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Editor-in-Chief's Message

I am delighted to be the editor-in-chief of the volume 2 of the Journal of the Dry Zone Agriculture, which is published by Faculty of Agriculture, University of Jaffna. Faculty of Agriculture was established in 1990 in Kilinochchi and underwent several displacement and finally settled in its own location of Kilinochchi in 2014. Education without innovative research and development is meaningless for the community as such faculty decided to publish a Journal named as Journal of Dry Zone Agriculture (JDZA). The objective of JDZA is to publish peer reviewed high-quality research papers and the Volume 1 was released in 2012 while faculty was functioning at Jaffna. Due the shifting and resettlement, the faculty was unable to continue to release the journal annually as planned. At present the faculty was well settled at Ariviyal Nagar, Kilinochchi with adequate infrastructure and human resources, the faculty decided to recommence the publication of the JDZA. The volumes 2, 3 and 4 will be released in 2018 and I am proud to be function as the Editor in chief for Volumes 2 and 3.

JDZA is a multidisciplinary, peer-reviewed journal that publishes original research in dry zone agriculture and other associated fields The JDZA provides platform to publish the research work of the students, scholars and academicians. Our primary role as editors is to encourage the best work to be submitted and to manage a fair process of review. All submissions will be subject to the journal's well-established system of peer review, which is rigorous and expeditious.

Volume 2 of JDZA is compiled with five papers which were subjected to the journal's double-blind review process. I would like to hear your valuable suggestions on improving our journal further. I sincerely extend my thanks to contributors, editorial board members and reviewers and looking forward for your continuous support. I offer my profound thanks to the co-editors and the members who contributed towards the quality publication of the JDZA.

Dr.(Mrs.) S. Sivachandiran
Editor-in-Chief

Co-Editors' Message

We are delighted to celebrate the launch of Volume 2 of the Journal of Dry Zone Agriculture (JDZA), at the Fourth International Conference on Dry Zone Agriculture (ICDA 2018) on 1st and 2nd of November, 2018 published by the Faculty of Agriculture, University of Jaffna, Sri Lanka. The JDZA is a multidisciplinary, peer-reviewed journal that publishes original research articles especially on dry zone agriculture and on the associated fields such as Agronomy, Animal production, Plant protection, Soil chemistry, Food science, Agriculture economics and extension, Agriculture engineering, Crop modelling and statistics, Plant physiology, Weed science, Forestry and Agroforestry, etc.

In Sri Lanka, Dry zone has widespread landscape diversity occupying two third of its land. Its richness is uncovered. The role of different stakeholders in dry zone landscape is essential for the conservation of dry land ecosystem in a sustainable manner. However, active and mutual involvement of multi stakeholders are still lacking towards good governance and maintenance of dry zone landscape. In Sri Lanka, each stakeholders on different landscapes are functioning independently. It is the biggest threat and challenge to formulate the policies and legislation for good governance of dry zone tropical landscape. Consequently, the country faces huge environmental problems at alarming rate. Therefore, there is an urgent need to address these multi issues in dry zone landscapes for conservation of natural resources. It includes different disciplines such as biodiversity of flora and fauna, water bodies, soils, vegetation, agriculture crops and other land uses.

We extend our sincere thanks to the authors for their submission of research articles and the reviewers for their timely response and for their critical assessments on the manuscripts and valuable comments. The recommencement of the release of JDZA is a joint venture of several key personnel within and outside the faculty. The credit goes to the Editorial Committee of JDZA, the Dean of the faculty of Agriculture, Staff of the Faculty of Agriculture, Organizing committees of ICDA 2015 and 2016 for their untiring support in releasing the journal in time as planned on the 1st day of ICDA 2018. We are confident that the future editorial committees also follow the tradition of releasing JDZA in the first day of ICDA in forthcoming years. We wish to thank the Vice Chancellor of University of Jaffna and the University administration for granting the financial assistance from the University Research Grant to release the journal. Quality is the important aspects of any final product which will be assessed by the consumers. This credit goes to Harikannan Printers and we wish to record of sincere thanks to them for their editing and attractive printing in time.

We are also happy to launch the website for the Faculty Journal at www.jdza.jfn.ac.lk and this website is linked with the faculty homepage: <http://www.agri.jfn.ac.lk/>. The editorial committee urges the research community to publish original research articles related to dry zone agriculture and other related fields in the future volumes of JDZA as hard copy or as soft copy to the email of jdzajournal@gmail.com.

We look forward to welcoming your submissions to the forthcoming volumes.

Jeyavanan, K. & Venugoban, K.
Co-Editors

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Fermentation of High Gravity Glucose by Free and Immobilized *saccharomyces cerevisiae* S1 by Cell Recycling

Balakumar, S.* and Arasaratnam, V.

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Abstract: The potential economic benefits in ethanol production could be realized by conducting fermentation at 40°C and above. In this study, thermotolerant yeast isolated and developed in our laboratory was used in the cell recycle operations to find the possibility to improve the high gravity fermentation. Fermentation of glucose (300 gL⁻¹)–PYN medium by free and agar immobilized *Saccharomyces cerevisiae* S1 cells took 36 h to utilize glucose completely and produced 100 and 143 gL⁻¹ ethanol, respectively. When the free and agar immobilized *Saccharmyces cerevisiae* S1 cells were subjected to repeated batch operation in glucose (300 gL⁻¹)–PYN medium, it was possible to continue the process with 2 and 37 cycles respectively. When the free cells were used in the repeated batch fermentation with glucose (300 gL⁻¹)–2PYN medium supplemented with 26.8 gL⁻¹ soy flour, it was possible to continue the process for 7 repeated cycles. However, stuck fermentation was observed with advancing cycles with increasing residual sugar with free cells. Thus, cell recycle batch process was possible with additional nutrients in the medium while with immobilized cells such additional nutrients were not necessary.

Keywords: : Stuck fermentation, high gravity fermentation, *Saccharomyces cerevisiae*, cell recycling, free cells

1. Introduction

Traditional fermentation system use freely suspended yeast cells in batch bioreactors (Verbelen *et al.*, 2006). Recycling of yeast cells (Matano *et al.*, 2013) and continuous alcohol fermentation (Deshphande, 2002; Vasconcelos *et. al.*, 2004) are useful to reduce the fermentation time and to increase the ethanol productivity. Immobilization of microbial cells showed certain technical

and economical advantages over free cell fermentation (Verbelen *et al.*, 2006). Several theories have been put forward to explain the enhanced fermentation capacity of immobilized microorganisms (Ivanova *et al.*, 1996; Lin and Tanaka, 2006).

The potential economic benefits in ethanol production could be realized by conducting fermentations at 40°C and above. This has

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generated considerable interest in the selection of thermotolerant yeast strains (D'Amore, *et al.*, 1988). It has been estimated that the cooling cost during fermentation was reduced by 10 % when the fermentation was carried out at 37°C instead of 32 °C (Anderson *et al.*, 1985). Further the ethanol recovery cost can also reduced considerably when operating at high temperature. Certain difficulties such as high ambient temperatures, coupled with an exothermic fermentation reaction lead to inhibition of the fermentation ability of yeast (Anderson *et al.*, 1985). This further emphasized the need for thermotolerant strains. In the present study the efficiency of a locally isolated thermotolerant yeast strain (*Saccharomyces cerevisiae* S1, Balakumar *et al.*, 2001) for the fermentation of high concentration of glucose at 40 °C was investigated, by comparing the recycling of the cells in the free and immobilized forms to improve the high gravity fermentation at 40°C to reduce the distillation cost.

2. Materials and Methods

Materials: Soybean from local market was powdered and dried at 80 °C. Culture media & Technical agar were purchased from Oxoid Limited, UK, and all the other chemicals were purchased from Sigma-Aldrich, USA.

Saccharomyces cerevisiae S1: *Saccharomyces cerevisiae* S1 is a locally improved thermo tolerant strain (Balakumar *et al.*, 2001), maintained in glucose (50 gL⁻¹) -peptone, yeast extract and nutrient (PYN)-agar slants in refrigerator at 4 °C.

Analytical methods: Glucose (Miller, 1959), ethanol (Varley *et al.*, 1980) and viable cell

count (Sami *et al.*, 1994) were determined by standard methods.

Peptone, Yeast extract and Nutrient (PYN) medium: The PYN medium contained (gL⁻¹) peptone, 3.5, yeast extract, 3.0, MgSO₄·7H₂O, 1.0, KH₂PO₄, 2.0; and (NH₄)₂SO₄, 1.0 at pH 5.0 (Balakumar and Arasaratnam, 2009). Based on the needs, different amounts of glucose were added to the medium and represented as glucose (amount in gL⁻¹) – PYN medium (Balakumar and Arasaratnam, 2009). When double the amount of the nutrients of PYN medium was used, the medium is presented as glucose (amount in gL⁻¹) – 2PYN medium'.

Inoculum: The yeast cells were grown in sterile (100 in gL⁻¹) – PYN medium, for 18h at 40 °C. Total cell count of the inoculum was taken before sedimentation and allowed to sediment at 4 °C for 6h. Sedimented cells were used as free cell inoculum or taken for the preparation of immobilized cells.

Immobilization of yeast: Sterile aqueous agar (40 gL⁻¹, 75 mL) was cooled to 50 °C and the sedimented cells, re-suspended in sterile normal saline (25 mL) were vigorously mixed by shaking at 50 °C in a water bath for 10 min. The liquid agar / yeast cell mixture was trickled via a syringe (0.7 mm) into sterile paraffin oil (300 mL) at 4 °C. After 2 h, the beads were washed with distilled water.

Comparison of the performance of free and agar immobilized *Saccharomyces cerevisiae* S1 in glucose (300 gL⁻¹)-PYN medium . Agar immobilized *Saccharomyces cerevisiae* S1 cells were inoculated to 100 mL glucose

(300 gL⁻¹)–PYN medium and incubated at 40 °C with shaking (100 rpm). Residual sugar and ethanol were monitored. Free sediment cells from 100 mL of 18 h old inoculum was treated instead of immobilized cells.

Cell Recycle Batch Fermentation

Repeated batch operation of free and agar immobilized *Saccharomyces cerevisiae* S1 in glucose (300 gL⁻¹) – PYN medium

The first batch operation was started as said above. At the end of the first batch, after complete utilization of glucose, the agar immobilized *Saccharomyces cerevisiae* S1 was recovered from the spent medium by allowing the beads to settle and decanting the spent medium. Then 100 mL of glucose (300 gL⁻¹)–PYN medium was added to the settled beads and incubated. This process was repeated till the immobilized cells ceased to produce ethanol. In addition to the cells and ethanol the cells leaked into the medium was monitored.

Sedimented free cells from 100 mL of 18 h old inoculum was treated as of immobilized cells. Upon reaching the complete utilization of glucose, cells were allowed to sediment and the supernatant was decanted. Fresh medium (100 mL) was added to the sedimented cells and this cycle was repeated till the free cells ceased ethanol-producing ability.

Recycling of free *Saccharomyces cerevisiae* S1 in (300gL⁻¹)–2PYN medium supplemented with soy flour

The glucose (300 gL⁻¹)–2PYN medium supplemented with soy flour (26.8 gL⁻¹) was prepared. After sterilization, the medium

was allowed to settle and the clear medium was used (to prevent the accumulation of soy flour particles in the subsequent cycles). The medium was inoculated with 10 % (v/v) *Saccharomyces cerevisiae* S1 inoculum and, aerated for 12 h and then incubated at 40 °C with shaking (100 rpm). Residual sugar, ethanol and biomass were measured with time.

4. Results and Discussions

4.1 Performance of free and agar immobilized *Saccharomyces cerevisiae* S1 in glucose (300 gL⁻¹)–PYN medium

Sediment cells from 100mL inoculum or agar immobilized yeast was used as inoculum for the fermentation of glucose (300 gL⁻¹)–PYN medium at 40 °C. Ethanol produced was 100 and 143 gL⁻¹ with free and agar immobilized yeast, respectively (Table 1) at 36 h. Decreased production of ethanol by free cells could be attributed to increased nutritional demands (Alvers *et al.*, 2009). In one of the previous study, fermentation of glucose (300 gL⁻¹)–PYN required the supplementation of the medium with double the concentration of the nutrients of PYN or soy flour (26.8 gL⁻¹) to produce 140 gL⁻¹ ethanol at 36 h (Balakumar and Arasaratnam, 2014). Residual sugar in the spent medium where agar immobilized yeast cells was used, complete utilization of glucose as observed (Table 1). Ethanol production rate was 2.8 and 3.9 gL⁻¹h⁻¹, respectively with suspended free cells and agar-immobilized yeast, respectively. Ethanol production rate was increased by 39 % by immobilizing the cells. This study seems to confirm the early findings (Holcberg and Margalith,

Table 1: Ethanol production in glucose (300 gL⁻¹) – PYN medium by free and immobilized yeast cells at pH 5.0 and 40 °C. The ethanol and residual glucose were measured at 36 h.

Parameter	Ethanol (gL ⁻¹)	
	Free cells	Immobilized cells
Ethanol (gL ⁻¹)	100	143
Residual sugar (gL ⁻¹)	80	0.0
Glucose utilization (%)	73.3	100
Ethanol production rate (gL ⁻¹ h ⁻¹)	2.8	3.9
Ethanol production efficiency (%)	65.2	65.2

Efficiency was calculated as the amount of alcohol produced / Theoretical amount of ethanol that could be produced from the added amount of glucose x 100; Theoretically 1g glucose gives 0.511g alcohol.

1981; Verbelen *et al.*, 2006; Lin and Tanaka, 2006). The membrane properties would have been altered by the immobilization process, affecting the permeability of the cells towards higher penetration of the substrate as well as faster removal of the end products from the cells (Verbelen *et al.*, 2006). Further glucose in the solution diffuses through the gel matrix, reaches the cells and disappears when fermented into ethanol and CO₂. This creates a gradient of glucose, which permits its flow from the solution into beads.

As long as the rate of diffusion is lower than the rate of fermentation, the local concentration of the sugar would be non inhibitory to the reaction. Further, continuous ethanol removal from the fermentation site is important to establish a non-inhibitory level of ethanol (Holcberg and Margalith, 1981). The free and immobilized cells were used for the cell recycle batch fermentation.

4.2 Cell Recycle Batch Fermentation

Repeated batch operation of free and agar

immobilized *Saccharomyces cerevisiae* S1 in glucose (300 gL⁻¹) – PYN medium

The free and immobilized cells recovered from the first batch operation were used for subsequent repeated cycle (Figure 1). At 36 h fermentation in the first batch the free count was taken in the medium. Initial free cell number in the free suspended medium was 2 x 10⁸ cells mL⁻¹ and at 36 h fermentation it was 2.8 x 10⁸ cells mL⁻¹. In the first and 2nd batch operations with the immobilized cells, the cell leakage was insignificant. When the free cells were used the process was active only for two repeated batches. Whereas when the immobilized cells were used they performed in a prolific manner up to 30 cycles with constant production of ethanol (139 ± 3.0 gL⁻¹). Carragenan entrapped yeast cells completely fermented 300 gL⁻¹ glucose, while 138 gL⁻¹ residual glucose was obtained with free cells (Barros *et al.*, 1987). The difference in fermentation behavior may be due to the higher cell viability in the immobilized cell system than their free cell

counterpart (Holcberg and Margalith, 1981). No cell leakage was observed till 10th batch and from batch 11 to 16th batches, very few free cells appeared in the medium. At 17th

batch the cell leaking was prominent and 1 x 10⁵ cells appeared in the medium and steady increase in the free cells was observed with the subsequent recycles.

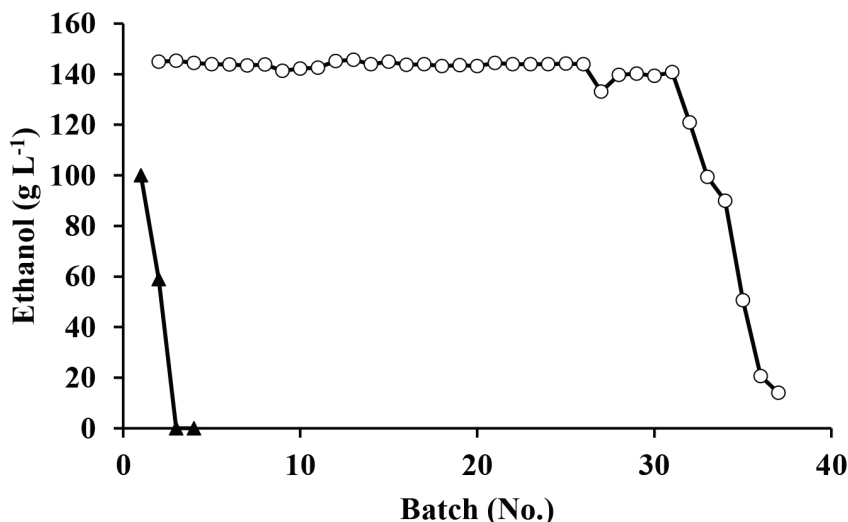


Figure 1: Repeated cell recycle ethanol production in glucose (300 g L⁻¹) PYN medium by (○) free and (▲) agar immobilized *Sacharomyces cerevisiae* S1 at 40 °C.

After 20th batches of operation clear micro-colonies were found on the immobilized yeast-agar beads. From 30th cycle the immobilized cells showed decreasing performance up to 36th batch of the operation and afterwards the immobilized cells were inactive. Cell recycling of *Saccharomyces cerevisiae* CAIM13 was possible for four repeated cycle (Al-Judaibi, 2011)

The use of agar immobilized yeast cells was economical in terms of less nutritional demands and the free cell operation requires fresh inoculum for every third batch operation. For example to carry out 30 repeated cycles free cell inoculum should be prepared for 15 cycles. Hence, the use of immobilized cells

for the fermentation of glucose (300 g L⁻¹)–PYN medium is more economical than free cell fermentation. In cell recycle operations yeast cells are centrifuged and re-inoculated into other fermentors (Ingledew, 1993). In this experiment, cells were recovered by keeping the fermented medium at 4 °C for 3 h. The unproductive down time could be reduced by the use of centrifuges. In this experiment by approximately 10 fold increase in cell density (the inoculum was obtained by sedimentation of 100 mL inoculum), increased ethanol production (from 82 to 100 g L⁻¹), with reduction in fermentation time (from 48 to 36 h) were observed. Therefore, by increasing the inoculum potential, ethanol production efficiency could be increased.

However, in the second cell recycle batch ethanol produced was 71.2 gL^{-1} and the third batch of cell recycle was inactive. The decrease in the performance of free cells could be attributed to the cell death with the repeated cycles. The viabilities in the second and third cycles were 58.7 and 7.1 %, respectively. Further with the removal of the spent medium, the young cells which would not have sedimented also been removed. This is not possible with the alginate-entrapped cells. Bakers' yeast (Fermipan) on simultaneous saccharification and fermentation was reported with intermittent addition of nutrient medium where liquefied rice flour starch was used (Arasaratnam *et al.*, 2012). In the report only liquefied rice flour starch was used and the ethanol yield was 91.9 % in the first cycle and it decreased to 22.3 % in the 9th cycle (Arasaratnam *et al.*, 2012). However, intermittent addition of fresh nutrient medium has helped to continue the recycling of free cells for 9 cycles (Arasaratnam *et al.*, 2012). There was no well-defined method available for the determination of viability of the agar immobilized yeast cells. However, the appearance and growth of micro-colonies within the agar beads from 20th batch operation reveals that the cells are still active. Alginate trapped *Saccharomyces cerevisiae* was able to perform in the repeated continuous process for 6 cycles from 95 to 85 gL^{-1} (Ivanova *et al.*, 2011).

Recycling of free *Saccharomyces cerevisiae* S1 in glucose (300 gL^{-1})–2PYN medium supplemented with soy flour

It has been observed that in the previous experiment, the nutrients of the glucose

(300 gL^{-1})–PYN medium were insufficient to continue the recycle process with free cells. Hence, the free cell recycling was carried out with the medium having double the concentration of PYN medium supplemented with soy flour (26.8 gL^{-1}), where the nitrogen content of the soy flour was equal to the nitrogen content of PYN medium. The first batch fermentation took 36h to produce 139.09 gL^{-1} ethanol with complete glucose utilization (Figure 2). The biomass has increased from 0.75 to 8.0 gL^{-1} at the end of the first batch operation. When the sediment from the first batch was mixed with fresh medium, the residual glucose reached 5.0 gL^{-1} at 20 h and 142 gL^{-1} ethanol was produced. In five batches of subsequent cycles 118, 98, 90, 79 and 51 gL^{-1} ethanol was produced with 50.0, 88.0, 111.0, 134.0 and 190 gL^{-1} residual glucose. Stuck fermentation was observed with advancing cycles with increasing residual sugar. No fermentation was observed after 7th batch of cell recycle operation. In the first batch 10.67 fold increase in the biomass was observed and in the second batch the increase was from 6.6 to 9.3 gL^{-1} . Even though the increase in biomass in the second batch was less than the first batch, the alcohol produced was higher in the second cycle (142 gL^{-1}). This could be due to the higher inoculum size (6.6 gL^{-1}) obtained at the second batch. With the advancing cycles, the increase in the biomass and the ethanol produced were reduced simultaneously. Figure 3 shows the sugar utilization, residual sugar, ethanol production rate and ethanol production efficiency with cell recycle operation. Sugar utilization was 97 % for the first two cycles. It has gradually

decreased to 36.72 % at the 7th batch. Ethanol production rate was 3.86 and 7.1 gL⁻¹h⁻¹ for 1st and 2nd cycles respectively and dropped to 1.6 at the 7th cycle. Ethanol production efficiency (% of theoretical maximum) was

96.5 and 97 % for the first two cycles and decreased to 33.26 % at the 7th cycle. The best performance of the cells was observed in the 2nd cell recycle batch of fermentation.

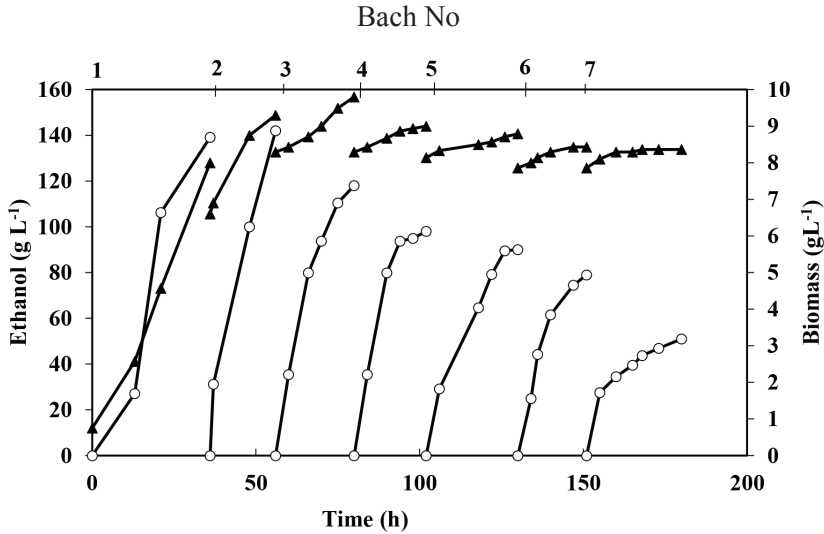


Figure 2: The time course on (O) ethanol and (▲) biomass production of the repeated batch cell recycle operation with *Saccharomyces cerevisiae* S1 at 40°C in glucose (300 gL⁻¹) – PYN medium supplemented with soy flour (26.8 gL⁻¹).

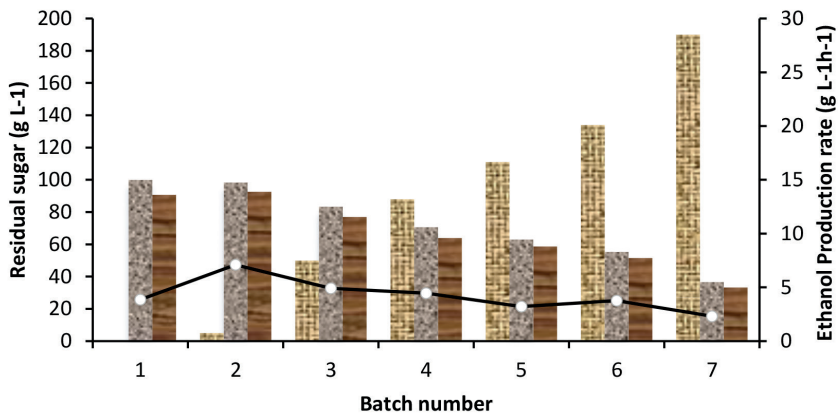


Figure 3: Cell recycle operation in glucose (300gL⁻¹)-2PYN medium supplemented with soy flour (26.8 gL⁻¹) at 40°C. (▨) Residual sugar; (O) ethanol production rate; (▤) glucose utilization and (■) ethanol production efficiency were obtained at the end of each batch operation.

4. Conclusions

The strain is capable of producing alcohol at 40°C, and thereby eliminates the problem of cooling the fermenter, particularly in large-scale fermentations. *Saccharomyces cerevisiae* S1 was able to perform better in the immobilized form (37 cycles) than in the free form (2 cycles). The free cells required additional nutrients for cell recycling process.

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Development of Bilimbi (*Averrhoa bilimbi* L.) Powder: Physico-chemical, Microbial and Organoleptic Qualities

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Abstract: Underutilized plant species are defined by their unexploited economic potential, making them an appropriate focus for commercialization. Fruits of these plants are an important supplement to human diet as they provide the essential minerals, vitamins and fibre required for maintaining health. A research was conducted to develop bilimbi fruit powder with good nutritional qualities and to assess the shelf life. Unripe mature bilimbi fruits were collected, washed and cut into 12 mm thick slices. The slices were dipped into potassium metabisulphite solution of 0.05 % (w/v) for 5 minutes. These slices were placed in a single layer on trays, dried in sun for 18 hours and in dehumidifier at 40 °C, 50 °C, 60 °C and 70 °C for 16, 14, 12 and 10 hours, respectively and ground into powder. The developed powders were assessed for physico-chemical, microbiological, sensory qualities and shelf life. Physico-chemical analyses revealed that among the tested treatments the powder developed from the unripe bilimbi dried at 50 °C had 3.4 % of moisture content, 1.4 % of fibre, 10.9 % of tritrate acidity (as % oxalic acid), 20.8 mg/100 g of ascorbic acid and 11.3 % of total sugar content. Microbiological analysis indicated that there was no total plate count observed in the tested treatments. Sensory attributes showed that powder developed from the unripe bilimbi dried at 50 °C was most preferred based on the organoleptic characteristics. Among the treatments, the bilimbi powder obtained from the unripe bilimbi dried at 50 °C for 14 hours had the highest shelf life which could be preserved minimum of six months without any significant changes in the quality characteristics.

Keywords: Bilimbi powder, dehydration, organoleptic quality, physico-chemical characteristics.

1. Introduction

The perishable fruits and vegetables are available as seasonal surpluses during certain parts of the year in different regions

and are wasted in large quantities due to absence of facilities and know-how for proper handling, distribution, marketing and

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storage. Furthermore, massive amounts of the perishable fruits and vegetables produced during a particular season results in a glut in the market and become scarce during other off-seasons. Quality of fruits in pre and postharvest influences the consumer acceptance. The changes that occur in various physical and chemical characters determine the quality and in turn the economic returns to the producers and processors (Agrawal and Mangaraj, 2005). Fruits and vegetables needed simple technologies for processing, preservation and transport to various places of need, have suffered postharvest losses, estimated to nearly 40–60 % (Ikram *et al.*, 2009).

Food preservation has an important role in the conservation and better utilization of fruits in order to avoid the glut and utilize the surplus during the off-seasons. It is necessary to employ modern methods to extend storage life for better distribution and also processing techniques to preserve them for utilization in the off-seasons in both large scale and small scale. Preservation processes include: heating to kill or denature organisms (e.g. boiling), oxidation (e.g. use of sulphur dioxide), toxic inhibition (e.g. smoking, use of carbon dioxide, vinegar, alcohol etc), dehydration (e.g. drying), osmotic inhibition (e.g. use of syrups) and low temperature inactivation (e.g. freezing). One of the oldest methods of food preservation is by drying, which reduces water activity sufficient to delay or prevent bacterial growth.

Historically, food was dried in the sun. Nowadays, we can sun dry or dry in an oven

or a dehydrator that is especially designed for home drying (Loesecke, 1999). Most fruits such as berries, cherries (pitted), seedless grapes, melons, prunes and plums require no pretreatment before drying. Light-coloured fruits especially apples, apricots, peaches, nectarines and pears, tend to darken during drying and storage. This process, called oxidation, drops the fruit of flavour, colour and vitamins A and C. To preserve the colour of these fruit, the most effective pretreatment is “sulfuring” (Srilakshmi, 2001).

Averrhoa bilimbi L., commonly known as bilimbi, belongs to the family of the Oxalidaceae and it is widely cultivated in the tropics. Bilimbi fruits are very sour and used in the production of vinegar, wine, pickles and in the preparation of Hindu dishes. The mature fruits can be eaten as raw or processed into jams and jellies. The dried powder is one of the value added product of unripe bilimbi fruit used in curries, dishes and powdered drinks etc. Medicinal uses are attributed to bilimbi, which include mixtures against cough, mumps, pimples and scurvy. The fruit juice has high levels of oxalic acid (Wong and Wong, 1995) and it is a good source of vitamin C. Most of the raw fruits are wasted due to their sour taste and shorter shelf life due to rotting and decay.

As bilimbi is a highly under-utilized fruit, it can be preserved by making it as a processed food to use it during off-seasons. Realizing the importance of fruit, as a cheap, highly nutritious and because of perishable nature and seasonally available it was decided to make a preserved product for human

consumption throughout the year. Therefore, this study was carried out keeping in view the nutritional importance of bilimbi, to utilize the fruit by preserving as dried powder and to evaluate the quality characteristics of developed bilimbi powders.

2. Materials and Methods

2.1 Material Collection and Sample Preparation

Unripe mature bilimbi fruits were obtained from the Regional Agricultural Research Station, Batticaloa. They were washed and cut into 12 mm thick slices. Potassium metabisulphite solution of 0.05 % (W/V) was prepared and the slices were dipped into the solution for 5 minutes. These slices were placed in a single layer on trays. The following dehydration methods and temperatures were used to dry the bilimbi slices: sun drying for 18 hours and in dehumidifier (ANDEN Model 1830) at 40 °C, 50°C, 60 °C and 70 °C for 16, 14, 12 and 10 hours, respectively. The temperature and duration for dehydration were identified by preliminary experiments carried out in the laboratory. The dried slices were ground into powder using a grinder for 3 minutes and the resultant powder was sieved into a particle size of 100 µm. The powders were stored in airtight plastic containers at ambient temperature of 30±1°C and Relative humidity of 80–85 % (R.H) for further analysis.

The treatments are listed as follows:

T₁ - Bilimbi powder obtained from unripe bilimbi dried in sun for 18 hours

T₂ - Bilimbi powder obtained from unripe bilimbi dried at 40 °C for 16 hours

T₃ - Bilimbi powder obtained from unripe bilimbi dried at 50 °C for 14 hours

T₄ - Bilimbi powder obtained from unripe bilimbi dried at 60 °C for 12 hours

T₅ - Bilimbi powder obtained from unripe bilimbi dried at 70 °C for 10 hours

2.2 Physico-chemical Analysis of Developed Bilimbi Powders

Physico-chemical qualities of dried bilimbi powder such as moisture, fibre, tritrateable acidity (as % oxalic acid), ascorbic acid and total sugar content were analyzed using recommended standard AOAC methods (2002). Three replicates were used from each treatment during the physico-chemical assessment.

2.3 Microbial Test

The microbial assessment was carried out by estimating total plate count according to the method described by Arachchi (2003) in raw mango powder. The total plate count was determined by observing the colonies formed especially bacteria.

2.4 Organoleptic Assessment

Organoleptic assessment was carried out by a panel consisting of 30 untrained members to determine consumer preference using a nine-point hedonic scale in 1 is denoted as “dislike extremely” and 9 denoted as “like extremely”. The organoleptic parameters such as taste, colour, flavour, absence of off-flavour and overall acceptability of the dried bilimbi powder were judged by the panelists.

2.5 Shelf Life Evaluation

The developed powders were stored in airtight plastic containers at ambient temperature of $30\pm1^{\circ}\text{C}$ and 80–85% RH for shelf life evaluation. The samples were nutritionally tested for fiber, tritratable acidity, ascorbic acid, total sugar and moisture at 2 weeks interval and observations were made on samples to evaluate the shelf life of the development of powders.

2.6 Statistical Analysis

Data obtained in physico-chemical analysis were subjected to Analysis of Variance (ANOVA) and mean separation was done with Duncan's Multiple Range Test (DMRT). Descriptive statistics was done on sensory attributes and the means were compared using the Tukey's test ($p<0.05$).

3. Results and Discussions

The main objective of drying the fresh material is to prolong its shelf life by inhibiting the growth and development of

pathogenic and spoilage micro-organisms. The unripe mature bilimbi slices were dried at different dehydration temperatures until the slices became dry but not sticky and leathery. Srilakshmi (2001) reported that the optimal temperature and time for drying of fruits is 50°C and 6–24 hours, respectively. Changes in the nutritional quality of foods may occur as a result of drying.

3.1 Physico-chemical Parameters of Dried Bilimbi Powders

The results of physico-chemical analysis with respects to moisture, fibre and tritratable acidity of developed bilimbi powder samples are as presented in Table 1. There were significant differences ($p<0.05$) in moisture content of dried bilimbi powders obtained by different dehydration temperatures. The products had moisture contents ranging from 3.4 to 4.1 %, in which the dried powder obtained from unripe bilimbi dried at 50°C had the lowest moisture content (Table 1).

Table 1: Moisture, Fibre and Tritratable acidity of Dried Bilimbi Powders

Treatment Code	Moisture (%)	Fibre (%)	Tritratable acidity (% oxalic acid)
T ₁	3.8 ± 0.05^c	1.2 ± 0.006^b	10.1 ± 0.02^b
T ₂	3.9 ± 0.05^b	1.3 ± 0.005^a	10.6 ± 0.01^a
T ₃	3.4 ± 0.03^e	1.4 ± 0.010^a	10.9 ± 0.03^a
T ₄	3.7 ± 0.01^d	1.1 ± 0.003^c	9.8 ± 0.05^c
T ₅	4.1 ± 0.03^a	0.9 ± 0.005^d	8.6 ± 0.01^d

Values are means of triplicates $\pm$ standard error. Means in the each column followed by the same letters are not significantly different at $p<0.05$. (T₁ - Bilimbi powder obtained from unripe bilimbi dried in the sun for 18 hours; T₂ - Bilimbi powder obtained from unripe bilimbi dried at 40°C for 16 hours; T₃ - Bilimbi powder obtained from unripe bilimbi dried at 50°C for 14 hours; T₄ - Bilimbi powder obtained from unripe bilimbi dried at 60°C for 12 hours, T₅ - Bilimbi powder obtained from unripe bilimbi dried at 70°C for 10 hours).

The moisture content for the dried and powdered products should be less than of 3.5 % and therefore exceeding the limit will directly impact on shelf life of the product (Mallawa, 2001). If the moisture contents of the products are within the acceptable limits and then, this can be considered as safe in terms of shelf life.

Fibre is a measure of the quantity of indigestible cellulose, pentosans, lignins and other components of this type present in foods. Aurand *et al.* (1987) stated that these components have little food value but provide the bulk necessary for proper peristaltic action in the intestinal tract. Among the treatments bilimbi powder obtained from the unripe bilimbi dried at 70 °C shows lowest fibre content than the dried powder obtained from the unripe bilimbi dried at 40 °C, 50 °C, 60 °C and in the sun (Table 1). This is due to the higher extent of thermal degradation of fibres at high temperature than that of low temperature (Brennan, 1994). Statistical analysis conducted on the data indicated that there were significant difference ($p < 0.05$) among treatments except the treatments of bilimbi powder obtained at 40 °C and 50 °C (T_2 and T_3).

The titratable acidity content of fresh unripe bilimbi was 16.4 % which was higher than the dried powder of bilimbi. This is due to the evaporative losses of acid during drying. The titratable acidity in bilimbi dried powders ranged between 8.6 and 10.9 % as oxalic acid. Manjunath *et al.* (1991) reported that the percentage of total acidity in commercial samples of powder was in the

range of 8.7–11.1 % as citric acid. The powder obtained from the unripe bilimbi dried at 70 °C showed low acid content than the other treatments (Table 1). This is supported by Josyin (1980) high temperature treatment cause high losses of acids in fruits.

The ascorbic acid content of bilimbi powder is shown in Figure 1. This is to compare the effects of dehydration temperatures on the ascorbic acid content of bilimbi powder.

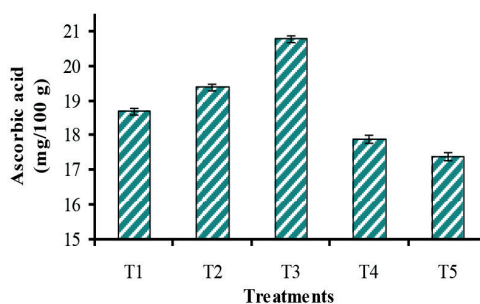


Figure 1: Ascorbic acid Content of Bilim– bi Powders

Values are means of triplicates. Vertical bars indicate the standard errors. (T_1 - Bilimbi powder obtained from unripe bilimbi dried in the sun for 18 hours; T_2 - Bilimbi powder obtained from unripe bilimbi dried at 40 °C for 16 hours; T_3 - Bilimbi powder obtained from unripe bilimbi dried at 50 °C for 14 hours; T_4 - Bilimbi powder obtained from unripe bilimbi dried at 60 °C for 12 hours, T_5 - Bilimbi powder obtained from unripe bilimbi dried at 70 °C for 10 hours).

The bilimbi powder obtained from the unripe bilimbi dried at 70 °C showed minimum amount of ascorbic acid content than the powder obtained from the unripe bilimbi dried at 40 °C, 50 °C, 60 °C and in the sun.

This is due to the more oxidative losses of ascorbic acid by thermal degradation. This was supported by Watada *et al.* (1991). According to the statistical analysis there were significant differences ($p < 0.05$) between the treatments for the ascorbic acid content of bilimbi powder.

The total sugar content of the dried bilimbi powders showed differences between the various treatments at 5 % significance level except the treatments that the bilimbi powder obtained from the unripe bilimbi dried at 60 °C and in the sun. Among the treatments the bilimbi powder obtained from the unripe bilimbi dried at 70 °C contains low total sugar content than the bilimbi powder obtained from the unripe bilimbi dried at 40 °C, 50 °C, 60 °C and in the sun (Figure 2). High losses of sugars due to browning reactions and caramalization process at high temperature than that of low temperatures. This is supported by Peacock *et al.* (1990). Saltveit (1984) also reported that high caramalization of sugars taken place at high temperature than that of low temperature.

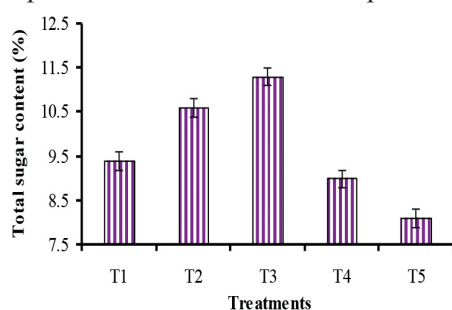


Figure 2: Total Sugar Content of Bilimbi Powders

Values are means of triplicates. Vertical bars indicate the standard errors. (T₁ - Bilimbi

powder obtained from unripe bilimbi dried in the sun for 18 hours; T₂ - Bilimbi powder obtained from unripe bilimbi dried at 40 °C for 16 hours; T₃ - Bilimbi powder obtained from unripe bilimbi dried at 50 °C for 14 hours; T₄ - Bilimbi powder obtained from unripe bilimbi dried at 60 °C for 12 hours, T₅ - Bilimbi powder obtained from unripe bilimbi dried at 70 °C for 10 hours).

3.2 Microbiological Test

The microbiological examination, in terms of total plate count revealed that there were no any microbes observed after 6 months of storage in the developed bilimbi powders because low pH value of 1.3–1.5 inhibited bacterial growth. This was supported by Manjunath *et al.* (1991) in tamarind powder. Therefore, these samples are suitable for consumption. Micro-organisms play significant role in the determination of shelf life of food products. They are usually responsible for spoilage of many food items. A high total plate count could indicate the presence mixed population of micro-organisms, which may consist of spoilage types. Limits of microbial counts (Aerobic plate count/g at 30 °C should be <10) have been recommended in most foods to keep them safe for consumption (Ogunjobi and Ogunwolu, 2010).

3.3 Organoleptic Assessment

The mean values of organoleptic qualities for treatments are shown in Table 2. Results revealed that there were significant differences between the treatments for bilimbi dried powder in terms of taste, colour, flavour, absence of off-flavour and overall acceptability at 5 % level of significant.

Table 2: Mean Values of Organoleptic Qualities of Dried Bilimbi Powders

Treatment Code	Taste	Colour	Flavour	Absence of off-flavour	Overall acceptability
T ₁	7.50±0.25 ^a	6.95±0.34 ^{bc}	6.95±0.29 ^c	7.70±0.26 ^a	6.55±0.21 ^d
T ₂	7.35±0.13 ^b	7.25±0.14 ^b	6.60±0.15 ^{cd}	7.60±0.21 ^a	7.50±0.12 ^b
T ₃	7.50±0.11 ^a	7.75±0.20 ^a	7.25±0.18 ^b	7.45±0.11 ^a	7.70±0.11 ^a
T ₄	7.20±0.21 ^c	6.95±0.19 ^c	8.40±0.27 ^a	7.70±0.26 ^a	7.45±0.15 ^b
T ₅	6.20±0.09 ^d	6.60±0.15 ^d	6.30±0.13 ^d	6.40±0.28 ^b	6.35±0.20 ^c

Values are means of 30 replicates $\pm$ standard error. Means in the each column followed by the same letters are not significantly different at $p < 0.05$. Nine point hedonic scale: 1- dislike extremely, 9- like extremely.

Taste is the primary factor, which determines the acceptability of any product, which has the highest impact as far as market success of product, is concerned. In case of taste, the experimental results designated that, all the products were significantly differed ($p < 0.05$), and the bilimbi powder obtained from the unripe bilimbi dried at 50 °C occupied the first rank, followed by dried powder obtained from the unripe bilimbi dried in sun. One of the most important sensory quality attribute of a food is colour. As the results of sensory analysis shown in Table 2 the scores ranged from 6.60 to 7.75; and the products significantly differed at 5 % significance level. The bilimbi powder obtained from the unripe bilimbi dried at 50 °C ranked as first, where as the bilimbi powder obtained from the unripe bilimbi dried at 70 °C ranked as last.

Flavor has a great impact on the consumption of food products. According to Tukey's test, there were significant differences between treatments at 5 % significance level. The treatment that the dried powder obtained from the unripe bilimbi dried at 60 °C had the highest mean value and the bilimbi powder obtained from the unripe bilimbi

dried at 70 °C had the least mean value. In the case of absence of off-flavor, there was no significance difference ($p < 0.05$) among treatments except the bilimbi powder obtained from the unripe bilimbi dried at 70 °C. Overall acceptability assessment showed that the bilimbi powder obtained from the unripe bilimbi dried at 50 °C had the most superior quality among the tested samples while the bilimbi powder obtained from the unripe bilimbi dried at 40 °C was rated as sub-superior.

The bilimbi powder obtained from the unripe bilimbi dried at 50 °C for 14 hours had the highest shelf life based on the physico-chemical, microbial and organoleptic quality characteristics compared to other treatments which could be preserved for a minimum period of 6 months at ambient conditions of $30 \pm 1^\circ\text{C}$ and 80–85 % R.H without any significant changes in quality characteristics.

4. Conclusions

This research was designed to utilize the bilimbi which is still largely regarded as an under-utilized fruit and thereby increasing the shelf life of bilimbi through the process of development of dried powder. The findings

of the study showed that the bilimbi powder obtained from the unripe bilimbi dried at 50 °C for 14 hours was found to be most suitable temperature and treatment based on the physico-chemical, microbial and organoleptical point of view. Based on the shelf life evaluation, bilimbi powder obtained from the unripe bilimbi dried at 50 °C for 14 hours could be kept at ambient conditions of 30±1°C and 80–85 % R.H for a minimum period of 6 months without any significant changes in quality attributes.

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***In-vitro* Assessment of Antifungal Activity of *Aloe vera* Leaf Powder Extracts Against Banana Pseudostem Rot Fungi, *Marasmiellus* spp.**

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Abstract: Being an important medicinal plant, *Aloe vera* is important in industrial perspective as well as traditional usage. The antifungal activity of *A. vera* leaf powder extracts were assessed against banana pseudostem rot fungi, *Marasmiellus* spp. Leaf powder with acetone and ethanol extracts of 20, 200, 400, 1000 and 2000 µL were administered to assess the inhibition of colony growth of *Marasmiellus* spp. The experiment was conducted in a CSompletely Randomized Design. In *A. vera* acetone extract, first day after inoculation, inhibition percentage of the *Marasmiellus* spp was higher (74.53 %) in 2000 µL and the lowest percentage (23.53 %) of inhibition was obtained in 20 µL of *A. vera* acetone extract. The similar highest and lowest percentage of inhibition were observed in second, third and fourth day after inoculation. In *A. vera* ethanol extract, complete (100 %) inhibition was observed in 1000 µL and 2000 µL extracts. The lowest inhibition (2.26 %) was recorded in 20 µL of *A. vera* ethanol extract. Among the 1000 µl and 2000 µL leaf extracts, ethanol extract had the highest inhibition percentage compared to *A. vera* acetone extract. These findings are useful to prepare the extracts of *A. vera* leaf powder for the management of *Marasmiellus* spp.

Keywords: : *Aloe vera*, Antifungal activity, Banana pseudostem rot, Colony inhibition, *Marasmiellus* spp

1. Introduction

Aloe vera has the inherent ability to induce toxic effects on mycelial growth and proliferation of selected fungi by producing aromatic substances, most of which are phenols or their oxygen substituted derivatives.

These substances serve as plant's natural defense mechanisms against predation by microorganisms (Prashar *et al.*, 2011). The antimicrobial effects of *A. vera* have been attributed to the plant's natural anthraquinones such as aloe emodin, aloetic acid, aloin,

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anthracine, anthranol, barbaloin, chrysophanic acid, ethereal oil, ester of cinnamonic acid, isobarbaloin, and resistannol. In relatively small concentrations together with the gel fraction, these anthraquinones provide analgesic, antibacterial, antifungal, and antiviral activity, in high concentrations they can be toxic (George *et al.*, 2009). Antifungal activity of *A. vera* was reported against *Aspergillus flavus* and *Aspergillus niger*. Arunkumar and Muthuselvam (2009) reported that maximum antifungal activity is observed in acetone extracts when compared to aqueous and ethanolic extracts. Among the two fungal organisms maximum growth suppression was observed in *A. flavus* than *A. niger*.

Bajwa and Shafique (2007) investigated that, the highest inhibitory effect of fungal (Genus *Alternaria*) biomass is achieved with aqueous extract of the tested plant species than n-hexane extracts that may be attributed to the presence of main active constituent of *A. vera* plant extract, the aloine, an anthraquinoneheteroside. The n-hexane extract exhibited least inhibitory activity. This was associated with the presence of nutritional compounds present in the n-hexane extract that stimulated fungal growth and masked the inhibitory effect. Antifungal activity of *A. vera* against *A. flavus* was investigated with six different solvents such as acetone, ethanol, water, methanol, chloroform and ethyl ether have been used for the extraction from *A. vera* fresh leaves. Acetone extract of *A. vera* is used as an effective antifungal agent to inhibit the growth of *A. flavus*

compared to other solvents (Babaei *et al.*, 2013). In agriculture, the banana crop loss due to *Marasmiellus* spp. has become major concern. Increased usage of different chemicals based products to control these pathogens has resulted in problems like residual effect of chemicals in agri-based products, increased resistance for chemicals in target pathogens and environmental pollution. To rectify this detrimental effect *Aloe vera* leaf powder extracts are used to control some of the plant pathogens as an eco-friendly manner.

2. Materials and Methods

2.1 Initial Preparation of Plant

Freshly collected *A. vera* leaves were surface sterilized with 70 % ethanol. Later, they were chopped into small pieces without removing gel and were allowed to sun drying for 3 days. After drying, leaf parts were powdered using an electric grinder.

2.2 Separation of Extracts

A. vera leaf powder of 30 g was mixed with 100 mL of ethanol and acetone separately and kept in room temperature for 72 h. Each mixture was filtered through Whatman No .1 filter paper.

2.3 Fungus Species

Marasmiellus spp. fungus was isolated from diseased banana pseudostem in Thirunelvely. Fungus was cultured in vitro on Potato Dextrose Agar (PDA).

2.4 Evaluation of Antifungal Activity of *A. vera* Extracts by Diffusion Plate Method

$$C.G.I. = \frac{\text{Colony diameter in control group} - \text{Colony diameter in the treatment}}{\text{Colony diameter in control group}} \times 100$$

Where, C.G.I (%)= Colony Growth Inhibition Percentage

To evaluate the antifungal activity of ethanol and acetone extracts of *A. vera*, diffusion plate method (Babaei *et al.*, 2013) was used. Different quantities (0, 20,100, 200,1000 and 2000 µL) of each extract (ethanol and acetone) were added separately into 20 mL PDA culture plates before solidifying of the medium and mixed well. After solidification of plates, by using cork borer of (0.5 mm diameter), *Marasmiellus* spp. was transferred to the center of the plate of each concentration and incubated at 28 °C. Three replicates were maintained. The growth of fungi (diameter in cm) was measured from one day after inoculation up to four days (Thiruchchelvan *et al.*, 2012). The fungal (colony) growth

inhibition (CGI) was measured following Kawai *et al.*, (1998) formula as indicated below and the data were statistically analyzed using SAS 9.0 statistical software package following LSD mean separation procedure at 95% probability level.

3. Results and Discussions

Significant reduction of *Marasmiellus* sp. fungal growth was obtained in *A. vera*-acetone extract in comparison to control. In the experiment, 2000 µL of *A. vera* – acetone extract had the most positive impact on inhibition of *Marasmiellus* sp. among the treatments (Table 1; Plate 1).

Table 1. Colony growth inhibition percentage with different levels of *A. vera*- acetone extract up to 4 days after inoculation.

<i>A.vera</i> -acetone extract	Days After Inoculation			
	1	2	3	4
20µL	23.53 ^e	26.61 ^e	23.01 ^e	7.43 ^e
100µL	27.47 ^d	37.41 ^d	34.09 ^d	11.88 ^d
200µL	37.25 ^c	48.92 ^c	42.05 ^c	18.13 ^c
1000µL	55 ^b	65.46 ^b	51.42 ^b	49.58 ^b
2000µL	74.53 ^a	74.81 ^a	71.59 ^a	59.79 ^a

All the values are means of three replicates; Mean values having same alphabet in a column indicate the values are not significantly different according to the LSD at 95 % confidence interval.

For all concentrations of *A. vera* acetone extract, the highest colony growth inhibition was obtained one day after inoculation. Inhibition of colony growth was gradually increased while increasing the concentration of extract. All treatments were significantly different among each other.

One day after inoculation, the highest colony growth inhibition (74.53 %) was obtained in 2000 µL of *A. vera* – acetone extract whereas

the, lowest colony growth inhibition (23.53 %) was recorded in 20 µL *A. vera* – acetone extract. Similar pattern of change was observed two, three and four days after the inoculation. Considering the ability of rapid growth rate of the fungus *Marasmiellus* spp. in banana plants grown in Jaffna peninsula, inhibition of fungal growth can greatly help to reduce the disease incidence of banana. Findings of this study would help to provide the usefulness and effectiveness of extract of

A. vera to reduce the fungal infections. Some specific compounds are isolated from the *A. vera* using particular solvents, due to their different solubility of various compounds. Different solvents have the specified fungal activity (Babaei *et al.*, 2013) thus, another experiment was set with *A. vera* ethanol extract.

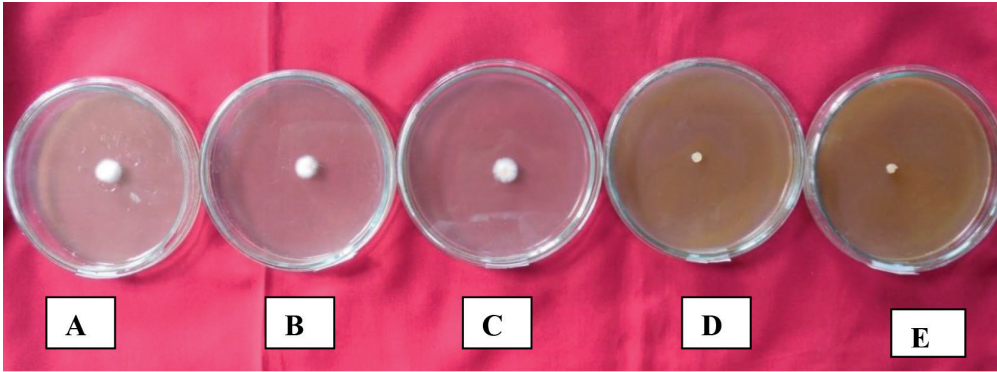


Plate 1 : Colony growth of *Marasmiellus* sp. in different quantities of *A. vera*- acetone extract; A– *A.vera* acetone leaf powder extract 20 μ L; B –*A.vera* acetone leaf powder extract 100 μ L; C–*A.vera* acetone leaf powder extract 200 μ L; D – *A.vera* acetone leaf powder extract 1000 μ L; E– *A.vera* acetone leaf powder extract 2000 μ L.

Table 2. Colony growth inhibition percentage with different levels of *A. vera* –ethanol extract up to 3 days after inoculation.

<i>A. vera</i> ethanol extract	Days After Inoculation		
	1	2	3
20 μ L	2.26 ^d	8.42 ^d	7.48 ^d
100 μ L	17.29 ^c	20.15 ^c	13.67 ^c
200 μ L	30.075 ^b	31.5 ^b	20.28 ^b
1000 μ L	100.00 ^a	100.00 ^a	100.00 ^a
2000 μ L	100.00 ^a	100.00 ^a	100.00 ^a

All the values are the means of three replicates; Figures having same alphabet in a column indicate the values are not significantly different according to the LSD at 95 % confidence interval.

Marasmiellus sp. growth was suppressed in ethanol extract than control. Ethanol extracts of *A. vera* at 1000 μ L and 2000 μ L showed complete inhibition of *Marasmiellus* spp. among the treatments. For all concentrations of *A. vera* ethanol extract, the highest colony growth inhibition was obtained one day after inoculation. Inhibition of colony growth was gradually increased while increasing the concentration of leaf powder extracts. One day after inoculation, the highest colony growth inhibition (100 %) was obtained in 1000 μ L and 2000 μ L of *A. vera* - ethanol extracts and the lowest colony growth inhibition (2.26 %) was obtained in 20 μ L *A. vera*-ethanol extract. Similar pattern of

change was observed two, three and four days after inoculation (Table 2).

While comparing the results of the two experiments, *A. vera* ethanol extract had the highest colony inhibition than acetone extract. In Jaffna peninsula, banana is the one of main orchard crop grown by the farmers and fungal diseases are severe and affect the yield. Therefore, management of *Marasmiellus* sp. is essential to obtain high yield of banana. Results obtained from this study will help to formulate better management strategy to inhibit the fungal growth and finally to reduce the disease incidence of banana aiming at improving the economic status of the people in Jaffna peninsula.

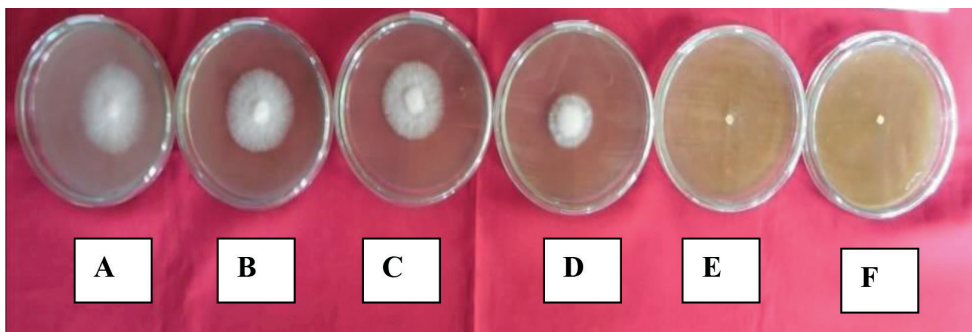


Plate 2: Colony of *Marasmiellus* sp. in *A. vera*- ethanol extract; A–Control; B–*A. vera* ethanol leaf powder extract 20 μ L; C–*A. vera* ethanol leaf powder extract 100 μ L; D–*A. vera* ethanol leaf powder extract 200 μ L; E–*A. vera* ethanol leaf powder extract 1000 μ L; F–*A. vera* ethanol leaf powder extract 2000 μ L

4. Conclusions

The results obtained from in vitro assessment of antifungal activity of *A. vera* extracts showed varying levels of antifungal properties against banana pseudostem rot fungus, *Marasmiellus* spp. The complete colony growth inhibition was achieved in 1000

μ L of ethanol- *A. vera* leaf powder extract. These antifungal properties of *A. vera* extracts indicate that *A. vera* is a valuable natural resource of antifungal agent in addition to other properties. Further studies should be carried out to analyze phytochemical compounds present in various *A. vera* leaf

powder extracts and to detect minimum inhibitory concentration of extracts against major pathogenic fungi.

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Impact of Alternative Wetting and Drying on the Soil Surface Organic Matter in a Lowland Paddy Field

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Abstract: Pondered water in lowland paddy cultivation has a role on soil biomass accumulation, decomposition, and nutrient availability. However, alternative wetting and drying (AWD) is a common phenomenon under minor irrigation systems due to scarcity of water. The AWD process may have an effect on the soil organic matter (SOM). Therefore, the effect of several cycles of varying length of AWD conditions on SOM content at the soil surface was investigated by using Lysimeter for a period of 98 days. The experiment design was complete randomized design with 4 treatments; i.e. 4 days dry spell (T_1), 12 days dry spell (T_2), 20 days dry spell (T_3) and 4 days dry spell with paddy (T_4). Soil samples from the surface were collected at 14 days interval and the SOM contents were measured. Results show significant differences among the treatment combinations. The accumulation of SOM after AWD water management practices is higher for T_1 followed by T_2 , T_3 and T_4 . The surface SOM content has reduced by 19 %, 53 %, 86 % and 49 % of the initial SOM content for T_1 , T_2 , T_3 and T_4 , respectively. Shorter dry spells enhance the organic matter accumulation compared to longer dry spells by creating anaerobic condition. On the other hand, organic matter degradation is higher in longer dry spells due to aerobic condition. This finding may help to take decisions on correct water management practices to optimize organic matter dynamics in lowland paddy fields.

Keywords: AWD, Decomposition, Low land paddy, SOM, Water management

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1. Introduction

Ponded water in lowland paddy cultivation plays a crucial role in soil biomass accumulation, decomposition, nutrient availability, weed control, crop growth and ultimately the yield. However, water availability under minor irrigation systems are not reliable to have continuous ponded water in the fields due to high rainfall variability and less water storage capacity of the tanks. Alternative wetting and drying is therefore a common phenomenon under minor irrigation systems. An extension of a dry spell will cause irregular and extreme water stresses for soil organisms and plants. Accumulation of organic matter (OM) in soils is controlled by environmental and pedogenic processes. Soil organic matter (SOM) content is a function of climate, parent material, time, organisms and topography (Jenny, 1941). It was shown that SOM content varies with fertilizer application and age of the crop in lowland paddy cultivation (Sellathurai *et al.*, 2015). The rate and products of OM was different in flooded and non-flooded soils (Alexander, 1961). The alternate wetting and drying (AWD) process, which creates anaerobic and aerobic conditions, may have an effect on the SOM content and its bio-availability. Therefore, a study was conducted to identify the effect of several cycles of AWD at varying lengths on surface SOM content in lowland paddy field.

2. Materials and Methods

Plastic containers, each with 54 cm length, 36 cm width and 30 cm depth were used as Lysimeter to simulate the field condition in the laboratory at Department of Agricultural

Engineering, Faculty of Agriculture, University of Peradeniya, Sri Lanka. Soil was collected from lowland paddy fields of the Bayawa minor irrigation system located in Kurunegala district. The soil was air dried, sieved (2 mm) and analyzed for texture, OM content (%), ammonium-N, nitrate-N and saturated hydraulic conductivity. A 3 cm depth from bottom of the Lysimeter was filled with aggregates to facilitate the drainage. Then, the Lysimeter was filled with sieved soil up to 15 cm. A piezometer was installed in order to monitor the water level in the Lysimeter. The Lysimeter were allowed to settle by adding water from the bottom. Then the experiment was conducted with the following treatment combinations for 98 days from 2nd September to 8th December, 2014.

- T₁ - 4 days dry spell without paddy
- T₂ - 12 days dry spell without paddy
- T₃ - 20 days dry spell without paddy
- T₄ - 4 days dry spell with paddy

The periods for dry spells were selected based on the probability analysis of rainfall for the period from 1981 to 2010 in this study area. The probabilities for 4, 12 and 20 days dry spells were 90, 70 and 50 %, respectively. Each treatment received an artificial rainfall (6 mm) to break the dry spell; which was the most frequent rainfall event in the study area during the 2013/2014 *Maha* season. A 10 cm depth of irrigation was provided at 0, 15, 30, 75 days and urea fertilizer was also applied on the same days at the rate of 125 kg/ha (Department of Agriculture Sri Lanka, 2006) to simulate the field condition. The experimental design adopted was factorial

complete randomized design with three replicates.

SAS statistical software at 95% probability level.

Soil samples at the surface were collected at 14 days interval. The SOM content was estimated by modified Walkley and Black method (1934). The temperature data for the study period was obtained from nearby meteorological station in Wariyapola. Statistical analyses were performed using the

3. Results and Discussions

The Lysimeter were subjected to several cycles of varying length of alternate wetting and drying condition. Figures 1 -3 show the management practices; irrigation and fertilizer application and climatic condition for T_1 , T_2 , T_3 and T_4 , respectively.

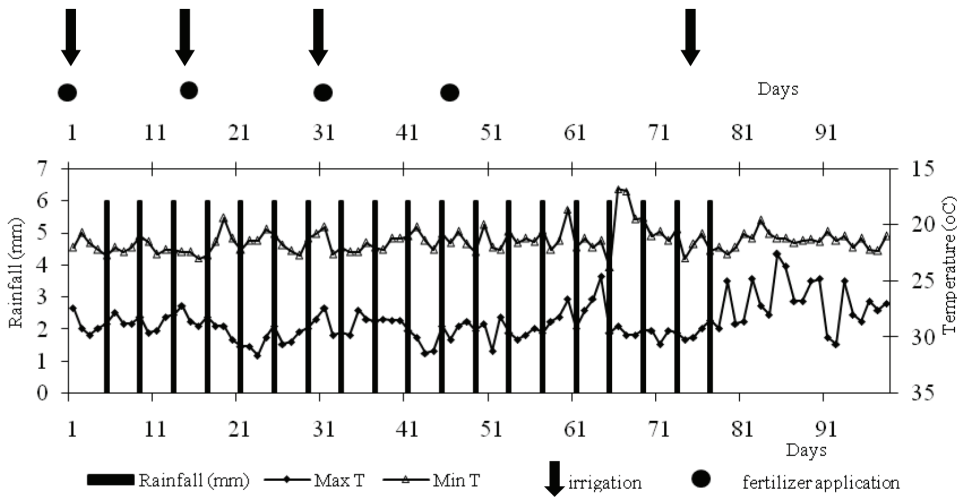


Figure 1: The climatic conditions and management practices for T_1

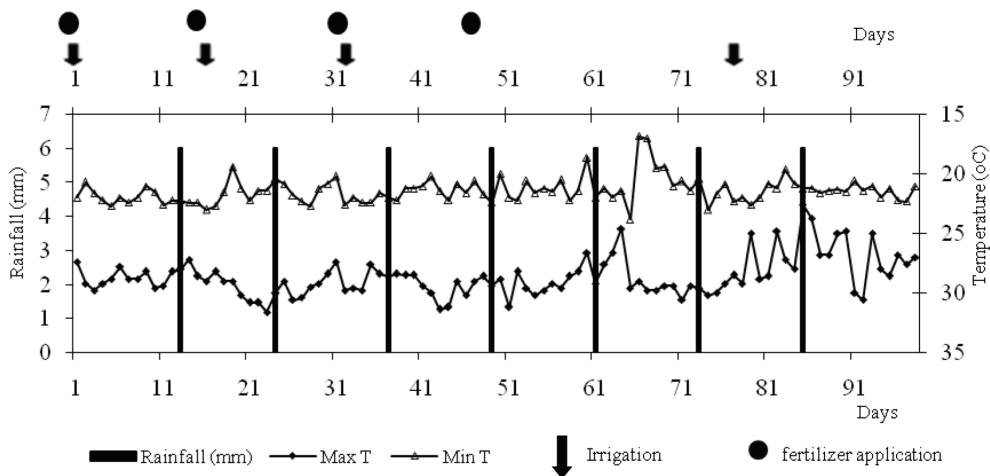


Figure 2: The climatic conditions and management practices for T_2

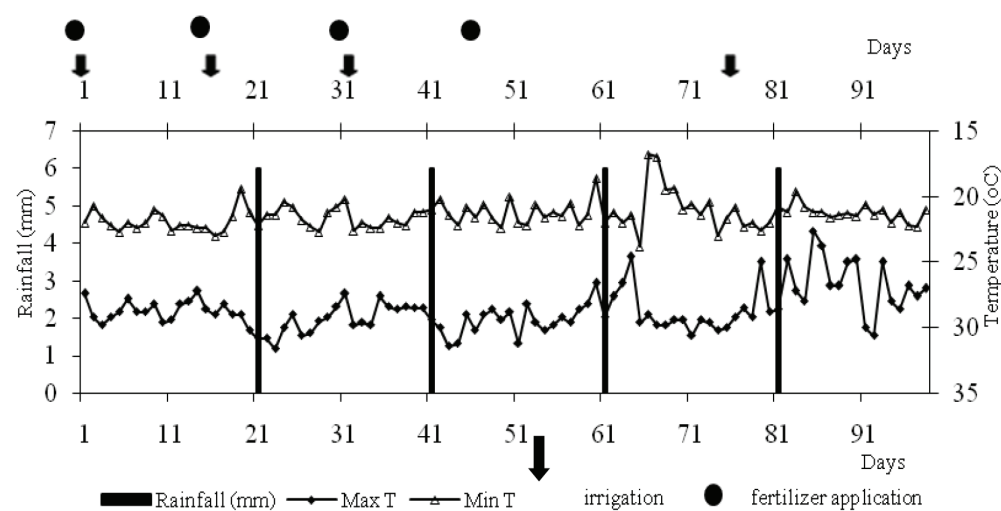


Figure 3: The climatic conditions and management practices for T₃

Table 1: The variation of surface SOM content with time (days)

T	Days							
	0	15	29	43	57	71	85	91
T ₁	0.80	1.02	0.55	0.56	0.90	1.02	1.02	1.29
	±0.13 ^{bc}	±0.31 ^{ab}	±0.16 ^c	±0.36 ^c	±0.26 ^{bc}	±0.48 ^{ab}	±0.3 ^{ab}	±0.00 ^{ab}
T ₂	0.64	0.92	0.81	1.01	0.49	0.84	1.19	0.64
	±0.47 ^{bc}	±0.46 ^{abc}	±0.13 ^{bc}	±0.61 ^{abc}	±0.30 ^c	±0.04 ^{abc}	±0.11 ^{ab}	±0.00 ^c
T ₃	0.88	0.93	0.41	0.61	0.67	0.85	1.20	0.49
	±0.81 ^{bc}	±0.04 ^{bc}	±0.44 ^c	±0.56 ^{bc}	±0.24 ^{bc}	±0.40 ^{bc}	±0.32 ^b	±0.00 ^c
T ₄	1.07	1.20	0.70	0.71	0.63	0.51	0.70	0.48
	±0.09 ^{ab}	±0.52 ^a	±0.31 ^{bcd}	±0.13 ^{bcd}	±0.44 ^{bcd}	±0.11 ^{cd}	±0.14 ^{bcd}	±0.00 ^d

T: treatments; the means with same letters are not significantly differ at $\alpha=0.05$ the mean comparison is in row wise.

Table 1 show how SOM temporally varied for each treatment. There are significant differences observed within the treatment with time. The results also show a significant difference among the treatment combination ($p<0.0001$). The AWD creates anaerobic and aerobic conditions that will effect on SOM accumulation and decomposition,

respectively resulting significant differences among treatments.

The cumulative surface SOM content for various treatments is shown in Figure 4. According to Brich (1958) the carbon mineralization rate generally increases for a few days following rewetting of a dry

soil. All treatments show a reduced surface SOM content after application of irrigation water. The T_1 has higher SOM than T_4 , but two values were not significantly differed

($P=0.07$). The SOM content increases in the latter part after the cessation of irrigation in T_1 , but it decreases for T_4 (Table 1 and Figure 4).

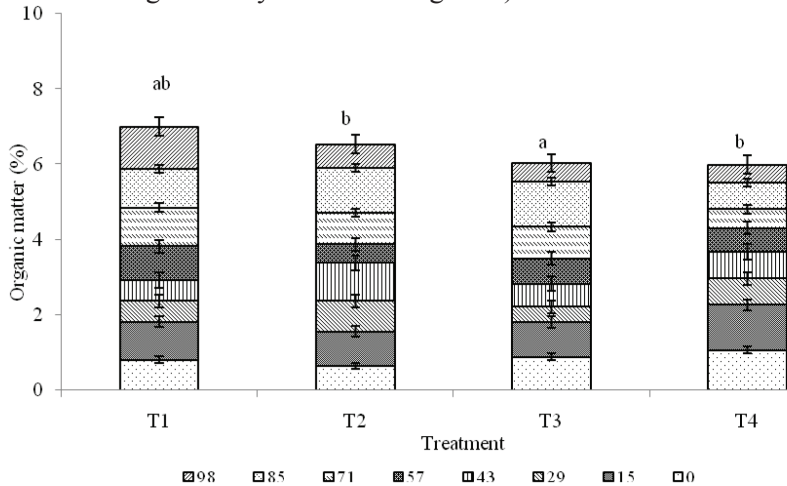


Figure 4: The surface SOM contents for various treatments

The cumulative organic matter accumulation after water application is higher for T_1 followed by T_2 , T_3 and finally T_4 . This may be due to the aerobic and anaerobic conditions and relevant microbial activity in the soil. Under the aerobic conditions, the mineralization process is high and therefore the T_3 condition shows the least amount of SOM. Because,

the interval between the two wetting cycle was high and it received only four rainfall events that creates favorable conditions for the microbial growth. The Figure 5 shows the box and Whisker diagram for the SOM content for each treatment. There are no mild out layers and extreme out layers found.

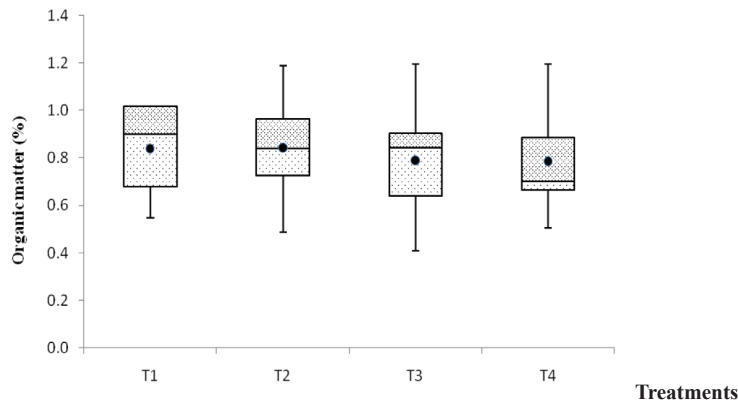


Figure 5: The Box and Whisker diagram for each treatment

4. Conclusions

Surface SOM content fluctuates with time as well as with wetting and drying cycles. The shorter dry spells (T_d) have high amount of surface SOM accumulation compared to other treatments. Longer dry spells have aerobic degradation resulting in less SOM accumulation. There is no significant difference among the measured SOM at 4 days dry spell with plant and without plant. This finding may help to decide on correct water management practices to optimize organic matter dynamics in lowland paddy fields.

Acknowledgements

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Determination of Quality of Parboiled Rice by Adapting Different Processing Practices in Jaffna Peninsula

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Abstract: Parboiling is a hydrothermal treatment applied before milling in order to increase milling recovery by minimizing broken rice percentage in the end product. Parboiling has three stages namely soaking, steaming and drying. Soaking and steaming play a key role to harden paddy grains by changing physical properties. Therefore, quality determination of end product is important. Effect of soaking and steaming duration on rice quality was investigated with respect to parboiling process. One kilogram of paddy was taken as sample. There were five treatments assigned with three replicates such as un-soaking and un-steaming, soaking and un-steaming, soaking and steaming an hour, soaking and steaming 2 hours soaking and steaming 3 hours. Soaking duration 12 hours at 30 °C was kept constant for all treatments. Machines were used on husking, milling and polishing of rice for analytical grade to evaluate the percentages of de-husked yield, head rice yield and broken rice yield, respectively. Lab scale detectors were used to count the quality attributes of white belly, heat damaged grains and whiteness value, which reflected the appearance of final processed product in local markets. Among the treatments, higher yield of de-husking rice, milling rice recovery and less percentage of broken rice were 78.4 %, 65.18 % and 11.9 %, respectively and obtained from the treatment of soaking 12 hours at 30 °C and steaming 2 hours of duration. The results revealed that steamed with two hours duration had given very less white belly, whereas un-soaked steamed rice kernel had highest percentage of white belly. The percentage of damaged grains by heat increased with the increasing the duration of steaming. There was significant difference on degree of de-husking, milling and broken percentage at 5% alpha level among treatments in un-soaked, un-steamed, soaked-un-steamed and soaked-steamed. Sample of Un-soaked with un-steamed had highest whiteness value while soaked with steamed samples yielded lowest whiteness values. From these results, it can be concluded that the parboiling process has to be designed with soaking and steaming strategies to minimize the percentage of broken rice at the end product.

Keywords: Brown Rice Parboiling, Bulk soaking, Cottage milling and Traditional steaming

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1. Introduction

Rice parboiling is a hydrothermal process applied as a treatment prior to the normal milling stage. Paddy parboiling is certainly the most ancient process, which has been applied to thresh paddy. It was probably invented to facilitate the easy removal of husk by pounding in the pestle and mortar. Practical advantages are edible rice contains less broken and good taste, flavor and hardness for cooked product to suit the consumer, and that can be stored for longer time without being sticky and rancid. The parboiling process is to produce physical, chemical and organoleptic modification in the rice with economic and nutritional advantages. Whatever may be the method of parboiling some of the main advantages are, the milled rice yield and head rice yield are higher than raw paddy milling and keeps longer and more resistance to insect attack (Pillaiyar, 1988).

There are three important steps involved in paddy parboiling: soaking, steaming and drying. The main objective of soaking is to achieve quick and uniform water absorption. The lower the water temperature is the slower the soaking process (Athapool, 2000). Paddy soaked in water at ambient temperature (20-30 °C) takes 36 to 48 hours to reach 30 % moisture content, whereas in hot water (60 to 65 °C), it takes only 2 to 4 hours. Generally saturated steam at a pressure of 1 to 5 kg/cm² is used for steaming, steaming duration depends on the steaming arrangement, and steam temperature at atmospheric pressure is always higher than that at which needed for gelatinization. The purpose of steaming is

to increase the milling yield and to improve storage characteristics and eating quality. It improves the firmness after cooking and achieves better vitamin and salt retention in the milled rice. During steaming process the moisture content of the paddy rice increases because of the extra water formed by condensation. Water soluble substances spread inside the paddy grain, the granular texture of the endosperm becomes pasty during gelatinization of starch. The cracks in the caryopsis become sealed and the texture of the endosperm becomes more compact. Paddy should be dried to 14 % moisture for safe storage or milling. If drying is done too fast, internal stress develops in the grain and causes breakage during milling. After drying is completed, the paddy should be allowed to stand for at least several hours preferably 1 or 2 days, before it is milled, to permit internal moisture difference and stresses to equalize. This study aims to determine the quality standards of parboiled rice through the degree of soaking and steaming.

2. Materials and Methods

2.1 Materials

Raw paddy (Moddai Karuppan – Jaffna Traditional Variety), Soaking water bath, Electric steam cooker, Hot air circulatory Oven, Lab scale electric Huller, Lab scale electric polisher, Lab scale electric grader, and Whiteness tester (Reflection meter) were used.

2.2 Methods

Sample Preparation: The paddy sample (10 kg) was cleaned before soaking. Paddy was soaked in a hot water bath for 12 hours at

30 °C. After soaking the excess water was drained-off then soaked paddy sample was steamed in an auto-clave at the pressure of 1.0 kg/cm² for 1hr, 2 hours and 3 hours durations. Triplicate was made for each treatment groups. Finally drying was done at two stages; first paddy sample was tempered under sun drying for a day and then aged at atmospheric temperature (25 °C) until the paddy sample reached a moisture content of 14 %. The quality parameters obtained from milling test of three different steaming duration of parboiled (1 hour, 2 hours and 3 hours) and two control (un-soaked & un-steam and soaked & un-steamed) paddy.

Milling and Polishing: Before milling, the moisture content of sample was measured by conventional oven at 30 °C. Then 500 g of each sample was milled by Satake huller and polished for 2 min by using Satake Polisher. The weight of milled rice was recorded. The following parameters for evaluating the quality of parboiled rice were determined which analyzed by statistically using standard statistical packages version 2000.

- Milled yield refers to the amount of rice obtained after the milling process. This was expressed as percentage (%)
- Head yield refers to the head rice obtained after milling ($> \frac{3}{4}$). This expressed as percentage (%)
- Broken refers to the pieces of rice kernel ($< \frac{3}{4}$) that are less than the size of the head rice (%).
- White belly grains refer to the un-gelatinized starch indicated by a white opaque spot at the centre of the kernel (%).

- Heat damage grains refer to the discolored rice kernel (%).
- Whiteness refers to the degree of whiteness of the sample surface with reference to the standards.

3. Results and Discussions

Results of the different parboiled treatments on rice quality parameters are shown in Table 1. It was found that there were no significant differences found between un-soaked and soaked un-steam. But milling yield was significantly increased in the treatment of soaked with steaming and was higher while increasing steaming time because of splitting of husk during steaming Therefore, parboiled rice was easily de-husked and yield more milling rice.

Head rice yield was increased with increasing in steaming time and was more at 2 hours steaming. More treatment (3 hours) could conclude the optimum steaming time. During steaming, parboiled rice kernel gelatinized and sealed the cracks inside, therefore kernel become compact and harder, therefore, less rice was broken and increased head rice yield during milling. Broken rice yield was inversely proportional to head rice yield and was found more with the paddy without steaming.

Parboiled rice is translucent because any chalkiness inside rice kernel disappears during steaming. Therefore no light scattered and diffracted during detector process (Hall, 1980).

Table 1: Quality parameters obtained from milling test of parboiled rice

Parameter	Rep.	Sam.	DH. (%)	MD. (%)	HR (%)	BR (%)	WB (%)	HD (%)	WV (%)
Un soaked	R1	500 g	72.51	52.33	32.17	20.16	8.6	0.66	51.90
Un steam	R2	500 g	73.77	52.99	33.29	19.70	8.51	2.00	50.00
paddy	R3	500 g	75.46	51.90	32.46	19.43	9.2	2.00	49.70
Soaked	R1	500 g	76.66	44.77	26.89	17.88	45	0.85	44.50
Un steamed	R2	500 g	77.59	44.91	25.72	19.19	31.2	0.80	43.80
	R3	500 g	77.54	44.19	26.93	17.26	27.2	0.90	45.50
Soaked	R1	500 g	78.76	48.18	36.17	12.02	26.5	1.00	36.00
Steamed	R2	500 g	78.77	49.70	37.05	12.66	29.5	1.50	36.40
1 hour	R3	500 g	78.75	47.87	34.61	13.26	27.5	1.20	36.20
Soaked	R1	500 g	81.60	64.21	52.31	11.90	2.00	4.50	21.50
Steamed	R2	500 g	76.41	65.18	53.05	12.13	1.00	6.00	20.60
2 hours	R3	500 g	77.22	66.10	54.27	11.83	1.00	5.30	21.10
Soaked	R1	500 g	78.76	56.46	43.42	13.04	16.5	3.30	33.50
Steamed	R2	500 g	78.34	58.57	44.33	14.24	15.0	2.60	35.00
3 hours	R3	500 g	78.85	57.16	45.78	11.38	18.5	2.20	33.50

Note: Rep: replicates; Sam: samples; DH: Dehushed; MD: Milled; HR: Head rice; BR: Broken rice, WB: white belly; HD: heat damage; WV: Whiteness value

The result reveals that steaming with 2 hours duration had almost very less white belly and un-soaked steamed rice kernel had highest white belly. The heat damaged grains were increased with the increase of steaming time was found to be more in 2 hours of steaming. There was significant difference on quality parameters of milled rice yield and head rice yield are higher than raw paddy milling among treatments in un-soaked un-steamed, soaked un-steamed and soaked steamed. Highest whiteness value was obtained from Un-soaked un-steamed sample while lowest whiteness values was obtained from soaking and steaming. This may be due to get rid off of many water soluble pigments from the kernel surface and make parboiled rice less white during soaking and steaming get them out (Pillaiyar, 1998).

4. Conclusions

Parboiling process (soaking and steaming) could improve milling characters of paddy,

it resulted in more milling yield, more head rice yield, lesser broken rice and lower white belly percentage. From this study, steaming with two hours was found to be best for this selected variety. Result from this study reveals that soaked but un-steamed paddy showed no significant difference with un-soaked un-steamed paddy regarding white belly and whiteness and a significant difference was obtained in terms of milling yield and head rice yield and this might be due to the soaking and subsequent drying resulted in swelling and shrinking of cells which made cracking of grains. Though most parameters showed that parboiled rice was better than un-parboiled rice, the only one drawback was whiteness.

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Annexure 1: Result of ANOVA, Two-Factor with replication

Summary	Count	Sum	Average	Variance
Row 1	7	238.91	34.13	679.56
Row 2	7	246.43	35.20	582.41
Row 3	7	241.02	34.43	635.61
Row 4	7	235.07	33.58	991.02
Row 5	7	246.48	35.21	731.35
Column 1	5	387.00	77.40	4.15
Column 2	5	268.17	53.63	63.82
Column 3	5	192.97	38.59	109.07
Column 4	5	75.36	15.07	12.91
Column 5	5	87.74	17.55	194.45
Column 6	5	10.94	2.19	3.34
Column 7	5	185.73	37.15	121.68

Annexure 2: Result of the ANOVA Table

Source of Variation	SS	df	MS	F	P-value	F crit
Rows –Replicates	13.90	4	3.47	0.04	1.00	2.78
Columns - Treatments	19695.98	6	3282.66	38.93	0.00	2.51
Error	2023.77	24	84.32			
Total	21733.64	34				

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Manuscript Layout: Manuscript for article should be organized as follows: Title, Abstract, Keywords, Introduction, Materials and Methods, Results and Discussion, Conclusion, Acknowledgement and References. Pages should be numbered consecutively and arranged in the following order.

Page 1

Title Page: Include the title of paper, names and affiliations of all authors including e-mail address, telephone and fax number of corresponding author. Submitted manuscripts must list full names for all authors; that is, full first/given name(s), middle initial(s), and last/surname(s). Title should be concise, informative and typed in 'Sentence case' bold letters.

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Keywords: A maximum of six keywords may include the name of an organism (common and scientific), method or other words or phrases that represent the subject of the study.

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New methods may be described in detail with an indication of their limitations. Established methods can be mentioned with appropriate references. Sufficient detail should be included

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The results should be concisely and logically presented. Only data essential for the main conclusions emerging from the study should be included. Repetition of the same results in figures, tables or text should be avoided. Long discussions should be avoided. The discussion should deal with the interpretation of results. It should logically relate new findings of earlier ones.

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Cite references in the text by name and year in parentheses. Some examples:

...fully hydrogenated fats are blended with liquid oils as the feed stocks of interesterification (Zhang *et al.*, 2000).

.... with low or zero trans fatty acids and the results were promising (Zhang *et al.*, 2001; Zhang *et al.*, 2006; Goli *et al.*, 2008).

.....trans-free margarine formulations and most widely used enzyme for the interesterification is Lipozyme TL IM (Ferreira-Dias, 2013).

.....fatty acids can be obtained by enzymatic interesterification (Huang and Akoh, 1994). This result was later contradicted by Becker and Seligman (1996).

Reference List

Journals Articles

1. Single author

Gil, A. 2002. Polyunsaturated fatty acids and inflammatory diseases. *Biomedicine and Pharmacotherapy*, 56:388–396.

2. Two Authors

Huang, K.H. and Akoh, C.C. 1994. Lipase-catalyzed incorporation of n-3 polyunsaturated fatty acids into vegetable oils. *Journal of the American Oil Chemists' Society*, 71:1277–1280.

3. More than two Authors

Zhang, H., Xu, X., Mu, H., Nilsson, J., Adler-Nissen, J. and Høy, C.E. 2000. Lipozyme IM- catalyzed interesterification for the production of margarine fats in a 1 kg scale stirred tank reactor. *European Journal of Lipid Science and Technology*, 102:411–418.

Books and other Monographs

4. Chapter in Book

Gordon, M.H. 2001. The development of oxidative rancidity. In: Pokorny, J., Yanishlieva, N. and Gordon, M. (eds) *Antioxidants in Food – Practical Applications*. CRC Press, Washington, pp 7-225.

Web References

The full URL should be given with the date when the reference was last accessed. Any further information, if known (DOI, author names, dates, reference to a source publication, etc.), should also be given.

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