have prescribed publication fees while a few have some consideration for the economic status of the researcher. There are some journals who ask for the fees after the review is conducted and the paper is recommended for publication like this journal. To some extent, from academic point of view, this approach is appropriate. What about those who do not have enough fees to pay? There are several journals now which publish the papers to earn money. The question arises why there is publication fees? The answer is simple when the libraries do not subscribe the journals who will fund the publisher for the publication cost. The libraries also do not subscribing the journals due to several reasons like lack of funds or diversion of funds for computerization so on and so forth. To cite, digital subscription has become the need of most of the libraries so they opt for the same. Thus, even the paucity of the funds and stress for the computerization, have contributed to this phenomenon. So what is the solution?

The academicians and all those involved in domain of education and research should think if a person is not having money will he not have any publication? While business community even in the education domain would enjoy the privilege of high quality publication the person without money would lag behind. This is something which is indigestible to hard core academicians.

These days, focus is on the publications of the results in the high impact factor journals. It means that the research should be of same standards as that of Oxford university, Yale university, MITS, BITS, IITs, Pu, DU, JNU etc.. One can understand easily that the prestigious institutes with large infrastructure and capability can produce these results. But would happen to new institutes who are growing? In many cases, this has been made as the basis for promotion to next level or even for the yearly appraisal. In any institution, there are many people with average intelligence or research capability or those doing teaching are not pure researchers or do not have facility of that level where would they go? When

institute is opened in specific area like the ICAR institutes, it does research for the areas where they work, for the sake of growth of general people of the area, how their research then, can be published in so prestigious journals with high impact factor. Moreover, their research is of regional importance so cannot be published in the journal with international standing. Even the departmental promotions have been made like so and no body is seeing the utility of the papers and the impact that these would make on the society.

Another issue of relevance is that the researchers submit their papers to a journal. If the same is extensively edited and made suitable for publication, it is published in some other journal. The journal that did lot of efforts is denied the paper. What policy is appropriate for such authors which do such practices. Black listing would not help the publisher or the editor? What solution could be there?

Cordially, I invite the academicians, the educators, the researchers, students and the public to open up their mind, express their views and send these to the Editor-in-Chief. These response would be published as letters to editor. They can also give their views in the form of article. Of course, no fee would be charged for the publication of letters to the editor.

Prof V.K. Joshi

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REVIEW PAPER

Food Allergy: An Important Health Hazard

Ghan Shyam Abrol^{1*}, Krishan Datt Sharma², Amit Kumar Singh¹,
Ranjit Pal¹ and A.K. Pandey¹

¹College of Horticulture and Forestry, Rani Lakshmi Bai Central Agricultural University, Jhansi, India

²Department of Food Science & Technology, Dr Y.S. Parmar University of Horticulture & Forestry, Solan, HP, India

*Corresponding author: ghanshyamabrol@gmail.com

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ABSTRACT

An adverse reaction to a food or food component that involves the immune system is called as food allergy. Food allergies are most often immunoglobulin E (IgE) mediated but may also be non-IgE or cell-mediated. On exposure of a sensitive individual to a particular food, the allergen stimulates lymphocytes to produce the IgE-antibody and attached to the surface of the mast cells in different tissues of the body. However, non-IgE mediated food allergy is observed in the first few years of life and it is an easily treatable clinical entity. Food allergy is of several types but Type I, Type II, Type III and Type IV are of key importance. The most common foods having allergens are peanuts, milk, eggs, tree nuts, fish, soybean, wheat etc and these foods account for about 90% of all allergic reactions in humans. People suffering from food allergy can undergo several problems such as digestive disorders, respiratory and circulatory symptoms, skin reactions and sometimes anaphylactic shock. Food processing such as ultrafiltration in milk, steam cooking of kiwi fruits and peeling of peaches may reduce the food allergens up to a certain extent. Therefore, it is utmost important to create awareness in people towards food allergy and save the precious life.

Keywords: Food allergy, Allergen, Food intolerance, Allergy types, IgE-antibody

Food allergy, an adverse reaction to a food or food component that involves the immune system, affecting a higher percentage of population of world affecting as many as 6% of young children and 3–4% of adults all over world (Sicherer and Sampson, 2009). Food allergy is increasing day-by-day but still the difference between the prevalence of clinically proven food allergy and the public's perception of the problem is misinterpreting with food intolerance or other adverse food reactions to food looks like as food allergy (Young *et al.* 1994). A true food allergy is an abnormal response to specific food protein that is triggered by a specific reaction in the immune system and expressed by certain, often characteristic, symptoms (Sicherer and Sampson, 2006). Food allergies are most common among children up to the

age of two with weak immune system and then, the incidence decreases with age (Wang and Sampson, 2007). It is much more common and rising in developed countries (Allen and Koplin, 2012). The most common food allergies are from milk, egg, peanut, soybean, wheat, tree nuts, fish and shellfish. The individual food allergy does vary by culture and population (Cianferoni and Spergel, 2009). This suggests that the factors, such as air pollution or environmental allergen exposure outflank the hygiene hypothesis (Wood and Kraynak, 2007). The genetically modified crops get its foreign gene from bacteria. They can

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produce such a protein, which provoke an adverse reaction (Anonymous, 2007). People having food allergies must identify and prevent them because even mild allergy can cause serious illness and in rare instances even death. However, understanding the food intolerance is as much as important that of food allergy. There are various new and quick methods which have been evolved recently (Rady and Guyer, 2015; Maet *et al.* 2017; Pasquini, 2018).

All these and related aspects have been discussed herein.

FOOD ALLERGIC REACTIONS

The allergens are the proteins in food responsible for allergic reaction (Clark *et al.* 2005; Sicherer, 2000). These proteins are usually resistant to the cooking heat, the acid in the stomach and the intestinal digestive enzymes (Brown *et al.* 2001; Ekramirad *et al.* 2017). As a result, the allergens survive and enter the bloodstream and go to the target organs resulting in allergic reactions (Sampson *et al.* 2006; Rady *et al.* 2020). Food allergy is either IgE mediated or non-IgE mediated. The food allergy is a response of poor immune system and heredity. Types of adverse food reactions are described in Fig. 1.

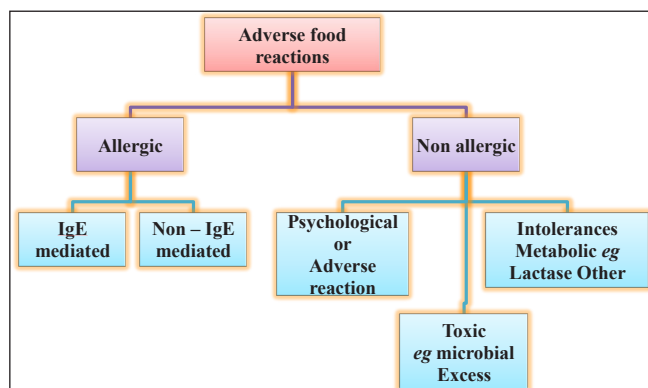


Fig. 1: Types of adverse food reactions

IgE mediated food allergy

Immune system: An allergic reaction to food involves two components of the immune system, immunoglobulin E (IgE) and the mast cells. When IgE antibodies on the surfaces of basophils and

mast cells come into contact with food allergens, it result in the release of histamine, prostaglandins and leukotrienes, which cause allergic symptoms (Sicherer and Teuber, 2004; Mari *et al.* 2005).

Heredity: Generally, heredity plays an important role in allergies to people and not necessarily to food but perhaps allergies to pollen, fur, feathers or drugs. Robert *et al.* (2007) predicted that if that no parents is allergic then the chances of food allergy are very less while that if both the parents have the same type of allergy then the chances of food allergy in their offspring are very high.

Mechanism: Food allergy is a hypersensitivity reaction that occurs even before the food is taken. At the initial exposure, the allergen stimulates lymphocytes (specialized white blood cells) to produce the IgE-antibody that is specific for the allergen. This IgE is then, released and get attached to the surface of the mast cells in different tissues of the body. The next time the person eats or even sees that food, it hone in on the specific IgE-antibody on the surface of the mast cells and prompts the cells to release chemicals such as histamine (Taylor and Hefle, 2001, Sicherer and Teuber, 2004) and results in various symptoms of food allergy in human body.

Non-IgE mediated food allergy

Some adverse reactions do not involve the immune system as seen with disorders such as coeliac disease (Troncone *et al.* 2008). These sensitivities may be attributed to the existence of metabolic disorders or the occurrence of reactions with unknown mechanism (Taylor and Hefle, 2001). Both IgE and non-IgE mediated food allergy is frequently seen during this period. Unlike IgE mediated food allergy, non-IgE mediated food allergy is rarely life-threatening; and has critically been reviewed by Jyonouchi (2008).

SYMPTOMS OF FOOD ALLERGY

The food allergy symptoms occur within a few minutes to an hour of eating (Stejskai, 1999; Ebo and Stevens, 2001). Symptoms may be limited or more generalised with involvement of the skin, nose, eyes,

and/or lungs. If allergens reach the skin, induce hives or eczema and when they reach the airways, cause asthma (Sampson, 1999). As allergens travel through the blood vessels, cause light headedness, weakness and anaphylaxis, which result in sudden drop in blood pressure and if not treated quickly, can bring death. The complete clinical symptoms of food allergy are shown in Table 1.

Table 1: Clinical symptoms of food allergy (WHO, 2009)

Site	Symptoms
Skin	Angioedema, urticaria, flushing, warm feeling
Respiratory	Chest tightness, wheezing, cough, tongue, palate, or uvular swelling
Upper airway	Stridor, hoarseness, sneezing
Gastrointestinal	Vomiting, nausea, abdominal pain, diarrhea
Cardiovascular	Tachycardia, hypotension, syncope, arrhythmias
Neurologic	Headache, altered mental status, seizure, dizziness
General	Anxiety, pallor, weakness, feeling of impending doom

In a study of self-reported food allergies, 3–38% answer that they have food allergies, but only 1–11% have their food allergy confirmed clinically (Rona *et al.* 2007). Most of the studies in which food allergy is clinically proven report percentages between 1% and 5% having any food allergy (Zuidmeer *et al.* 2008). Food allergies symptoms related to skin and gastrointestinal generally decreases however, respiratory symptoms increases with age (Saarinen and Kajosaari, 1995).

Table 2. Increase in prevalence over past 20 years (Source: Sampson, 2004)

Food	Children	Adults	Outgrown
Milk	2.5%	0.3%	80%
Egg	1.3%	0.2%	60-70%
Peanut	0.8%	0.6%	20%
Tree nut	0.2%	0.5%	No
Fish	0.1%	0.4%	No
Shellfish	0.1%	2.0%	No
Other	6%	3.7%	—

Table 2 shows that the milk, egg and peanut allergy is very common among children and out grown in last 20 years however, tree nut, fish and shellfish is common in adults and there is no increase in these.

TYPES OF FOOD ALLERGIES

Several types of food or metal allergies are there but the major ones include Type I, Type II, Type III and Type IV (Shamberger, 2008).

Type I is common in children, but rare in adults. The IgE-antibodies attach to mucous membranes, which release histamine. Allergic reactions may occur within 1-60 min and affect the skin, airway, and digestive tract, causing classical allergies such as rhinitis, urticaria, angioedema, vomiting, diarrhea and anaphylaxis (Stejskai, 1999). These are more dangerous and can be life-threatening.

Type II food allergy involves lectin allergens, which bind to ABA markers on cells that include red blood cells, mucous membranes, intestinal lining and other cells. It affects IgG-antibodies and killer cells, which will destroy red blood cells, thereby causing anaemia. There are about 70 known food lectins, which could attach specifically to A, B, or O blood types. However, most lectins are destroyed by cooking and digestion and therefore, rarely cause serious problems (Gold and Balding, 1975).

Type III immune reactions are more frequently involved in food allergy than Type I reactions. About 45-60% of the population has been reported as having Type III food allergies. Up to 70-80% of Americans who do not respond well to medical treatment may be suffering from IgG-mediated, delayed-onset food allergies (Power, 1991).

Type IV reactions are involved in many autoimmune and infectious diseases, but may also involve contact dermatitis (e.g. poison ivy). These reactions are mediated by T cells in the skin and soft tissues, monocytes. The best test for Type IV allergy is the ELISA/ACT LRA blood test.

COMMON FOOD ALLERGIES

In children, milk, egg, peanut and tree nut allergies

where as in adults, shellfish, fish, peanut, walnut, and egg are very common food allergies. Most food allergy is acquired in the first 1 to 2 years of life (Robert and Wood, 2003). Cow's milk allergy develops in the 1st year of life, but about 85% become tolerant by the 3rd year, egg allergy develops in the 2nd year of life and half of it become tolerant in 3 years, and up to 66% become tolerant in 5 years. Peanut allergy tends to persist throughout adulthood, although up to 20% of peanut-allergic children lose their allergy (Osterballe *et al.* 2005)

Cow milk allergy

Cow's milk allergy is a common allergy in early childhood, with a prevalence of 2–6% and 90% naturally acquiring tolerance by the age of 5 year (Crittenden and Bennett, 2005). Adulthood incidence remains only 0.1–0.5 per cent. Some patients may be extremely sensitive to milk and even one drop has been reported to cause generalized reactions (Hill *et al.* 1988). It mainly causes hives and asthma but rarely leads to colic, sleeplessness and perhaps blood in the stool or poor growth. Infants are susceptible to allergic syndrome because their immune and digestive systems are immature. It is associated with the development of regulatory CD4⁺CD25⁺ T lymphocytes (Karlsson *et al.* 2004). The main allergens associated with cow milk allergy are Casein, β -lactoglobulin and α -lactalbumin. Lee *et al.* (2010) studied about the specific allergen in cow's milk allergy responses of late eczematous reactions B cell subset in atopic dermatitis. Glucocorticosteroid medicines are prescribed to treat infants with very severe GI reactions to milk formulas (Ascherio and Munger, 2007). Breast feeding to infant is another way to check allergy to milk but some studies indicate that breast feeding has been associated with a reduced incidence of allergy (Gruskay, 1982; Oddy, 1999; Saarinen 2000), while other do not support this (Wright, 2001; Sears, 2002).

Wheat allergy

Wheat allergy involves IgE and mast cell response. The allergy is due to proteins of wheat for example

serine protease inhibitors, glutelins and prolamins and different responses are often attributed to different proteins (Naoko, 2009). The most severe response is exercise/aspirin induced anaphylaxis attributed to one omega gliadin that is a relative of the protein that causes coeliac disease (Varjonen *et al.* 2000; Akagawa *et al.* 2007). The common symptoms include nausea, urticaria, atopy gastrointestinal manifestations and coeliac disease (Constantin *et al.* 2005; Perr, 2006). In a study, only 1% children were found to have wheat allergy out of 1612 children when they were exposed to cereals after 6 months of age (Poole *et al.* 2006). Patch test is no more reliable diagnosing method for this allergy compared to other methods (Majamaa *et al.* 1999). In most of cases wheat allergy is reduced with age. In a study, 65% children were found to be resistant to wheat allergy by the age of 12 (Keet *et al.* 2009).

Nut allergy

Peanut and tree nut allergies occur in 0.4-1.3% of children and 0.5-1% of adults (Hourihane *et al.* 1998; Hourihane, 2011). Usually, this type of food allergy onset by the age of 5 years and remains for lifelong. But recent studies show that out of this 20-25% may resolve for peanut and around 10% for tree nut (Taylor *et al.* 2010). Allergy to cashew ranks second among the tree nut allergies (Sicherer *et al.* 2003) and has been reported to cause allergic responses in sensitive individuals exceeding those observed for peanut (Venugopal, 2006; Clark *et al.* 2007). The major classes of tree nut allergens include 7S globulins (vicilins), 11S globulins (legumins), and 2S albumins, all of which are classified as food storage proteins (Roux *et al.* 2003; Sathe *et al.* 2005).

Fish allergy

Shellfish allergy persists throughout the life and is often associated with severe reactions, including life-threatening anaphylaxis (Steensma, 2003). Tropomyosin, a heat-stable muscle protein, is the major allergen in shellfish and seafood, with highly homologous proteins being found in the commonly edible crustaceans (WHO, 2001). These homologies

are responsible for the cross-reactive allergies. In addition to being stable to cooking, the allergen leaches from shellfish and seafood into cooking water (Bush and Hefle, 1996).

Green bean allergy

Green bean allergy an IgE mediated allergy, has been reported by various workers (Bernhisel-Broadbent *et al.* 1989; Ibanez *et al.* 2003) but none of these reported the types allergens behind it. Zoccatelli *et al.* (2010) in oral provocation test confirmed green bean allergy is a result of nsLTP and similar structure rLTPs (named LTP1a and LTP1b) and these share 61.3% amino acids. Further, the IgE-binding and histamine release assays provided evidence that rLTPs and nLTPs possess different allergenic potency.

DIAGNOSES OF FOOD ALLERGY

To diagnose food allergy, first step is to see whether presence of hive wheal or any other adverse reaction to a particular food (Lack, 2008). To establish food allergy, a detailed history from the patient, the patient's dietary diary, or an elimination diet *etc.* are required. He or she then, confirms the diagnosis by the more objective skin tests, blood tests, or food challenges (Sampson, 1999). Recently, new methods other than traditional ones are now being used to diagnose food allergy and reviewed by Caubet and Sampson (2012).

History: The history usually is the most important diagnostic tool in diagnosing food allergy. But in many cases infants do not have a family history of food allergy (Sears, 1996; Tariq, 1998). Food allergy is clearly suspected more often than it is found by accurate diagnostic procedures and is confirmed by challenges in less than 20% of the time (Cianferoni and Spergel, 2009). In general, the history can be more helpful in IgE-mediated disorders, because these reactions occur so soon after food ingestion and because multiple target organs are affected. In history certain questions are very common for example; did the reaction come on quickly after eating the food, is the reaction always associated with a certain food and how was the food prepared.

Dietary diary: Relining on a history alone cannot establish a true food allergy. So, keeping a record of the contents of each meal and whether reactions occurred that are consistent with allergy is important. Moreover, the dietary diary provides more details than the oral history to establish a relationship between a food and the allergic reactions (Young *et al.* 1994).

Elimination diet: When multiple food allergens are suspect, an elimination diet is used (Feeney, 1969). It is very important to avoid food allergy for which person is susceptible. If the patient resumes eating the food and the symptoms return, this sequence confirms the diagnosis (Sheldon *et al.* 1967). This technique is however not suitable if the allergic reactions have been infrequent (Young *et al.* 1994).

Skin test: It is also known as scratch-the-skin test. A dilute extract of the suspected food is placed on the skin and then, this portion of the skin is scratched with a needle and observed for swelling or redness. A positive scratch test indicates that the patient has the IgE-antibody. Skin tests are rapid, simple, and relatively safe (Rosen *et al.* 1994). If, food allergens eliciting a wheal at least 3 mm or greater than it indicate positive food allergy while less than 3 mm considered to be negative (Bock *et al.* 1978). In the evaluation of IgE-mediated food allergy, specific tests can help to identify or exclude responsible foods. One method of determining the presence of specific IgE-antibody is prick-puncture skin testing (Sampson and Albergo, 1984; Spergel, 2007). In some highly allergic people, however, especially if they have had anaphylactic reactions, skin tests should not be done because they could provoke another dangerous reaction. Skin tests also cannot be done in patients with extensive eczema.

Blood tests: In those situations where skin tests cannot be done, blood tests such as the RAST, the ELISA and improved ELISA methods are used (Takagi *et al.* 2006). These tests measure the presence of food-specific IgE-antibodies in the blood of patients, but they cost more than skin tests, and the results are not available immediately. As with positive skin

tests, positive blood tests make the diagnosis of a specific food allergy only when the clinical history is compatible (Young *et al.* 1994).

Food challenge: The double-blind food challenge has become the gold standard for objective allergy testing (Bock and Atkins, 1990; Sampson, 2005; Caubet and Sampson, 2012). Various foods, some of which are suspected of allergic reaction, are placed in individual opaque capsules. Both the patient and the doctor are blinded, so that neither of them knows which capsules contain the suspected allergens. The patient swallows a capsule and the doctor then observes whether an allergic reaction occurs. This process is repeated with each capsule. Alternatively, the food to be tested may be disguised in another type of food to which the person is not allergic (Young *et al.* 1994).

TREATMENT

To avoid food allergy now-a-days genetic engineering is gaining popularity for removal of food allergens (Singh and Bhalla, 2008) for example; different workers have gained a success in removal of allergens from tomato. Different therapies available for prevention of food Allergies are summarized in Table 3.

Table 3: Therapies to treat or prevent food allergy (Sicherer and Sampson, 2009)

Standard subcutaneous immunotherapy (native allergens)
<ul style="list-style-type: none"> • Antigen presentation in non-mucosal site results in Th1 skewing
Sublingual/oral immunotherapy
<ul style="list-style-type: none"> • Antigen presentation to mucosal site provides desensitization and may induce tolerance
Modified protein vaccine
<ul style="list-style-type: none"> • Avoid activation of IgE by mutation of binding sites but maintain T cell responses
Peptide vaccine (overlapping peptides)
<ul style="list-style-type: none"> • Avoid activation of IgE by lack of peptides large enough to cross-link IgE but maintain T cell responses
Conjugation of immune stimulatory sequences to allergen
<ul style="list-style-type: none"> • Enhance Th2 response by activating innate immune receptors, possibly hinder IgE binding

Plasmid DNA encoded vaccines

- Endogenous production of allergen may result in tolerance

Anti-IgE antibodies

- Bind and inactivate IgE while it is not bound to high-affinity IgE receptors

Chinese herbal medicine

- Mechanism unknown, not simply immune suppression, not steroid effect

Cytokine/anti-cytokine

- Interrupt inflammatory signals

Source: Printer Bring the source here below the every table.

Dietary avoidance: Avoiding the allergen in the diet is the primary treatment of food allergy. Person sensitive to particular food must be removed from the diet (Pereira *et al.* 2005). But it is not so easy, as peanuts often are used as protein supplements, eggs are found in some salad dressings, and milk is in bakery products. The FDA requires that the ingredients in a food be listed on its label so that they should avoid the food (Altschul *et al.* 2001).

Cross-reactivity: Cross-reactivity is caused by homologous proteins containing IgE-binding epitopes (Ivanciuc, 2009; Bernhisel and Sampson, 1989). If someone has a life-threatening reaction to a certain food, the doctor will counsel that patient to avoid related foods, which also might induce the same reaction. For example, if a person has a history of a severe allergy to shrimp, he or she can also possibly be allergic to crab, lobster, and crayfish another example, allergy to fenugreek evolves mainly from primary peanut allergy (Fæste *et al.* 2010).

Exercise-induced food allergy: Exercise can induce an allergic reaction to food. The usual scenario is that of a person eating a specific food and then, exercising. Body temperature increases with exercise and a results in allergic reactions of hives, asthma, abdominal symptoms, and even anaphylaxis and is most common in females 15–35 years of age (Horan and Sheffer, 1991). So it is better to avoid eating for at least 2 h before exercising.

Use of medicines: Several medicines are available in the market for initial immediate treatment of food allergy antihistamines can relieve gastrointestinal symptoms, hives, sneezing, and a runny nose. Bronchodilators can relieve the symptoms of asthma (Wüthrich, 2000). They are not effective however, in preventing an allergic reaction when taken prior to eating the food (Varjonen *et al.* 1997).

New therapies for the treatment of food allergy: Various new therapies are also available to prevent the food allergy and to treat the food allergy (Sicherer and Sampson, 2009). A complete list of these along with their benefits and limitation are given in Table 3.

Effect of processing on food allergy: Processing can eliminate allergens from food and make food eatable to allergic patients.

Milk: In case of cow's milk low heat treatment ensures the bacteriological safety but not reduces allergenicity. However, a slight decrease in IgE binding seen between unheated and heat-treated milk at 74°C. But a more pronounced decrease was found at 90°C (Sathe *et al.* 2005).

Fruit: Two important types of proteins are responsible for food allergy in fruit are profilins, reduces after cooking whereas, Lipid transfer proteins are more stable to heat. To eliminate kiwi fruit allergy steam cooking at 100°C for 5 min and homogenization is done commercially (Fiocchi *et al.* 2004). Peach allergens are mainly concentrated in the peel therefore, peeling of a peach reduces the allergenic properties of the fruit juice (Brenna *et al.* 2000). Dry heat is also found effective in minimizing the cherry allergen Pru av 1 (Gruber *et al.* 2004). In another study conducted by Hansen *et al.* (2003) reported that patients having hazelnut birch pollen allergy (Cor a 1.04 and Cor a 2), roasting found to decrease the hazelnut allergenicity.

Fish: The purified tropomyosins had a typical alpha-helical secondary structure and the stability of boiled tropomyosin has lower than that of raw tropomyosins. Extracts from boiled shrimp produce lower IgE binding than extracts from raw shrimp, which suggest that boiling can be used as a tool in attempting to reduce shrimp allergenicity (Liu *et*

al. 2009). Cooking processing may destroy existing epitopes on a protein or may generate new ones (neoallergen formation) as a result of change in protein conformation. (Maleki *et al.* 2000; Lehrer *et al.* 2003; Taylor, 2008).

Food grain: Allergens in food grains can also be degraded easily by processing and germination process. Yamada *et al.* (2005) found that germination and heat processing can result in degradation of some of food allergens (14-16-kDa, 26-kDa type) along with soluble proteins in brown rice.

Ultrafiltration: Ultrafiltration followed with proteases treatment removes the traces of intact proteins and suppresses the allergenicity of infant formulas based milk for children (Lehmann *et al.* 2006).

Fermentation: Effects of fermentation on food allergens is rather scant. Soy sauce is an example of a fermented food product containing both wheat and soy. It has recently been demonstrated that soy allergenicity is retained in the finished product (Hefle *et al.* 2005). However, the retained immunoreactivity as assessed by inhibition assays of β -lactoglobulin was significantly impaired in some fermented acidified milk products such as yogurt as compared to that in non-fermented milk (Ehn *et al.* 2004).

7. Probiotics: Probiotic bacteria may promote endogenous barrier mechanisms in patients with atopic dermatitis and food allergy and by alleviating intestinal inflammation, may act as a useful tool in the treatment of food allergy (Majamaa and Isolauri, 1997). *Lactobacillus casei* strain Shirota found to be very effective against IgE and IgG1 mediated food allergy (Shida *et al.* 2002).

SUMMARY

An allergy is a specific reaction to an allergen characterized by a specific IgE response. Common allergies include allergic rhinitis or hay fever, asthma, eczema or atopic dermatitis as discussed above. Allergies affect more than 20 per cent of people and the prevalence of allergic diseases may be increasing. In India, it has become an alarming situation in metro cities more particularly among the

children and infants, because of modern life style that results in weak immune system. So, it is important to create awareness between true food allergy and food intolerance.

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REVIEW PAPER

Present Status and Future Prospects of Traditional Fermented Beverages of Himachal Pradesh, India

Savitri, Navdeep Thakur and Tek Chand Bhalla*

Department of Biotechnology, Himachal Pradesh University, Summer Hill, Shimla, India

*Corresponding author: bhallatc@rediffmail.com

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ABSTRACT

Alcoholic beverages form an important component of culture and traditions of many civilizations for thousands of years. The people in tribal and rural areas of Himachal Pradesh still practice the preparation of many traditional fermented beverages that form an important component of their socio-cultural life. *Chhang, sura, chakti, kinnauri, chulli*, etc. are some popular beverages that are quite unique in terms of raw material and the fermentation process. Despite of immense social, economic and medicinal importance as described by the local consumers, most of these beverages are prepared at home scale, limiting their popularity to particular region only. Commercialization of these products with scientific interventions for improvement in quality and methods of production may help in bringing these beverages from household confines to population at large. This will provide an opportunity to the locals to generate a tradable commodity and income generation.

Keywords: Income, civilizations, rural areas, beverages, *Chhang, sura, chakti, kinnauri, chulli*

Human civilizations have been practicing the method for production of fermented foods and beverages for many centuries, even before the microbes were discovered and before the term fermentation was coined. Traditionally, fermented beverages have been a part of many cultures for thousands of years and still form an important component of the traditional knowledge in some human communities. There are reports of consumption of these beverages since pre-Vedic times in India and specific reference to their consumption was mentioned in Ramayana (300-750 BC). Indigenous fermented products form an important part of human diet in all the continents and Asians are considered as the pioneers in the development of indigenous fermentations. In India, Himalayan states have their unique ethnic fermented

foods and beverages (Savitri and Bhalla, 2007). Himachal Pradesh is a state with diversity in climate and geographical conditions. In Tribal and rural areas of Lahaul & Spiti, Kinnaur and Kullu districts of Himachal, many fermented beverages are produced and consumed. A number of cereal (rice/barley), fruit (grapes/wild apricot/apple), jaggery and millet-based indigenously made fermented beverages are popular among the people of these areas of Himachal Pradesh. Most of these traditionally fermented beverages in tribal and rural areas of Himachal are prepared by using '*phab*' as traditional inocula except '*sura*' which is a product of natural fermentation (Thakur, 2013).

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TRADITIONAL ALCOHOLIC BEVERAGES OF HIMACHAL PRADESH

Most cultures throughout the world have traditionally consumed some form of alcoholic beverages for thousands of years, and local specialty alcoholic beverages still account for the majority of all those that exist. A variety of traditionally fermented alcoholic beverages are produced and consumed in tribal and rural areas of Himachal Pradesh by indigenous people (Thakur et al. 2004; Joshi et al. 2012; Kumari et al. 2015). Fruits (apple, pear, grapes, apricot), medicinal herbs and local cereals (*ragi*, barley, wheat and rice) are available in plenty in these regions. These beverages can be classified into various types such as cereal based, millet based, fruit based and distilled on the basis of use of raw material and distillation process. The traditionally fermented alcoholic beverages of Himachal Pradesh are quite unique in terms of both starting material and the process of fermentation from those made throughout the country and the rest of the world.

Traditional fermented beverages of Himachal are mostly based on locally available raw materials such as rice, barley (locally called *Jau*), jaggery, *ragi*, apple, wild apricot (*chulli*) and grapes. The traditional starter '*phab*' is generally used as source of fermenting microorganisms. Some of the very popular beverages are *chhang*, *sura*, *lugri*, *angoori*, *daru*, *chulli*, *ark*, *chakti* etc (Table 1).

Cereal based alcoholic beverages

Among the cereal based alcoholic beverages, *chhang* prepared from barley (*nechhang* or *jauchhang*) and rice (*drechhang*), *lugri* and *chakti* are popular undistilled beverages (Fig. 1 a, b, c). *Chhang* is a turbid, acidic and an alcoholic beverage prepared by solid-state fermentation of cooked rice/barley with the traditional inoculum called '*phab*' (Thakur et al. 2004; Kanwar et al. 2011). The solid material formed before filtration of *chhang* is called *lum* and can be consumed as such. In most of the tribal districts of Himachal Pradesh, all social activities require provision and consumption of appreciable quantities of alcoholic beverages. *Chhang* is consumed mainly during winter months,

as it is believed to provide protection against cold. *Arak*, *sura* or *ara* are the distilled beverages made by fermentation followed by distillation of *chhang* (Fig. 1 g) and have a higher quantity of alcohol. The distilled form of *chhang* is also called as *daru* in some parts of the state. However, similar beverage made from a mixture of Barley, apple, *chulli* (apricot) and pear called as *ark* or *arais* very popular in Kinnaur district of Himachal Pradesh and is prepared especially during traditional festivals (Thakur et al. 2004). *Chakti* or *jhol* is a jiggery based traditional fermented beverage prepared and consumed in rural areas of Shimla, Kullu and other regions of Himachal Pradesh. *Babool* wood locally called as *kikar* (*Acacia nilotica*) is added to give appealing taste and aroma to *chakti*.

Millet based alcoholic beverages

Sura is a unique product of natural fermentation prepared by using finger millet (*Eleusine coracana*). It is mainly produced in lug valley of Kullu district of Himachal Pradesh (Fig 1 f). This beverage is peculiar in the fact that no specific inoculum is used for its preparation. It is fermented in two stages in first primary stage, natural fermentation takes place and during secondary fermentation, *dhehli* the traditional inoculum with various bioactive herbal components is added (Thakur, 2013; Thakur et al. 2015; Joshi et al. 2015).

Fruit based alcoholic beverages

Apart from cereal based alcoholic beverages, a wide variety of fruit based beverages are also prepared in Himachal Pradesh. *Kinnauri/angoori* is an alcoholic beverages prepared by the fermentation of red and white varieties of grapes that are cultivated in Kinnaur district of Himachal Pradesh. This is one of the very popular traditional beverages consumed during the local festivals and marriage ceremonies by the tribal people of Kinnaur. *Chulli*, is a popular fruit-based alcoholic beverage, indigenous to the tribal district of Kinnaur commonly made from wild apricots (Thakur et al. 2004). *Rak* is another fruit based fermented beverage made from fermentation of mixture of dried apple, *chulli*, barley, pear and jaggery is also consumed in Kinnaur. It is also known

by other names like *phasur* and *raakt* in Kinnaur district. This is one of the popular alcoholic beverages prepared in the rural areas especially for entertaining guests during local festivals and marriage ceremonies in Kinnaur (Kumari *et al.* 2015). *Ghanti* made from fermented must of apple and apricot is also prepared and consumed by the people in Kinnaur district of Himachal Pradesh.

ROLE OF FERMENTATION

Fermentation has been shown to improve the nutritional value of grains such as wheat and rice,

basically by increasing the content of the essential amino acids viz., lysine, methionine and tryptophan. In case of *chhang* and *sura*, fermentation resulted in a significant increase in B vitamin and essential amino acid levels (Thakur *et al.* 2004). The shelf-life of different products is also increased by fermentation that is attributed to the production of different metabolites by fermenting microorganisms which inhibit the growth and activities of harmful microorganisms. In addition, fermented foods and beverages contain beneficial microorganisms that can help in maintaining a healthy gut.



Fig. 1: Plates showing (a) Unfiltered rice *chhang* (also called *lum*), (b) filtered rice *chhang*, (c) unfiltered barley *chhang* (*lum*), (d) *dhehli* preparation practiced by villagers in lug valley, Kullu district (e) dried *dhehli*, (f) *sura*, (g) distillation in progress, (h) traditional vessels *chhapkian* and (i) *batig* used to serve *chhang*

TRADITIONAL INOCULUM USED IN FERMENTATION

Traditional fermentations generally utilize mixed cultures (yeast and bacterial) and lead to nutrient enhancement as well as alcohol production. Most of the beverages of Himachal Pradesh are produced by using solid state fermentation, in which substrate is allowed to ferment either naturally or by adding traditional starter. In the preparation of all the traditional fermented beverages except *sura*, 'phab' is the inoculum used for fermentation. It is a traditional starter having consortium of different microorganisms which mainly consisted of lactic acid bacteria, yeasts and moulds. *Dhehli* is the starter used in the fermentation of *sura* which is prepared by the elderly people of the Kullu valley during the *Bhadpadra* month i.e. rainy season (Fig. 1 d, e). It is a bioactive mixture of 36 different herbs and roasted barley flour (*sattu*) made in the shape of bricks, which imparts additional flavor to the beverage (Thakur et al. 2015a).

Pediococcus pentosaceus, *Enterococcus faecium* and *Lactobacillus plantarum* are the main lactic acid bacteria identified in both *phab* and *dhehli*. Among the yeasts *Saccharomyces cerevisiae*, *Saccharomyces fibuligera* were the major yeasts present in these starters (Thakur et al. 2015b,). Among the moulds, the species of *Aspergillus*, *Mucor* and *Rhizopus* were commonly found (Thakur, 2013).

CULTURAL SIGNIFICANCE OF TRADITIONAL BEVERAGES

Alcoholic beverages have played an important role in spiritual and cultural life of people in rural districts of Himachal Pradesh. Most of the traditional beverages prepared by the local people are generally associated either with festivals or with the seasons. For example, *chhang* is considered essential to solemnize the marriage ceremony of *Lahaulis* where it is sprinkled on guests as *shagun* (traditional custom) and served in special traditional vessels called *chapkian* and *batig* (Fig. 1 h, i). It is also offered to deities and exchanged as an important gift during local festivals like *phagli* and *halda* in Lahaul and has also been incorporated as an important component of any religious activities (Savitri and Bhalla, 2007). Another traditional beverage, *sura* is prepared during special local festivals like *seori sajja* and marriage ceremonies of locals of *lug* valley in Kullu district.

SCOPE OF TRADITIONAL ALCOHOLIC BEVERAGES

Some advantages of traditional fermentations are that they are labor-intensive, integrated into village customs and manners, familiar, utilize locally produced raw materials, inexpensive, have better potential. Many of the traditional alcoholic beverages of the tribal and rural belts of the Himachal Pradesh

Table 1: List of popular traditional fermented beverages of Himachal Pradesh

Fermented beverages	Substrate used	Starter used	Area
Cereal and millet based			
Chhang	Rice/Barley	Phab	Lahaul Spiti, Kullu and Kinnaur
<i>Sura</i>	Millet flour	Natural fermentation and <i>dhehli</i> as additive	Kullu (<i>lug</i> valley)
Fruit based			
Angoori/Kinnauri	Grapes	<i>Phab</i>	Kinnaur
<i>Chulli</i>	Wild apricot	<i>Phab</i>	Kinnaur
<i>Arak/lara</i>	Apple, pear, barley, etc., <i>Chhang</i>	<i>Phab</i> / distilled	Kinnaur, Lahaul Spiti
<i>Rak/phasur/raakt</i>	Wild almond, apple	<i>Phab</i>	Kinnaur
Others			
Chakti/jhol	Jaggery (<i>gur</i>)	<i>Phab</i>	Shimla, Kullu
<i>Daru</i>	<i>Chhang</i>	Distilled	All over Himachal

are made at home scale only by the rural folks and is little known in outside world. These traditional beverages especially, *chhang* and *sura* are widely believed by the local people to be of immense social, economic and medicinal importance to its consumers. A concerted effort should therefore, be made to improve the quality and production techniques of these indigenous exotic beverages so that the large scale production for export outside the tribal and rural belts could be done. This may offer a reliable and effective way for small-scale farmers to provide opportunities to generate a tradable commodity and contribute to income generation.

CHALLENGES AHEAD

Though there are a number of traditional fermented beverages that are prepared and consumed in Himachal Pradesh, yet their popularity is limited to areas where they are produced. In spite of the nutritional benefits along with use as alcohol source these have not reached general population for commercial exploitation. Limited scientific explorations have been carried out. Much effort is still needed to put these on scientific lines. In addition, many people, especially the younger members of society, are continuously migrating to the cities looking for job opportunities and better facilities. Along with a declining rural population, many traditional lifestyles and related traditional knowledge are also disappearing. This depopulation has adversely affected the time tested traditional technologies for preparation of fermented foods and beverages. Our indigenous traditional knowledge will disappear before it could be validated and improved on the scientific criteria if we ignore it. Documentation and characterization has been done to some extent by some groups but improvement of inoculum on scientific lines is needed to prevent spoilage of these fermented beverages for commercialization.

Future prospects of traditional fermented beverages of Himachal Pradesh

Traditional alcoholic beverages are important component of socio-cultural life of the people in Himachal Pradesh. Most of the traditional

alcoholic beverages prepared in different districts of Himachal are made on household level by the women. Throughout the world, fermented products are gaining importance as functional foods due to increased awareness of such products among the common man. So there is a need to provide scientific base for commercialization of these traditionally fermented beverages and improvement by quality addition. Further research should be directed towards developing defined inoculums geared toward maximizing desirable quality attributes in these beverages and minimizing any undesirable attributes. Intentional incorporation of beneficial or probiotic microorganisms into fermented products will further improve the overall quality of these products with desirable health benefits. In addition, manipulation of environmental factors and choice of good raw material for fermentation may help in improving the quality and quantity of fermented beverages. These improvements if implemented can bring these from household confines to population at large. Beverages with predictable and consistent quality thus, can be produced on a large scale that will further help in revenue and employment generation. Developments in the fields of industrial microbiology and biotechnology can further facilitate transforming home scale art of preparation of fermented products into small to medium industry and lead to a more standardized product with unique characteristics and improved quality along with better storage properties and enhanced nutrition value.

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REVIEW PAPER

Indigenous Fermented Foods of India with Special Reference to Andhra Pradesh and Adjoining Areas

Lebaka Veeranjanya Reddy^{1*} and V.K. Joshi²

¹Department of Microbiology, Yogi Vemana University, Kadapa, Andhra Pradesh, India

²Former Department of Food Science and Technology and Management, Y.S. Parmar University of Horticulture and Forestry, Solan, India

*Corresponding author: lvereddy@yahoo.com

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ABSTRACT

Indigenous or traditional fermented foods have been prepared and consumed world over including South Asia for hundreds of years and are strongly linked to cultures and traditions of millions of people around the world, especially in rural communities. A food is considered fermented when one or more of its constituents have been acted upon by selected microorganisms or their enzymes to produce a significantly altered final product desirable for human consumption. Most fermentations are caused by molds, yeasts, or bacteria, either singularly or in combination. The origins of most fermentation technologies have been lost in the mists of history. Some products and practices no doubt fell by the wayside; those that remain today have survived the test of time. Indigenous Fermented food products are important components of the diet as staples, adjuncts to staples, condiments, and beverages. There is a need to protect our traditional technology and foods that are very valuable. In this review we have given a brief account on traditional/indigenous fermented foods (preparation methods, microbes involved, biochemical changes and preservation techniques) especially of Andhra Pradesh (AP), India.

Keywords: Fermented foods, olds, yeasts, bacteria, traditional technology

Traditional foods are generally categorized into fermented foods and non-fermented food. Fermented foods are prepared by the action of microorganism(s), either naturally or adding starter culture (s), which modify the substrates biochemically and organoleptically into edible product, and are thus, generally palatable, safe and nutritious (Pederson, 1960; Tamang and Holzapfel, 1999; Hansen, 2002; Campbell-Platt, 1987; Achaya, 1998; Joshi, 2016; Molinos *et al.* 2016). These inexpensive culturally acceptable traditional foods provide basic diet as staple, pickle, confectionery, condiment and alcoholic beverages, which supplement enhanced nutrition, palatability, wholesomeness of the product with acceptable flavor and texture (Steinkraus, 1967;

Joshi, 2012). Most of them are common while other fermented foods are less familiar and confined to particular community and places. For food fermentations by molds, yeasts, or bacteria, either singularly or in combination are used. Indigenous or traditional fermented foods have been prepared and consumed by the people around the world, especially in rural communities. The origins of most fermentation technologies have been lost in the mists of history. Some products and practices no doubt fell by the wayside; those that remain today have survived the test of time. Fermented food products

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are important components of the diet as staples, adjuncts to staples, condiments, and beverages.

FERMENTED FOODS

A food is considered fermented when one or more of its constituents have been acted upon by selected microorganisms or their enzymes to produce a significantly altered final product desirable for human consumption. This oldest form of food biotechnology originated as a necessity for enhancing the keeping quality of diverse plant and animal food materials through organic acid, alcoholic, and alkaline fermentations. Fermentation, a relatively efficient low-energy preservation process, also improves digestibility, flavor, appearance, nutrient contents, and other quality attributes and reduces antinutritional components of the substrates and cooking time (Nouts, 2001). Many fermented foods are now receiving global attention for their health-promoting or disease-preventing or -curing effects. Whereas a considerable number of food fermentation processes have been scaled up for commercial purposes, most types of fermented foods are still produced on a home scale. Such products often contain mixed genera and populations of microorganisms because of the lack of controlled processing facilities. In view of larger-scale industrialized food fermentation, microbial ecology and metabolic activities of functional microorganisms must be investigated. Present-day developments in molecular methods enable accurate characterization of strains and development of tailor-made fermented food products (Adams 1999).

Three major types of microorganisms are associated with traditional fermented foods and beverages (Ray and Joshi (2014) and these are:

Microorganisms associated with fermented foods

- ❑ **Filamentous fungi:** Species of *Aspergillus*, *Amylomyces*, *Aciinomucor*, *Monascus*, *Mucor*, *Neurospora*, *Penicillium* and *Rhizopus*.
- ❑ **Yeasts:** Species of *Candida*, *Debaryomyces*, *Georichum*, *Hansenula*, *Kluyveromyces*, *Pichia*, *Saccharomyces*, *Saccharomycopsis*, *Torulopsis* and *Zygosaccharomyces*.
- ❑ **Bacteria:** Species of lactic acid bacteria

(LAB)- *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Enterococcus*, *Pediococcus*, *Tetragenococcus* and *Streptococcus*, Species of *Acetobacter*, *Citrobacter*, *Klebsiella*, *Bacillus*, *Brevibacterium* and *Propionibacterium*.

Types of Fermented foods

A variety of fermented foods can be found widespread over the world and the foods are prepared in India are presented in Table 1, some of them, made in Andhra Pradesh are described in this review. Indigenous fermented foods can be divided into the following types depending up on the substrate used:

- ❑ Fermented milk products
- ❑ Fermented cereal-legume/legume based products
- ❑ Fermented cereal foods
- ❑ Fermented vegetables and fruits
- ❑ Fermented beverages

FERMENTED MILK PRODUCTS

Fermented milk products have nutritive and medicinal value. Being tropical region in the south India, the sour milk preparation started ages ago because of that it is more stable and contain high quality nutrients compared to the fresh milk (Mann 1977).

Dahi/Curd

This is one of the most popular and oldest Indian fermented milk products, known for its milk acidic taste and pleasant flavour and is equivalent to western yogurt (Laxminarayana and Shankar, 1980). The Aryans made a virtue of it so much so that it is must in the Indian daily menu. Curd is said to create relish for food, promote appetite, operate as emollient and increase strength. A good quality *dahi* is firm and of uniform consistency with sweet flavor and acidic taste. The surface is smooth and glossy and usually a cut surface is trim ad free from cracks and gas bubbles.

Preparation of curd/*dahi* is probably originated for the preservation of surplus milk. The inocula

Table 1: Common traditional fermented foods/beverages of India

Food	Substrate	Microorganism	Nature and use
Traditional Fermented Products of South India			
<i>Ildi</i>	Rice-black gram	LAB, yeasts	Steamed, spongy cake; breakfast food
<i>Dosa</i>	Rice-black gram	LAB, yeasts	Spongy pan cake, shallow fried staple food
<i>Dahi</i>	Milk	<i>B. amyloliquefaciens</i>	Sweet/sour beverage use to take along with rice
<i>Butter milk</i>		LAB	
<i>Lassi</i>	Millet, rice	LAB, <i>Bacillus</i> spp	Sweet and flavoured beverage
<i>Ambali</i>		LAB, yeast and <i>Bacillus</i> spp	Steamed sour cake, staple food
<i>Kanji</i>	Carrot or beet roots	Starter culture used is TORANI Which contains LAB, Yeasts	Strong flavored alcoholic beverage's
<i>Kali</i>	Black gram	LAB	
<i>Papadam</i>			
<i>Pickles</i>	Matured fruits and vegetables		
<i>Toddy/Palm wine</i>	Sap from inflorescence/trunk of palm plants		
<i>Sara</i>	Black jiggery and plant bark	Yeast, LAB and <i>Acetobacter</i>	
Traditional fermented products of North India			
<i>Ballac</i>	Black gram	LAB, Yeasts, <i>B. subtilis</i>	Deep fried patties, snack
<i>Vadai</i>	Black gram	LAB, Yeasts, <i>B. subtilis</i>	Deep fried patties, snacks
<i>Papad</i>	Black gram	LAB, Yeasts.	Circular wafers, snacks
<i>wari</i>	Black gram	LAB, Yeasts.	Ball like hollow, brittle, condiment
<i>Bhatura</i>	Wheat	LAB	Flat deep fried, leavened bread, snack
<i>Nan</i>	Wheat	Yeasts, LAB	Leavened flat baked bread, staple food
<i>Jalebi</i>	Wheat	LAB, Yeasts	Crispy, deep fried, pretzel sweet confectionery.
<i>Paneer</i>	Milk	LAB	Soft mild flavored cheese, fried, curry
Traditional fermented products of western regions of a India			
<i>Dkokla</i>	Bengal gram	LAB, yeasts	Steamed, spongy cake; snack
<i>Khamam</i>	Bengal gram	LAB	Spongy cake; breakfast food
<i>Rabadi</i>	Wheat/pear-millet/ maize/ barley buttermilk mixture	LAB, <i>Bacillus</i> spp.	Cooked paste; staple food
<i>Sl,rikjrand</i>	Milk	LAB	Concentrated sweetened, savory
Traditional Fermented Products of Eastern Regions of India			
<i>Mistidalji</i>	Milk	LAB	Thick gel, sweet savory
<i>Tari</i>	Datepalm	Yeasts, LAB	Sweet cloudy white alcoholic beverage

Traditional Fermented Products of the Himalaya			
<i>Kinema</i>	Soybeans	<i>Bacillus subtilis</i> , <i>Enterococcus faecium</i> , yeasts	Sticky with typical flavor; side-dish curry
<i>Hawaijar</i>	Soybeans	<i>Bacillus spp.</i>	-do- Fish substitute
<i>Gundruk</i>	Leafy vegetables	LAB	Sun-dried, sour-acidic taste; soup/pickle
<i>Sinki</i>	Radish tap root	LAB	-do-
<i>Mesu</i>	Bamboo shoot	LAB	Sour-acidic pickle
<i>Laanr</i>	Finger-millet/rice maize/ barley	Starter culture used is <i>Mrrcha</i> which contains Filamentous moulds, Yeasts, LAB	Mild alcoholic, slightly sweet-acidic beverage

Source: Soni and Sandhu 1986.

used in preparation are mixture of *Lactobacillus* and *Streptococci* spp. Generally, a small quantity of the curd from previous fermentation is used as inoculum. Acceptable quality *dahi* could be obtained with the application of acid producing as well as flavor (primarily diacetyl)-producing micro-organisms and adopting two-stage fermentation. Biotechnological innovations suggest inclusion of certain probiotic and beneficial bacteria for further enhancement in the dietetic properties of traditional *dahi*. Application of bio-preservatives and thermization (mild heat-treatment) may be recommended for shelf-life extension of *dahi* to extend the market reach (Gandhi, 1989). The commonly associated lactic microflora in the inoculum are *Lactobacillus lactis*, *Lactobacillus delbrueckii*, *L. acidophilus*, *L. helveticus* and *S. thermophilus*. Extensive research is carried out in NDRI (Karnal and Bangalore) covering important aspects such as determining lactic microflora in homemade and commercial samples; isolation of lactic culture from *dahi* and suitability of lactic culture in getting stable product of acceptable quality attributes; good quality; biochemical and microbiological changes occurring during fermentation (Sreenivasan and Benarjee, 1946; Laxminarayana *et al.* 1952; Attaie *et al.* 1987; Gandhi, 2002). Composition and quality of *dahi* vary widely from one locality to another as it is being prepared under different market and domestic conditions as well as types of milk, with variable chemical and bacteriological quality used for the preparation. However, the chemical composition of *dahi* has been reported as fat ranging from 5-9%, protein 3.3-3.4%, lactic acid 0.5-1.1%, calcium 0.12-0.14% and ash 0.75 – 0.79%

Butter

Butter is one of the important fermented dairy products, although little is consumed directly (Rangappa and Achaya, 1974). It is used in small quantities as table butter and the rest of the major portion is used in the preparation of *ghee* and clarified butterfat. From the fresh milk first curd is prepared and then, the curd is churned with wooden or metal churns, during which butter rises to the top and is removed. Unlike the trained west farmer, the Indian farmer knowledge is purely traditional and handed down from the ancient times like Vedas. After the cooperative movement in Gujarat, now the butter is preparing in many dairy farms in large quantities commercially. Most predominant microorganisms that are present in butter are *Lactobacilli* followed by *Streptococci*, *Coli*-forms and aerobic spore formers. Yeasts and moulds also present in considerable number and contribute to acidity, colours and off-flavor. *S.lactis* subssp *diacetylactis* produces the flavor compounds diacetyl, acetaldehyde, acetoin and carbon dioxide (Gandhi 1989). Butter has moisture content of 18-20%, fat 78-81%, non-fatty solids 0.7-1.5%, acidity 0.5-0.8% (as lactic acid), energy 680-760 Kcal, Ca 400mg, Mg 30mg, Fe 0-15 mg, riboflavin 0.5 mg, thiamine 0.1 mg and niacin 2.5 mg.

Ghee

It is the Indian name for clarified butter fat and is mainly prepared from cow, buffalo, sheep or goat's milk. There is no cold storage for this and it has good shelf-life. It is prepared by heating the butter

or churned curd at 100-140°C for 5-10 min, which removes the water through evaporation. Then, is clarified using muslin cloth and cooled to solidify.

Ghee prepared from buffalo's milk has white colour but due to the presence of carotinoids *ghee* prepared from cow's milk is yellow in colour. All the microorganisms present in curd and butter are killed during heating process. So the *ghee* is free of microorganisms (Campbell-Platt 1987; Aneja 2002). *Ghee* contains water 0.1-1%, protein 0.5-1 g, ash 1-2 g, energy 880 Kcal, Na 100 mg, K 20 mg, Ca 20 mg, Mg 2 mg, P 30 mg, S 10 mg, Niacin 0.2 mg, retinol 900 ug, carotene 550 ug and vitamin E 1.8 ug (Campbell-Platt 1987; Aneja 2002).

Lassi

It is also a popular fermented beverage made in India. *Lassi* is appreciated throughout the country as a beverage for its palatability and as thirst quenching/refreshing drink. It is also known for its therapeutic values. The palatability and wholesomeness of the product depend on the quality of curd churned and temperature of churning. An average curd obtained by fermentation with contaminants produces a highly sour or off-flavored *lassi*, unfit for human consumption (Gandhi, 2002). In general, the preparation of *lassi* there are few steps such as:

- ❑ Add a little quantity of water to well set-curd in a container,
- ❑ Addition of water will depend on the composition of curd.
- ❑ Homogenize the contents using curd beater,
- ❑ Dilute the contents with 1-2 parts of cold water
- ❑ Add sugar at the rate of 14 to 20 per cent or add 1-2 per cent powdered common salt and mix the content.

The commonly associated lactic microflora in *lassi* include *Lactobacillus lactis*, *Lactobacillus delbrueckii*, *L. acidophilus*, *Lueconostoc mesenteroides*, *L.diacetyllactis*, *L. helveticus* and *S. thermophilus*. To get good quality *lassi*, restricted fermentation with cultured

microorganisms produces *lassi* with byproducts such as enhanced acetaldehyde and diacetylyle (Gandhi, 1989). Composition of *lassi* vary widely with types of milk, with variable chemical and bacteriological quality used for the preparation.

Butter milk

It is a well known and highly consuming non-alcoholic dairy beverage in Andhra Pradesh and adjoining areas. Butter milk is prepared as homemade and commercial product as a by - product of butter making. Sometimes it contains small butter flakes (Lindsay *et al.* 1965). Because of its taste, nutrients and medicinal properties is a must with south Indian meal. It is prepared through natural fermentation of sour cream butter or from sweet and non-fermented cream. Traditionally it is prepared from churned *dahi*/curd by diluting with water (Sarkar *et al.* 1996). The commonly associated lactic microflora in buttermilk are *Lactobacillus lactis*, *Lactobacillus delbrueckii*, *L. acidophilus*, *Lueconostoc mesenteroides*, *L.diacetyllactis*, *L. helveticus* and *S. thermophilus*. To get good quality buttermilk restricted fermentation with cultured microorganisms such as enhanced acetaldehyde and diacetylyle producing organisms are using. Generally the chemical composition of butter milk depends on *dahi* that used in preparation and large extent on butter making technology and season. It contains 9% dry matter, lactose 4%, lactic acid 0.5-1%, nitrogenous compounds 3.3-4%, ash 0.7% and fat content ranges from 0.3-0.6% (Agarwala and Sharma, 1961).

FERMENTED CEREAL AND LEGUME BASED PRODUCTS

Dosa/Dosai

Dosa is one of the very popular traditional fermented foods of South India. First reference to *dosa* occurs in Tamil Sangam literature from around 6th century AD and Chalukya king Somaesvara III describe the *dosa* in 1051. Eminent food scientist K.T. Achaya said that *dosa* have a history of two thousand years in Dravidian region (Achaya, 1998).

It is prepared from naturally fermented rice (*Oryza sativa*) and black gram (*Phaseolus mungo*) batter spreading 1:1 batter on a hot greasy griddle. More rice flour is used in *dosa* batter than idli batter. *Dosa* is prepared by the following steps:

- ❑ Soaking 3 parts rice and 1 part of black gram in water for 4-6h in ambient temperature 27-30 degree centigrade's,
- ❑ Grinding to a fine paste and mixing both to get a free running batter,
- ❑ 1% of salt is added to the batter and allowed to ferment overnight (10-16h). The natural microflora of both *rice* and *dhal* act as starter and bring about the fermentation.
- ❑ About 50-80 ml of batter is poured on to a hot, oiled pan and fried until crisp and brown colour on one side then turned to second side, cook well and rolled.
- ❑ This crispy and roasted *dosa* served with either coconut chutney or with lentil-vegetable curry (Soni *et al.* 1985).

Traditional *dosa* batter fermentation has revealed the occurrence and role of several bacteria alone or in combination with yeasts in bringing about various biochemical changes (Sandhu *et al.* 1986; Soni *et al.* 1986). *Leuconostoc mesenteroides*, *Streptococcus faecalis* and *Lactobacillus fermentum* are predominant among bacteria while *Saccharomyces cerevisiae*, *Debarvomvces hansenii*, *Trichosporon*, *Candida glabrata*, *C. tropicalis*, *C. sake*, *C. kruzsei*, *Hansenula* and *Trichosporon beigeli* are the common yeasts involved in fermentation. Microbes present in *dosa* batter and the microbes that contributed from two substrates are as shown in Table 2. *Dosa* fermentation is accompanied by an increase in total acids, total volume, total solids, soluble solids, non-protein nitrogen, free amino acids, amylases, proteinases, vitamins B1 and B2 (Soni *et al.* 1985; 1986; Sandhu *et al.* 1986). Total nitrogen and total proteins do not vary significantly where as reducing sugars and soluble proteins after declining initially tend to increase afterwards with the progress in fermentation. *Dosa* contains 35-40% water (on dry

matter basis), 15-20%, fat 15-25%, carbohydrates 40-50% fiber 5-8%, ash 4-6% and energy 400-450 Kcal, vitamin B, niacin, thiamine concentrations increased during fermentation (Soni *et al.* 1985).

Biochemical changes and Improvements

With the advent of fermentation acidity is increased and carbon dioxide is produced. The production of acid and gas in *dosa* and *idli* is dependent upon bacteria while the yeasts contribute enzymes and esters which impart desirable flavours (Radhakrishnamurthy *et al.* 1961; Steinkraus *et al.* 1967).

Table 2: Prevalence of different bacteria and yeasts in fermented *dosa* batter

Microorganism	No.	%
Bacteria		
<i>Leuconostoc mesenteroides</i>	33	94
<i>Streptococcus faecalis</i>	30	85
<i>Lactobacillus fermentum</i>	25	71
<i>Bacillus amyloliquefaciens</i>	20	57
<i>Lactobacillus delbrueckii</i>	13	37
<i>Bacilluspolymyxa</i>	5	14
<i>Pediococcus cerevisiae</i>	5	14
<i>Bacillus subtilis</i>	5	14
<i>Micrococcus varians</i>	3	8
<i>Enterobacter sp.</i>	3	8
Yeasts		
<i>Saccharomyces cerevisiae</i>	18	51
<i>Debaryomyces hansenii</i>	15	43
<i>Trichosporon beigeUii</i>	9	26
<i>Hansenula anomala</i>	6	17
<i>Torulopsis candida</i>	5	14
<i>Oosporidium margaritifarium</i>	4	11
<i>Candida robusta</i>	3	9
<i>Trichosporon pullulans</i>	2	6
<i>Kluyveromyces marxianus</i>	2	6
<i>Candida kefir</i>	2	6
<i>Candida krusei</i>	2	6
<i>Rhodotorula mucilagimosa</i>	1	3
<i>Rhodotorula glutinis</i>	1	3

Source: Soni and Sandhu 1986.

The biochemical and physiological characterization of the predominant microorganisms that yeasts *Saccharomyces*, *Debaryomyces*, *Trichosporon* and *Hansenula* help in the degradation of starch into

maltose and glucose by producing extracellular amylolytic enzymes which are utilized by developing microbial load. Bacteria along with some yeast are mainly responsible for the production of acid and gas from simple sugars like glucose and maltose and thus account for its fall in pH and rise in volume with the progress in fermentation. Some strains of *Saccharomyces cerevisiae* also produce acid and gas from starch itself and may thus play a major role in *dosa* fermentation. Due to the ability of some strains to grow at high temperature (42° C), *Saccharomyces* and *Trichosporon* along with bacteria also play a role in fermentation during summers when the temperature of the atmosphere exceeds 40°C.

Table 3: Volatile flavour compounds identified by GC-MS analysis in 2 day stored *Idli* batter cultures

No	Heterocyclic compounds	Retention time (Min)
1	Piperazinetrione	11.5
2	Benzitriazole	25.5
3	Imidazole	37.0
4	Cyclohexylamine	42.3
5	Chlorothiazide	46.0

Source: Renu Agarwal *et al.* 2000.

Further, it was observed in case of experimental fermentations carried out in our laboratory by self inoculating with different groups of micro-organisms that *Leuconostoc mesenteroides* and other acid producing bacteria mainly caused the acidification and leavening of the *dosa* batter while the yeasts *Saccharomyces cerevisiae* and *Debaryomyces hansenii* improved the nutritional quality of the batter in terms of flavour, total proteins, amino acids, vitamins and enzymes (unpublished data). According to Mukherjee *et al.* (1965), Steinkraus *et al.* (1967), the acid and gas required for souring and the leavening action in the *idli* batter are produced exclusively by the activity of hetero fermentative lactic acid bacteria *Leuconostoc mesenteroides* although there is a sequential change in the bacterial flora. Venkatasubbaiah *et al.* (1985) recently indicated that the gas production during *idli* batter fermentation depends upon the activity of the yeast flora belonging to *Hansenula anomala*, *Candida*

glabrata, *C. tropicalis*, *C. sake*, *C. kruzsei*, *Torulopsis candida*, *T. holmii* and *Trichosporon pullulans*.

The values of nutritional constituents and organoleptic characteristics of *dosa* reveal that the product is very nutritious. There are many ways to improve its physical and biochemical characteristics by replacing the black gram, rice and restricted fermentation using selected known microorganisms. Attempts have been made to prepare *dosa*-like products with the replacement of black gram with sorgam, maize and bajra (Ragavendra Rao *et al.* 1979; Soni and Sandhu 1990). Especially *S. cerevisiae* in combination with the natural bacterial flora can play a significant role in further improving the nutritional quality and acceptability of *dosa* (Soni *et al.* 1989a and b). Further, Soni *et al.* (1989b) studied the replacement of the legume component and fermentation and fermentation can be satisfactorily performed in *dosa* batter by choosing suitable proportions of the ingredients. Mung beans, for example, rich in soluble compounds, and soybeans rich in proteins and enzymes, and they stated that can be used in combination with black grams which impart attractive organoleptic characteristics for further improving the digestibility and nutritional value of traditional *dosa*.

Idli

Idli is a very popular traditional fermented breakfast food staple consumed in the Indian subcontinent, especially in southern parts (Joshi *et al.* 2011). The major ingredients are rice (*Oryza sativum*) and black gram (*Phaseolus mungo*). *Idli* (and the process of steaming) was known in Southern India by as early as 700 CE. The process of steaming was influenced from Indonesia subsequently between 800-1200 CE, giving rise to the modern day *Idli*. Traditional *Idli* preparation is depicted in Fig. 1, and briefly as follows: 1. Soaking the *polished parboiled* rice and *decorticated* black gram separately for 4 – 5h at 30 ± 1C in water. 2. After draining the water, grind the rice and black gram separately to 0.5- to 0.7-mm particle size batter with occasional addition of water during the grinding process. 3. Mixing rice and black gram

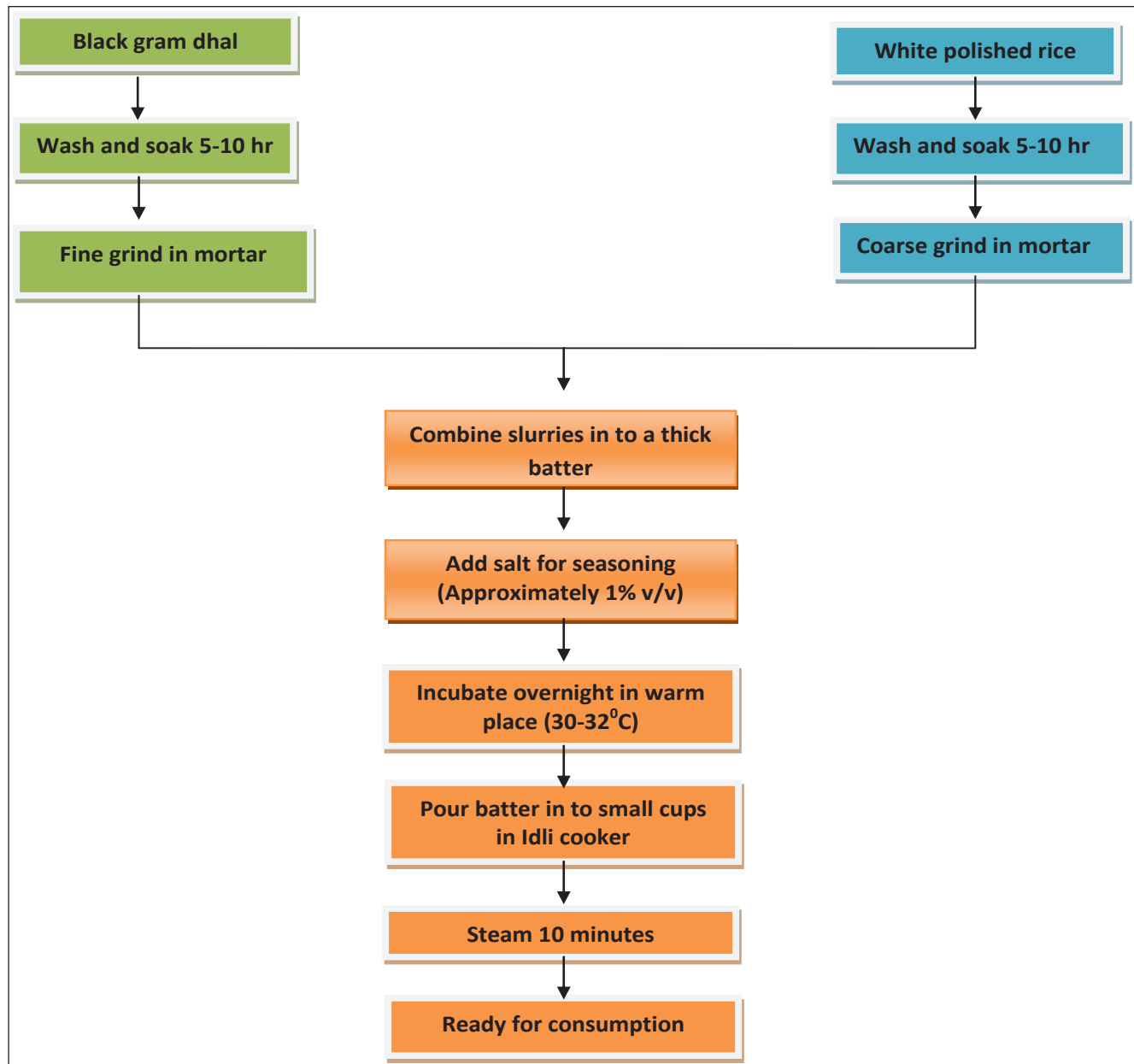


Fig. 1: Flow chart for *Idli* production

batters together in ratios of 2:1, 3:1 and 4:1 (v/v) batter were allowed for fermentation (0, 6, 12, 18 and 24 h) adding 2% of salt. 4. Allowing to ferment overnight at room temperature. 5. Depositing the fermented batter in special *Idli* pans and steaming for 5±8 min (Balasubramanium *et al.* 2006). It may have been found that the rice helped speed the fermentation process. Although the ingredients used in preparing

idli have changed, the preparation process and the name have still remained the same.

The production of acid and gas in *idli* is dependent upon bacteria while the yeasts contribute enzymes and esters which impart desirable flavours (Radhakrishnamurthy *et al.* 1961; Steinkraus *et al.* 1967). The lactic acid bacteria *Leuconostoc mesenteroides*, *Streptococcus faecalis*, *Lactobacillus*

delbrueckii, *Lactobacillus fermenti*, *Lactobacillus lactis* and *Pediococcus cerevisiae*, *Pediococcus pentosaceus* have been found to be responsible for the fermentation process, although *L. mesenteroides* and *S. faecalis* are considered to be the microorganism essential for leavening of the *batter* and for acid production in *idli* (Purushothaman *et al.* 1993; Ramakrishnan 1993). Yeast fermentation occurs overnight by the joint action of *Torulopsis candida* (Saito) *Trichosporon pullulans* (Lindner). In large cities, baker's yeast is used. *Idli* batter fermentation has been the subject of many research investigations covering aspects of optimization of ingredients, microbiological, physico-chemical and nutritional (Steinkraus *et al.* 1967; Ramakrishnan 1979; Venkatasubbaiah *et al.* 1984; Thyagaraja *et al.* 1991 and 1992). Two significant changes occurring in *idli* batter fermentation are leavening and acidification. These two parameters have been used as the criteria for judging the progress of fermentation. The percent total acidity of *idli* batter at different period of fermentation ranged between 0.44 to 0.91% (Ghosh and Chattopadhyay, 2010). Black gram, the leguminous component of *idli* batter serves not only as an effective substrate but also provides the maximum number of microorganisms for fermentation. These levels have been reported as ideal parameters reached in 12±14 h of fermentation for obtaining acceptable products (Soni & Sandhu, 1990). In naturally fermented *batter*, within a period of 2 days, the pH and acidity levels were not organoleptically acceptable. The surface-active proteins and polysaccharides of black gram are well conditioned to retain a large volume of gases to give soft and fluffy texture.

Idli contains protein 14%, carbohydrates 35-50%, fat 0.2%, Ca 80 mg, Fe 3.5 mg. The vitamins content of *idli* batter per 100 g are 0.59 mg riboflavin, 0.59 mg thiamine and 0.76 mg folic acid and energy 65 Kcal/*idli*. It has also been reported that during fermentation, vitamins B and C increase, and also phytate is hydrolyzed almost to 50%. *L. mesenteroides* (leavening) and *S. faecalis* (acid production) develop concomitantly at soaking stage and continue to multiply following grinding (Mukherjee *et al.* 1965).

These changes during fermentation are highly significant for nutritional point of view. An increase in methionine, a limiting essential amino acid in legumes, greatly improves protein value.

Papadam

These are flat, spicy, circular wafers from South India. Prepared from flour paste of dehulled black gram and fermented 4 to 6- hours. These are thin, dried, crispy, circular tortilla like wafers made up of legumes, cereals or starch crop flour eaten as snake or with meal by deep fried or roasted (Sankaran 1998). The dough is prepared and rolled into wafers of around 10-20 cm in diameter and 0.5 mm thickness. These are then fermented and dried in open air for 4-6 h. Soni and Sandhu, (1990) isolated bacteria and yeast from papdams. There are three different types of bacteria present and *Leuconostoc mesenteroides* is the most common along with *Lactobacillus fermentum* and *S. faecalis*. Fermentation increase the soluble solids and decrease in reducing sugars. The batter amount increases with duration of the fermentation. It contains the PH 5, total acids 0.7%, total nitrogen is remain same, amylase is decrease and vitamin B1, B2 and B12 increased.

FERMENTED CEREAL FOODS

Ambali

Fermented product of South and East Indian cereal grass called *ragi* [*Eleusine coracana* (L.) Gaertn.] rice. In olden days it was taken as breakfast. People think this is very energetic and good for health. In villages of Andhra Pradesh people prefer this at working places where they need energy. Sometimes this takes with butter milk, green chilli, onions and pickles which give better taste (Ramakrishnan, 1979). *Ragi* flour is made into a thick batter with water and allowed to ferment for 14 hours. The fermented *ragi* batter is then added to cooked rice while stirring to avoid lump formation. It is allowed to cool, mixed with sour buttermilk, and consumed. The microorganisms are *Leuconostoc mesenteroides* (Tsenk.) V. Tieghem, *Lactobaci fermentum* Beij., *Streptococcus faecalis* and

Saccharomyces sp. The pH usually decreases from 6.4 to 4.0 and volume increases by about 20%, indicating CO₂ production (Ramakrishnan, 1979).

Kali

It is one of the indigenous fermented rice products in AP, India. To leftover cooked rice, water is added, and the mixture is allowed to ferment overnight. This generally prepare in summer season because of the high temperatures in the nights. People in AP believes that Kali cools the body and very good for health (Sekar and Muniyappa, 2007). The microorganisms are *Leuconostoc mesenteroides*, *Lactobaci fermentum* Beij. Yeast such as *Hansnula*, *Candida*, *Saccharomyces* sp.

FERMENTED FRUITS AND VEGETABLES

Pickles

Pickling is one of the oldest methods of food preservation. Pickles are one of the important consumer products (Joshi *et al.* 2013). The important pickles in India are mango, drumstick, brinjal, gongura, mirchi pickle, chicken pickle, mutton pickle, fish pickle and prawn pickle. Pickles are one of the earliest commercial products appeared in the market (Verma and Joshi, 2000). In Orissa and Andhra Pradesh, the people are consuming the various pickles in daily diet. Pickles are consumed along with staple food such as rice, *chapathi*, bread, *samosa*, *upma*, etc. Particularly in both states particularly in tribal areas, raw material availability is high. Thus pickles making units can be promoted in tribal areas (Sekar and Muniyappa, 2007). Fully matured fresh vegetables, fruits, are washed and cut into required sizes and add salt, mirchi, oil and other ingredients in required quantity. The preparation of all pickles is similar and very popular mango pickle in AP is depicted in Fig. 2. The freshly prepared pickles are to be cured for a week and packed in different sizes with labeling and dispatched for market in commercial preparations. The same process also for non-veg pickles. The durability of the non-veg pickles is very short. The addition of salt to the pickles restricts gram negative bacteria and enhance the growth of lactic

acid bacteria. In all types of pickles the dominant organisms are *Leuconostoc mesenteroides*, *L.pallax* and *Lactobacillus plantarum* including these *Staphylococcus aureus*, *Saccharomyces cerevisiae* and *A. niger* also present when pickle spoiled.

INDIGENOUS FERMENTED BEVERAGES

Toddy and palm wine are alcoholic beverages that are made by fermenting the sugary sap from various palm plants. Virtually any sugary plant sap can be processed into an alcoholic beverage – it just needs the correct yeasts, temperature and processing conditions (Joshi, 2016). Throughout the world, alcoholic drinks are made from the juices of locally grown plants including coconut palm, oil palm, wild date palm, nipa palm, raphia palm, Caryota palm and kithul palm.

Palm Wine

Palm wine is the fermented sap of certain varieties of palm trees including raphia palm (*Raphia hookeri* or *R. vinifera*) and the Caryota palm (*Jeeluga*). It is collected by tapping the top of the trunk. There are many variations of the same product and no individual method or recipe. Palm wine is particularly common South India (Shamala and Sreekantiah, 1988). The sap use to collect from a growing palm. It is collected by tapping the palm. This involves making a small incision in the bark of the palm, about 15 cm from the top of the trunk. A clean gourd is tied around the tree to collect the sap, which runs into it. The sap is collected each day and should be consumed within 5-12 hours of collection. Fresh palm juice is a sweet, clear, colourless juice containing 10-12 percent sugar. The sap is not heated and the wine is an excellent substrate for microbial growth. It is therefore essential that proper hygienic collection procedures are followed to prevent contaminating bacteria from competing with the yeast and producing acid instead of alcohol (Fig. 3). Fermentation starts soon after the sap is collected and within an hour or two becomes reasonably high in alcohol (up to 4%). If allowed to continue to ferment for more than a day, it starts turning into vinegar. The quality of the final wine is

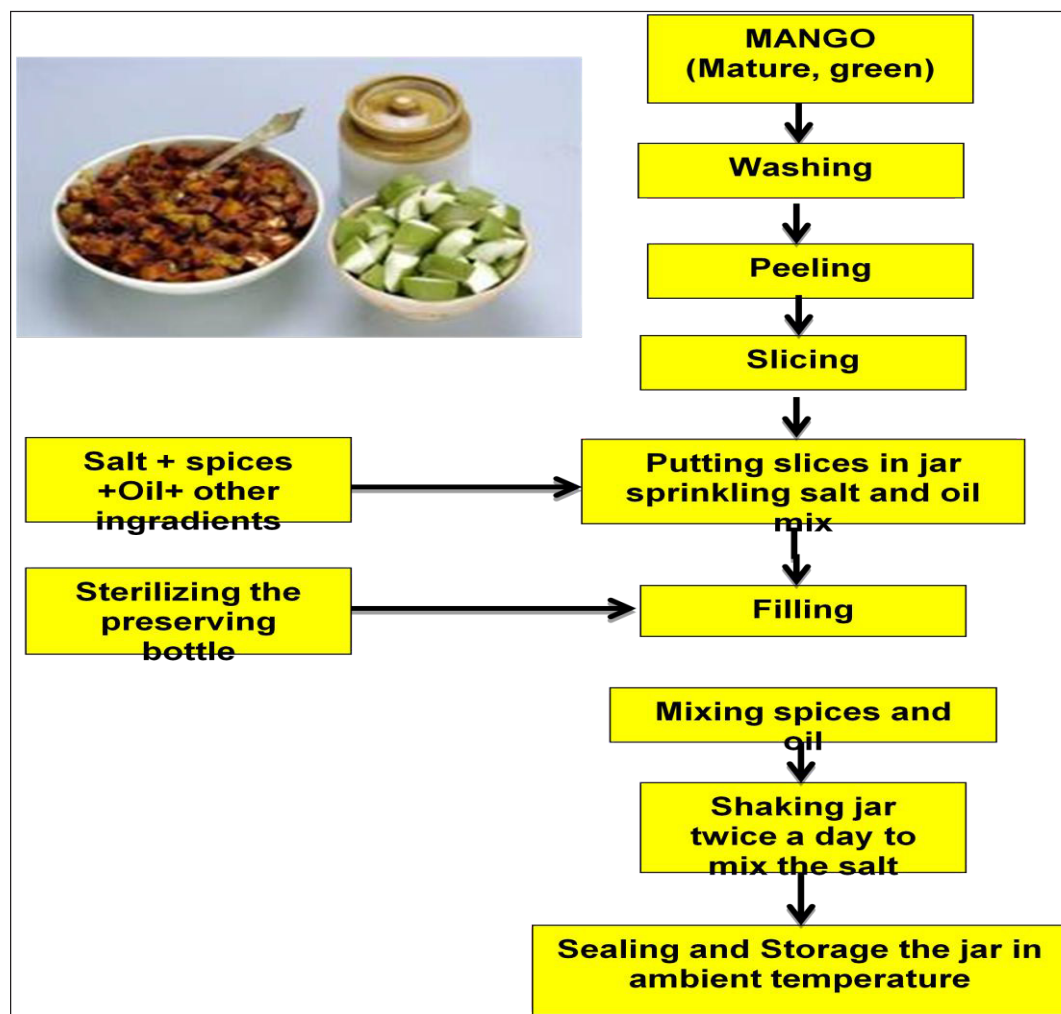


Fig. 2: Flow chart of mango pickle preparation

determined by the conditions used for the collection of the sap. Often the collecting gourd is not washed between collections and residual yeasts in the gourd quickly begin the fermentation. This is beneficial as it prevents the growth of bacteria which can spoil the sap (Grimwood and Ashman, 1975). Packaging is usually only required to keep the product clean and to transport it for its relatively short shelf life. Clean glass or plastic bottles should be used. The product should be kept in a cool place away from direct sunlight.

Toddy

Toddy is an alcoholic drink made by the fermentation

of the flower sap from a coconut palm (*Cocus neusifera*). Fresh coconut sap contained 12-15% of sucrose (by weight) and trace amount of reducing sugar including glucose, fructose, maltose and raffinose. The sap contains approximately 0.23% protein, 0.02% fat. Half of the total sugars are fermented during first 24 hours and ethanol content of the fermented palm sap reaches maximum of 5.0 – 5.28% (v/v) after 48 h (Sekar and Mariappan 2005 and 2007). After fermentation it is white and sweet with a characteristic flavour and contains between 4 and 6% alcohol. There are two main types of *kallu* in Andhra Pradesh, namely *Thadi kallu* (from Toddy Palmyra trees) and *Eetha kallu* (from silver date palms). *Eetha Kallu* is very sweet and less

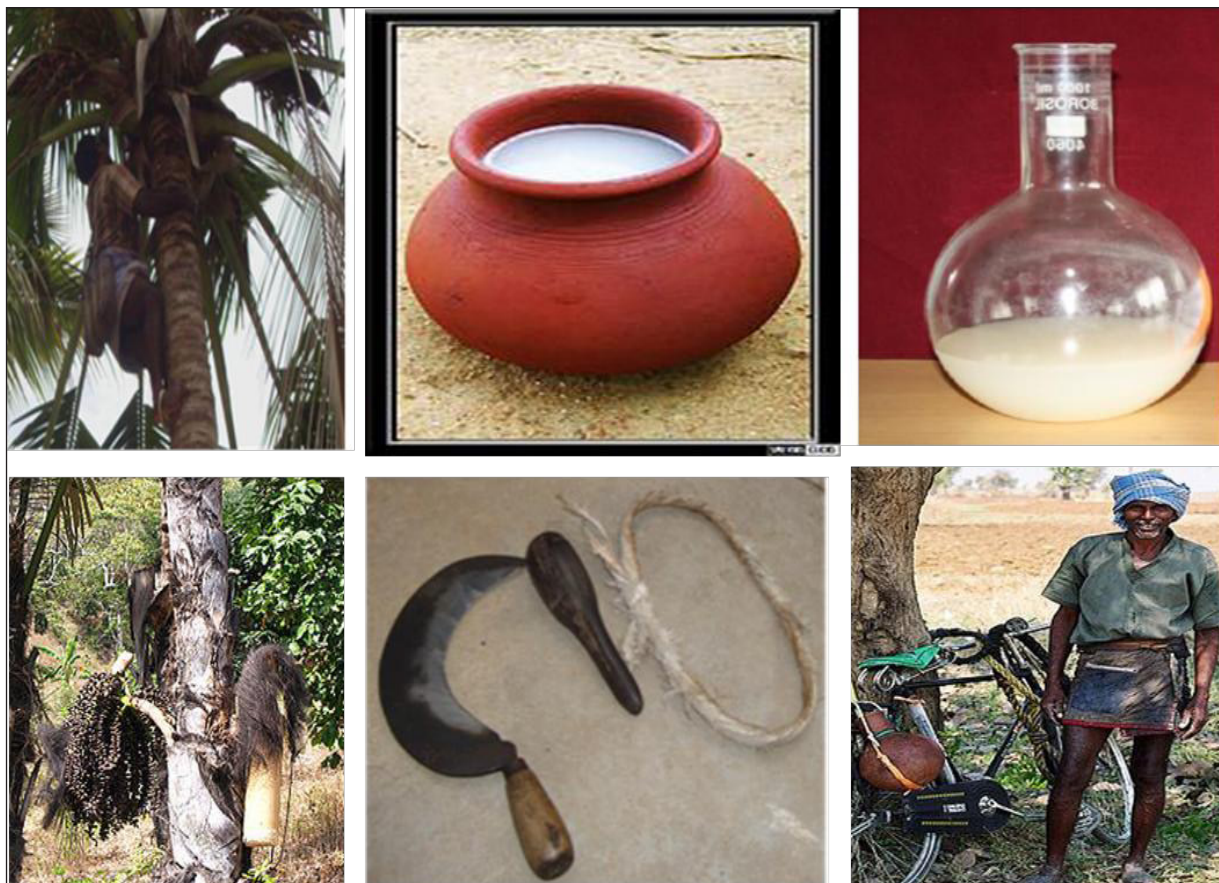


Fig. 3: Palm /Toddy wine collection and Toddy collector in Andhra Pradesh

(From left to right 1. Coconut tree, 2. Toddy wine in earthen pot, 3. palm wine collected in glass beaker, 4. Palm tree, 5. Instruments used to collect palm/toddy wine and 6. Toddy collector)

Source: Half yearly report on standardization of selected ethnic fermented foods and beverages by rationalization of indigenous knowledge C30031.

intoxicating, whereas *Thati Kallu* is stronger (sweet in the morning, becoming sour to bitter-sour in the evening) and is highly intoxicating. A small amount of toddy from the previous day's fermentation should be left in the pot to start the fermentation (Fig. 3). A natural fermentation takes place due to yeasts that are present in the sap and those that are added from a previous batch of toddy. The sugar in the sap is partly fermented to alcohol, which helps to preserve the product. It requires refrigeration if it needs to be stored for more than 24 hours. The collecting pots should not be washed out. That way they retain a small amount of starter inoculum for future fermentations. However, it is important to

ensure that they are kept clean and do not become contaminated by other bacteria. The fermentation vessels should be kept clean and the product must be hygienically handled (Law *et al.* 2011). Shamala and Sreekantiah (1988) reported that the fermentation produces mainly ethanol, acetic acid and lactic acid. pH of the sap rapidly dropped from around 7.2 to 5.5 due to formation of acetic acid and ethanol content drastically increased to 5% (v/v) within 8 hours. Palm wine contains 5% (v/v) ethanol, lactic and acetic acids 1-1.5%. Palm wine was found rich in mineral as it contains 0.019 – 0.028% (w/v) of vitamin B-12 and 0.008% of ascorbic acid (Van Pee and Swings, 1971; Steinkraus, 1994).

The palm sap fermentation involved alcoholic–lactic–acetic acid fermentation, by the presence of mainly yeasts and lactic acid bacteria. Aidoo *et al.* (2006) concluded that *Saccharomyces* spp. present in the natural fermented palm sap and are important for the formation of characteristic aroma of the palm wine. *S. cerevisiae* and *S. pombe* have been reported to be the dominant yeast species (Odunfa and Oyewole, 1998). Other yeast species such as other *Candida* spp and *Pichia* spp. are also present (Atacador-Ramos, 1996). Lactic acid bacteria and other bacteria such as *Lactobacillus plantarum*, *L. mesenteroides*, *Acetobacter* spp. and *Zymomonas mobilis* are also present. The microorganisms are reported to originate from the palm tree, the gourd used for sap collection and fermentation, or the tapping equipment. Study done by Atputharajah *et al.* (1986) reported the presence of seventeen (17) species of yeasts and seven (7) genera of bacteria in the natural fermented coconut palm sap. Yeast species found include the *Candida paropsilosis*, *C. tropicalis*, *C. valida*, *K. javanica*, *Pichia etchellsii*, *P. farinose*, *P. guilliermondi*, *P. membranaeficiens*, *P. ohmeri*, *Rhodotorula glutinis*, *Saccharomyces chevalieri*, *S. ludwigii*, *S. bailii*, *Schizosaccharomyces pombe*, *Sporobolomyces salmonicolor* and *Torulopsis* spp. *Bacillus* is the predominant bacteria genus while others included *Enterobacter*, *Leuconostoc*, *Micrococcus* and *Lactobacillus*.

Sara

Sara is a distilled liquor and very popular alcohol beverage/drink in villages of Andhra Pradesh which also called *Naatusarai* (country *Sara*). It is used to prepare in festive seasons like *Sankranti*, *Ugadi* and *Vijayadasimi*. It contains high alcohol concentration around 20-25% (v/v). Rice flour, black jaggery and bark of white acacia tree are used in the preparation of *Sara*. It is prepared by anaerobic fermentation with in porous earthen pots that kept in deep soil for 10-15 days. After the fermentation it is distilled using pot distillation method. In some varieties different fruits/ and nuts banana, orange, guava, mango also used to get best quality *Sara*. But after the fermentation the *Sara* prepared with the addition of fruits and nuts

is filtered through sieves and resulting filtrate is consumed directly.

CONCLUSION

Fermented foods is important to human's diet and possess a natural and healthy image. Traditional fermented foods of Andhra Pradesh (AP) are prepared at the house hold level and only few are commercialized. There are very few reports on traditional fermented foods of Andhra Pradesh. Most of the countries have been well investigated and documented their traditional fermented foods and beverages. In India and in Andhra Pradesh, most of the traditional fermented foods and beverages are yet to be investigated. Only a few common fermented foods such as *idli*, *dosa*, *dahi* have been studied so far. Isolation, purification and identification of dominant microorganisms involved in traditional fermented foods and beverages are important aspects of such studies. Identification of predominant microorganisms through phenotypic and genotypic molecular methods such as 16S rRNA sequences, FISH, metagenomics tools with the aid of computer informatics, chemotaxonomic tools such as cell wall studies, cellular fatty acids and isoprenoid, quinones are helpful, when the conventional methods of identification is not possible. Due to the presence of various ethnic groups in India we have a diversified food habits. There is a need to study the microbial diversity associated in fermented foods may contribute a significant gene pool, which must not be lost in this generation. This is right time to commercialize our popular and acceptable traditional fermented foods, some of which may present unknown potent microorganisms which could be of scientific interest or may have potential industrial application.

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REVIEW PAPER

Recent Advances in Preparation and Functional Properties of Smoothie as Food: A Review

Aanchal Srivastava, Rajeev Kumar*, Akansha Arora, Jyoti Joshi and Sanchit Vishnoi

Department of Food Technology, Uttarakhand University, Dehradun, Uttarakhand, India

*Corresponding author: rajeev.kumar267@gmail.com

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ABSTRACT

The aim of this study was to know the scenario of habit of consumption of fruits and vegetables amongst consumers and implement the concept of smoothie as an easy, nutritive and innovative form of their consumption. Smoothie is a liquid refreshing drink usually taken as health-conscious supplement for a healthy living. Smoothie contains either whole or different parts of fruit and vegetable. Fiber and juice are not separated from each other thus, increasing nutritive as well as digestive properties of the smoothie. Fruits and vegetables have low energy value but high nutritive physiological value. They are mainly constituted of starch and dietary fibers. They contain appreciable amount of micro-nutrients such as iron, calcium, phosphorus, magnesium, sodium and chromium. Fruits and vegetables are also rich in phytochemicals. These phytochemicals help in reducing the possibilities of cancer. To increase the uptake of phytochemicals, food industries offer an alternate source of utilization of fresh fruits and vegetables as smoothie. To create distinct flavour and ease in consumption corresponding fruit juices or concentrates can also be used. The natural pigments and antioxidants present in fruits and vegetables also help in glowing and smooth skin, also increasing better functioning and immunity of the body.

Keywords: Fruits, Smoothie, Consumption, Functionality, Nutritive value, etc.

Smoothie is an excellent and convenient substitute which promotes the intake to fresh fruits and vegetables on daily basis (Rodriguez-Verastegui, *et al.* 2015). They are non-alcoholic liquid refreshment drink usually made out of fresh or frozen fruits or vegetables. They are mixed together without straining and often served with crushed ice. Some smoothies may also contain other ingredients such as yoghurt, milk, ice-cream, lemonade, tea or spices and condiments. Their consistency is slightly concentrated than slushie (Castillejo *et al.* 2015). Smoothies have been mostly encouraged as fitness freak beverage option for wellbeing and healthy lifestyle. Considering the storage period,

non-thermally processed smoothies are stored for shorter duration only due to possibility of increased microbial growth. The longer storage time may also result in degradation of natural colour and total phenols (Cano-Lamadrid, *et al.* 2018).

CATEGORIES OF SMOOTHIES

Smoothies can be sub-divided into three main categories viz. fruit only, Fruit and dairy product

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and functional smoothie. Functional smoothies are considered as the very recently available product in the market, usually containing probiotics amongst itself. Various types of Smoothies are described in Table 1. The advancement of consumption of fruits and vegetables is getting pronounced more than ever. This is caused by the desire of consumers for less processed, healthy and nutritive foods (Banigo *et al.* 2015; Zlabur *et al.* 2017). Smoothies are considered to contain one portion of fruit. Many commercially available smoothies are rich in calories and added sugars. Syrup-based fruit concentrates are being utilized instead of natural fruits, thereby resulting in a product high in sugar and lower in macro-nutrients especially vitamin C. The concept of consuming smoothie is not well known amongst citizens; almost more than a half of population prefers fruit juices over smoothies.

ADVANTAGES OF SMOOTHIES

Foods grown from the ground are considered as one of the basic piece of human sustenance (Boeing *et al.* 2012). They contain huge measure of small scale supplements making them progressively significant regarding nourishing physiology. They are rich water-solvent nutrients provitamin A, phytosterols and assortment of minerals (Gebbers, 2007). Various investigations have been recorded which shows that foods grown from the ground help in counteraction of certain chronic sicknesses for example hypertension (Dauchet *et al.* 2007), coronary heart maladies (Dauchet *et al.* 2006; He *et al.* 2007), and the danger of stroke (Dauchet *et al.* 2005; He *et al.* 2006). Regardless of the way that admission of leafy foods diminishes the event of illnesses, the utilization of products of the soil stay beneath the suggested levels (Mytton

Table 1: Different types of smoothies

Sl. No.	Constituents	Formulation	Analysis	Reference
1	Apple Smoothie	Apple phenolic extract, CMC, citric acid	Apple Fibre, N ₂ Physisorption, FTIR, Total Dietary Fibre, Total Extracted Polyphenol Content, HPLC, Rheological measurement	Sun-Waterhouse <i>et al.</i> 2013
2	Carrots Smoothie	Carrot: Water= 1:1,	pH, TSS, titrable acidity, Browning index, Phenylalanine Ammonia-Lyase, Total antioxidant capacity, TPC	Formica-Oliveira <i>et al.</i> (2017)
3	Fruit Smoothie	Entire apple-29.5% Squeezed apple concentrates-29.5% Strawberry-21% Banana-12% Orange-8%	Polyphenols, HPLC-DAD (diode ray detector), Hunter Lab, Dynamic Oscillatory measurement, Sensory	Keenan <i>et al.</i> (2011)
4	Pomegranate Smoothie	Pomegranate: Figs + Jujubes + Quinces Puree (60:40 & 40:60), Rhubarb Juice (5%)	Hunter Lab, UPLC-PDA (Ultra-performance Liq. Chromatography-Photodiode Array), Polymeric Procyanidins, Antioxidant Capacity, Four-way Anova	Cano-Lamadrid, <i>et al.</i> (2018)
5	Red and Green Smoothie	26% Cherries, 8% Tomatoes, 5% Blackberries, 31% Prunes for Red Smoothie 40% Kiwi, 7% Fennel, 8% Spinach, 15% Papaya for Green Smoothie White grape juice- 20% Aloe vera- 10%	Microbiological, pH, soluble solids, titrable acidity, apparent viscosity, colour, browning index, soluble dietary fibres, total polyphenolic compounds, Organic acids and sugars, ascorbic acid, antioxidant activity	Cagno, <i>et al.</i> (2011)

6	Purple Smoothie	Beetroot- 12% Purple grapes- 45% Cucumber- 35% Broccoli- 8%	Sensory, Microbial, Vitamin C, TPC, Total antioxidant content (TAC), Anthocyanin	Gonzalez-Tejedor <i>et al.</i> (2017)
7	Fruit Smoothie	Apple Juice- 33% Orange Juice- 33% Strawberry- 14% Whole Apple- 10% Banana- 10%	Sensory, Peroxidase enzyme activity, Polyphenol oxidase enzyme activity, Pectin methyl esterase enzyme activity, Antioxidant, Density, Viscosity, Total soluble and insoluble solids, Turbidity and Transmittance, Hunter Lab, Browning Index, Total Acidity, pH, Vitamin C, Total Phenols and Flavonoids, Sugars, Microbial	Hurtado <i>et al.</i> (2015)
8	Kiwi Smoothie	Kiwi- 3kg Sugar- 0%, 10%, 15%, 20%, 25%	Bioactive compounds, TAC, Total Chlorophyll, Total Carotenoid, Total Ascorbic Acid, Fluorometric measurement, FTIR	Park <i>et al.</i> (2016)
9	Mixed Fruit with Coconut Milk Smoothie	Pineapple- 50g Banana- 28g Apples- 12g Orange- 3g Coconut milk- 7g (per 100g smoothie)	Microbiological, Shelf-life analysis, Sensory analysis, Heat and Pulsed electric field and thermal pasteurization for preservation	Walkling-Ribeiro, <i>et al.</i> (2010)
10	Aloe gel- spiced ripened papaya Smoothie	Papaya pulp- 15% Sugar Syrup- 20% Aniseed, Ginger and Pepper- 5% Citric Acid- 0.1% Aloe gel- 10%, 20%, 30%	pH, Titrable acidity, TSS, non-enzymatic browning, Total and reducing sugar, Hunter Colour Lab, Vitamin C, Total flavonoids, Total polyphenols, Microbial analysis, Sensory acceptability	Ramachandran & Nagarajan (2014)
11	Dehydrated Smoothie	Dehydrated Banana, Dehydrated Strawberries, Dehydrated egg white, Demerara sugar (2%) Cold water/cold whole milk	Sensory, Viscosity, Hunter Lab	Guazi <i>et al.</i> (2019)

et al. 2014). Accordingly, to take out this propensity and present a sound living, exchanges, for example, juices, mixes, smoothies and matured and sustained refreshments are a well-known path for foods grown from the ground utilization (Wootton-Beard and Ryan, 2011; Corbo *et al.* 2014; Marsh *et al.* 2014; Ramachandran and Nagarajan, 2014 and Hurtado *et al.* 2015).

Due to health-promoting properties, diets rich in fruits and vegetables are highly recommended. They

possess a superior place due to their concentrations of vitamins, especially water soluble; minerals, especially electrolytes; and more commonly phytochemicals such as antioxidants (Slavin and Lloyd, 2012). Avocado, potatoes, dried beans and corn are wealthy in starch while yams are nearly sucrose and not starch. Natural products, aside from banana, and dull green vegetables contain next to zero starch. It is important to understand the categories of fruits and vegetables to ensure proper dietary intake in accordance with dietary recommendations. Fruits

and vegetables are usually low in energy density and excellent wellsprings of fiber and potassium (Hornick and Weiss, 2011).

Natural products generally contain sugars and fibers, for example, gelatin, that are widely matured in the internal organ. Natural products, for example, apples and pears, are packed in fructose (Southgate *et al.* 1978). Apples display 6% fructose and 3% sucrose though pears contain about 6.5% fructose and 1.3% sucrose; these qualities would be predictable in their particular juices. Admission of foods grown from the ground decreases the event of oxidative pressure related infections (Escudero-Lopez *et al.* 2016). Smoothies are a great and appropriate choice to advance the everyday usage of products of the soil (Rodriguez-Verastegui *et al.* 2015). Epidemiological examinations show that there is an away from between admission of products of the soil and diminished pace of heart infections mortality, normal malignant growths and other degenerative illnesses including maturing (Steinmetz and Potter, 1996; Garcia-Closas *et al.* 1999; Joseph *et al.* 1999; Dillard and German, 2000; Prior and Cao, 2000; Wargovich, 2000). It mainly helps in reducing the risk of cancers of the mouth and pharynx, oesophagus, lung, stomach and colon.

Blending is an alternate to reduce under-utilization of vegetables, fruits, spices and condiments. It may be attributed to alter dietary intake, taste preference and way of life of consumers. The demand of blended smoothies has been increasing with each passing year due to their health benefits and amazing taste. Therefore, to enhance taste, flavor, palatability, aroma and nutritive properties of any particular fruit or vegetable, it is aimed to be convenient to blend it with highly nutritive fruits and vegetables of other variety.

BEVERAGES

Water balance inside human body can be maintained by consuming an adequate amount of water which is equal to amount of water being excreted out. Human body keeps water balance in harmony state

by controlling admission and discharge (Sayed and Abdellatif, 2018). Refreshments add to prerequisites of water in human. Troiano *et al.* 2000 detailed that around 20-24% of vitality consumption originates from drinks. Notwithstanding satisfying essential prerequisite, drinks turned into a piece of culture of human culture. Assortment of drinks taken influences arrangement of present day diet (Stubbs and Whybrow, 2004). These drinks are in top ten contributing nourishments for a few supplements. Natural product juices adds to vitality, calcium, iron, vitamin C, vitamin A and fiber consumption while milk adds to vitality, protein, fat, calcium, and nutrient An admission (Zohouri, *et al.* 2004).

SMOOTHIE

The concept of smoothies came into vision in 1990s and is one of the major growing sectors of beverage industry (Mordor Intelligence, 2017). Smoothies are progressively famous method of expending organic product. It should contain minimum of one portion of fruit. To increase the consumption and eating interest of consumers, it is the best way to process fruits and vegetables through different methods (Bates and Price, 2015). Smoothies are generally made up by joining entire foods grown from the ground which keeps up fiber despite the fact that squeezing will in general abandon a mash containing fiber however in any case holds its dietary benefit (Clemens *et al.* 2015).

As of late, smoothies have increased a lot of notoriety for example development of item ascends from 2.39 occasions from 2002 to 2007 according to food merchandiser (Lal, 2007). Smoothies are a superb and helpful source to improve the day by day utilization of products of the soil (Rodriguez-Verastegui *et al.* 2015). Comfort and fitness food properties are two most significant variables for high-pace way of life. Changes in dietary admission are most earlier treatment for development of human wellbeing (Williams *et al.* 2004; Sabbe *et al.* 2009; Verbeke *et al.* 2009; Faresjo *et al.* 2010; Krystallis *et al.* 2010).

PREPARATION AND PRESERVATION OF SMOOTHIES

The time span of usability of unpasteurized smoothies is relatively shorter because of microbial development accordingly negligible degree of preparation are related with these items. These are ordinarily expended either new or protected for brief timeframe (1–3 weeks) by putting away them under refrigeration conditions. A few specialists proposed that it could be worth to utilize a gentle warm sanitization treatment (Walkling-Ribeiro, *et al.* 2010) or a warm and high hydrostatic weight handling to degree their time span of usability and for predominant shading maintenance, polyphenols and other quality characteristics, ensuring purchasers acknowledgment and sanitation (Keenan, *et al.* 2011; Tiwari, 2018).

Low thermal processing is projected which enables retaining colour, consistency, fresh flavour, and ascorbic acid content. Acidification may convert low acid juice to an acidic juice allowing milder thermal process conditions (Agarwal & Kumar, 2017).

FUNCTIONAL PROPERTIES OF SMOOTHIE

The rule job of products of the soil was by and large generally explored from most recent a very long while because of its high fiber substance and wealth of nutrients and minerals. It has been acknowledged because of its recipient impact on human wellbeing, introducing general wellbeing endeavors, for example, '5 per Day for Better Health', DASH diet and incorporation in the American Heart Association (AHA) dietary rules to upgrade utilization to improve human wellbeing (Tucker, 2004). Expanded admission of products of the soil helps in counteraction of constant and degenerative illnesses. In this way products of the soil are accepting a nickname as "useful nourishments" (Whitney and Rolfes, 2008).

Useful non-mixed drink industry is a quickest developing division and it is ceaselessly getting new items and bundling styles advertise. Increment in purchaser's interest for helpful beverages and

its suggestion on solid way of life is the impetus of industry's development. Being non-improved included characteristic flavors and enhanced with nutrients and minerals is a sound option in contrast to sodas (Suhag and Singh, 2017). Certain practical drinks have been created to give explicit health advantages. Numerous epidemiologic and clinical researches depicts multi advantages of products of the soil in human day by day diet for avoidance from intense and interminable afflictions (Lock *et al.* 2004).

Smoothies are generally made up from products of the soil which are wealthy in phenolic mixes. These phenolic mixes are acceptable wellspring of cell reinforcements liable for wellbeing advancing properties, for example, calming, antitumoral, forestalling neurodegenerative and interminable infections (El Gharras, 2009). Essential (shikimate) and auxiliary (phenylpropanoid) pathways are most regular pathways that occurred for the biosynthesis of polyphenolic mixes which happens within the sight of key chemical phenylalanine smelling salts lyase (PAL) (Dixon and Paiva, 1995). It was proposed that PAL movement can be expanded by a variety of biotic and abiotic stress-actuated components, for example, injuring, radiation presentation, hyperoxia capacity, chilling injury, water pressure, low minerals, hormones, ultrasounds, and pathogen assault (Cisneros-Zevallos, 2003; Cuellar-Villarreal *et al.* 2016).

Apple is considered as a best source of a healthy diet. They contain high amount of polyphenolic compounds and dietary fibers. It helps in preventing digestive problems, chronic disorders, respiratory cancer, asthma, diabetes type 2, thrombotic stroke and ischaemic heart problems (Lee *et al.* 2003; Boyer and Liu, 2004; Schulze *et al.* 2004; McGhie *et al.* 2005; Okoko *et al.* 2007; Hansen *et al.* 2009; Sun-Waterhouse *et al.* 2008a, b, 2011; Chai *et al.* 2011). Fresh apple prevents constipation, reduces dental caries, controls obesity and provide extra energy for heavy exercise. The apple fruit pulp is found to be richest source of phytochemicals like catechin, quercetin, phloridzin and chlorogenic acids. They are strong antioxidants which decrease the risk of cancers, cardiovascular

diseases, asthma and diabetes. These phytochemicals inhibits cancer cell proliferation, reduces lipid oxidation and lower cholesterol (Boyer & Liu, 2004).

Green leafy vegetables are separated and investigated independently from fruits and vegetables. There are few studies according to which green leafy vegetables possess protective effects and studies have shown that including them in regular diets is beneficial as due to their superiority in nutritive quantity amongst fruits and vegetables (Hung *et al.* 2004; Johnston *et al.* 2000; Zhang *et al.* 2011).

Milk is considered as a rich source of calcium, which is an essential nutrient for growth and development of strong bones and teeth, muscle movement and nerve signals. Many related organizations exclaim that getting enough amount of calcium in daily diet results in preventing bone fracture and osteoporosis. It is also a good source of potassium which helps blood vessels dilate and reduce blood pressure. Increased level of potassium rather than sodium intake helps in reducing blood pressure, lowering risk of heart disease and stroke (Miller *et al.* 1995; McCarron, 1998). The components such as calcium, vitamin D, lactic acid bacteria, linoleic acid and bioactive peptides present in dairy products could protect against colon cancer (Mobarhan, 2009; Garland *et al.* 1991; Ouwehand *et al.* 1999; McIntosh, *et al.* 1998).

Smoothies are made up by combining together fruit, fruit juice, ice, and yoghurt or milk. Between 2002 and 2006, the market share increased to 214% in Republic of Ireland (Mintel Group, 2008). Oey *et al.* (2008) have investigated various possible substitutes to warm preparing of natural product beverages to limit the conceivable negative effect that warm handling may have, coming about in negligibly prepared new items without concession towards timeframe of realistic usability. The execution of high hydrostatic weight (HHP) (up to 700 Mpa) can impact in protein inactivation and a gentle sanitization to food items at encompassing temperatures (Meyer *et al.* 2000; Weemaes *et al.* 1999).

Patras *et al.* (2009) suggests that, HHP can be used

to retain nutritional attributes of antioxidant rich fruits due to its property of affecting high atomic weight sub-atomic structure, for example, sugars and proteins in food. It doesn't influence littler particles connected along with organoleptic, dietary and wellbeing advancing properties. Smoothies are considered as a mainstream method of devouring organic product homogenates containing mash. They contribute towards a healthier diet and lifestyle. It comprises of numerous ingredients from fruit to dairy products, from pulp to yoghurt and sometimes sweeteners (Walkling-Ribeiro *et al.* 2010).

According to Hurtado *et al.* (2015) studies published up till now on high pressure processing (HPP) was carried out on individual fruit products. In recent years, some studies and researches have also been developed on blended fruit mixtures. Different pressurizing conditions were trialed to obtain products with organoleptic and nutritional characteristics normally in relation with "minimally processed" fruit.

Balaswamy *et al.* (2013) in their study regarding advancement of smoothies from chosen organic products pulps/juices aimed to blend different fruit pulp i.e. mango, banana, sapota, papaya and juices viz. grapes, phalsa, pineapple, watermelon and pomegranate of optimal quantities. The fruits were mixed in a manner that it provide required consistency as well as was compliant to thermal preservation processes in glass bottles. The blending also helped in manageable sugar acid ratio together with better colour and flavour without using preservative. Combining and mixing various fruit pulps and juices helps utmost use of natural product pulps and squeezes and furthermore help to sustain wholesome status of the populace.

MARKET SCENARIO

It is important for the consumers to increase their awareness level followed with new trends and innovations. Awareness regarding ingredients associated with product and nutritional and adverse effects of the final product. It is an important criteria as some ingredients can be consumed by every age

group while some may cause fatal symptoms on wellness of the person (Olivo, 2015). Consumption of fruits and vegetables either in their original form or other variety is directly in relation to prevention and treatment of any acute or chronic diseases. As functional foods provide more than just nutrition thus it is gaining popularity around the consumers. According to Levy & Tapsell, (2007), due to emerging trends and consumers' specific needs, new variety of products could be witnessed in the market with natural and functional ingredients.

All around, admission of foods grown from the ground by larger part of individuals is underneath the day by day suggested dietary necessities. Researchers exclaimed that in developed countries such as Australia, Europe, Canada, UK and USA a large gap is observed between actual intake of fruits and vegetables to recommended servings. From an investigation inside 52 low and center salary nations about 77.6% men and 78.4% ladies are expending not as much as that of suggested dietary cutoff points. According to the same study, 74% low consumption is reported amongst adult of India (Hall *et al.* 2009).

India being the second largest producer of fruits and vegetables in the world grows almost all varieties of fruits and vegetables which contribute about 92.3% of total horticultural production within the country. Yearly utilization (kg/individual/annum) of organic products was 9.6 for provincial, 15.6 for urban and generally speaking India is 11.8 though if there should arise an occurrence of vegetables 74.3 (rustic), 79.1 (urban) and 76.1 (India) (NSSO). From the aggregate

of green creation, just 2% of these yields are prepared into esteem included items, for example, sticks and jams, squeezes and thinks, canned and dried out items, mash, pickles and chutneys. In the contrary, processing of these value-added products ranges between 80% to 30% in countries like USA, China, South Africa, Brazil, Chile, Philippines, Malaysia, Thailand, etc. (Kaul, 1998; Goyal, 2006).

McCartney *et al.* (2018) designed an online survey among Australian adults constituting about 79% female to recognize the smoothie consumption pattern and individual motives behind its intake. Candidates reported consuming 98% fruit, 46% fruit juice, 66% yoghurt, 52% honey and 58% milk and 46% nutritional supplements in their smoothies. Visit smoothie purchasers were found to devour more center food and rather less intentional things, for example, nectar, frozen yogurt, and so on in contrast with rare smoothie buyers. These frequent smoothie consumers perceives smoothie as nutritious (84%) while infrequent smoothie consumers regarded smoothie as indulgent beverages (62%).

RECENT TRENDS AND INNOVATIONS

Varous recent trends and Innovations made in Smoothies preparation are summarized in Table 2.

Limitations

The study regarding smoothie and its consumption is limited because of a little awareness about physiological and nutritional value of ingredients and amount present in final product among

Table 2: Recent Trends and Innovation

Sl. No.	Constituents	Formulations	Observations	Reference
1	Cereal-milk fruit smoothie	Full Cream Milk- 42.4% Banana-26.2% Blueberry- 14.5% Yoghurt-11.3% SMP- 3.0% Psyllium husk- 2.5% Water	Comparative survey on difference in dietary behaviour	McCartney <i>et al.</i> (2019)

2	Detox Smoothie	Mixed e.g. Green and red juice smoothies, Wellness shots, Nuts shakes and filling smoothie	Recipe Book consisting of different types of healthy smoothies	Maranik, E. (2015)
3	Flour-Milk based smoothie	Dairy animals milk: 3% Fat, 8.5% SNF Sprouted finger millet flour, Germinated sorghum flour, Germinated green gram flour, Germinated chickpea flour, flour went between 2-6% Mango mash 10% 15% and 20%, Sugar-9% 10% and 11%	Sensory evaluation, Effect of apple juice and mango pulp on sensory attributes, Effect of flour levels on sensory attributes, Effect of sugar level on sensory attributes	Rani <i>et al.</i> (2016)
4	Green coconut smoothie	Solid albumen of green coconut-20% Acerola pulp, pineapple pulp and coconut water- different ratios	Vitamin C content, Antioxidant capacity, Sensory acceptability, Total Phenolic Compounds, pH, Titrable acidity, TSS	Teixeira <i>et al.</i> (2019)
5	Chikoo Chia Smoothie	Chikoo – 50g Curd- 25g Honey- 10g Milk- 125ml Chia seeds in ratios 4g, 8g and 12g	Colour, Consistency, Taste, Overall acceptability	Battalwar & Shah (2015)
6	Pumpkin leaves fortified Smoothie	Pineapple, Banana, Apple, Pumpkin leaves (4.5%, 3% and 1.5%)	Proximate analysis, Vitamin C, Mineral content, Total flavonoids, Total Phenolic, Antioxidants, Sensory	Aderinola (2018)
7	Jamun Synbiotic Smoothie	Skim milk, SMP, Yoghurt, Sugar, Jamun juice	Proximate analysis, Shelf life at refrigerated temperature, Sensory evaluation, Prebiotic effect for microencapsulation, pH, acidity, viscosity, total viable count, antioxidant, total phenolic compounds, ascorbic acid.	Saranyambiga, <i>et al.</i> (2017)
8	Soy/Carrot Flavoured with Beetroot	Soybean milk: water= 1:10 Beetroot/Carrot pulp: water= 1:4	Viscosity, pH, TSS, Sensory Evaluation, Proximate Analysis	Banigo, <i>et al.</i> (2015)
9	Olive Leaf Extract fortified smoothie	Sodium cyclamate, sucrose, sodium chloride, citric acid, Strawberry-Banana smoothie, Olive leaf extract with oleuropein content, modified starch	Threshold Test, Ranking Test	Kranz, <i>et al.</i> (2010)

consumers. The smoothie is not well differentiated from the term soft drinks and health beverages. Numerous researches are available on consumption of whole fruit in its actual form or alternatives like juices, concentrates, etc. but no specific clinical health research is exhibited regarding consumption of smoothies. Smoothies being available in market are

high in sugar content and thus making it unhealthy in perspective of energy and low calorific food product.

CONCLUSION

The review paper highlights the preparation of various smoothies depending on availability of seasonal fruits and the taste of consumers. Various

types of smoothies, market scenario, limitations and functional properties of smoothie are well covered in this review paper. Numerous researches are available on consumption of whole fruit in its actual form or alternatives like juices, concentrates, etc. but no specific clinical health research is exhibited regarding consumption of smoothies. Furthermore, many of the constraints in smoothies can be minimized through its commercialization and research that makes in utilizing various perishable seasonal fruits into value added nutritive products, which in turn enhances its economic demand.

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REVIEW PAPER

Value Added Products from Lignocellulosic Agricultural Residues: An Overview

Sanjeev Kumar Soni^{1*}, Om Parkash², Ritika Manhas¹, Rupinder Tewari² and Raman Soni³

¹Department of Microbiology, Punjab University, Chandigarh, India

²Department of Microbial Biotechnology, Punjab University, Chandigarh, India

³Department of Biotechnology, D.A.V. College, Chandigarh, India

*Corresponding author: sonisk@pu.ac.in

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ABSTRACT

Cellulose is the most abundant biopolymer found on earth present in the form of agricultural, agro-industrial and food waste residues which also have hemicellulose and lignin associated with them. This is also known as lignocellulosic biomass and its disposal is a major global concern as most of it is presently subjected to burning or buried in soil thus leading to environmental pollution and global warming. Some residues are traditionally used as animal fodder, substrate for mushroom cultivation, pulp and paper making, composting, amalgamation and mulching in soil, fuel for domestic and industrial applications etc. It has the potential to act as a renewable and near zero cost substrate for the economical production of high value compounds. They can act as sustainable alternative sources of bioenergy to replace fossil fuels and the production of value added products including industrial enzymes, organic acids, microbial polysaccharides, biofertilizers and fine chemicals is likely to promote bioeconomy by valorisation of lignocellulosic biomass. This piece of work discusses the traditional ways of management that are employed for making use of the agricultural residues. The potential of lignocellulosic resources for bioconversion into high value compounds emphasizing on its economic importance for production of fuels and chemicals by simultaneously minimizing the contribution to environmental pollution because of burning and land filling has also been reviewed.

Keywords: Lignocellulosic biomass, agro-industrial waste, biomass conversion, fermentation, value added products

Rapid depletion rates of fossil fuels coupled with energy crisis and other environmental concerns are the major factors stressing upon an urgent need for development of sustainable sources of energy. Indiscriminate use of the fossil fuels have numerous disadvantages including environmental pollution, high cost, non-uniform distribution and negative impact on economy. Biofuels have potential to replace them. First generation biofuels are derived from edible part of the plant biomass with high starch content, which can be converted to glucose to be utilized for fermentation. But the use of high

starch plant biomass, which is intended primarily for human consumption, gives rise to food security concerns (Singhania *et al.* 2014). On the other hand, non-edible part of the plant biomass derived in the form of agricultural waste including wheat straw, rice straw, bagasse, corn stover, husk and grasses, etc can also be used for production of second generation biofuels (Avci *et al.* 2013). They have

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the clear advantage of being low cost substrates as are produced in large quantities and considerable amount has been found as waste (Lynd *et al.* 2002). Major component of this biomass is lignocellulose, which is composed of cellulose, hemicellulose and lignin. Cellulose comprises the largest fraction (30-50%) of total biomass and thus makes this biomass as an attractive resource for microbial transformations to generate value added products. Fractions of hemicelluloses and lignin in lignocelluloses are variable. Use of this energy rich source is restricted by complex composition of lignocellulose and crystalline nature of cellulose which makes it difficult to hydrolyze.

A huge amount of agricultural and agro-industrial residues are produced annually through processing of food crops including wheat, rice, corn, barley, sugar cane, sugar beet, apple, carrot, orange, date, grape, peach and potato etc. In general, most of these residues have no economic value despite their richness in cellulose, hemicellulose, soluble sugars, and soluble fibers which can potentially be exploited as feedstocks for biofuel industry (Panahi *et al.* 2020).

LIGNOCELLULOSIC BIOMASS: A RESOURCE FOR VALUE ADDED PRODUCTS

Lignocellulosic biomass is a heterogeneous complex of polysaccharides and lignin (a complex polymer of phenylpropanoid units). It is considered as the major resource left on earth for the production of value-added chemicals, enzymes, and energy (Guldhe *et al.* 2017; Pandey *et al.* 2000). Annually 1.81×10^{11} tons of biomass is generated making this biopolymer most abundant on earth (Paul and Dutta, 2018). It is generated by photosynthesis consuming atmospheric CO₂, water and sunlight. A large portion of this biomass is generated as waste in agricultural activities (Pérez *et al.* 2002) and has the potential act as the feedstock for conversion to high-value compounds after enzymatic hydrolysis.

COMPOSITION OF LIGNOCELLULOSIC BIOMASS

The main components of lignocellulosic biomass include cellulose, hemicellulose, and lignin

generally in a 4:3:3 ratio but the actual proportion varies depending on the source (Bajpai, 2016). Most agricultural residues have 40-50% cellulose, 15-25% hemicellulose and 20-25% lignin (Zing *et al.* 2017). Table 1 summarizes the approximate compositions of various lignocellulosic feedstocks and the general structural composition of its various components is summarized in Table 2.

Table 1: Composition of various lignocellulosic feedstocks

Lignocellulosic residues	Composition (% dry wt)		
	Cellulose	Hemicellulose	Lignin
Agricultural byproducts			
Rice straw	29.2–39	15–25.9	10–19
Wheat straw	33–39	22–30	12–25.5
Sugarcane bagasse	25–45	25–32	12.2–25
Corn stover	35.1–39.5	19.1–24.6	11–19.1
Rapeseed stover	27.6	20.2	18.3
Oilseed rape	27.3	20.5	14.2
Oat straw	27–35	20–37	10–19
Corn cob	32.3–45.6	35–39.8	6.7–15
Rice husk	24–36.1	12–29.3	11–20
Wheat bran	10.5–14.8	35.5–39.2	8.3–12.5
Sorghum straw	32–35	24–27	15–21
Cotton stalk	31	11	30
Cotton seed hairs	80–95	5–20	0
Nut shells	25–30	22–30	30–40
Bamboo	49–50	18–20	23
Rye straw	33–35	27–30	16–19
Jute fibers	45–53	18–21	21–26
Barley straw	31–43	24–33	6.3–15
Switch grass	25–45	22–31.4	12–20
Alfalfa	21.8	12.4	9.7
Waste woody biomass			
Loblolly pine	35	16.8	29
Lodgepole pine	44.9	22.6	26.8
Monterey pine	41.7	20.5	25.9
Douglas fir	44.6	19.4	31.5
Hybrid poplar	40	22	24
Willow	49.3	14.1	20
Hardwood stems	40–55	24–40	18–25
Softwood stems	45–50	25–35	25–35
Eucalyptus	45–51	11–18	29

Municipal solid waste			
Swine waste	6	28	NA
Cattle manure- Solid	1.6–4.7	1.4–3.3	2.7–5.7
Waste paper- chemical pulp	60–70	10–20	5–10
Waste water solids- Primary	8–15	NA	24–29
Banana waste	13.2	14.8	14

ECONOMICS IMPORTANCE OF LIGNOCELLULOSIC BIOMASS

Lignocellulosic mass possesses an immense potential to be used as the substrate with several applications in addition to the production of high-value compounds and products. It is considered as a sustainable organic carbon source for fuel and chemical production with net zero carbon emission and thus considered as a suitable candidate to compete with crude oil and reduce environmental

pollution. Lignocellulosic biomass is basically a byproduct recovered from forestry, agricultural and agro-industries and its disposal is a great challenge. It has an added advantage of being a nearly zero value resource as a considerable amount of this it is discarded as waste in the form of agricultural residues, sugarcane bagasse, straws, grasses etc. (Abu Yazid *et al.* 2017). These residues can be directly used as the raw materials with several applications and generation of useful products or may be used as substrates for the growth of suitable microorganisms for transformation into value added products. Hence, using these waste byproducts as the substrates for transformation into value-added products signifies the economic importance of lignocellulosic biomass. The range of the value added products which can be obtained by conversion of lignocellulosic biomass at industrial scale and its employing in the field of agriculture again by using traditional methods is depicted in Fig. 1.

Table 2: General composition of cellulose, hemicellulose, and lignin in lignocellulosic biomass

	Cellulose	Hemicellulose	Lignin
Subunits	D-Pyran glucose	D-Xylose, L-Arabinose, Glucose, Mannose Galactose, glucuronic, and galacturonic acids	Guaiacyl propane (G), Syringyl propane (S), p-Hydroxyphenyl propane (H)
Bonds between the subunits	β -1,4-Glycosidic bonds	β -1,4-Glycosidic bonds in main chains; β -1.2-, β -1.3-, β -1.6-Glycosidic bonds in side chains	Various ether bonds and carbon-carbon bonds, mainly β -O-4 ether bond
Degree of polymerization	Several hundred to tens of thousands	Less than 200	4,000
Type of polymers	β-Glucan	Polyxylose, Polyarabinose, Polymannose, Polygalactan, Galactoglucomannan, Galactomannan, Glucomannan	G lignin, GS lignin, GSH lignin
Composition	Three-dimensional linear molecular composed of the crystalline and amorphous regions	Three-dimensional inhomogeneous molecular with a small crystalline region	Amorphous, Inhomogeneous, Nonlinear three-dimensional polymer
Bonds between three components	Without chemical bond	Contains chemical bond with lignin	Contain chemical bond with hemicellulose

Cited from Chen, 2014.

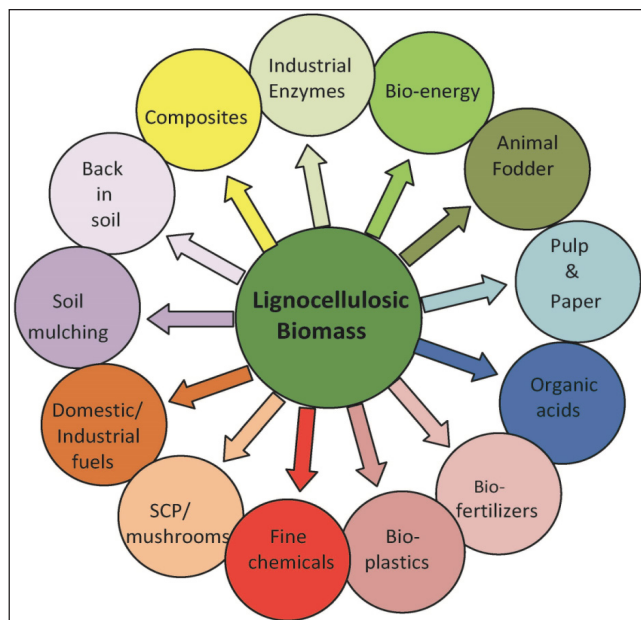


Fig. 1: Range of the applications and high-value products that can be obtained from lignocellulosic biomass

USE OF LIGNOCELLULOSIC BIOMASS IN AGRICULTURE AND ANIMAL WELFARE

The main applications of agricultural residues where they are used directly are discussed hereafter:

Mushroom farming

Paddy straw is the main substrate for the cultivation of temperate mushrooms (paddy mushroom) in Punjab (Choudhary *et al.* 2009). However, wheat straw is used for the cultivation of button and oyster mushrooms. For the production of button mushroom, wheat straw is first subjected to composting while the oyster mushrooms are cultivated directly on properly cut and moistened wheat straw.

Incorporation back in soil

Addition of agricultural residues back in soil increases the organic carbon content by 14–29% (Singh *et al.* 1996), but at least 3 weeks must be given for in-situ decomposition before the sowing of the next crop (Singh *et al.* 1992). This decomposes the residues to some nutrients and also enhances the pH value, organic carbon, infiltration rate, and water holding capacity of the soil (Gupta *et al.* 2004). This

practice is especially important considering the carbon deficiency levels observed in the soils due to continuous cultivation of crops year after year.

Mulching of soil for improving soil health

Mulching of soil with agricultural residues especially rice straw is used to conserve and maintain the moisture levels of the soil. It may be used for the crops like wheat, maize, sugarcane, sunflower, soybean, potato, and chilli production since these crops require wetland (Arora *et al.* 2011; Kumar and Singh, 2020). It also maintains the minimum soil temperature essential for crops in the winter and gives a shading effect in the summer.

Composting

Composting is the decomposition of rice straw to enable recovery of portions of its nutrients and organic components if the feedstock materials have a high nitrogen content to obtain a better carbon-to-nitrogen ratio. Rice straw takes a longer time to decompose it may take a year. Scientists have already developed a rapid composting technique to convert huge piles of rice straw into the organic-rich soil. Generally, it takes about 45 days to prepare this rice straw compost (Kumar and Singh, 2020).

Animal fodder and shed preparation

Most of the agricultural residues may be used as animal fodder, and for the preparation of shed of cattle. The paddy straw is normally not used as fodder for cattle due to the presence of high silica and lignocellulosic content which is not easily digestible except straw of basmati rice and wheat straw (Jain, 2016). Wheat and basmati rice straws are frequently used as cattle fodder. The paddy straw is frequently used for making shed for the cattle in the winter, which protects the cattle from extreme cold and prevents the chances of injury (Kumar *et al.* 2015).

USE OF AGRICULTURAL RESIDUES IN HOUSEHOLD AND INDUSTRY

Discussed below are uses of agricultural residues as raw material, fuel etc in the various industries and household:

Domestic fuel

The people of the rural areas are still dependent on fuel wood and agricultural residues (combustion with dung cake/ wood/ coal) for cooking (Jain *et al.* 2014; Kumar *et al.* 2015).

Fuel for power plants

Several thermal power plants are using rice straw as fuel for power production. Fly ash and bottom ash as the by-products of fuel combustion in power plant may be used in the cement and brick manufacturing industry. The paddy straw may be used in the form of bales directly in the furnace or in the form of shredded straw with pulverized coal. The handling and storage of straw in the form bales become easy (Kumar and Singh, 2020).

Pellets for use as refuse derived fuel (RDF)

The crop residue may be used as a fuel in the pellet form. The pellet mill is used to crush, press, compact and form the straw, peanut shell, cob, cotton bar, soybean rod, weeds branches, leaves, sawdust, bark, and other solid wastes to prepare the pellets. This kind of fuel has high efficiency and is easy to store. It can also be used as main fuel for the industrial boilers (Verma 2014; Kumar and Singh, 2020).

Paper and pulp industry

A pulping technology eliminates agricultural waste by converting into paper. The best method extracts cellulose from the straw to make paper and natural phenolic materials (Kumar and Singh, 2020).

Mixing with plastics

The paddy straw can also be used as reinforcing the material in plastics. The paddy straw is shredded into small pieces of 1.5–2 mm size and mixed with polypropylene. The mixture is then extruded into granules using a twin screw extruder (Verma 2014). Studies are being conducted to assess the lignin reinforced matrices and lignocellulosic matrices for their application in bioplastics (Yang *et al.* 2019).

Composite materials

In India, rice husk, jute, stalk, and coconut fibre are produced in large amount. All these natural fibres have excellent physical and mechanical properties and can be utilized more effectively in the development of composite materials for various applications like hard board (Pandey and Sujatha 2011).

VALUE-ADDED PRODUCTS FROM LIGNOCELLULOSIC BIOMASS

Use of lignocellulosic biomass as a feedstock for the growth of microorganisms is a sustainable approach which is cost effective as well as eco friendly for the production of a wide range of value added products (Kumar *et al.* 2016). Potential uses of lignocellulosic biomass are in composting, manufacturing paper, production of biofuel and animal feed. Bio-transformation may be used for production of ethanol, acetone, butanol, bio-hydrogen, bio-methane, bio-fertilizers, acetic acid, citric acid, fumaric acid, lactic acid, sugar alcohols (xylitol, arabitol, etc.), hydroxymethyl furfurals, phenol, vanillin, etc. The range of various value added products which can be obtained upon the bioconversion of lignocellulosic biomass is illustrated in Fig. 2 and some of these are discussed hereafter.

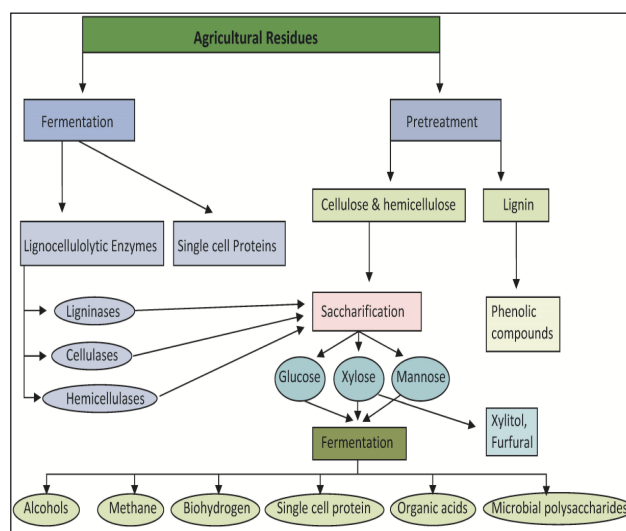


Fig. 2: Value added products from agricultural biomass conversion

INDUSTRIAL ENZYMES

As the enzyme cost is the most important factor in determining the commercial viability of a process using the rice straw as the feedstock, it is desirable to develop the low-cost processes for the enzyme production using the abundantly available agricultural residues. Since, the industrial demand for cellulases, xylanases, lignases and pectinases is extremely high, lignocellulosic biomass derived from agro-industrial residues are appropriate substrates for production of these enzymes on a large scale. Of the various fermentation technologies available for bulk enzyme production, solid-state fermentation appears to be most promising as it can use the lignocellulosic residues for supporting the growth of the microorganisms because of high carbohydrate content. This also establishes a better contact of the microorganism with the substrate for inducing higher titers of enzymes (Bansal *et al.* 2012; Chugh *et al.* 2016). Since, the industrial demand for cellulases, xylanases, lignases, pectinases and proteases is extremely high, lignocellulosic biomass derived from agro-industrial residues are appropriate substrates for production of these enzymes on a large scale. Several attempts have been made during the last few years to produce the cellulases and hemicellulases by solid-state fermentation of agricultural residues including wheat bran (Chakraborty *et al.* 2016; Hemansi *et al.* 2018), rice bran (Chugh *et al.* 2016), kitchen waste (Janveja *et al.* 2013), brewer's spent grain (Rana *et al.* 2013), rice straw (Aggarwal *et al.* 2017; Kaur *et al.* 2020), a combination of rice straw and wheat bran (Sandhu *et al.* 2013), sweet sorghum bagasse (Bagewadi *et al.* 2018), and banana peels (Rehman *et al.* 2014). Rastogi *et al.* (2016) reported the co-production of laccase, xylanase and mannanase by a natural variant of *Pyrenophora phaeocomes* on various agricultural residues. Solid state fermentation of rice straw induced the highest productivities corresponding to $10,859.51 \pm 46.74$, 22.01 ± 1.00 and 10.45 ± 0.128 IU gds⁻¹ for laccase, xylanase and mannanase respectively after 4 days.

BIO-ENERGY

Bio fuels produced from renewable sources may

replace the dependence on fossil fuels in the near future, hence, the production of bioethanol, biobutanol, biomethane and biohydrogen from lignocellulosic biomass is a sustainable alternative (Fatma *et al.* 2018).

Bioethanol: The conventional fossil fuels are detrimental in terms of future security, pollution, geopolitical instability and thus derive the need for alternative fuels. Bioethanol can be used as blend with gasoline or directly as fuel. Use of starchy food crops in first generation of biofuels is in direct conflict to the food needs of population. Second generation biofuel uses lignocellulosic biomass which is full of possibilities even upto level of replacement of fossil fuels. Market share for ethanol is largest due to its use as chemical feed stock coupled with fuel use (Gamage *et al.* 2010).

Many of such residues like wheat straw (Qui *et al.* 2017), sugarcane bagasse (Pitarelo *et al.* 2016), corn stover (Chen *et al.* 2016), corn cob (Zerva *et al.* 2014), brewers spent grain (Zerva *et al.* 2014), rice straw (Molaverdi *et al.* 2019a,b), kitchen waste (Janveja *et al.* 2013b, Karimi and Karimi, 2018), have been worked upon by various research groups for ethanol production using different pretreatment strategies, varying doses cellulases and different consortia of pentose and hexose fermenting yeasts revealing different degrees of success. Chandel *et al.* (2009) studied the fermentation of acid hydrolyzed deoiled rice bran by *Pichia stipitis* NCIM3499 and reported the release of 38.50 g/l of reducing sugars. The same, when subjected to fermentation, yielded 12.47 g/l of ethanol after 72h with fermentation efficiency of 81.74%. Rana *et al.* (2014) observed ethanol concentration of 15.6 g/L from wet exploded corn stover after enzymatic hydrolysis by cellulases from *Trichoderma reesei* RUT C30 and *Aspergillus saccharolyticus* at an enzyme dose of 15FPU/g at 5% solid concentration after 72h of hydrolysis. Singhanian *et al.* (2014) carried out SHF of 2.5% H₂SO₄ pretreated wheat straw by in-house produced cellulases from *P. janthinellum* EMS-UV-8 and observed ethanol concentration and yield corresponding to 12.0 g/l and 30.2% respectively. Yu *et al.* (2016) resulted 160 g/kg of

ethanol productivity from corn stover pretreated with magnesium bisulfate after enzymatic hydrolysis with two commercial enzyme preparations (Celluclast 1.5 L and Novozyme 188) at enzyme dose of 15 FPU/g after 48 h of hydrolysis. Nghiem *et al.* (2016) observed ethanol conc. of 39.5 g/L from low moisture anhydrous ammonia (LMAA) pretreated corn stover by using CTec2 and HTec2 at 7.5 % solid loading after 72 h of hydrolysis. Zhao *et al.* (2018) reported ethanol yield of 21.7 g/l and Molevardi *et al.* (2019b) obtained 83.9 g/l ethanol with Na₂CO₃ pretreated rice straw. Ethanol concentration of 6.47 g/l was also reported by simultaneous delignification, saccharification and fermentation of rice straw (Bhardwaj *et al.* 2019). Prasad *et al.* (2020) reported ethanol yield of 25.3 g/l using *P. stiptis* NCIM 3186 from 2% NaOH pretreated rice straw. Bhardwaj *et al.* (2020) and Jin *et al.* (2020) observed ethanol yield of 11.9g/l and 9.45 g/l from KCl-H₃PO₄ and NaOH pretreated rice straw respectively. Chohan *et al.* (2020) optimized bioethanol production from potato peel wastes on inputs of temperature, pH and solid loading using simultaneous saccharification and fermentation. Subsequently, the kinetics of yeast growth and bioethanol formation under the optimized conditions were assessed using the logistic and modified Gompertz models, respectively. Maximum bioethanol concentration (22.54 g/L) and yield (0.32 g/g) were observed under optimal process conditions of 40 °C (temperature), 5.78 (pH) and 12.25% w/v (solid loading). Kaur *et al.* (2020) in a recent study saccharified 0.25N NaOH pretreated rice straw using inhouse produced cellulase-hemicellulase consortium from *A. niger* P-19 releasing 70 g/l of reducing sugars with 10% solid loading and the fermentation of C6 sugars resulted in an ethanol yield of 15.6 g/l .

Biobutanol: Biobutanol has potential to replace even gasoline in future. Its salient features include high energy density and miscibility with gasoline in all ratios. It is less corrosive and has low vapor pressure allowing transportation by existing structure. It has lesser solubility in water reducing ground water contamination risk (Rajagopalan *et al.* 2016). Lignocellulosic biomass has been

used for biobutanol production (Mahapatra & Kumar, 2017). The main drawback with the use of biobutanol is its low production rate at industrial level (Jin *et al.* 2011). Usually, the production under fermentation processes is carried out by bacteria, such as *Clostridium* spp (Gottumukkala *et al.* 2013; Grassi *et al.* 2018) which may use lignocellulosic biomass (Phanchan *et al.* 2017). Magalhães *et al.* (2018) reported the improved n-butanol production from lignocellulosic hydrolysate by *Clostridium* strain and culture-medium optimization.

Biomethane: Biogas generation has been used for meeting thermal and electrical needs at local level. It is a mixture of methane and carbon dioxide. Lignocellulosic biomass comprising fallen leaves, stover, straws etc has been used for production of biomethane (Kumar *et al.* 2016; Petersson *et al.* 2007). Few recent studies have also explored the potential of potato peels as feedstock for the production of biogas. Achinas *et al.* (2019) examined the performance of anaerobic digestion of potato peels in different inoculum-to-substrate ratios without and with dilute sulphuric acid pretreatment. In addition, the impact of combined treatment with cow manure and pretreatment of potato peels was examined. Comparing the co-digestion to mono-digestion of potato peels, co-digestion in PPW/CM ratio of 60:40 increased the methane yield by 10%. In addition, grinding and acid hydrolysis applied to potato peels were positively effective in increasing the methane amount. Compared to untreated potato peels, pretreatment led to an elevation of the methane amount by 9% and 17% respectively and alleviated the kinetics of biogas production.

Bio-Hydrogen: Hydrogen as a fuel has advantage that it has very high energy density and the only byproduct is water (Dada *et al.* 2013). Wheat bran/straw, corn cob/stalk, potato peels have been used for hydrogen production (Cheng *et al.* 2011; Soares *et al.* 2020) with the productivities ranging from 12 to 7019 mL H₂/L. This wide variation is due to the source of lignocellulosic, its pretreatment method and the operational conditions of fermentation.

BIOFERTILIZERS

Lignocellulosics can be subjected to hydrolysis for the release of sugars that can be readily utilized for the production of plant growth promoting rhizobacteria (PGPR). Many bacteria and fungi have been known to produce extracellular hydrolytic enzymes that can efficiently hydrolyse lignocellulosic biomass. In a study the effectiveness of poultry manure (PM) and banana waste (BW) as carriers for biofertilizer consortium of *Azospirillum*, *Azotobacter* and P-solubiliser was carried out. The plant's physical and soil's physical and microbiological properties were studied for six months and the biofertilizer prepared on BW was found to be better than the PM but the benefits appeared to be restricted to moderate doses of application ($\leq 3\%$) (Riveracruz *et al.* 2008). *Aspergillus niger* P-19 strain has been studied for production of hydrolytic enzymes to hydrolyse rice straw, municipal solid wastes, etc which can be used as compost or as carrier for adsorption of PGPRs for the production of a carrier based biofertilizer (Kajal, 2020; Manhas, 2020; Dhiman, 2020).

ORGANIC ACIDS

A significant effort is being made to find bioconversion methods to utilize the bulk lignocellulosics being generated for the production of organic chemicals. Organic acids are used for synthesis of value added chemicals. A range of acids have been produced using lignocellulosic biomass (Prado *et al.* 2005).

Citric Acid: After ethanol, the 2nd largest fermentation product is citric acid. It is used for medicinal purposes, heavy metal bioremediation and nanotechnology. Production of citric acid using lignocellulosic biomass including cotton waste, pomace, cobs, husk etc. has been reported (Kumar *et al.* 2016).

Succinic Acid: It is used in agriculture and pharma industries as well as for production of methyl ethyl ketone, adipic acid, 1,3-butadiene, ethylene diaminedisuccinate and 1,4-butanediol. It is conventionally produced by petro based chemicals but with poor efficiency (Cheng *et al.* 2012). *Actinobacillus succinogenes* 130Z, readily converted

29.2 g/L of cellulosic glucose to 17.8 g/L of succinic acid using pre-treated sweet sorghum bagasse as the substrate (Dogaris *et al.* 2020).

Lactic Acid: It has uses in chemical, food, textile and pharmaceutical industries. Its demand is expected to rise as it is used in poly-lactic acid and lactate solvents. Poly lactic acid is environmentally safe polymer which can be used as alternative to plastics (Cui *et al.* 2011). Various lignocellulosic biomass sources have been used for lactic acid production. Currently lactic acid is produced by microbial route as it has better environmental impact (Cubas-Cano *et al.* 2018).

Acetic Acid: It is famously known as vinegar which is used as a preservative in the food industry, has anti-septic usage in the field of medicine and has found pivotal roles in many industrial processes. A study using *Moorella thermoacetica* (strain ATCC 39073) was conducted for bioconversion of sugars obtained from steam pre-treated lignocellulosic biomass into acetic acid a yield of 17 g/L acetic acid from 24 g/L total sugars was obtained (Ehsanipour *et al.* 2016). Another organism *Acetobacterium woodii* was examined for growth on lignocellulosic substrates for the production of acetic acid, with an initial 10 g/L glucose concentration a yield of 0.76g acetic acid per g glucose was recorded from 6.919 g/L acetate production (Karekar *et al.* 2019).

Levulinic Acid: It has extensive use in the fragrance and perfume industry and is used in multiple other industries as it is a precursor for numerous compounds of industrial relevance in pharmaceutical products, as herbicide, as plasticizer, etc. Levulinic acid can be produced from agricultural wastes subjected to acid hydrolysis under high pressure using the carbohydrates that are released. Sugarcane bagasse was bioconverted into levulinic acid using acid catalysed hydrolysis by determining optimum conditions and 194 Kg of levulinic acid was obtained from 1 tonne of dry sugarcane bagasse. The kinetic model thus used can predict the levulinic acid yield under different temperature and acid concentrations (Girisuta *et al.* 2013). Another study recorded a yield of up to 47.52% levulinic acid at 100 °C in 60 mins

from bamboo biomass (Khan *et al.* 2018). Lately instead of acid hydrolysis use of organic solvents as the medium of reaction are being studied for the production of levulinic acid (Li *et al.* 2019).

Formic Acid: It has found its use as a preservative, anti-bacterial agent and decalcifying agent in several leather tanning industries, textile dyeing industries, etc. Due to its high demand the conventional production of formic acid as a byproduct of the acetic acid production has proven insufficient. Therefore, exploring other potential substrates for the production of formic acid is required. Lignocellulosics have been used as a substrate for the production of formic acid as a byproduct of levulinic acid from cellulose although in a study conducted the yield of formic acid was found to be highest from glucose followed by cellulose after a hydrothermal treatment (Yun *et al.* 2010). Another study used a Lindqvist type catalyst $K_5V_3W_3O_{19}$ successfully converted all the hemicellulose and lignin model compounds into formic acid but the yields were very low (Albert, 2017). A latest study a crude formic acid (CF) solution was produced having formic acid yield of 17.62 g/L using a mild reaction of combined hydrolysis/oxidation of lignocellulosic feedstock (Park *et al.* 2019).

HYDROXYMETHYL FURFURAL (HMF)

It is the primary product formed from the hydrolysis of lignocellulosics and has been vastly in use as a platform chemical as it acts as the intermediate in the production of multiple chemicals of interest for the industry. It is primarily used on the food industry as a food additive. The hexose sugars released from the lignocellulosics under acidic dehydration conditions lead to the production of HMF. With a combined acid/base treatment followed by catalytic conversion a maximum HMF yield from biomass is 79 mol% was achieved (Nguyen *et al.* 2016). Various minerals and organic solvents have been used as catalysts to increase the yield (Zhang *et al.* 2017).

Sugar Alcohols

Another class of organic compounds that are produced directly from lignocellulosic biomass is a

variety of sugar alcohols. They occur naturally and are produced industrially by hydrogenation of sugar molecules, this process of hydrogenation can be hastened by employing metal catalysts in presence of acids. To list a few xylitol, maltitol, arabitol and mannitol are all sugar alcohols. Xylitol is an artificial sweetener equivalent to sucrose and used among diabetic patients and considered among top twelve value-added chemicals produced from plant biomass (Martin *et al.* 2009). Xylitol is produced by costly process resulting in low yields. Xylose present in biomass can be converted to xylitol using *Candida* spp., (Damião Xavier *et al.* 2018; Zhang *et al.* 2014). Metal catalysts such as Pt, Pd and Ru have been studied and conversion of 60% sugars was observed in an hour (Palkovits *et al.* 2010). In a study using zirconium phosphate the highest sugar alcohol yield of 70% was recorded utilizing the cellulosic and hemicellulosic fractions of raw lignocellulosic biomass (Liu *et al.* 2017).

PHENOL

Lignin present as a major fraction in the lignocellulosics biomass available is known to be converted into phenol and phenolic compounds that are used in the production of various chemicals. This is attributable to its complex structure. To convert lignin into value added products processes such as hydrogenolysis, pyrolysis, oxidation and depolymerization have been found to be very promising. Kraft lignin pyrolysis assisted with formates gives monophenols and polyphenols as the main components. Using alkylated lignin for the production of phenolics by exploring supercritical conditions of methanol or ethanol to obtain catechol is also a very promising method (Tang and Zhou, 2015). Amongst formates the nickel formates have shown high selectivity for polyphenols 21.95% (w/w) was recorded and 23.7% (w/w) guaiacol was obtained from pure lignin (Wang *et al.* 2019). In a catalytic transfer hydrogenolysis process using isopropanol as H-donor solvent 98.1% highest activity was achieved giving phenolic compounds as major products making it a promising strategy for valorisation of lignin (Guan *et al.* 2020).

VANILLIN

It is widely used in the food, cosmetics and perfume, and pharmaceutical industry as a flavouring agent. Vanillin forms a major fraction of the phenol aldehydes obtained from lignin especially from the guaiacyl and syringyl units (Luo *et al.* 2016). The linkages present in the lignin used for the production of vanillin play a vital role in determining the yield of vanillin. The lignin high in content of β -O-4 linkages showed higher vanillin yield (Wang *et al.* 2018).

MICROBIAL POLYSACCHARIDES

Bio based polymers are used in different industries like chemical, food, petroleum, health, and bionanotechnology due to diverse functionality, rheological and physicochemical properties (Özcan & Öner, 2015). Fermentation is used for production of polysaccharides like schizophyllan, xanthan, dextran, pullulan, curdlan etc. Microbial species belonging to *Xanthomonas*, *Leuconostoc*, *Sphingomonas*, and *Alcaligenes* are used industrially for production of xanthan, dextran, gellan, and curdlan. Hyaluronan is used in cosmetics and in regenerative medicine as it has high immune compatibility, water binding ability and retention properties. Alginate has a wide spread use as thickener, stabilizer and as gelifying thickening, stabilizing, and gelifying agent (Özcan & Öner, 2015). Municipal solid waste (MSW) including sugarcane bagasse (Hilares *et al.* 2017), kitchen waste (Rishi *et al.* 2020), sugar cane baggase (Hilares *et al.* 2017; 2019), Asian palm kernel (Sugumaran *et al.* 2013), cassava bagasse (Sugumaran *et al.* 2014) have been exploited as cheap sources of carbon for production of pullulan by *A. pullulans* after the enzymatic hydrolysis. A study conducted using spent/damaged wheat grains as source of sugars for growth of *Aureobasidium pullulans* 3B2 obtained highest yield of polysaccharide production of 44.4 mg/gds. It can further be used for film casting and 2.5% and 5% conc of eps gave good and properly peelable film that can be used as bioplastics (Armaan, 2018).

Single-Cell Proteins

Single cell proteins are extracts or whole microbes

used as a dietary supplement or ingredient rich in proteins. They are used for human and animal feed. They have advantage over agricultural practices as they have low water consumption, lesser use of land, low greenhouse gas production and an all-weather production (Ivanovs *et al.* 2018). Lignocellulosic biomass can be used for single cell protein production due to their low cost. A study using lignocellulosic hydrolysates for production of single cell protein by *Candida intermedia* FL023 reported high protein content (Ivanovs *et al.* 2018). Rice straw pulp has been used as substrate for single cell protein production using *Trichoderma reesei* by solid state fermentation (Ivanovs *et al.* 2018). Also, *Chaetomium* spp. are well recognised among the group of cellulolytic fungi for their potential to produce single cell protein (SCP) (Darwish *et al.* 2019).

CONCLUSION

The entire concept of lignocellulosic biomass conversion has been described with an attempt to formulate the idea of valorization of biomass residues into value-added products. In this regard, a detailed analysis on the economical significance of lignocellulosic biomass has been carried out displaying it as a near zero cost substrate. The traditional and conventional ways already in place for the use of the lignocellulosic residues have also been discussed. An effort has also been made to specify the various effective processes to produce various value-added products from lignocellulosic feedstock. The important steps in the bioconversions are the pretreatment and enzymatic hydrolysis making use of a consortium of cellulases and hemicellulases which are produced commercially and marketed by several industries globally, but these are very expensive and the cost of these is another crucial factor in making the bioprocesses using agricultural residues a reality. The conversion of lignocellulosic biomass by on-site production of cellulases employing suitable micro-organisms is a potential, sustainable and economically viable approach to develop novel bioprocesses using cellulosic residues. Further, cellulase research has been concentrated

mostly in fungi, though many bacterial cellulases have also been isolated but they are reported to have lower yields as compared to fungi so there is also a need to genetically engineer such bacterial isolates for high productivities or cellulase genes from efficient fungi need to be cloned and expressed in fast growing bacteria. Further advanced biotechnologies are crucial for discovery and characterization of new enzymes, and production in homologous or heterologous systems which will ultimately lead to low-cost conversion of lignocellulosic biomasses into bio-fuels and bio-chemicals.

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RESEARCH PAPER

Cluster Analysis of Quality Characteristics of Seedling Mango Fruits Collected from Different Locations of Himachal Pradesh, India for the Development of Pickle

N.S. Thakur¹, Dharmender Kumar¹, V.K. Joshi², Abhimanyu Thakur^{1*} and Hamid¹

¹Department of Food Science and Technology, Dr YS Parmar University of Horticulture and Forestry, Nauni, Solan, HP, India

²Formerly of Department of Food Science and Technology, Dr YS Parmar University of Horticulture and Forestry, Nauni, Solan, HP, India

*Corresponding author: abhimanyuthakurprashar@gmail.com

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ABSTRACT

Seedling mango (*Mangifera indica* L.) fruit is one of the important wild fruits which have got commercial importance due to its utilization in pickle preparation and other culinary purpose. In Himachal Pradesh (HP), it is widely distributed in the lower hills and plain areas with significant difference in the quality characteristics of fruits from location to location. The fruits of seedling mango were harvested from 24 different locations of six districts (Kangra, Una, Hamirpur, Bilaspur, Mandi and Sirmour) of HP and the results of their physical, chemical, sensory, physico-chemical and combined physico-chemical and sensory characteristics were analyzed using cluster analysis (SPSS). On the basis of this analysis of quality characteristics including higher acid-edible content indicator (2.36) and sensory quality, fruits of 10 locations were found suitable for pickle preparation viz. Kangra (Sulah and Bhawarna), Una (Una Sadar and Amb), Hamirpur (Bhoranj), Bilaspur (Ghumarwin), Mandi (Gopalpur and Pandoh) and Sirmour (Nahan and Ponta Sahib).

Keywords: Cluster analysis, Locations, Pickle, Quality, Seedling mango

Mango (*Mangifera indica* L.) known as the king of fruits is one of the popular tropical fruits which belong to family Anacardiaceae. The other edible species belonging to genus are commonly known as wild mango or seedling mango. The wild forms are found in various countries like Malaysia, Indonesia, Malaya, Borneo, Sumatra, India, China, Mexico, Pakistan, Nigeria, Thailand, South Central America, Philippines, Brazil, Australia and Egypt (Kumar *et al.* 2001 and Singh *et al.* 2016). In India, the wild forms are widely distributed throughout the subtropical Himalayas, hills of Western and

Eastern Ghats and in lower hills or plain areas of Himachal Pradesh (Singh *et al.* 2009 and Thakur *et al.* 2017a). Seedling mango fruit is a rich source of various nutrients like carbohydrates, vitamins, organic acids, fibres, proteins, fats and minerals like potassium, magnesium, sodium, phosphorous and sulphur (Knight, 1997 and Singh *et al.* 2012).

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These nutrients along with other antioxidants are known to have beneficial effect against nutritional and neurodegenerative disorders such as beri-beri, bronchial disorders, kidney stones, insomnia, brain fatigue, mental depression, Alzheimer’s disease and strengthen the immune system (Singh *et al.* 2009; Kashyap *et al.* 2017 and Thakur *et al.* 2019). Due to the acidic taste, wild mango fruits are utilized for the culinary purpose and mostly for the pickle preparation in India (Thakur *et al.* 2017b). Because of the greater variation in the quality characteristics of wild mango fruits collected from different locations of HP, cluster analysis of quality characteristics was carried out for the preliminary selection of the mango fruits for pickle preparation.

MATERIALS AND METHODS

Procurement of raw material: The mature green fruits of seedling mango were harvested from six districts (24 different locations) of Himachal Pradesh given in Fig. 1.

Physico-chemical and sensory characteristics

The length and diameter of fruit samples were measured with the help of digital vernier calliper whereas, digital weighing balance was used to weigh the fruit samples. Water displacement method was used to determine fruit volume and weight divided by volume of fruit gave specific gravity of fruit. The edible content and stone content of the samples were observed on weight basis (%). Moisture, TSS, sugars, titratable acidity, ascorbic acid, chlorophyll, starch and ash content of samples were determined according to the standard methods (Ranganna, 2009). The pH of fruits was determined by using a digital pH meter (CRISON Instrument, Ltd, Spain). Total phenols and crude fibre content of fruit samples were determined by standard procedures (Singleton and Rossi, 1965 and Gould, 1978). The sensory evaluation of samples was carried out by hedonic rating test and the samples were evaluated for sensory qualities based on appearance, texture, taste and overall

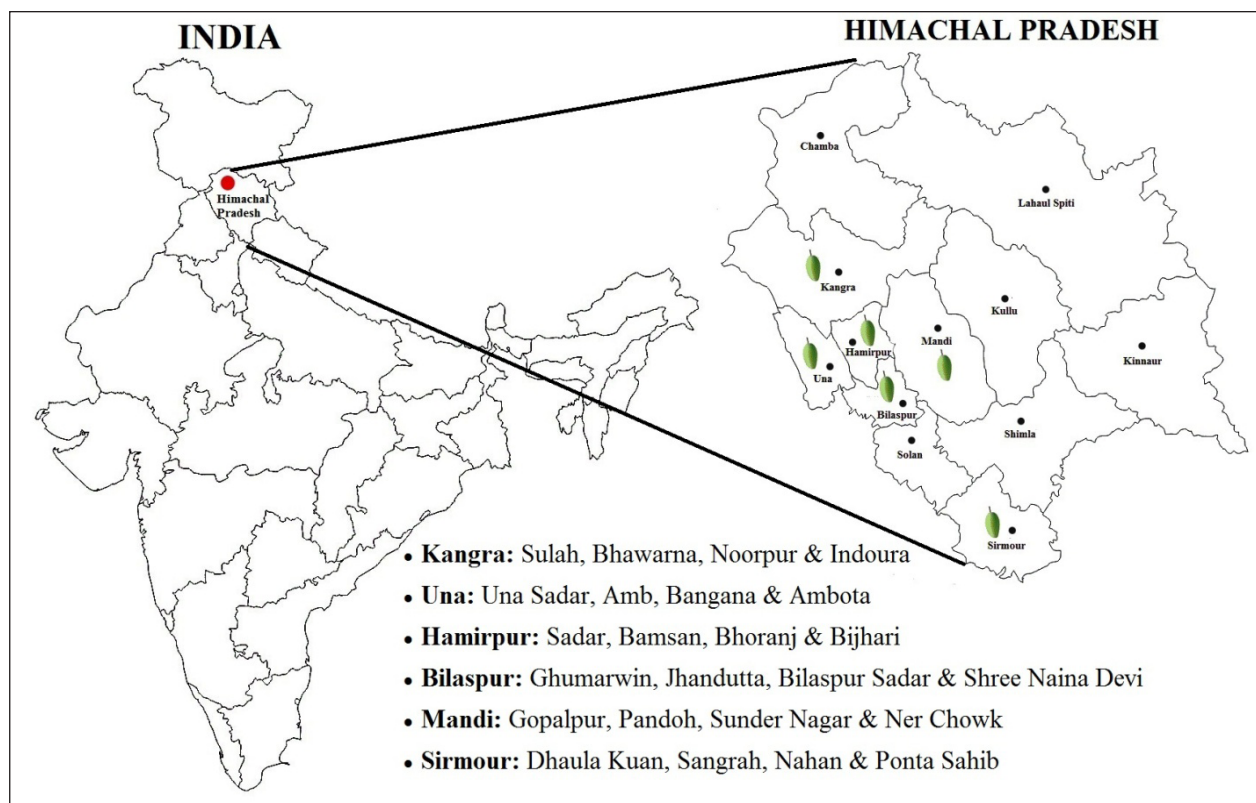


Fig. 1: Map of the locations for procurement of seedling mango fruits

acceptability (Joshi, 2006). The data for cluster analysis was adapted from Thakur *et al.* (2017c).

Cluster analysis: The physical, chemical, sensory, physico-chemical and combined physico-chemical and sensory characteristics of 24 locations were analyzed by cluster analysis (SPSS). The technique is a multivariate technique and is capable of clustering the samples or varieties with similar characteristics into group(s).

Cluster analysis of the samples from different locations of HP was performed using rescaled distance cluster with respect to physical parameters, chemical parameters and sensory characteristics individually, physico-chemical combined and physico-chemical and sensory scores characteristics combined together.

Based on different parameters of fruits of different locations separate clusters were formed as discussed in the Results and Discussion.

RESULTS AND DISCUSSION

Cluster analysis of physico-chemical and sensory characteristics of seedling mango fruits

Cluster analysis of the samples from different locations of HP was performed and the results obtained have been presented in Fig. 2-6. On the basis of Dendrogram of physical characteristics (Fig. 2) the locations have been separated into two broad clusters with 10 (7 and 3 locations in sub-clusters) and 14 locations (4, 5 and 5 locations in sub clusters), respectively. Cluster analysis of chemical characteristics (Fig. 3) however reveals that locations were separated into two broad clusters with 18 (3, 2, 3, 2, 2, 4 and 3 locations in sub clusters) and 6 locations (2 and 4 locations in sub clusters), respectively. The Dendrogram in Fig. 4 shows that 24 locations were separated into two broad clusters with 10 (5 locations in 2 sub clusters) and 14 locations (4, 6 and 4 locations in sub clusters), respectively on the basis of

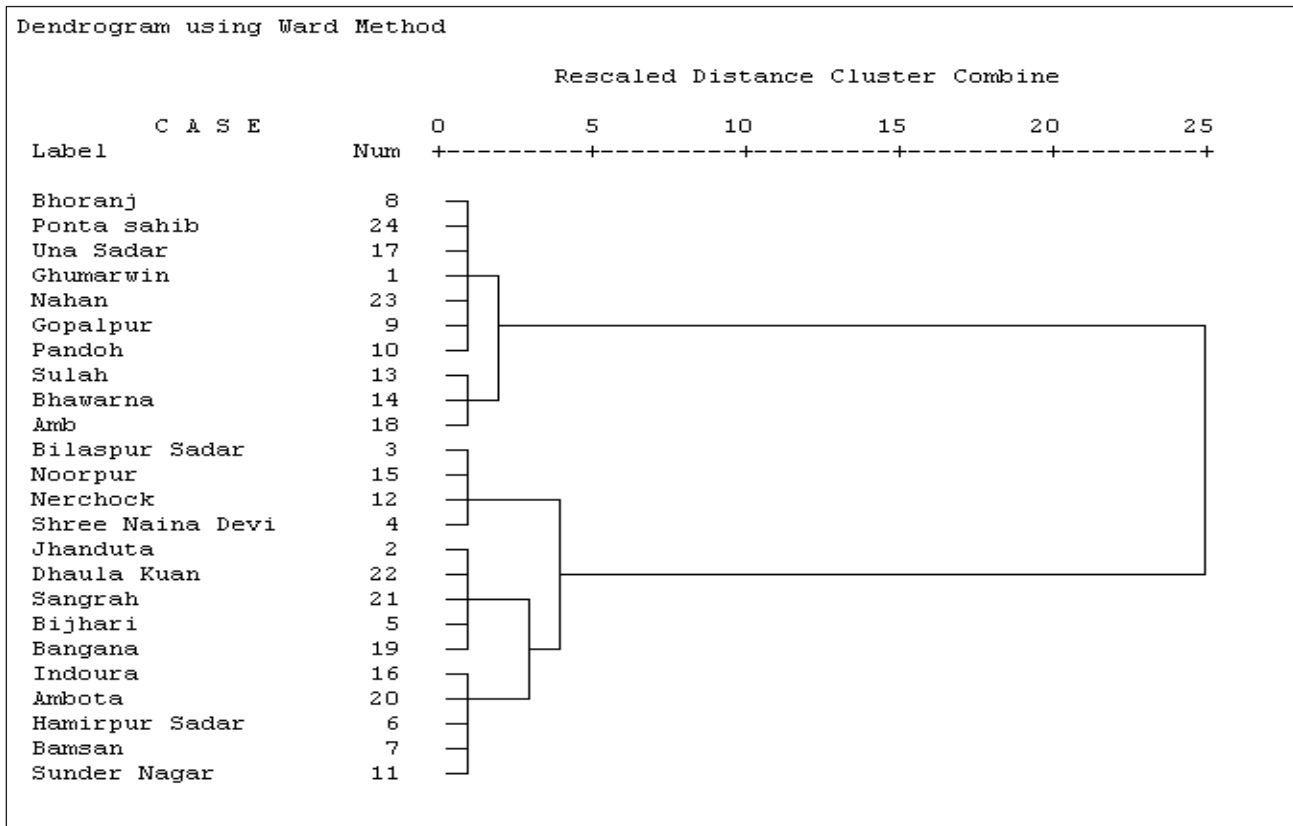


Fig. 2: Dendrogram of physical characteristics of seedling mango fruits of different locations of HP

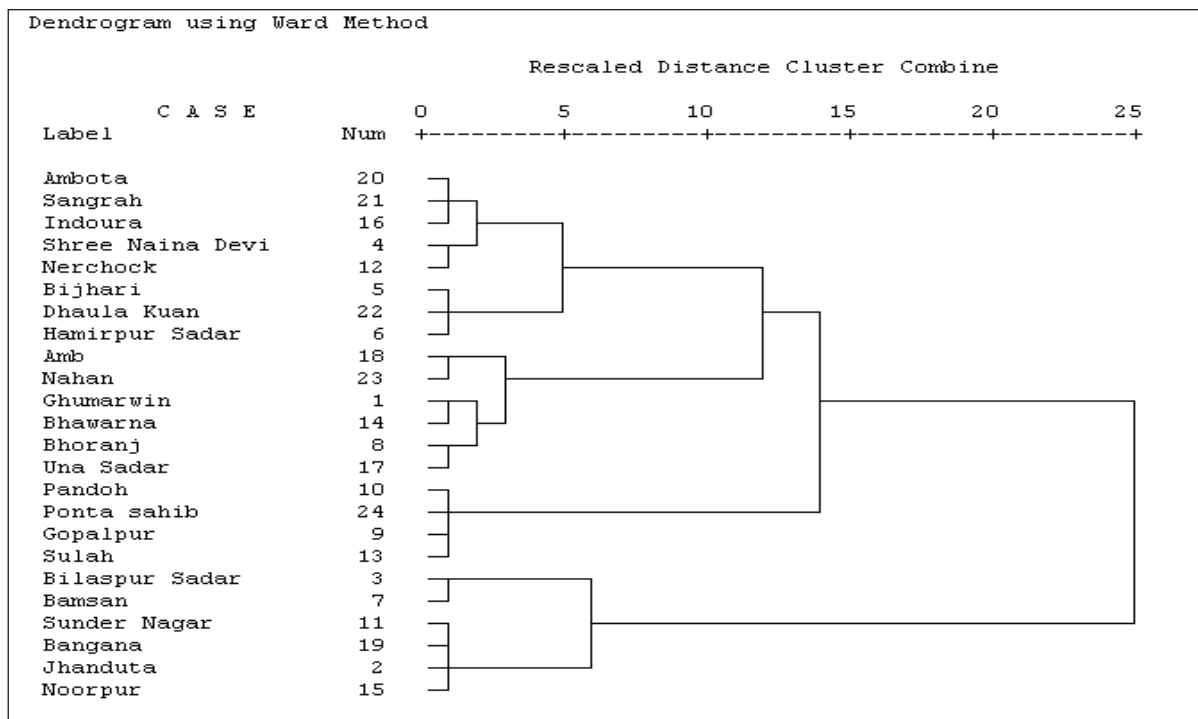


Fig. 3: Dendrogram of chemical characteristics of seedling mango fruits of different locations of HP

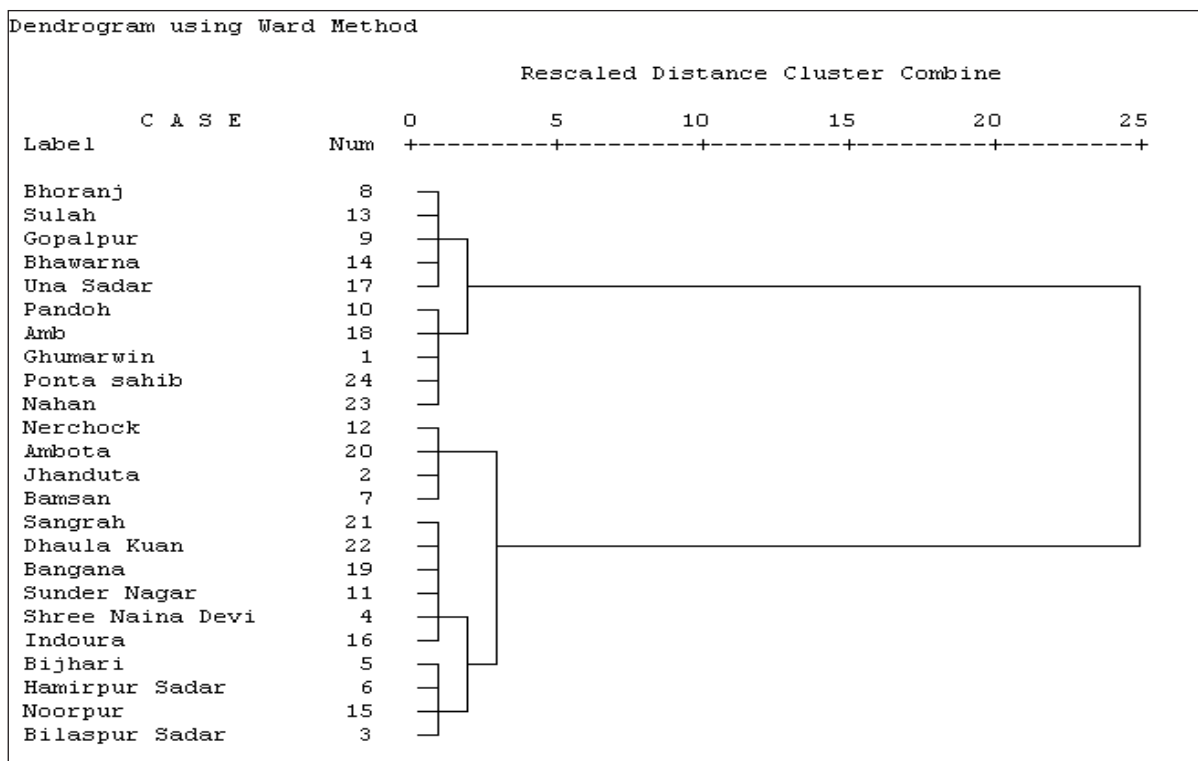


Fig. 4: Dendrogram of sensory characteristics score of seedling mango fruits of different locations of HP

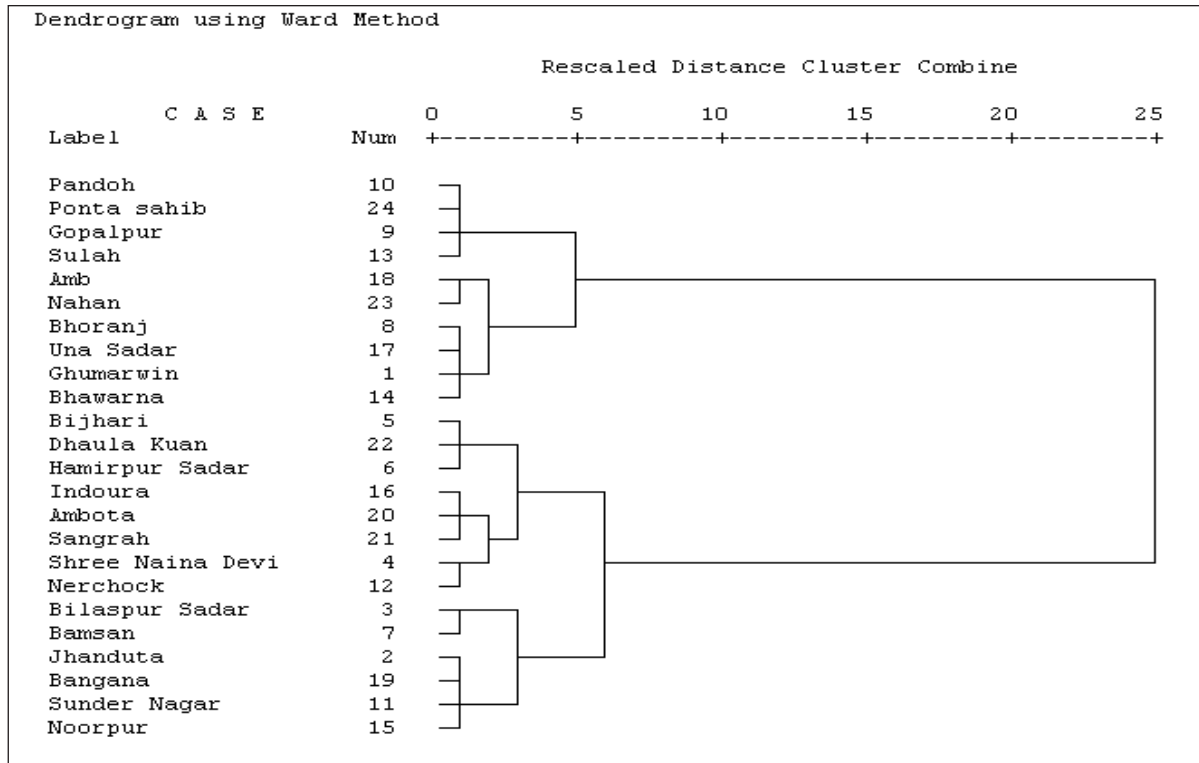


Fig. 5: Dendrogram of physico-chemical characteristics of seedling mango fruits of different locations of HP

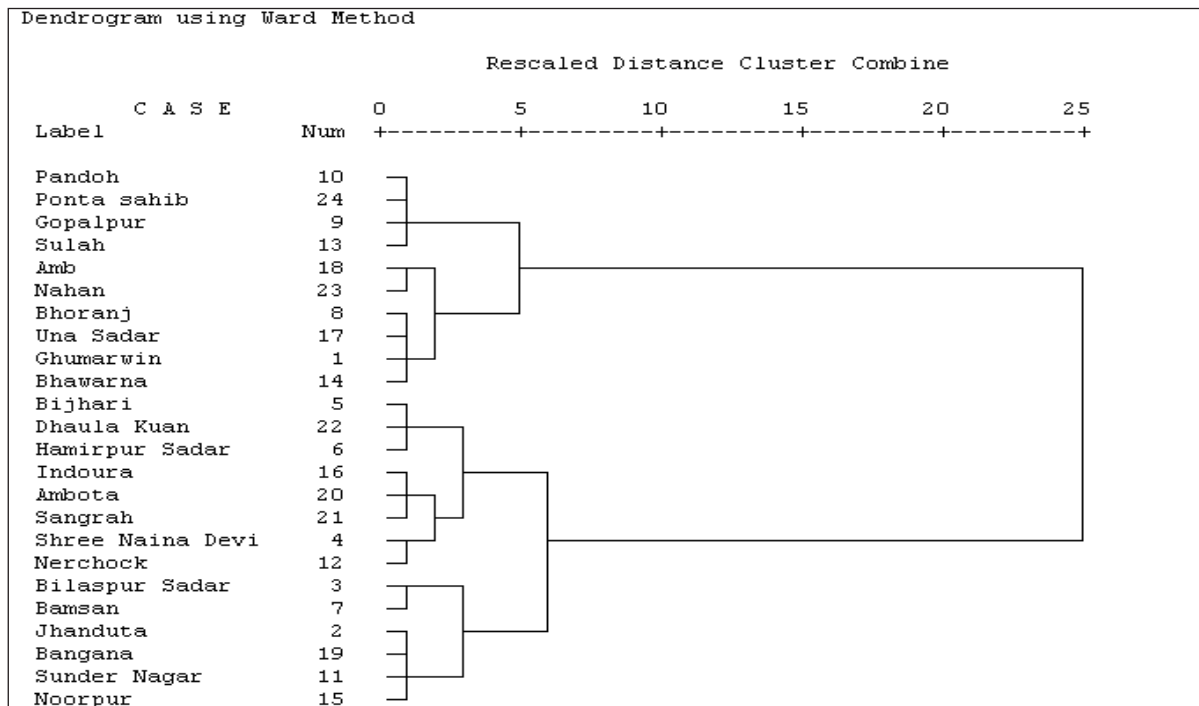


Fig. 6: Dendrogram of physico-chemical and sensory characteristics score of seedling mango fruits of different locations of HP

sensory characteristics. On the basis of Dendrogram of physico-chemical characteristics and physico-chemical and sensory characteristics (Fig. 5 and 6) the locations were separated into two broad clusters with 10 (4, 2 and 4 locations in sub clusters) and 14 locations (3, 3, 2, 2 and 4 locations in sub clusters), respectively. It is apparent that cluster analysis of physical and chemical characteristics individually didn't separate the different locations in distinct clusters. However, it is very much clear that cluster analysis has successfully separated distinct locations in different clusters based upon physico-chemical and physico-chemical sensory characteristics of fruits. The cluster analysis has broadly separated the locations into two clusters shown as cluster-1 (Gopalpur, Bhoranj, Ghumarwin, Pandoh, Sulah, Bhawarna, Una Sadar, Amb, Nahan and Ponta Sahib) and cluster-2 (Noorpur, Indoura, Bangana, Ambota, Hamirpur Sadar, Bamsan, Bijhari, Jhandutta, Bilaspur Sadar, Shree Naina Devi, Sunder Nagar, Ner Chowk, Dhaula Kuan and Sangrah). Based on the acid-edible content indicator as well as the cluster analysis of physical; chemical; sensory; physico-chemical; and physico-chemical and sensory; characteristics, fruits from the locations of cluster-1 were selected.

Similar to these results cluster analysis has successfully been employed to separate different isolates, products or locations based upon their physico-chemical and sensory characteristics (Headly *et al.* 1989). Cluster analysis (multivariate technique) separated the similar groups based on different physico-chemical and sensory characteristics in the form of dendrogram (Kaiser, 1960 and Ludwig *et al.* 1988). So, on the basis of cluster analysis of data of physico-chemical, sensory characteristics scores and acid-edible content indicator, fruits of 10 locations *viz.* Gopalpur, Bhoranj, Ghumarwin, Pandoh, Sulah, Bhawarna, Una Sadar, Amb, Nahan and Ponta Sahib were selected.

CONCLUSION

Out of 24 locations, fruits from 10 locations were selected preliminarily on the basis of cluster analysis of some physico-chemical and sensory characteristics.

These locations were Gopalpur, Bhoranj, Ghumarwin, Pandoh, Sulah, Bhawarna, Una Sadar, Amb, Nahan and Ponta Sahib from the major wild mango growing districts of Himachal Pradesh

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RESEARCH PAPER

Optimization of Nutritional Health Drink Developed from Guava, *Jamun* and *Aloe vera*

Sakshi Sharma and Rakesh Sharma*

Department of Food Science and Technology, Dr YS Parmar University of Horticulture and Forestry, Solan, HP, India

*Corresponding author: drrakes@gmail.com

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ABSTRACT

Guava, *jamun* and *Aloe vera* are valued for their nutritive and medicinal properties, but their utilization as drink is limited due to certain consumer repelling factors like poor appearance and grittiness of guava juice, high astringent taste of *jamun* juice and bitter taste of *Aloe vera* juice. Therefore, present study was designed to optimize the blending of guava pulp with *jamun* and *Aloe vera* juice for developing nutritious health drink. Different formulations, where *jamun* juice concentration varied from 10-50 per cent with two levels of fruit part (10 and 15%) and two levels of TSS (10 and 15°B) were tried for optimizing guava-*jamun* blended drink. Further, *Aloe vera* juice from 1 to 9.0 per cent was added to enhance the nutritive and functional value without compromising overall acceptability of the developed beverage. Out of different formulations, the drink prepared by blending guava-*jamun* in the ratio of 60:40 with 15 per cent fruit part (blended) and 15°B TSS was found the best on the basis of nutritional and sensory characteristics. Herbal fortification with *Aloe vera* juice @ 5 per cent was optimized for improving nutritional and medicinal properties of the developed beverage. Results revealed that the *Aloe vera* fortified guava-*jamun* health drink contained higher values of ascorbic acid (33.91 mg/100g), total phenolics (36.12 mg/100g) and antioxidant potential (13.40 % free radical scavenging activity) compared to control the sample (100% guava drink).

Keywords: Guava, *jamun*, *Aloe vera*, health drink, functional beverages

Fruit, vegetable and medicinal herbs act as protective foods and their consumption has increased because of their disease combating properties (Adefegha 2018; Dukhi and Taylor, 2018; Sharma *et al.* 2019). Recently, increased health awareness around the globe has increased the demand for functional foods especially the beverages which provide necessary nutrients, prevent nutrition-related diseases and improve physical and mental well-being of the consumers (Raj *et al.* 2017; Kaur and Sigh 2017; Sharma *et al.* 2018; Palamthodi *et al.* 2019). Among many fruits, guava (*Psidium guajava* L.) and *jamun* (*Syzygium cumini* L.) are reported to contain several bioactive compounds and have been widely used to treat diabetes by the traditional practitioners over

many centuries (Kapoor and Ranote, 2015; Joshi *et al.* 2019). Guava is often included among super fruits, being rich in dietary fibres, vitamin A and C, folic acid and dietary minerals (Singh and Tiwari, 2019). It is a hypoglycaemic fruit and generally, provides less energy (38-57 Kcal/100g) as compared to other fruits like mango, banana etc. and hence suitable for diabetics and for those involved in weight management (Rai *et al.* 2007; Joseph and Priya, 2011). Similarly, *jamun* (Indian blackberry or black plum) is reported to contain vitamins, amino acids, minerals

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and other phyto-chemicals (Kannan and Puraikalan, 2016). It has antioxidant, anti-inflammatory, antimicrobial, antibacterial, antifungal, free radical scavenging, gastro-protective and anti-diabetic properties (Sagrawat *et al.* 2006; Joshi *et al.* 2019). In spite of great nutritional significance of these fruits; cloudy appearance, grittiness and poor taste of guava juice/pulp and high astringency, blunt taste of *jamun* juice are some of the consumer repelling factors and major problems to the juice industry (Sharma *et al.* 2014; Joshi *et al.* 2012; Raj *et al.* 2017). Hence, finding right blend of juices/ pulps to maximize consumer appeal for health and sensory attributes is the alternative for utilization of such fruits (Bhardwaj and Pandey, 2011; Singh and Gaikwad 2012; Heena *et al.* 2017). Furthermore, fortification of beverages with herbal extracts has been reported to improve nutritional and health properties besides enhancing storage/microbial stability (Jairajpuri and Qadri, 2015; Sharma *et al.* 2018). *Aloe vera* (*Aloe verabarbadensis* L.) also known as miracle plant, possess a wide range of medicinal and therapeutic properties such as reducing blood sugar, easing intestinal problems, ulcers etc. (Akinnyele and Odiyi, 2007; Sharma *et al.* 2015). Keeping the above facts in view, the present study was conducted to optimize blending of guava, *jamun* and *Aloe vera* for developing a nutritious health drink.

MATERIALS AND METHODS

Materials

Fresh guava and *jamun* fruits were procured from the local fruit and vegetable market, Solan (HP) and brought immediately to the fruit processing unit of Department of Food Science and Technology for further studies. Whereas, *Aloe vera* leaves were procured from the the Herbal Garden, Department of Forest Products, Dr Yashwant Singh Parmar University of Horticulture and Forestry, Nauni, Solan (HP).

Extraction and preservation of pulp/juice

Guava fruits were sorted, washed, cut and heated along with water (1:1) in a pressure cooker for 15

minutes prior to passing through the pulper (BSB, New Delhi) for extraction of pulp. Similarly, *jamun* pulp was prepared by hot break pulping method (Bons and Dhawan, 2013). However, *Aloe vera* juice was extracted as per the method given by Sharma *et al.* (2015). The pulp and juice so extracted were heat preserved and stored under refrigerated temperature till used for product development.

Guava-*jamun* and *Aloe vera* fortified guava-*jamun* drink

Different combinations of guava and *jamun* pulp with varying levels of fruit part and total soluble solids (TSS) were tried for optimization of a suitable combination for the preparation of palatable guava-*jamun* blended drink. The drinks were prepared as per standard methods and specifications of FSSA-2006. The acidity (as % citric acid) was kept constant (i.e. 0.30 %) in all the treatments. The treatment T₁ was kept control which was having 100 per cent of guava pulp (10 % fruit part; 10 °B TSS). The rest of the treatments (T₂ to T₂₁) were prepared by blending different ratio of *jamun* pulp in guava pulp (10, 20, 30, 40 and 50) with two levels of fruit part (10 and 15%) and two levels of TSS (10 and 15 °B). Further, five levels of *Aloe vera* juice fortification i.e. 1, 3, 5, 7 and 9 per cent were tried for optimization of a suitable level for the development of *Aloe vera* fortified guava-*jamun* health drink.

Physico-chemical, nutritional and sensory quality analysis

All the beverages were evaluated for their physico-chemical characteristics viz. TSS, titratable acidity, total sugars, reducing sugars, ascorbic acid, total phenols and total anthocyanins as per the standard analytical methods (Ranganna, 2009). Antioxidant activity (Free radical scavenging activity) of juice and beverage was measured as per the method of Brand-Williams *et al.* (1995) by using DPPH (2, 2-diphenyl-1-picrylhydrazyl) as a source of free radical. Sensory evaluation of the product was conducted by a panel of ten judges using 9- point hedonic scale system for different parameters viz. appearance, body, flavour and overall acceptability (Amerine *et al.* 1965).

STATISTICAL ANALYSIS

All the analytical parameters were recorded in triplicates and the mean values of each parameter were described with standard error (SE). The data pertaining to quantitative estimation of physico-chemical characteristics were assessed by factorial CRD, whereas the data regarding sensory evaluation of products were analysed by RBD as described by Cochran and Cox (1967).

RESULTS AND DISCUSSION

Physico-chemical characteristics of fresh guava, *jamun* and *Aloe vera* juice/pulp

Data pertaining to the physico-chemical characteristics of fresh guava, *jamun* and *Aloe vera* juice: Pulp revealed that guava pulp contained 5.02 °B total soluble solids (TSS), 0.38 per cent titratable acidity, 120.19 mg/100g ascorbic acid, 1.26 per cent reducing sugars, 2.70 per cent total sugars and 3.42 pH (Table 1). Whereas, the total phenolic contents and antioxidant activity in guava pulp were observed as 180.42 mg/100g and 72.80 per cent, respectively. Fresh *jamun* pulp was found to be a rich source of total phenolics (424.24 mg/100g) besides containing 9.97°B total soluble solids, 0.75 per cent titratable acidity, 2.79 per cent reducing sugars and 6.52 per cent total sugars, respectively. While, ascorbic acid, antioxidant potential and total anthocyanins were

estimated as 27.28 mg/100g, 75.56 per cent and 112.50 mg/100g, respectively. Data presented in Table 1 further revealed that *Aloe vera* juice contained 2.20°B total soluble solids, 0.35 per cent titratable acidity and 102 mg/100g ascorbic acid. Similar findings of these parameters were also reported by Jain and Asati (2009), Abed *et al.* (2012) and Bons and Dhawan (2013) for guava; Chaudhary and Mukhopadhyay (2012) and Kapoor and Ranote (2015) for *jamun* and Sharma *et al.* (2015) for *Aloe vera*.

Optimization of guava-*jamun* and *Aloe vera* fortified guava-*jamun* health drink

The effects of blending of guava pulp with different proportions of *jamun* pulp on sensory attributes of the developed drink are presented in Table 2. It was observed that with the increase in the proportions of *jamun* pulp up to 40 per cent the score for colour and body increased, beyond which it decreased due to cloudy appearance of the drink. The highest colour score (7.80) and body (7.70) was obtained by the drink having 60 per cent guava pulp and 40 per cent *jamun* pulp with 15 per cent fruit part and 15°B TSS. It is evident from the data that flavour score for different treatments varied from 6.90 to 7.75 with maximum score obtained by the treatment T₁₇ (G₆₀ + J₄₀). Similar results were also recorded for overall acceptability (Table 2). Blending of guava pulp with appropriate proportion of *jamun* pulp might have enhanced the

Table 1: Physico-chemical characteristics of guava, *jamun* and *Aloe vera* juice/pulp

Parameters*	Mean ± SE		
	Guava pulp	<i>Jamun</i> pulp	<i>Aloe vera</i> juice
TSS (°B)	5.02 ± 0.10	9.97 ± 0.15	2.20 ± 0.10
Titratable acidity (%)	0.38 ± 0.02	0.75 ± 0.02	0.35 ± 0.02
Ascorbic acid (mg/100g)	120.19 ± 2.17	27.28 ± 1.70	102.00 ± 1.33
Reducing sugars (%)	1.26 ± 0.04	2.79 ± 0.26	0.42 ± 0.03
Total sugars (%)	2.70 ± 0.50	6.52 ± 0.36	1.64 ± 0.09
Total phenolics (mg/100g)	180.42 ± 1.86	424.24 ± 1.84	198.38 ± 1.45
Antioxidant potential (% free radical scavenging activity)	72.80 ± 1.25	75.56 ± 1.92	84.68 ± 0.92
Total anthocyanins (mg/100g)	ND	112.50 ± 0.13	ND

*Each value is average of three determinations; SE = Standard error; ND = Not detected.

Table 2: Sensory evaluation of different guava-jamun blended drinks

Treatment	Detail of treatments		Sensory Score*			
	Fruit part (% blended pulp)	TSS (°Brix)	Colour	Body	Flavour	Overall acceptability
T ₁ (G ₁₀₀) Control	10	10	7.00	7.00	6.90	7.00
T ₂ (G ₉₀ :J ₁₀)	10	10	7.15	7.11	6.95	7.10
T ₃ (G ₉₀ :J ₁₀)	10	15	7.20	7.18	7.00	7.15
T ₄ (G ₉₀ :J ₁₀)	15	10	7.22	7.21	7.15	7.20
T ₅ (G ₉₀ :J ₁₀)	15	15	7.22	7.22	7.15	7.25
T ₆ (G ₈₀ :J ₂₀)	10	10	7.28	7.28	7.18	7.28
T ₇ (G ₈₀ :J ₂₀)	10	15	7.32	7.32	7.25	7.35
T ₈ (G ₈₀ :J ₂₀)	15	10	7.38	7.35	7.30	7.42
T ₉ (G ₈₀ :J ₂₀)	15	15	7.40	7.35	7.30	7.48
T ₁₀ (G ₇₀ :J ₃₀)	10	10	7.50	7.38	7.35	7.55
T ₁₁ (G ₇₀ :J ₃₀)	10	15	7.55	7.40	7.40	7.60
T ₁₂ (G ₇₀ :J ₃₀)	15	10	7.60	7.45	7.50	7.66
T ₁₃ (G ₇₀ :J ₃₀)	15	15	7.63	7.48	7.55	7.66
T ₁₄ (G ₆₀ :J ₄₀)	10	10	7.60	7.50	7.56	7.70
T ₁₅ (G ₆₀ :J ₄₀)	10	15	7.65	7.62	7.60	7.80
T ₁₆ (G ₆₀ :J ₄₀)	15	10	7.70	7.67	7.70	7.72
T ₁₇ (G ₆₀ :J ₄₀)	15	15	7.80	7.70	7.75	7.85
T ₁₈ (G ₅₀ :J ₅₀)	10	10	7.78	7.68	7.72	7.71
T ₁₉ (G ₅₀ :J ₅₀)	10	15	7.79	7.65	7.60	7.69
T ₂₀ (G ₅₀ :J ₅₀)	15	10	7.78	7.55	7.55	7.67
T ₂₁ (G ₅₀ :J ₅₀)	15	15	7.79	7.55	7.55	7.70
CD _{0.05}	—	—	0.15	0.13	0.18	0.10

*Sensory score recorded on 9-point hedonic scale; 9=like extremely, 1=dislike extremely; G= guava; J= jamun.

flavour profile of the product and hence rated highest by the panellist on the basis of overall acceptability of the beverage. Tiwari and Deen (2015; Sharma *et al.* (2016) and Sharma *et al.* (2018) had observed similar findings in Bael-Aloe vera RTS, Jamun- mango squash and Aloe vera-aonla squash, respectively.

Data pertaining to the effect of Aloe vera fortification on sensory quality of guava-jamun blended drink is presented in Fig 1. It was found that with the increase in proportion of Aloe vera juice up to 9 per cent, the colour score of prepared drink decreased, however all the treatments obtained colour score between 7.72 to 7.80, which were well with the acceptable range (more than 7.00). Whereas, with the addition of Aloe vera juice up to a level of 5 per cent, the score for flavour increased, which has resulted in higher overall acceptability score of the products. While, the addition of Aloe vera at higher levels (>5%) resulted in decline of overall acceptability score of the products

(Fig. 1). Our results are in conformity with earlier studies conducted by Tiwari and Deen (2015) and Pandey *et al.* (2019).

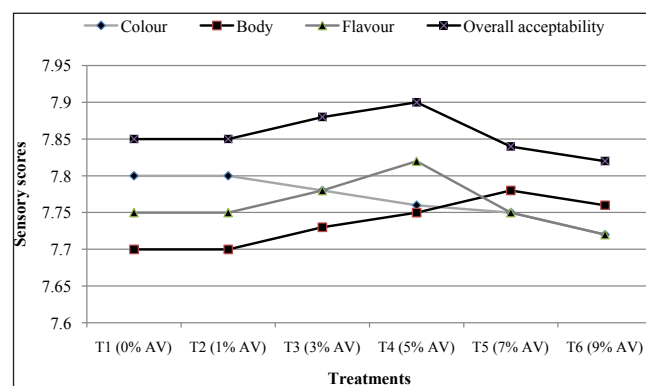


Fig. 1: Sensory evaluation of Aloe vera fortified guava-jamun blended health drink

Other researchers have also reported that fortification of beverages with herbal extracts improved physico-chemical, sensory, nutritional and microbial quality

of the prepared beverage (Boghani *et al.* 2012; Jairajpuri and Qadri 2015; Sharma and Tandon 2015). Hence, on the basis of sensory evaluation, *Aloe vera* fortification up-to 5 per cent was optimized.

Physico-chemical, nutritional and sensory characteristics of the developed beverage

The data presented in Table 3 revealed that the addition of *jamun* pulp had improved the nutritional quality of drink as evident from its higher total phenolic content (32.75 mg/100g), ascorbic acid content (33.91 mg/100g) and antioxidant potential (11.30 % free radical scavenging activity) compared to standard drink (18.09 mg/100g, 16.16 mg/100g and 8.77 % free radical scavenging activity), respectively (Table 3). Palamthodi *et al.* (2019) studied physicochemical and functional properties of ash gourd/ bottle gourd beverages blended with *jamun* and showed significant increase in bio-accessibility of polyphenols, flavonoids, and anthocyanins. Further, *Aloe vera* fortified guava-*jamun* blended drink so developed has also showed strong antimicrobial activity against human pathogen *E. coli* (Table 3). Similar results have been reported by Kapoor and Ranote (2015) and Sharma *et al.* (2018).

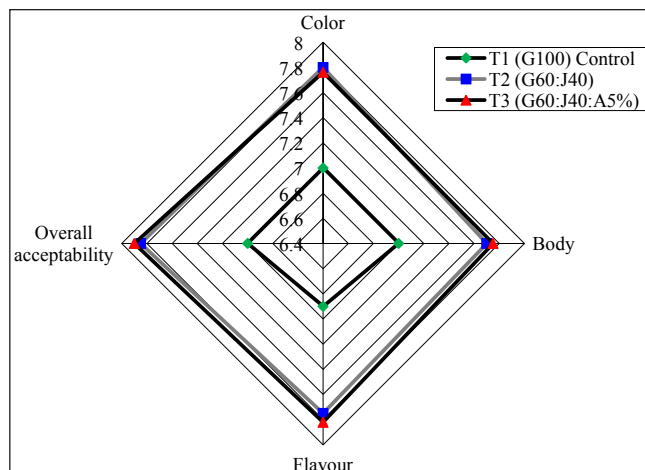


Fig. 2: Sensory attributes of guava drink, guava-*jamun* drink and *Aloe vera* fortified guava-*jamun* health drink

Further, blending has also exerted positive effect on sensory attributes of the beverages as the blended drink had recorded higher score for flavour (7.82) and overall acceptability (7.90) compared to guava drink (Fig. 2). Earlier, many workers have reported blending of two or more fruit pulp/juices in various proportions for making more palatable and nutritious beverages (Boghani *et al.* 2012; Mohamed *et al.* 2014; Sharma *et al.* 2016; Raj *et al.* 2017).

Table 3: Physico-chemical attributes of guava drink, best rated guava-*jamun* drink and *Aloe vera* fortified guava-*jamun* drink

Parameters*	Mean ± SE		
	Guava drink (G ₁₀₀)	Guava- <i>jamun</i> drink (G ₆₀ :J ₄₀)	Guava- <i>jamun</i> - <i>Aloe vera</i> drink (G ₆₀ :J ₄₀ :A _{5%})
TSS (°B)	10.00 ± 0.20	15.00 ± 0.20	15.00 ± 0.20
Tiratable acidity (%)	0.30 ± 0.01	0.30 ± 0.01	0.30 ± 0.02
Total sugars (%)	9.41 ± 0.16	14.06 ± 0.03	14.38 ± 0.13
Ascorbic acid (mg/100g)	16.16 ± 0.15	18.38 ± 0.08	33.91 ± 0.07
Total phenolics (mg/100g)	18.09 ± 0.10	32.75 ± 0.06	36.12 ± 0.02
Antioxidant potential (% free radical scavenging activity)	8.77 ± 0.06	11.30 ± 0.02	13.40 ± 0.02
Total anthocyanins (mg/100g)	1.05 ± 0.03	10.09 ± 0.03	10.02 ± 0.02
Antimicrobial activity (mm zone of inhibition)	6.5 ± 0.03	9.2 ± 0.04	14.5 ± 0.04

SE = Standard error; G= guava; J= *jamun*; A= *Aloe vera*; T= TSS.

CONCLUSIONS

Blending guava pulp with *jamun* pulp and *Aloe vera* juice have resulted in improvement of sensory, nutritional and functional properties of the drink. The blended drink prepared by using 60 per cent guava pulp and 40 per cent *jamun* pulp with 15 per cent fruit part and 15°B TSS was optimized for better sensory characteristics. Addition of *Aloe vera* juice up to 5 per cent was found acceptable. *Aloe vera* fortified guava- *jamun* drink contained higher amounts of ascorbic acid, total phenolic content, antioxidant activity and also showed strong antimicrobial activity against human pathogen *E. coli* compared to standard guava drink. So, it was concluded that, guava and *jamun* can successfully be utilized for the development of functional/health beverages by way of blending and/or fortification with *Aloe vera*. Hence, their availability in the market will definitely benefit the health conscious people.

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RESEARCH PAPER

Effect of Addition of Additives on Sequential Culture Lactic Acid Fermentation of Radish

Somesh Sharma and V.K. Joshi*

School of Bioengineering and Food Technology, Shoolini University, Solan, Himachal Pradesh, India

*Corresponding author: vkjoshipt@rediffmail.com

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ABSTRACT

Spices or aromatic herbs are added to most of the lactic acid fruit and vegetable fermentation preparations to improve the flavour of the finished products. Aromatic compounds in these spices (including mainly terpenes and polyphenols) often have an antimicrobial effect which means that they can exert a selective role in the growth and activity of microorganisms during fermentation. Hence, investigations were carried out to study the effect of different additives on the lactic acid fermentation of radish. It was concluded from the studies that the acid production pattern in sequential lactic acid fermentation of radish are influenced by the addition of additives. There were also differences in acid production right from the beginning and differences were quite evident after 5 days of fermentation on addition of mustard as compared to other additives. It is clearly evident that addition of 2 per cent mustard produced the highest titratable acid. The addition of mustard seeds correspondingly enhanced the lactic acid bacterial count along with the titratable acidity. Amongst the interactions, mustard (2 per cent) had the lowest (0.18%) reducing sugar followed by mustard 1 per cent. Based on the reducing sugar concentration it is concluded that, the use of mustard seeds had enhanced the fermentability of sugar into lactic acid. The low alcohol content in treatment with 2 per cent mustard might be due to its influence on the selective growth of lactic acid bacteria in mustard and its antimicrobial effect on other microorganisms thus, allowing lactic acid bacteria to grow and produce maximum lactic acid.

Keywords: Aromatic herbs, lactic acid, antimicrobial, fermentation, alcohol, bacteria, titratable acid

Among the different methods, preservation by fermentation is well known. It is one of the oldest method of food preservation in the world (Pederson, 1979). Although China is believed to be the birth place of the process of fermented vegetables, the preservation by fermentation began before the recorded history (Joshi and Thakur, 2000). Fermentation using natural or starter culture has emerged to be a cheap method of preservation to increase the shelf-life of the products, besides preparation of new products with diversified taste and flavour. Lactic acid fermentation is used for commercial bulk storage of seasonal vegetables to increase their availability and to obtain a desired

sensory quality of products (Frazier and Westhoff, 1998). This process apart from contributing certain desirable physical and flavour characteristics, ensures the availability of products at relatively low cost. If some of these fermented products can be made into food products which are being consumed or are nutritionally or microbiologically more stable, still retaining the advantages of lactic acid fermentation, it would be the most appropriate approach.

Fermented foods are more nutritious and have better

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acceptability than unfermented ones (Joshi *et al.* 1999; Sahlin, 1999; Hang and Jackson, 1967). One of the reasons for the increasing interest in fermented foods now-a-days is their recognition to promote the functions of the human digestive system in a number of positive ways. In 1900, Metchnikoff pointed out the use of fermented milk in the diet for prevention of certain diseases of the gastrointestinal tract and promotion of healthy day-to-day life. Since then, a number of studies have shown that the fermented food products do have a positive effect on health (Sahlin, 1999).

In recent years, increase in consumers demand for fresh like products, containing natural ingredients, changing food patterns and convenience have led to the development of minimally processed products using LAB culture. Lactic acid fermentation using LAB cultures is employed for the preparation of different products such as fermented grape juice, sauces, ready-to-serve drink, instant chutney, fermented peanut milk, *sogurt*, fermented corn meal *Kuhunzaki*, fermented radish pickle, and fermented beverages from wheat and maize (Joshi and Thakur, 2000; Joshi and Sharma, 2009; Sharma *et al.* 2008; Joshi *et al.* 2011). Kimchi and sauerkraut are the well known products made by fermenting vegetables. Some of these fermented foods have established a reputation of being beneficial to human health. Although, all food items contain different types of microorganisms and in different population, which microorganisms would dominate depends upon several factors. Sometimes, microorganisms are initially present in a very low number in the food, for example, lactic acid bacteria (LAB) in the favorable conditions, will outnumber the other organisms especially those of public health significance, inhibiting their growth. In contrast to fermented meat, fish, dairy and cereal products, fermented vegetables have not been recorded as a significant source of microbial food poisoning (Fleming and McFeeters, 1981). Several studies have shown improved nutritional value in grains as a result of lactic fermentation, principally through enhancement of essential amino acids. Further, fermentation of plant products reduces levels of anti-nutritional factors present in the natural

produce. Bacteriocins produced during lactic acid fermentation of fig have been also reported as a bio-preservative (Kumari *et al.* 2018).

In principle, most vegetables can be LAB fermented but, so far only beets, turnip, cauliflower, radish, carrot, cabbage, olives and cucumber are fermented on commercial scale for human consumption. However, only a few reports are available on LAB fermentation of carrot, radish and cucumber. Most of the studies have concentrated on the vegetables mentioned earlier using natural fermentation. Employing inoculated fermentation and investigating their sequence, and finally the physico-chemical and sensory quality would be an interesting aspect for more investigation both academically and practical utility point of view. Sharma and Joshi (2007) studied the influence of temperature and salt concentration on lactic acid fermentation of radish (*Raphanus sativus*). Even some of the studies done on probiotics reflected that these Lactic acid bacteria can also be probiotic and can be used in fruit juices after microencapsulation (Oberoi *et al.* 2019).

Spices or aromatic herbs are added to most of the lactic acid fruit and vegetable fermentation preparations to improve the flavour of finished products. Aromatic compounds in these spices (including mainly terpenes and polyphenols) often have an antimicrobial effect thus, they can have a selective role in the development of bacteria during fermentation. In the present investigations, effect of addition of different additives {lactose, magnesium sulphate, manganese sulphate and mustard (*Brassica juncea*)} on the LAB sequential culture fermentation was studied and the results have been discussed here.

MATERIALS AND METHODS

Lactic acid bacterial strains

The strains of Lactic acid bacteria (LAB) were procured from National Dairy Research Institute (NDRI), Karnal used in the study were *Lactobacillus plantarum* (NCDC 020), *Pediococcus cerevisiae* (NCDC 038) and *Streptococcus lactis var diacetylactis* (NCDC 061). These strains were first activated in MRS

broth for 24-32 hrs at temperature of 30-37°C. After activation, these were maintained on MRS agar slants under refrigerated condition and were re-cultured after every three months from the stock cultures.

Sequential fermentation

In sequential fermentation, three different microorganisms were added as starter culture in sequence. *Streptococcus lactis* was added after 24hrs of fermentation and after about 48 hrs *Pediococcus cerevisiae* was added and then, after 72 hrs *Lactobacillus plantarum* was added at the rate of 2 per cent each.

Experimental setup

The radish procured from the local market were washed, peeled and grated. To study the effect of additives, addition of different additives viz. lactose (1 and 2 per cent), mustard grounded (1 and 2 per cent) and magnesium sulphate + manganese sulphate (1 and 2 per cent) in combination were added to the grated radish along with 2.5 per cent salt. The fermentation was carried out at 26 °C with sequential culture as starter culture. During fermentation, acid production pattern was recorded. On completion of fermentation when no further increase in acidity took place, the fermented vegetables were filled in sterilized glass jars. Hot brine was added and the jars were exhausted to expel the air inside the product to avoid any oxidation process during the storage period. The packed product was then, pasteurized at temperature of 71 °C for 15 min.

Analysis

Chemical Analysis

Titrateable acidity was estimated by treating a known aliquot of the sample against N/10 NaOH solution using phenolphthalein as an indicator as per the standard method (A.O.A.C. 1980).

The pH was taken with HPG, G-2004 pH meter, after calibrating it with buffer solutions of pH 4 and 9.2 (Ranganna, 1986).

Determination of reducing sugars was based on

Nelson-Somogy method (Sadasivam and Manickam, 1996). Diluted sample was heated with alkaline copper tartrate reagent (1 ml), which is reduced to form cuprous oxide. Treated sample solution was boiled for 10 mins and cooled, arsenomolybdate (1ml) was added, which resulted in blue colour development. The colour was compared with a set of standards (glucose, 10-100 µg) in a spectrophotometer-20D, by measuring absorbance at 520 nm.

The calculations were made as per the standard curve in terms of glucose.

Lactic acid Bacterial Count

Lactic acids bacterial (LAB) count was taken according to the method given by Harrigan and McCance (1996). MRS agar media was prepared as per the composition and count was taken after incubation at 32°C depending upon the type of microorganism for 48-72 hrs and the number of microbial colony forming units as log CFU/g or ml of product was recorded.

Statistical analysis

The data of quantitative estimation of various chemical characteristics of different fermented products were analysed by Completely Randomized Design (CRD) while the data of sensory evaluation were analysed by Randomized Block Designs (RBD) as described by O'Mahony (1985).

RESULTS AND DISCUSSION

The results depicted in Fig. 1 show the acid production pattern in sequential lactic acid fermentation of radish as influenced by the addition of additives. There were differences in acid production right from the beginning due to addition of various additives. It is also clearly evident that addition of 2 per cent mustard produced the highest titrateable acid. The differences were prominent after 5 days of fermentation on addition of mustard as compared with other additives.

The results (Table 1) on the physico-chemical characteristics showed significant differences in the titrateable acidity among the various treatments, their

Table 1: Effect of addition of additives on sequential culture lactic acid fermentation of radish

Treatment	Conc. (%)	Titratable acidity (% LA)	pH	Reducing sugars (%)	Alcohol (% v/v)
Lactose	1	0.89	3.35	0.35	0.031
	2	0.97	3.39	0.31	0.044
	Control	0.94	3.41	0.39	0.049
	Mean	0.93	3.38	0.35	0.041
Mustard	1	1.12	3.29	0.23	0.028
	2	1.26	3.20	0.18	0.021
	Control	0.94	3.41	0.39	0.049
	Mean	1.10	3.30	0.26	0.032
MgSO ₄ + MnSO ₄	1	1.10	3.29	0.32	0.047
	2	0.96	3.26	0.35	0.042
	Control	0.94	3.41	0.39	0.049
	Mean	1.00	3.32	0.35	0.046
Mean conc.	1	1.03	3.31	0.30	0.035
	2	1.06	3.28	0.28	0.035
	Control	0.94	3.41	0.39	0.049
*Initial characteristics		0.16	5.87	2.10	–
CD (≤ 0.05)					
Treatment (T)		0.034	0.032	0.027	NS
Concentration (C)		0.034	0.032	0.027	NS
T×C		0.059	0.056	0.047	NS

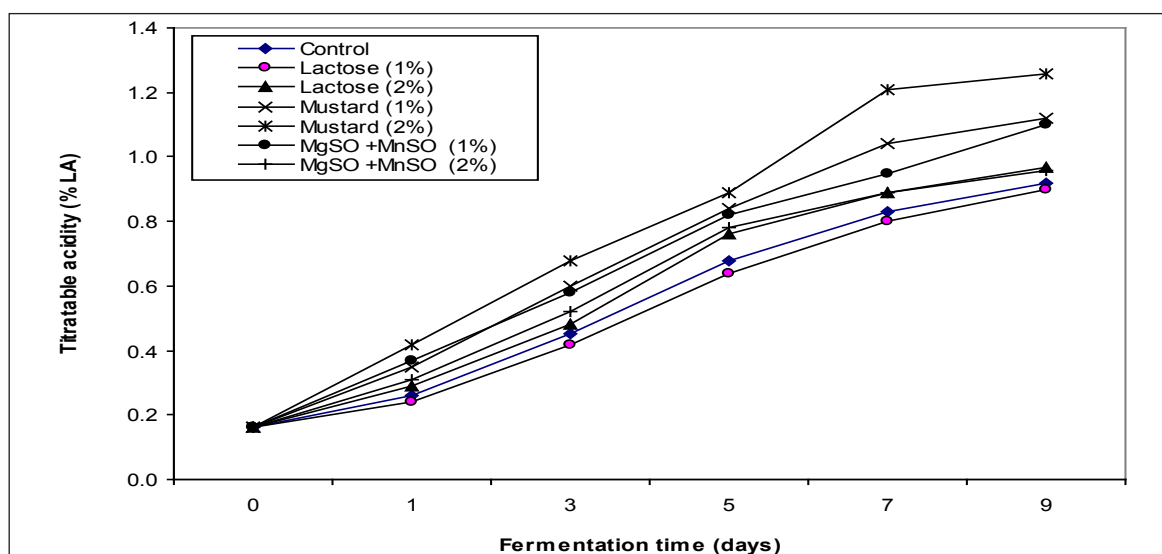


Fig. 1: Acid production pattern in sequential LAB fermentation of radish by addition of additives

concentration and the control. The titratable acidity ranged from 0.89 to 1.26 per cent. The highest (1.10% LA) titratable acidity was recorded in the treatment where mustard was added. Amongst the interactions, the highest titratable acidity (1.26%) was found in fermentation where 2 per cent mustard was added

and the minimum titratable acidity was documented in the control. It is clear that addition of additives had certainly increased the titratable acidity of fermented radish. The results are in consistence with the earlier results of acid production pattern. Our results are also consistent with the earlier findings (Anand and

Das, 1971) indicating that adding 6-10 g of mustard seed powder to turnip increased the lactic acid levels during fermentation. The results in Fig. 2 depicted the lactic acid bacteria (LAB) count in sequential culture fermentation of radish on addition of additives. It is clear that on addition of mustard, the lactic acid bacteria (LAB) count was more and corresponded to that titratable acidity. But the concentration 1 and 2 per cent did not effect the lactic acid bacterial (LAB) population. However, the lactic acid bacterial (LAB) count was comparable with other additives used in the fermentation.

The results (Table 1) reveal that pH values varied from 3.41 to 3.20. Among the treatments, the lowest (3.30) pH value was obtained in mustard and the highest (3.41) in control. However, among the interaction's lowest pH value (3.20) was recorded in treatment with 2 per cent mustard and highest (3.41) in the control. The pH values obtained are in corroboration with earlier results of titratable acidity. It is also discernible from the results that reducing sugar content ranged between 0.18 to 0.39 per cent.

Amongst the interactions, mustard 2 per cent had the lowest (0.18%) reducing sugar followed by mustard 1 per cent. Based on the reducing sugar concentration, it can be stated that the use of mustard had enhanced the fermentability of sugar into lactic acid.

During lactic acid bacterial (LAB) fermentation, the alcohol content ranged from 0.021 to 0.049 per cent with highest (0.049%) alcohol content in control and lowest (0.021%) in treatment with mustard 2 per cent. Further, amongst the treatments mustard addition had lowest (0.035%) alcohol content. It is desirable as the sugar was not diverted to alcohol production but was converted into lactic acid. However, the highest (0.58%) alcohol content was found in lactic acid fermentation of radish with addition of lactose. The low alcohol content in treatment with 2 per cent mustard might be due to its influence on the selective growth of lactic acid bacteria in treatment with mustard and its antimicrobial effect on other microorganisms thus, allowing lactic acid bacteria to grow and produce maximum lactic acid. It has been found that mustard seeds contain a volatile aromatic

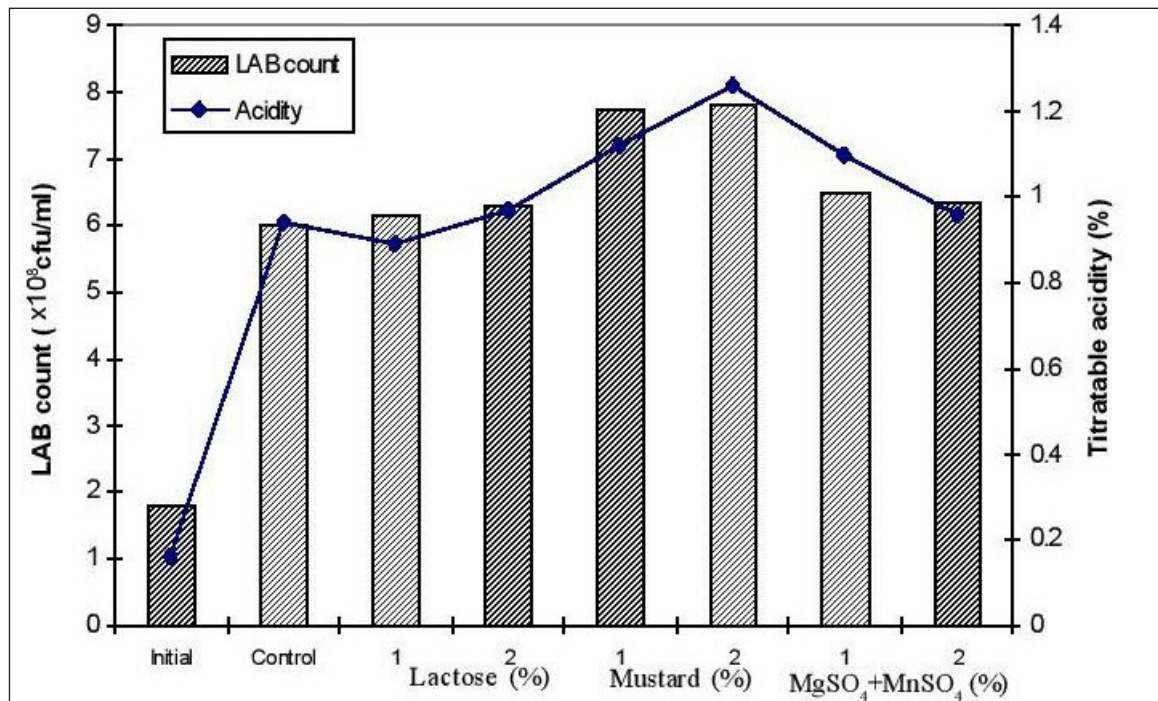


Fig. 2: Variation in LAB count and acid production on addition of additives during radish sequential culture fermentation

compound with antibacterial and antifungal properties (Anand and Das, 1971) and there it has been shown to have a selective preservative action, allowing lactic acid bacteria (LAB) to grow and suppress the surface yeasts (Sethi and Anand, 1984). The results are in corroboration with the results of lactic acid bacteria (LAB) count.

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RESEARCH PAPER

Process Development of Low Fat *Quarg* Cheese Using Fat Replacer

Kadpe, B.B.¹, Patange, D.D.^{2*}, Yadav, M.M.², Kamble, D.K.², Memane, C.V.² and Kamble, S.S.²

¹College of Agriculture, Nesri, Kolhapur, India

²Faculties of Division of Animal Husbandry and Dairy Science, RCSI College of Agriculture, Kolhapur, India

*Corresponding author: patangedeshmukh1@gmail.com

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ABSTRACT

Quarg is a sparkling cheese having a smooth texture, barely bitter flavor and is a type of natural un-ripened and clean cheese. *Quarg* cheese contains more fat and lacks in whey protein which affects its nutritional quality, therefore it is essential to produce low fat *quarg* cheese using fat replacers. Initially two fat replacers were tried i.e. carragennan and Simplese-100 @ 0.5 of *quarg* and after selecting one, its level was optimized. The optimized product was assessed for its storage stability. During investigation cheese was analyzed for its sensory, physico-chemical and microbial analysis. Maximum score was awarded to the *quarg* cheese prepared using 0.5 per cent Simplese-100 when added to *quarg* after removal of whey. The superior product contained 0.62±0.03, 12.68±0.08, 3.33±0.02, 1.32±0.04, 17.87±0.02 % fat, protein, lactose, ash and total solids, respectively whereas, its pH was 4.36±0.06. During the storage, there were significant (p<0.05) differences in change in all the sensory qualities, proximate composition of fat replacer added *quarg* cheese.

Keywords: *Quarg* cheese, fat replacer, storage study, sensory and proximate analysis

Fermentation is the oldest and most economical method in food preservation. It has been well documented that fermentation enhances mineral bioavailability and digestibility of proteins and carbohydrates as well as improves sensory qualities of the product. Numbers of fermented dairy products are being prepared all over the world including *dahi*, yoghurt, *lassi* and cheese. Cheese is a well known milk product that is valued for its nutritional superiority. Several types of chesses are manufactured with wide ranging flavours, texture, and forms. There are more than 2000 varieties of cheese, although many have little differences. The most popular varieties of cheese are *cheddar*, *mozzarella*, *feta*, cottage and *quarg* cheese.

Quarg is a sparkling cheese having a smooth texture and barely bitter flavor. It is a protein-rich, milky white or slight yellowish acidic coagulated and is a type of natural un-ripened and clean cheese which is most popular in countries like Germany. It is made from pasteurized skim milk or entire milk by incorporating *Streptococcus cremoris* and *Leuconostoc citrovorum* or any other starter culture and with a little quantity of rennet enzyme. Precipitation of the casein proteins occurs due to the combined action of these substances.

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The obtained coagulum is subsequently cooked and allowed draining the whey to get *quarg*. The moisture content in *quarg* is more (82%, w/w) (Yadav *et al.* 2019).

On an average, *quarg* cheese contain 11 per cent fat, 18 per cent protein, 4.5 per cent carbohydrate and 1.4 per cent ash. Presence of 18% protein is significant of *quarg* cheese, however presence of 11 per cent fat may increase its production cost and decreases consumption. As incidence of various chronic diseases such as elevated blood pressure, obesity and atherosclerosis is increasing rapidly and it has been found to be associated with high fat intake by people (Tiwari, 2005). Saturated fats are found mainly in foods of animal origin like whole milk, butter, cheese and also in coconut and palm oil. Nutrition experts recommend that the total intake of dietary fat should not exceed 30 percent of daily energy intake. Health conscious people look for the ways to improve nutritional habits without sacrificing psychological satisfaction so peoples prefer low fat diet. This has led to the increasing demand for low fat or no fat dairy food products with good taste. However, development in fat reduced products is associated with a number of difficulties. Defatted dairy products most often have a number of flaws: too hard, resilient of coarse consistency, poor taste and aroma (Simrnova, 2014).

In case of cheese, reduction in fat in also adversely affects its textural, functional and sensorial attributes such as rubbery texture, poor meltability, bitter taste, lack of flavor and undesirable colour (Alfonso and Norma, 2008). Few substances have been identified which can be incorporated in low fat food to improve its sensory properties, without affecting the aroma and flavor of foods formulated with the fat replacer.

Therefore, fat replacer possesses such potential to provide an alternative to high fat product to meet the expectation of the consumers' requirement for healthy food (Tiwari, 2005). Accordingly, food industries have focused on the production of low fat /low calorie, high fiber as a functional food in response to the public interest in the last couple of decades (Agarwal *et al.* 2016).

Fat replacer is a substance which chemically resembles fats, proteins or carbohydrates and possesses certain desirable physical or organoleptic properties of fat (Ahmed *et al.* 2015). These are of low calorie and possess some of functional properties of fat (Chavan *et al.* 2016). Fat replacers can be successfully utilized in the development of low fat cheese, yoghurt, spreads, frozen dessert, cream cheese and sour cream. It gives a sense of lubricity and creaminess in these products. Considering the health benefits of low fat *quarg* cheese and functional properties of fat replacer, the present investigation was undertaken. The objective of this study was to determine the effects of fat replacers on the physico-chemical, sensory and microbial quality of low-fat *quarg* cheese and also to find the storage stability of the product.

MATERIAL AND METHODS

Buffalo milk was procured from Dairy Farm, Rajarshi Chhatrapati Shahu Maharaj College of Agriculture, Kolhapur. Starter culture LF 40 was procured from National Collection of Dairy Culture, ICAR-NDRI, Karnal, Haryana (India) and microbial rennet was procured from CHR Hansen Ltd. Mumbai. Simplese-100 from CP Kelco, Denmark and Carragennan from Marine Hydrocolloids Cochin, Kerala (India) were utilized as fat replacer.

Preparation of low fat *quarg* cheese

Initially *quarg* cheese was prepared as per protocol developed by Patange *et al.* (2018) for spiced *quarg* cheese with suitable modification. Buffalo milk was separated to get the milk of desired fat per cent as per treatment. The milk was heated to 80°C for 10 min in SS made cheese vat of size 25×16×16 cm and then, cooled to 28–30°C followed by inoculation of LF-40 starter culture @1 per cent and allowed to hold in undisturbed condition for at least one hr. to lower down the pH to 6.2-6.3. Subsequently microbial rennet was added @ 0.5g per 100 ml of milk and mix was incubated for 14-16 hr at 31°C to achieve the pH of 4.9 to 5.0. Obtained firmed curd was then, cut using both vertical and horizontal MS framed knife with nylon wire followed by cooking it by keeping

cheese vat in water bath. The heating process was accomplished slowly upto 65°C (rate of rising 1°C / 2 min of time). After cooking the expelled whey was drained out by hanging the coagulum in doubled layered cotton muslin cloth to get *quarg*. The process is shown in Fig. 1.

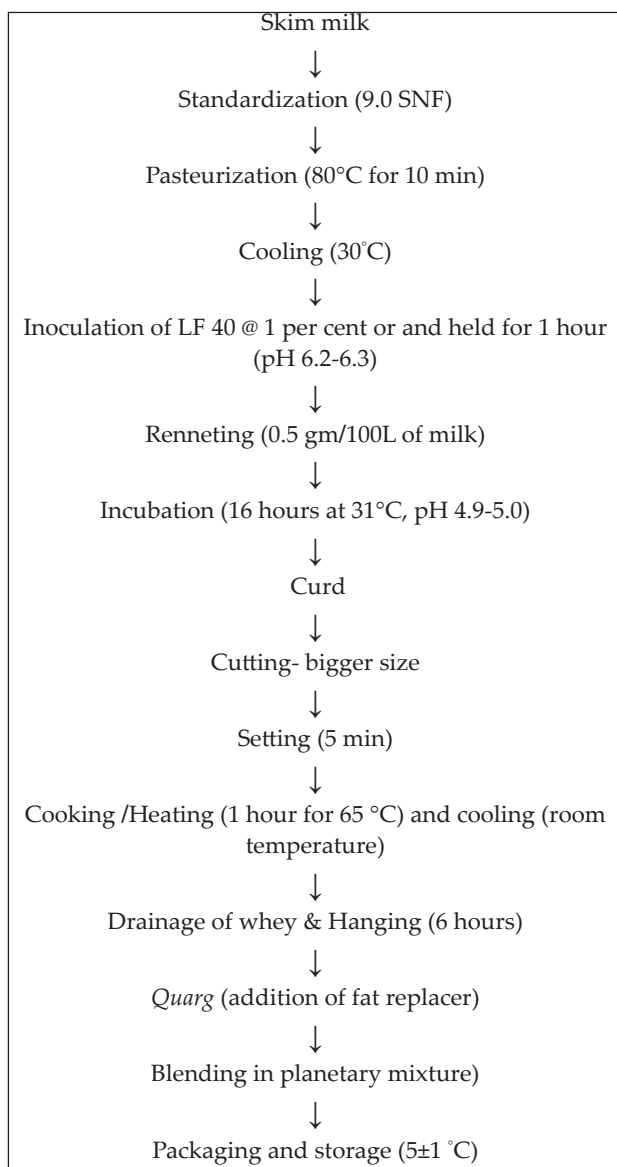


Fig. 1: Flow diagram of low fat *quarg* cheese added with fat replacer

Selection of type and stage of fat replacer in *quarg* cheese

Carragennan (T_2) and Simplesse-100 (T_3) fat replacer

were mixed in the *quarg* cheese (@0.5 % of *quarg*) after the drainage of whey and compared with *quarg* cheese made from standardized milk (T_0) and skim milk (T_1). One fat replacer was selected on the basis of sensory evaluation of *quarg* cheese.

Optimization of level of fat replacer in *quarg* cheese

From above trail, the selected fat replacer was tested @ 0.2 (S_1), 0.4 (S_2), 0.6 (S_3) and 0.8 % (S_4) of *quarg* and compared with the *quarg* prepared from standardized milk (S_0) was added at following level of *quarg* and analyzed for sensory and physico – chemical properties. The best level of fat replacer was optimized on the basis of sensory evaluation.

Storage stability study of low fat *quarg* cheese

The optimized product (P_2) was packed in food grade plastic sterilized container of 100 g size and stored at $5\pm 1^\circ\text{C}$ for storage stability study and compared with control (P_1) i.e. low fat *quarg* cheese without addition of fat replacer. The samples were evaluated after 3 days of interval for changes in sensory, physico-chemical and microbial qualities till their sensory acceptability.

Analysis of *quarg* cheese

Sensory evaluation was carried out with scoring test by five panelists who were the faculty members of the department. The panelists were selected on the basis of their interest in sensory evaluation of cheese. The different samples of cheeses were evaluated for flavour, colour and appearance, spreadability, body and texture and overall acceptability using a score from '1' to '9'. Approximately, 15 to 20 g of each sample was presented to panelists, to clean palates of panelists between samples potable drinking water and bread were provide. Panelists were also requested to tick-mark the defects on the scale in order to determine the reasons of decrease in scores. The samples of cheeses were analyzed for proximate composition as per AOAC, (2000); during the storage period along with sensory and chemical analysis microbial load in terms of total plate count, yeast and

mould coliform and count were enumerated as per manual of dairy bacteriology, ICAR (1982).

Statistical Analysis

All data are presented as mean \pm SD for three replicates for each sample. The experimental design was completely randomized design (CRD) in factorial arrangement using three replications as suggested by (Snedecor and Cochran, 1994).

RESULTS AND DISCUSSION

Effect of type of fat replacer on sensory properties of low fat *quarg* cheese

The diagrammatic representation (Fig. 1) of score for colour and appearance of low fat *quarg* cheese was significantly ($p < 0.05$) affected by the level of fat replacer. The highest score (8.25 ± 0.03) was obtained by *quarg* cheese where in simplese-100 was mixed. For this treatment, judges made the comments that the product had shiny and creamy colour with uniform appearance. The effect of simplese-100 was at par with the *quarg* produced from 4.5% milk. Nurcan and Mustafa (2004) also reported that the colour of the low-fat fresh *kashar* cheeses containing fat replacers was different from that of low-fat control cheese. Moreover, the use of simplese D-100 corrected all appearance defects which were determined in low fat fresh *kashar* cheese. Further they reported that the low-fat control cheese had a more translucent surface and denser colour than full-fat cheese. Fat replacers, can also act as light-scattering centers and increase the opaqueness of low fat cheese (Mcmahon *et al.* 1996). It was observed that the score of body and texture obtained was significantly ($p < 0.05$) highest (8.35 ± 0.02) for *quarg* cheese under treatment T_3 (Fig. 2) and it possessed soft body and smooth texture. The *quarg* prepared from skim milk was comparatively compact and had firm body and coarse texture. When tapioca starch and lecithin as a fat replacer were used to produce a reduced-fat Feta cheese, the hardness and yield loss values decreased and the moisture content were increased (Sipahioglu *et al.* 1999). The addition of some hydrocolloids, mainly

carrageenan, may replace the addition of fat and emulsifying salts. The *quarg* prepared by addition of carrageenan recorded significantly ($p < 0.05$) highest score for spreadability (score 8.21 ± 0.02) as compared to simplese-100 (Fig. 3). Processed cheese with added carrageenan was found to be hard and impossible to spread was reported by Cernikova *et al.* (2010). However, improvement in spreadability score of carrageenan added *quarg* cheese over cheese made from skim milk is supported by the facts of Blaszak *et al.* (2018) who reported that even small amounts of carrageenan can increase cheese firmness and maintain cheese structure after cheese curd heating. Further, they mentioned that any modifications in cheese composition can destroy the natural cheese structure, but the addition of carrageenan can be useful for creating modified cheese-like products with desirable attributes. The good quality *quarg* has characteristics like clean and mildly acidic flavour and has optimum sourness and acidic taste as good as *Chakka* and yoghurt. The effect of all treatments were statistically different ($p < 0.05$) with respect to flavour score, the *quarg* prepared by addition of Simplese-100 recorded highest score for flavour (score 8.10 ± 0.02), followed by the *quarg* prepared from the milk containing 4.5 % fat (score 8.00 ± 0.05). Improvement in flavor score was in accordance with the facts reported by Smirnova (2014), who found that the advantages of protein fat imitators are not only to increase the protein content but also to add a creamy flavor to nonfat products. The flavour score of *quarg* cheese with carrageenan and Simplese-100 were close to each other (Fig. 4). The mean flavour scores SimpleseD-100 added low fat cheese were closer scores to full-fat cheese was informed by Nurcan and Mustafa (2004). Among the four treatments under study, the *quarg* prepared from skim milk had lowest score which could be due to low-fat cheeses commonly present a poor aroma and a typical flavors caused by the lack of aromatic compounds that are produced by lower levels of lipolysis. In addition, their typical high moisture content cause excessive growth of the starter culture, which accelerates acid production, resulting in bitter flavors (Diamantino *et al.* 2014). Cheeses with lower fats usually have less

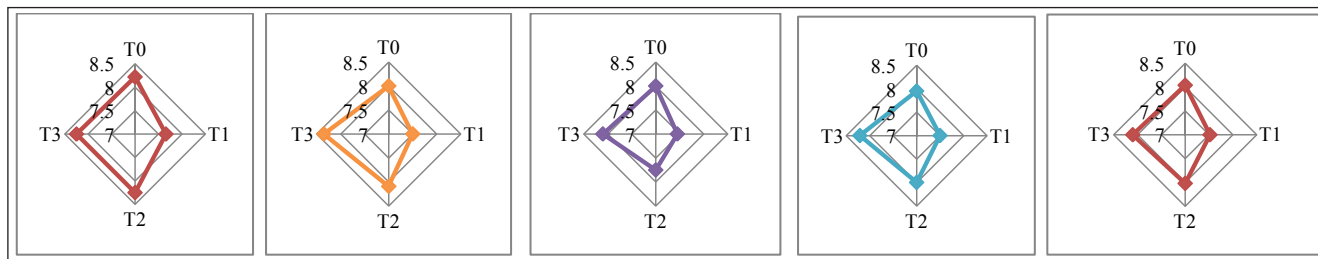


Fig. 1: Effect of type of fat replacer on colour and appearance score

Fig. 2: Effect of type of fat replacer on body and texture score

Fig. 3: Effect of type of fat replacer on flavour score

Fig. 4: Effect of type of fat replacer on spreadability score

Fig. 5: Effect of type of fat replacer on overall acceptability score

pronounced flavour, possibly because of its dilution in reduced and low-fat cheeses because of excessive moisture retention, and the fats in cheese are responsible for most of the flavors, therefore when fats are decreased, the cheeses flavour was decreased by as described earlier Tareq *et al.* (2016).

The effect of type of fat replacer was also examined on the overall acceptability of *quarg* cheese (Fig. 5) The overall acceptability score ranged from 7.52 ± 0.04 to 8.10 ± 0.02 . The maximum score was obtained by the product containing Simplese-100, it may be because of higher water holding capacity of carragennan resulted in too firm body of product along with resistance to spread and poor mouth-feel. The difference in the score of T_0 and T_1 were supported by the findings of Gokhan *et al.* (2004) who reported that in full-fat and low-fat white pickled cheeses added with fat replacer showed that, as the fat content of cheese milk decreased, fat in total solids, total solids and cheese yield of white pickled cheese significantly decreased but the moisture and total nitrogen values significantly increased which affected the sensory qualities of cheese. Tareq *et al.* (2016) also studied the effect of Kappa Carrageenan, locust bean and Xanthan gums had on sensory score of Domiati cheese and found that higher scores on the evaluated attributes of cheese were to the moisture content enhancement, thereby resulting in a decrease in the differences noted between the sensory evaluation of full and low-fat cheese. After looking into these results, in subsequence study the product was prepared by addition of Simplese-100 as a fat replacer.

Effect of level of fat replacer on sensory attributes of low fat *quarg* cheese

The score for colour and appearance of *quarg* cheese were significantly affected by the level of Simplese-100. The highest score (8.45 ± 0.03) was awarded to *quarg* wherein Simplese-100 was added @ 0.6 per cent (Table 2). For these treatment, judges made the comments that the product had shiny and slight creamy colour with uniform appearance. With the increasing quantity of WPC in *quarg* the improvement in colour and appearance was also observed by Patange *et al.* (2018) and the colour was shining and slight creamy colour with uniform appearance. Statistically, the colour and appearance difference within S_2 , S_3 , and S_4 were at par with each other. The *quarg* prepared by addition of Simplese-100 at 0.6 per cent recorded highest score for body and texture (8.50 ± 0.06). Lowest score was noticed for skim milk *quarg* cheese (7.50 ± 0.02). Statistically the body and texture difference within S_3 were at par with S_2 and S_4 . The score for these three treatments were in narrow range and ranged from 8.35 to 8.50. It is clearly illustrated from the Table 1 that at 0.6 per cent level of addition Simplese-100 the score for spreadability of *quarg* was highly significant ($p < 0.05$) compared to the treatments. The spreadability score for S_0 , S_1 , S_2 , S_3 and S_4 were 7.46 ± 0.02 , 8.07 ± 0.03 , 8.21 ± 0.02 , 8.30 ± 0.05 , 7.90 ± 0.01 . The score within S_1 , S_2 , S_3 were at par with each other.

The good quality *quarg* has characteristic clean and mildly acidic flavour and has optimum sourness and acidic taste as good as like *chakka* and yoghurt. It was observed that treatment differences are statistically

Table 1: Effect of level of fat replacer on sensory attributes (score) of low fat *quarg* cheese

Treatment	Sensory attributes				
	Colour and appearance	Body and texture	Spreadability	Flavor	Overall acceptability
S ₀ (Control)	7.66 ^a ±0.02	7.50 ^a ±0.02	7.46 ^a ±0.02	7.50 ^a ±0.02	7.53 ^a ±0.03
S ₁ (0.2)	7.92 ^b ±0.02	8.10 ^b ±0.05	8.07 ^b ±0.03	8.05 ^b ±0.03	8.05 ^b ±0.04
S ₂ (0.4)	8.32 ^c ±0.02	8.40 ^c ±0.05	8.21 ^c ±0.02	8.30 ^c ±0.03	8.32 ^c ±0.05
S ₃ (0.6)	8.45 ^c ±0.03	8.50 ^{cd} ±0.06	8.30 ^c ±0.05	8.40 ^d ±0.03	8.39 ^c ±0.02
S ₄ (0.8)	8.40 ^c ±0.02	8.35 ^d ±0.01	7.90 ^b ±0.01	8.50 ^e ±0.05	8.28 ^b ±0.05
SEm	0.01	0.01	0.09	0.01	0.01
CD(p<0.05)	0.16	0.15	0.27	0.04	0.10

Mean± of four replication within column followed by same letter are non-significantly different at p<0.05.

Table 2: Effect of fat replacer physico-chemical properties* of low fat *quarg* cheese

Treatment	Physico-chemical properties of low fat <i>quarg</i> cheese					
	Fat (%)	Protein (%)	Lactose (%)	Ash (%)	TS (%)	pH
S ₀ (control)	0.45 ^a ± 0.02	12.00 ^a ± 0.02	3.00 ^a ± 0.15	1.01 ^a ± 0.13	16.46 ^a ± 0.02	4.47 ^d ± 0.03
S ₁ (0.2)	0.53 ^b ± 0.03	12.33 ^b ± 0.05	3.17 ^b ± 0.03	1.20 ^b ± 0.06	17.17 ^b ± 0.06	4.45 ^c ± 0.05
S ₂ (0.4)	0.45 ^a ± 0.02	12.50 ^c ± 0.05	3.25 ^b ± 0.01	1.29 ^c ± 0.04	17.54 ^c ± 0.02	4.42 ^b ± 0.01
S ₃ (0.6)	0.62 ^d ± 0.03	12.68 ^d ± 0.08	3.33 ^b ± 0.02	1.32 ^d ± 0.04	17.87 ^d ± 0.02	4.36 ^a ± 0.06
S ₄ (0.8)	0.69 ^e ± 0.05	12.74 ^e ± 0.06	3.40 ^b ± 0.05	1.37 ^e ± 0.01	18.08 ^e ± 0.02	4.34 ^a ± 0.02
SEm	0.01	0.03	0.02	0.03	0.01	0.02
CD(p<0.05)	0.03	0.10	0.07	0.10	0.03	0.02

* Mean± of four replication within column followed by same letter are non-significantly different at p<0.05.

significant at 5 per cent level of significance. The perusal of data from showed that the *quarg* prepared by addition of Simplese-100 at 0.8 per cent was recorded highest score for flavour (8.50±0.05). The flavour score for S₁, S₂, and S₃ were 8.05±0.03, 8.30±0.03 and 8.40±0.03, respectively. Slightly sourish with an expressed flavor and odor of pasteurization with a slight cooked flavor of whey proteins was noticed in protein based fat replacer products by Smirnova (2014). From overall acceptability scores, it is clear that *quarg* prepared by addition of 0.6 per cent Simplese-100 had scored significantly highest score (8.39±0.02), followed by *quarg* of S₂ (8.32±0.05) treatments. Statistically there were no significant differences between S₂ and S₃. Study results of Nurcan and Mustafa (2004) indicated that SimplexesD-100 can improve the instrumental texture properties and sensory properties of low-fat fresh *kashar* cheese.

Fuangpaiboon and Kijroongrojana (2017) studied the effects of Simplese® 100 on the sensory and physical properties of reduced-fat (4%) and low-fat (2%) coconut-milk ice cream in comparison with a control containing 8% fat. The reduced-fat ice cream with Simplese 100 added exhibited the sensory characteristics (firmness, iciness, melt-down, mouth-coating, sweetness, coconut flavour and skimmed milk powder flavour) mostly similar to the control.

Effect of level of fat replacer on physico-chemical properties of low fat *quarg* cheese

The effect of level of fat replacer on physico-chemical properties of low fat *quarg* cheese were also measured and presented in Table 2. The average fat content of low fat *quarg* was 0.45±0.02, 0.53±0.03, 0.45±0.02, 0.62±0.03 and 0.69±0.05 for S₀, S₁, S₂, S₃ and S₄, respectively. The slight but significant rise

in the fat content of *quarg* cheese may be because of presence of fat in Simplese as reported by Smirnova, (2014). The highest (12.74%) level of protein content was noticed in low fat *quarg* cheese added with 0.8 percent Simplese-100. It was observed that protein content showed gradual but significant ($p < 0.05$) increase in the level of Simplese-100. Contradictory findings were reported by Tareq *et al.* (2016) who reported that adding mixture of hydrocolloids as fat mimetic didn't significantly affect the cheese protein content. However, lactose content in *quarg* cheese S_1 , S_2 , and S_3 were at par with each other and values for these chesses were 3.17 ± 0.03 , 3.25 ± 0.01 and 3.33 ± 0.02 , respectively. The Average ash content (%) in *quarg* were 1.01 ± 0.13 (S_0), 1.20 ± 0.06 (S_1), 1.29 ± 0.04 (S_2), 1.32 ± 0.04 (S_3), and 1.37 ± 0.01 (S_4). It was observed that the ash content of low fat *quarg* cheese gradual increase with increase in level of Simplese-100. It was observed that the TS content showed gradual increased with the increase in level of Simplese-100. Average TS was ranged from 16.46 ± 0.02 to 18.08 ± 0.02 %. Comparatively higher TS content in Sudanese low fat cheese was reported by Elkhidier and Hamid (2017) and Patange *et al.* (2018) in WPC increased *quarg* cheese. The acidity of low fat *quarg* in was ranged from 1.68 ± 0.09 to 1.80 ± 0.02 (% LA) whereas pH was ranged from 4.47 ± 0.03 , to 4.34 ± 0.02 .

Effect of storage period on sensory attributes (score) of low fat *quarg* cheese

As the storage period was increased, then score of colour and appearance decreased in both the cheeses. Initially the score for colour and appearance for control sample and *quarg* with fat replacer was 7.66 ± 0.02 and 8.50 ± 0.05 , respectively (Fig. 6). However, the rate of decline was higher in P_1 than P_2 cheese. Controlled sample was best up to 9 days while fat replacer *quarg* sample was up to 12 days and after that the microbial (yeast and mould) visible growth was observed in the product. The decline in body and texture scores during storage (Fig. 6) may be attributed to increase in the free moisture content in the product and contributed to the softness of the product. The average flavour score was decreased from 8.50 to 6.25 for P_2 (Fig. 7). Smirnova (2014) recorded decreasing flavour score

of dairy product added with Simplese- 100 had the main taste defect, which is intensified during storage, is a caramelization flavor, which is associated with a higher content of whey protein. Improvement in flavour score of low-fat kashar cheese added with Simplese- 100 up to 22 days of storage was observed by Nurcan and Mustafa (2004).

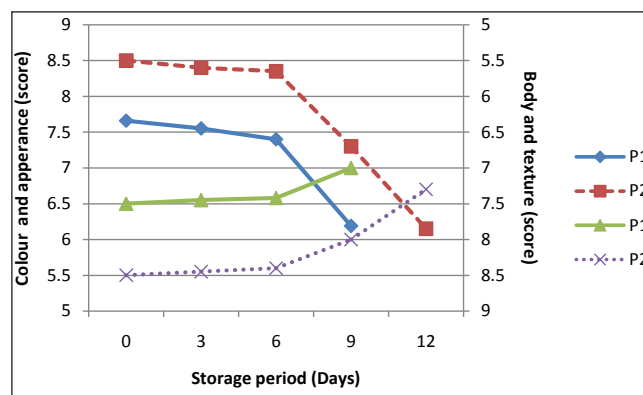


Fig. 6: Changes in color and appearance and body and texture score of *quarg* cheese during storage

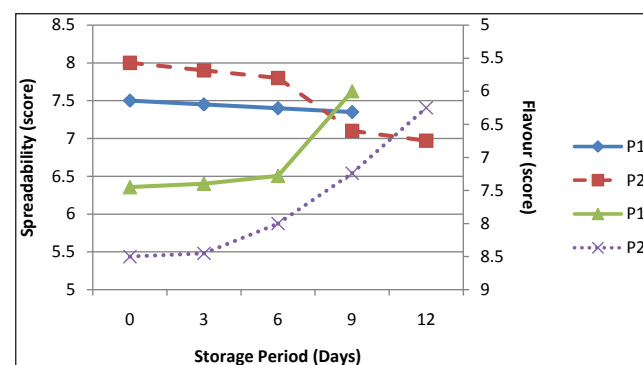


Fig. 7: Changes in spreadability and flavour score of *quarg* cheese during storage

During storage, development of stale bitter and metallic flavour was observed by Sachdeva *et al* (1993) in *quarg* cheese. Similar type of observation was also recorded by Kadiya *et al.* (2014) and who observed a prominent purification odour in *quarg* cheese due to yeast and mould growth. Spreadability score was ranged from 8.00 ± 0.05 to 6.97 ± 0.3 for same sample. The score for overall acceptability on last day of storage period were 6.55 ± 0.01 and 6.35 ± 0.01 for P_1 and P_2 samples (Fig. 7). Similar result was obtained by Yadav (2015) where author reported that the *quarg*

sample stored under refrigeration (4°C) was same in appearance up to six days, thereafter the quality decreased slightly and remained acceptable for 10 days.

Effect of storage period on physico-chemical properties of low fat *quarg* cheese

Fat content (%) of freshly prepared control sample and Simplese-100 added *quarg* cheese was found to be 0.47 ± 0.03 and 0.50 ± 0.02 . The fat content of P₂ sample was reduced to 0.32 ± 0.01 during 12th day of storage (Fig. 8). The decrease in fat content of *quarg* cheese during storage period might be attributed to the fact of lipolysis activity in the product. Similarly the protein content of P₁ and P₂ sample shown significant ($p < 0.005$) differences throughout the storage period. The protein content in P₂ sample was decreased up to 11.28 ± 0.08 on 12th day of storage (Fig. 8). Decrease in protein content in low-fat *kashar* cheese was also observed by Nurcan and Mustafa (2004). It was revealed from the Fig. 9 that there was a slightly decrease in lactose content during storage. The overall reduction in lactose content from 3.00 ± 0.05 to 2.76 ± 0.04 in P₁ while 3.25 ± 0.04 to 2.63 ± 0.05 in P₂. The average per cent ash content of control low fat *quarg* cheese was 1.00 ± 0.01 on 0 days whereas ash content in *quarg* of blended with fat replacer on same day was 1.29 ± 0.01 . The ash content of the *quarg* of blended with fat replacer increased to 1.45 ± 0.01 (Fig 9). Hence, it is clear that there was significant increase during storage period. The initial Total solid content in P₁ and P₂ was 17.18 ± 0.02 and 17.54 ± 0.02 per cent, respectively. The P₂ sample showed the significant decrease in total solid over a storage period. The average total solid per cent recorded was 17.46 ± 0.04 , 17.44 ± 0.02 , 17.40 ± 0.02 and 17.36 ± 0.04 per cent on 3rd, 6th, 9th and 12th day of storage respectively (Fig. 10). Somewhat similar trend in TS content of with and without fat replacement fresh low-fat *kashar* cheese was reported by Nurcan and Mustafa (2004). The result of Vajihel *et al.* (2012) are closely associated with the present finding where in they reported that moisture content was significant low or fat replacer added sample.

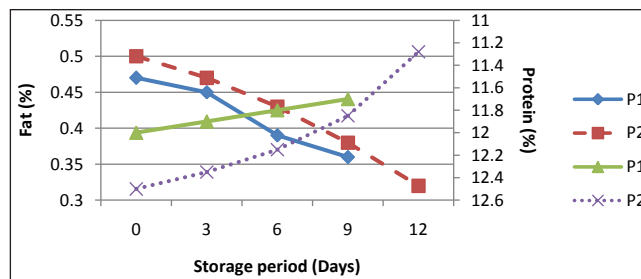


Fig. 8: Changes in fat and protein content (%) of *quarg* cheese during storage

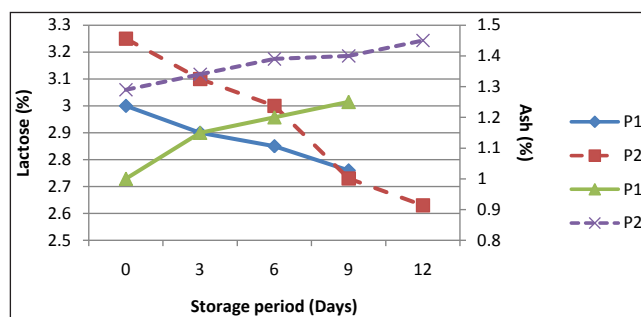


Fig. 9: Changes in lactose and ash content (%) of *quarg* cheese during storage

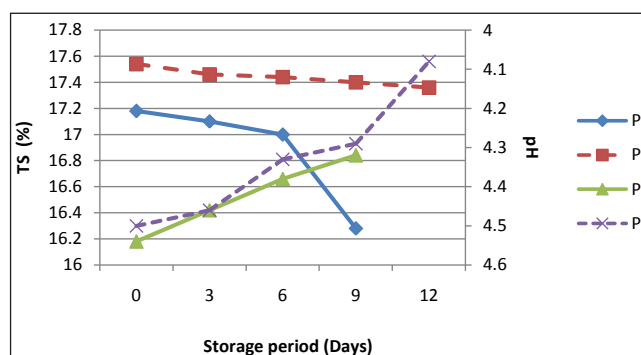


Fig. 10: Changes in TS (%) and pH of *quarg* cheese during storage

The pH of fresh *quarg* cheese sample was 4.54 ± 0.01 (P₁) and 4.50 ± 0.05 (P₂). It decreased during the storage period in all the samples but at different rate. The effect was found to be statistical significant ($p < 0.05$) on each day of evaluation. In the P₁ sample the rate of decrease was rapid as pH decreased from initial value of 4.54 ± 0.01 to 4.46 ± 0.02 , 4.38 ± 0.02 , and 4.32 ± 0.03 after 3, 6 and 9 days respectively. In case of P₂ cheese, pH was decreased from 4.46 ± 0.01 , 4.33 ± 0.02 , 4.29 ± 0.04 and 4.08 ± 0.12 after 3, 6,

9 and 12 days of storage respectively. Shekhar (2014) and Patange *et al.* (2018) also observed decrease in pH of *quarg* during storage.

Effect of storage on microbial load of low fat *quarg* cheese

The rate of growth of microorganism in control sample was significantly ($P < 0.05$) faster in P_1 than P_2 . The average of SPC count was observed to be 1.85 ± 0.01 , 2.25 ± 0.01 , and 3.60 ± 0.05 log₁₀ /g on 3rd, 6th and 9th day of storage respectively. In case of *quarg* cheese with fat replacer, the average SPC count observed was 2.16 ± 0.01 , 3.22 ± 0.01 , 3.42 ± 0.02 , 3.65 ± 0.01 log₁₀/g on 3rd, 6th, 9th and 12th day of storage respectively. In treatment P_1 the average YMC on 6th and 9th day of storage was observed to be 0.32 ± 0.01 and 0.54 ± 0.02 log₁₀/g. There was no effect of addition of fat replacer on coliform count in *quarg* cheese and it compared with control during storage.

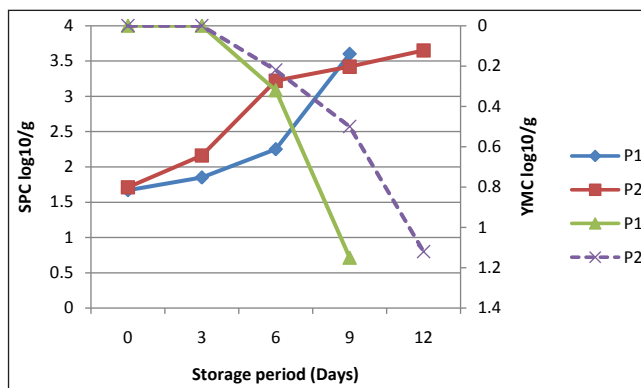


Fig. 11: Changes in SPC and YMC of *quarg* cheese during storage

CONCLUSIONS

From the study it is concluded that compared to carragennan, use of simpleese-100@ 0.6 %, as a fat replacer found to be the most suitable in terms of sensory qualities of low fat *quarg* cheese. The optimized *quarg* cheese scored 8.45 ± 0.03 , 8.50 ± 0.06 , 8.30 ± 0.05 , 8.40 ± 0.03 and 8.39 ± 0.02 for colour and appearance, body and texture, spreadability, flavour and overall acceptability, respectively. Whereas, it contained 0.62 ± 0.03 , 12.68 ± 0.08 , 3.33 ± 0.02 , 1.32 ± 0.04 and 17.87 ± 0.02 fat(%), protein(%), lactose (%), ash(%)

and TS (%), respectively. The product had 4.36 ± 0.06 pH. The experimental low-fat *quarg* cheese added with fat replacer could be stored upto 12 days without any marked deterioration.

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RESEARCH NOTE

Quality Attributes, Biochemical Profile and Calorific Status of Functional RTS Drink from a Blend of Mango and Tomato

Madhu Parmar¹, Sonia Johri^{1*}, Hradesh Rajput² and J.L. Bhat²

¹Department of Life Sciences, ITM University, Gwalior, Madhya Pradesh, India

²Department of Food Technology, ITM University, Gwalior, Madhya Pradesh, India

*Corresponding author: johrisonia@gmail.com

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ABSTRACT

Fruits are rich sources of nutrients, macro molecules, good source of energy and a reservoir of antioxidants. In the present study, an attempt has been made to prepare a RTS (Ready-to-serve) functional food. Sensory evaluation of various combinations, followed by screening of the best combination was performed. Proximate analysis such as pH, moisture content, ash content, total solid and Vitamin C content of the combination was estimated. Results of biochemical evaluation revealed that combination of mango: tomato in 50:50 followed by 60:40 proved to be the best combination. The phenolic and flavanoid content was the marker of the antioxidant status. The RTS proved to have a rich calorific value.

Keywords: Mango, RTS, tomato, fruits juice and phytochemicals

Fruits play a very important role in maintaining the nutritional status of the body. They are a reservoir of essential elements, such as water, vitamins (A, B₁, B₂, C, D and E), minerals (Ca, Mg, Zn, Fe, K etc.) and organic compounds which are very important for our body to make body function, (Okwu and Emenike, 2006; Dosumu *et al.* 2009). They are a good source of antioxidants which are the scavengers of free radicals. Because of rich perishability of fruits, they spoil quickly but can be preserved as beverages (Brett *et al.* 1996). Juices can be prepared by squeezing fresh fruits mechanically or by enzymatic extraction process. Fruit juices promote detoxification in the human body and contribute to good health (Minich and Bland, 2007). Natural sources of antioxidants from fruits are more advantageous to health than the synthetic counterparts or supplements (Liu, 2003). In the present era, consumer awareness towards the relationship between food and health has led to

an explosion of interest in “healthy foods”; which could be partly attributed to the increasing cost of healthcare, the steady increase in life expectancy, and the desire of older people for an improved quality of life (Granato *et al.* 2010). Ready-to-serve (RTS) beverages are made out of fruits, such as pineapple, orange, lime, banana, litchi, passion fruit, and other local fruits which can be used for ready-to-serve (RTS) beverages preparations.

Mango has high nutritional value and is an important source of dietary antioxidants such as carotenoids, ascorbic acid and phenolic compounds (Manthey and Perkins, 2009). The phenolic compounds include flavonoids, phenolic acids, xanthenes and

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gallotannins (Kim *et al.* 2010) (Manthey and Perkins, 2009). The ascorbic acid is an important micronutrient acting as an antioxidant in the human diet is also known as neuroprotective agent (Du *et al.* 2012).

Tomato is among the fruit of the numerous cultivated varieties of *Solanum lycopersicum*. It is a rich source of ascorbic acid (vitamin C). Tomatoes are known as a good source of phenolic compounds, pigments, like carotenoids antioxidants, and other nutrients in the human diet (Taveira *et al.* 2012).

The present study aims to prepare a ready-to-serve formulation (RTS) of combination of mango and tomato in different proportions.

MATERIALS AND METHODS

Preparation of mango pulp and tomato juice

Mango and tomato were procured from a local market of Gwalior, M.P. India. Fresh fully ripe mangoes were used for extraction of pulp. After washing the fruits were peeled cut into small pieces and then ground using laboratory grinder and the pulp was collected and heated at 80 °C for 5 minutes and filled in sterilized bottles for further preparations. For preparation of tomato juice, fresh and ripe tomatoes were washed, dipped in hot water for removing the peels followed by juice extraction using laboratory juicer. For preservation, it was heated at 80 °C for 5 min and stored in sterilized bottles for further use.

Preparation of Mango and Tomato RTS

Preparation of Mango and Tomato (ready-to-serve) RTS. After preparation of mango pulp and tomato juices RTS were prepared in different formulations. In all the different formulations sugar, citric acid, black paper and salt were added in same proportion but the amount of mango pulp and tomato juices were different (Table 1). The mixture was filtered through muslin cloth. The prepared product was filled in sterilized glass bottles, sealed and sterilized at 100 °C for 15 min, cooled and stored at room temperature for further study. The samples were screened on the basis of sensory evaluation and

were further analyzed for their physicochemical and phyto-chemical properties.

Table 1: Different combination of RTS prepared by mango pulp and tomato juice

Sample No	Mango pulp: tomato juice
S1	100 : 00
S2.	90 : 10
S3.	80 : 20
S4.	70 : 30
S5.	60 : 40
S6.	50 : 50
S7.	40 : 60
S8.	30 : 70
S9.	20 : 80
S10.	10 : 90
S11.	0 : 100

Physico-chemical Analysis of raw juice and RTS

Raw fruits and their processed products were analysed for various physico-chemical characteristics. Oven drying method described by the Association of Official Analytical Chemists (AOAC, 1990; Nielsen, 1998) was used for determination of moisture content and total solid. The Kjeldah method was used to determine the protein content (Rangana, 2005). The acidity was determined by titration using standard sodium hydroxide solution and expressed as anhydrous citric acid while pH was measured by a pH meter.

Dinitrosalicylic acid (DNSA) method was used to measure the reducing sugars as per the method of Miller (1959). Calorie content was calculated according to Recommended Dietary Allowance (RDA) as described here.

Calculation: Calorie (kcal/100 g) = (Protein % × 4) + (Fat % × 9) + (Carbohydrate % × 4)

Vitamin C was determined by the titrimetric method using dichlorophenol indophenol dye (Ranganna 2005). Total phenolic content was determined by Colorimetric method of Folin-ciocalteu reagent (Singleton *et al.* 1999) using Gallic acid as a standard.

Total flavonoid contents were measured by the aluminum chloride colorimetric method.

Sensory evaluation of ready-to-serve (RTS)

Sensory evaluation was done by 9 point hedonic scale. Panelists were selected from the teachers, student and employee of the department and were briefed before evaluating sensory quality of the samples. The panelists evaluated the color, flavor, mouth feel, and overall acceptability. The scale was arranged such that: 9 = like extremely, 8 = like very much, 7 = like moderately, 6 = like slightly, 5 = neither like nor dislike, 4 = dislike slightly, 3 =dislike moderately, 2 = dislike very much, 1 = dislike extremely (Joshi, 2006).

Statistical Analysis

Results obtained from the physicochemical, phytochemical and sensory analysis were analysed and the means were presented Means+Standard deviation (S.D).

RESULTS AND DISCUSSION

Sensory evaluation of ready-to-serve (RTS)

The mean scores of sensory evaluation are presented on Table 2. In case of color preference among the samples, Sample S6 secured the highest score (7.3)

and ranked as liked moderately while sample S7 score was 7.1. In case of flavor preference, sample S6 the most acceptable among two samples. Sample S6 secured the highest score 7.6 with moderately like ranking, followed by sample S7 (score = 7.4) From (Table 2), Overall acceptability of sample S6 (7.8) was more than S7 (6.4) thus, rendering it overall acceptability as highly acceptable. On the basis of sensory analysis it is conferred that mixed fruit juice (S6) containing 50% mango, 50% tomato juice secured the highest score followed by S7 in terms of color, flavor, sweetness and overall acceptability. This results are in confirmatory to those reported earlier (Sajeda Begum *et al.* 2018).

Physico-chemical properties of raw juice and selected RTS

The proximate analysis of the mango pulp revealed that, the moisture content to be 83%, ash content 0.55%, respectively and tomato juice contain moisture content 87.33% and total ash 0.45 %, respectively. Table 3 showed that sample S6 or S7 contained 92.4% and 95.46% water. This moisture value is less compared to the reported range of the mixed tropical fruit juice (Siti *et al.* 2017). The higher the moisture content of products, the more it is susceptible to spoilage by microbial action (Robert *et al.* 2002).

Table 2: Sensory evaluation of the different combination of RTS

Sample	Colour	Mouth feel	Flavor	Overall Acceptability
S1	6.05±0.42	5.91±0.97	5.28±1.13	5.01±1.13
S2	6.71±0.45	6.46±0.65	6.26±0.66	6.63±0.31
S3	6.11±0.77	5.95±0.43	6.08±0.92	6.1±0.72
S4	6.88±0.22	6.90±0.38	7.08±0.56	6.11±0.51
S4	6.85±0.40	6.95±0.37	7.28±0.53	6.16±0.52
S6	7.33±0.69	8.05±0.60	7.6±0.72	7.88±0.40
S7	7.01±0.42	7.06±0.36	7.4±0.48	6.43±0.61
S8	5.83±0.69	6.50±0.34	6.91±0.42	6.05±0.50
S9	5.23±0.54	5.98±0.51	6.63±0.35	5.5±0.50
S10	5.31±0.52	5.65±0.65	6.23±0.45	4.86±0.44
S11	4.31±1.16	4.13±0.43	5.48±1.11	5.50±1.36

Results are mean ± SD.

Table 3: Physico-chemical characteristics of mango pulp and tomato juice

Parameter	Mango pulp	Tomato juice	S6	S7
Crude Moisture (%)	83 ± 3.60	87.33 ± 2.08	92.40 ± 1.64	95.46 ± 0.50
Crude Ash (%)	0.55 ± 0.07	0.45 ± 0.110	0.37 ± 0.006	0.320 ± 0.010
Titrateable acidity (%)	0.3 ± 0.1	0.05 ± 0.01	0.27 ± 0.005	0.23 ± 0.01
Crude Total solid (%)	15.66 ± 1.52	12.66 ± 1.15	8.33 ± 1.53	4.78 ± 0.70
Crude Protein (%)	0.55 ± 0.9	0.116 ± 0.002	0.45 ± 0.01	0.31 ± 0.01
Crude Fat (%)	0.43 ± 0.05	—	0.30 ± 0.1	0.20 ± 0.1
Crude Carbohydrates (%)	14.21 ± 2.15	34.70 ± 8.62	13.55 ± 0.53	13.17 ± 0.85
pH	4.32 ± 0.39	4.13 ± 0.21	4.157 ± 0.232	4.280 ± 0.620
Calorie (kcal/100g)	63.3 ± 0.81	138.4 ± 0.91	59.03 ± 0.30	55.53 ± 1.30

Results are mean ± SD.

Ash content of combination S6 was 0.38 and of S7 was 0.32, respectively (Table 3). The results were more, similar to that reported by (Sajeda Begum *et al.* 2018) where 0.33% ash content was reported in orange juice. It was reported by (Harbers, 2002) that the ash contents of cherries, apple and tomatoes were 0.3, 0.5 and 0.6%, respectively. The total ash content in food can be referred to the residue of inorganic substances such as minerals in a food (Pomeranz and Meloan, 1994). The higher ash content indicates higher mineral content (Monti *et al.* 2008).

Generally, some nutrients such as protein, fat and fiber will be slightly lower in fruit juice compared to its fresh fruit because these nutrients were reduced during the processing of the fruit juice (Mercola 2014). Fortification and enrichment of any nutrient can be carried out, if needed. In our study protein content was found to be 0.55%, fat and carbohydrate content, ascorbic acid were 0.4%, 14.21%, 30.60 mg/100g respectively in mango pulp. The tomato sample was found to contain protein content 0.116%, carbohydrate 34.70%, ascorbic acid 23.6 mg /100g. pH 4.13. Further, these results are more and less similar to the values reported by earlier Bello *et al.* (2016). Moisture content and ash content of tomato juice were 87% and 0.45%, respectively. These results were in line with (Isabel Martinez-Valverde *et al.* 2002). Total solid content of tomato juice was 12.66%. Total solid and pH of the mango pulp were 15.60 %, 4.32,

respectively. The pH of the pulp was lower to the values of reported earlier (Ruhul Amin *et al.* 2018).

Tomato juice was also analyzed for its physico-chemical parameters. Protein for combination S6 was 0.45% while that of S7 was 0.31% (Table 3). The highest protein was observed in sample S6 (0.45%). Siti *et al.* (2017) reported a similar protein content in Mixed Tropical Fruit Juice. Fat content in combination S6 was 0.3 % while fat content in combination S7 was 0.2 g (Table 3). This also indicated that the combination of these fruit juices had very low fat content. The value of carbohydrate observed in combination S6 was 13.55% and S7 was 13.17 % (Table 3). The juice was also low in calories, suitable to be included in diet. This was in line with reported value by (Morton, 1987) for Mango pulp to range between 16.2-17.8%, respectively. (Ahmed and Ahmed, 2014) have reported that matured fruits were usually sweeter due to higher sugar content.

The carbohydrate content of any food product determines its energy (Nicolas *et al.* 2002). The pH value of the juice represents the degree of acidity and alkalinity of a substance. This indicated that the juice was in acidic condition and suitable to be served as ready to drink (RTD) beverages here.

Phytochemical properties of raw juice and selected RTS

Flavonoid and phenolic content of the mango pulp were 9.0 mg/100g and 116 mg/100g, respectively.

Phenolic content of tomato juice was 29.66 mg/100. The results more or less similar to those reported value earlier (Gurpreet Kaur *et al.* 2016). In our study, the Vitamin C content was 30.60 mg/100g in mango pulp. (Table 4). Vitamin C for combination (S6) was 15.33 mg/100g and combination (S7) was 13.25 mg/100g (Table 4). Higher Vitamin C content has been reported as 35.18 mg/100g earlier (Gurpreet Kaur *et al.* 2016).

The value of phenolic content in combination S6 was 82.63 mg / 100g and combination S7 was 66.78mg / 100g (Table 4). The result was more or less similar to that reported earlier (Gurpreet Kaur *et al.* 2016) where 84 to 98 mg /100g phenolic compound in mix fruits and vegetable juice were estimated, respectively.

Table 4: Phytochemical properties of selected ready-to-serve (RTS)

Parameter	Mango pulp	Tomato juice	S6	S7
Ascorbic acid (mg/100)	15.33 ± 1.15	13.25 ± 1.09	30.60 ± 1.15	23.6 ± 0.57
Flavonoid (mg /100g)	5.77 ± 0.40	4.76 ± 2.08	9.0 ± 0.20	1.2 ± 0.20
Phenolic (mg of /100g)	82.63 ± 0.33	66.78 ± 4.88	116 ± 2.08	29.66 ± 3.05

Results are mean + SD.

Generally it is known that phenolic content are highly correlated with antioxidant activity and bioavailability of polyphenols (Manach *et al.* 2005). Phenolic compounds play vital role in the quality of processed food products like flavor and taste, they have specific and important health-promoting properties (Sharma *et al.* 2012). Flavonoid observed in combination S6 was 5.77 mg / 100g and combination S7 was 4.76mg / 100g, respectively (Table 4). Leosava *et al.* (2018) reported about flavonoid content in commercial apple juice was found to range from 5.53 to 5.55 mg/l QE. Flavonoids are natural antidiabetic agents, which interfere with the production of free radicals and reduces oxidative stress.

The preparation of RTS drinks has been optimized

here. Due to its nutritional values which can act as efficient energy booster and a potent antioxidant.

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Book Review



The book “100 UNDER \$100: TOOLS FOR REDUCING POST-HARVEST LOSSES”, authored by Betsy Teutsch, technically edited by Lisa Kitinoja and published by The Post-harvest Education Founder, La Pine, Oregon, 2019 © Betsy Teutsch, has been divided into 8 sections viz., Farming, Packing house, Food Processing and Value Addition, Storage, Transportation, Markets: Local and Global, ICT and Financial, and Legal aspects. The book has highlighted the important pre- and post-harvest tools, essential to minimize the post-harvest losses in general food, and horticultural perishables, in particular.

Presently, the world produces enough food to every one, but more than 800 million people suffer from hunger and malnutrition, since more than 30% of the produced food is never consumed. In developing nations, the food loss is even more than 40% during harvesting, handling/ drying and pre-consumption storage and transportation, which is morally unacceptable, and yet practically addressable. By 2050, the global population is expected to reach 9.8 billion, but our food supplies will be under far greater stress, and the food demand will be 60% higher than it is today. Wasted food means wasted water, energy, land and

money besides harming the planet. Worldwide, food loss and waste impede food security and fuels climate change. This loss and waste accounts for 8% of greenhouse gas emissions, and is a huge waste of water and land. If food waste were a country, it would be the third-largest polluter in the world, releasing 3.3 gigatonnes of CO₂ to the environment. Overall reduction of food waste is challenging but achievable with systematic approach by fine-tuning the entire food chain through innovative research, and by stimulating collaboration amongst the scientists, policy makers and the business people. Particularly in developing and under-developed nations, reduction of unacceptable levels of food wastage is indispensable by investing more in smallholder friendly and gender sensitive post-harvest technologies in order to increase food supply to improve the quality of life in the communities. So the reason behind writing this book is very much valid.

A lot of scattered information pertaining to post-harvest technology was available, but systematically linked information on various aspects was missing. The contents accommodated in this book by author are very well linked in each other from production to consumption, which fulfill the objectives of book writing on such a need based subject. The author earned a vast experience in her life on post-harvest technology, travelled widely and worked with well known post-harvest technologists. She has attempted to share her earned experience in the compiled manuscript, making this book unique. The author has beautifully depicted the efforts made by marginal farmers and small scale industrialists to produce and handle the produce step-by-step.

The present book is very useful for farmers, food producers (including farm women), undergraduate students and trainees who are willing to start up his/her own business on pre and post-harvest management of food items.

The contents have been presented in cohesive manner with clarity and precise words, and supported by beautiful coloured photographs. Some of the aspects particularly marketing, use of electronic sources under ICT and financial, and legal matters have made the book as a unique source of information. The inclusion of sanitation issues are very well addressed in this manuscript which are the inseparable part of healthy food production.

I hope that this book will be of immense use in dissemination of technologies/informations in a comprehensive manner, and will serve the purpose of all stakeholders involved at the various levels of food industry.

Prof. R.M. Sharma

Principal Scientist

Fruits & Horticultural Technology

ICAR-Indian Agricultural Research Institute, New Delhi, India

E-mail: rmsharma345@gmail.com