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Journal of Agricultural Sciences

Editorial Policy

The Journal of Agricultural Sciences is a peer reviewed journal aiming to publish high quality articles on topical issues in Agriculture, and publish three issues of the journal annually; 1st January, 1st May and 1st September. The journal provides a forum for Sri Lankan and international scholars to publish authoritative and well referenced articles in agriculture related areas. The journal publishes original research works, book reviews, short communications and comparative articles.

Research papers submitted for publication should have a sound disciplinary basis, although cross disciplinary contributions are also accepted. Papers submitted for publication are mainly peer reviewed. However, guest papers from senior scholars are subject to the review of editorial board only.

Manuscripts submitted by the authors are subject to a preliminary screening based on appropriateness of the theme and quality of the content of the manuscript. Those manuscripts that are cleared the initial screening then undergo a double-blind peer review process. Two reviewers in the same or related field are assigned by Editor in Chief in consultation with Coordinating Editor to carry out the review based on the evaluation criteria. Based on the evaluation report of the reviewers, the Editor in Chief and the editorial team then make a final decision for acceptance or rejection of the paper.

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Editorial Foreword

I am very pleased that Journal of Agricultural Sciences is presenting its 1st issue of the volume 11 on time. Initiating a new journal is generally a difficult, brave and risky task and continuing it for more than a decade with regular on time publishing is even more difficult and challenging. We are very excited that the journal has been attracting papers from a variety of emerging economies such as India, Iran, Indonesia, South Africa, Nigeria, Pakistan, Bangladesh, China, Nepal etc. The variety of submissions from such countries will help the expected global initiatives of the journal. We are also pleased that the researchers from livestock development, crop science and agri economics demonstrate an interest to share their research with the readers of this journal. It is worth to mention here that SCOPUS is now in the process of evaluating the journal for adding it to their data base. We are looking forward to the possible announcement of this honor within this year.

This issue of Journal of Agricultural Sciences contains six outstanding articles which shed light on contemporary research questions in agriculture and agri business fields. JAS provides a platform for the publication of evidence-based studies on all scientific aspects of agriculture including the crop science, agricultural economics, biotechnology in agriculture, livestock production, agri business management, agricultural biology, agricultural engineering, agricultural extension, and many more related areas. JAS is a double blind peer reviewed and fully open access journal intended to maintain the highest possible global scientific standards.

Many authors in today’s publishing environment want to make their research freely available to all reader communities. It is the policy of open access. Open Access stands for unrestricted access and unrestricted reuse. There are number of benefits of Open Access publishing of research. They are accelerated discovery: with open access, researchers can read and build on the findings of others without restriction, public enrichment: most of this scientific research is funded by public funds and open access allows taxpayers to see the results of their investment, improved education: open access means that professors and their students have access to the latest research findings throughout the world without barriers of funds.

The Journal of Agricultural Sciences is a fully Open Access Journal. We do not charge publication fee from authors and there is no fee for downloading of the full papers in pdf format.

Today I am very happy and proud to say that JAS is making significant progress and receiving recognition from research community in its field of study. There were many people behind this success. This credit must go to Dr. Chandrika Dissanayake, Coordinating Editor of JAS, Mr Prasad C. Iddamalgoda, Ms Suiox Cummings of SLJOL, all the authors, reviewers and editorial committee members.

Prof Rohana P Mahaliyanaarachchi
Editor in Chief
01st January 2016
Agri Tourism as a Risk Management Strategy in Rural Agriculture Sector:
With Special Reference to Developing Countries

Rohana P Mahaliyanaarachchi

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ABSTRACT

This article reviewed literature on potential of agri tourism as a risk management strategy in rural agriculture sector with special reference to developing countries. Reviewing literature indicated that agri tourism as a risk management strategy in rural agriculture sector has an immense potential to contribute to manage the risk in agricultural economy. Evidence from the production and price fluctuations during last three to four decades in both conventional agriculture sector with intensive production of rice, vegetables, and other subsistence crops and plantation agriculture sector with intensive production of tea, rubber, coconut, coffee, etc shows that they are highly vulnerable to external factors such as internal & international politics, climate change and whether pattern changers, market and trade slumps, etc. Sudden and unanticipated influences of these external factors cause higher risks in sustainability of agriculture sector and it results in the collapse of both micro and macro economies in a country. This paper attempts to discuss how agri tourism can be introduced to agriculture sector as a supplementary income source as a risk management strategy which is less susceptible to above mentioned externalities. Further, agri tourism will motivate and encourage farming communities to raise their crops in eco friendly approach and to conserve the biodiversity of farms which will minimize the internal risk factors of farming such as pest and disease outbreaks, soil degradation, etc. Research studies has shown that a well-developed agri tourism industry would result in a market mechanism generating additional income of US $251 to US $364 million annually in counties like Dominican Republic. Agri tourism sector would improve sustainable agricultural practices by maintaining and increasing positive externalities and nonmarket services provided by agriculture. Agri tourism products and services would have the added benefit of promoting sustainable agricultural practices too. This is a good option for farmers and planters who are willing to diversify their farming operations that will help bringing more economic activities to rural areas sustaining livelihoods of the rural people. In addition, agri tourism not only allows farmers to enjoy greater economic benefits through managing risks, but also helps to retain the young generation of the farming community in the rural areas instead of migrating to urban areas for better livelihoods.

Keywords: Agri tourism, risk, management, rural agriculture, economic development

INTRODUCTION

This article reviewed literature on potential of agri tourism as a risk management strategy in rural agriculture sector with special reference to developing countries. It is also provided a review of agri tourism development particularly in emerging economies in Asia, Africa and Latin America. Reviewing literature indicated that agri tourism as a risk management strategy...
in rural agriculture sector has an immense potential to contribute to manage the risk in agricultural economy.

Evidence from the production and price fluctuations during last three to four decades in both conventional agriculture sector with intensive production of rice, vegetables, and other subsistence crops and plantation agriculture sector with intensive production of tea, rubber, coconut, coffee, etc shows that they are highly vulnerable to external factors such as internal & international politics, climate change and whether pattern changers, market and trade crumple, etc. Sudden and unanticipated influences of these external factors cause higher risks in sustainability of agriculture sector as a consequence both micro and macro economies in a country collapses.

**Types of risks faced by farming sector**

Farming activities are subject to wide range of risks due to biological, physical and economic environment in which farming operates. Most of these risks are specific to agriculture and they affect to the overall production and economic efficiency of agricultural production system. Further, these risks cause to fall of farm incomes, welfare of agricultural workers with potential to constraint future investment and growth of farm production. Therefore, it is important to understand how the presence of risks in agricultural production affects the economy and how these risks can be mitigated.

The main risks in farming can be categorized as follows (OECD, 2008).

a. Production or yield risk: this is uncertainty about the volume or quantity of agricultural production due to weather related factors such as heavy rains, floods, droughts, cyclones and typhoons, tornadoes, frosts, heavy snow falls, hails, etc, crops and livestock diseases, pest outbreaks and change of technology.

b. Market or price risk: uncertainty and fluctuations of prices of both inputs and outputs (agricultural production) due to market instabilities, trade policies of the governments, new markets, etc.

c. Regulatory risk: unexpected changers of national agricultural policies, environmental regulations, provincial government laws, and trade policies. This may happen due to change of rulers or any other political reasons.

d. Financial and management risk: changers of bank policies and its credit facilities, change of interest rates, fluctuations in the share market, international and national financial crisis, management change.

e. Personal risk: personal hazards such as illness, death, theft, injuries, family crisis, etc.

There are various other classifications for agricultural risks faced by farmers other than above given classification. Nevertheless, all the different types of risks given in the literature can be grouped into above given five categories and is shown in table 1.

According to OECD (2011) there are three different layers of agricultural risks and they require different mitigation strategies.

- **Normal risks**: They do not need any specific policy response from government or relevant authorities. They can directly managed by farmers as a normal business strategy.

- **Catastrophic risks**: Many or all farmers in a region or country get affected by these risk sources and usually are beyond farmers or markets capacity to cope. Examples for this type of risk sources are severe, prolonging and widespread droughts, outbreak and spread of a highly contagious and damaging diseases or pests and unexpected, severe floods. In such cases government invention is unavoidable.
** Marketable risks:** In between normal and catastrophic risk layers lies marketable risk layer that can be handled through market tools. These tools are crop insurance, extended markets, cooperative systems, guaranteed prices, etc.

Further, there is a difference between **systematic** and **non systematic** risks. Systematic risks repeat over time with a pattern of probabilities that can be analysed in order to have a good estimate of the actuarial probability. Non-systematic risks are very short or imperfect records of their occurrence and, therefore, difficulties in estimating an objective pattern of probabilities or distribution of outcome (Newbery and Stieglitz, 1981). If there is a high degree of correlation among individuals in the same region or country the risk is called **systemic** risk. An individual risk that is independent and uncorrelated with any other risks is called **idiosyncratic** risk. However, it is important to have an idea about degree of correlation among these different types of risk in finding solutions to mitigate them (Jorion, 2001).

**Factors influencing risks in farming sector**

The overall impact of risks both on individual farms and on whole farming sector in a region depends on the relationships between the different risk factors. In the broader sense, correlation between risk factors can differ significantly that affects the overall risk exposure of farm enterprises. When risks are not perfectly correlated at farm level, total risk exposure will be less than the sum of individual risks (OECD, 2009). Therefore it is important to consider that relationships between risk factors allows the possible effects on farm income to be determined more accurately and introduce risk management strategies more effectively.

**Table 01: Major risks faced by farmers**

<table>
<thead>
<tr>
<th>Risk Group</th>
<th>Type of risk</th>
<th>Examples</th>
<th>Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Production or yield risks</strong></td>
<td>Weather</td>
<td>Deficit or excess rainfall, extraordinary temperatures (both high and low), strong winds, hail storms</td>
<td>Loss of crops, lower yields, income loss</td>
</tr>
<tr>
<td>Natural disaster</td>
<td>Floods, prolonging droughts, cyclones, typhoons, volcanic activities, earth slips, earthquakes, tornados</td>
<td>Damage of crops and loss of animals, complete or partially loss of yields, infrastructure losses, totally or partially damage and loss of farms</td>
<td></td>
</tr>
<tr>
<td>Biological</td>
<td>Diseases and pest outbreaks (for both crops and livestock), contamination of micro organisms, heavy metals or chemicals</td>
<td>Damage of crops and loss of animals, complete or partially loss of yields, loss of income</td>
<td></td>
</tr>
<tr>
<td>Market</td>
<td>Unexpected changes of demand for certain products, changers of food safety requirements, delaying of delivery, damages during transport, changers of supply chain stakeholders</td>
<td>Loss of markets, lower prices, loss of production, loss of income,</td>
<td></td>
</tr>
<tr>
<td>Price</td>
<td>Low prices, volatility of prices</td>
<td>Loss of income</td>
<td></td>
</tr>
<tr>
<td>Policy and Institutions</td>
<td>Regulatory changers, uncertain legal policies, weak institutional capacities, unfavorable tax policies,</td>
<td>Fluctuations of prices, low income</td>
<td></td>
</tr>
<tr>
<td>Politics</td>
<td>Government related uncertainty, political upheaval, social unrest, wars , employees strikes of supply chain</td>
<td>Fluctuations of prices, low income</td>
<td></td>
</tr>
<tr>
<td>Financial and management risk</td>
<td>Logistics and infrastructure</td>
<td>Change cost of transportation, energy and communication, low quality transport facilities, energy and information</td>
<td>High COP, low profits or losses</td>
</tr>
<tr>
<td>Management and operations</td>
<td>Poor management decisions, poor quality control, poor forecasting of demand and supply,</td>
<td>Fluctuations of prices, low income</td>
<td></td>
</tr>
<tr>
<td>Personal risk</td>
<td>Labour and health</td>
<td>Illness, death, injury, theft, family crisis, labour scarcity</td>
<td>Loss of productivity, high COP, loss of income</td>
</tr>
</tbody>
</table>

*Source: Jaffee S. et al, 2010, and Author*
However, in practice calculating the effect and strength of the risks and interaction effect of different risk factors may be highly complex.

It is important to consider how risk factors affect different farmers. Systematic risks such as droughts, floods and price fluctuations which are specific to a particular region or country are highly correlated within the group of farmers. In other hand distinctive risks such as localized weather conditions (hail, frost, etc) and personal risks (death, illness, theft, etc) are unrelated to the farming community as a whole. They are affected only to individual cases.

Risks in agriculture are interconnected and sometimes merging or sometimes counteracting each other. As an example when production is declined due to risk factors related to production, these risks can be partially offset by price movements, if the prices of products are relatively high (OECD, 2011).

There are two major types of agricultural risk and factors influencing these risks are different to each other (Huirne et al, 2000; Hardaker et al, 2004). First category is business risk and it includes production, market, institutional and personal risks. Factors affecting production risk are mainly unpredictable weather and unexpected outbreaks of pests & diseases. They directly affect performance of crops and livestock. Market risk is related to uncertainty about the price of outputs and, sometimes also inputs, at the time production decisions are taken. Factors affecting market risk are sudden changers of markets, unusual changers of consumer behaviour, high fluctuations of supply and demand and unexpected crisis on food safety in the supply chain. Government actions and rules on food production and distribution, regulations on use of agro chemicals, tax provisions and payments, interest rates rise and unavailable of bank loan for agriculture.

External

External factors affecting risk of farming (both crop production and livestock) are mainly influenced to the farming process from outside of the farm. These factors are less controllable by the farmer. Nevertheless, farmer as an individual or farming community can take measures to mitigate the effect of these factors. Unpredictable weather is an external factor that cannot be controlled by the farmers. Deficit or excess rainfall, extraordinary temperatures (both high and low), strong winds, hail storms, tornados and such unfavorable weather conditions to farming cause for adverse effects on farm production and subsequently for total farm income and profitability. Other external factors affecting risk of farming are sudden changers of markets, unusual changers of consumer behaviour, high fluctuations of supply and demand and unexpected crisis on food safety in the supply chain, government actions and rules on food production and distribution, regulations on use of agro chemicals, tax provisions and payments, interest rates rise and unavailable of bank loan for agriculture.

Internal

Internal factors affecting risk of farming mainly influences the farming process from inside of the farm. Some of these factors are controllable by the farmers and some are not. Unexpected outbreaks of pest and diseases are mainly within the farming region. Reasons for pest and diseases outbreaks are different and some of them are controllable by the farmers some are not. Some of these reasons are deforestation, destruction of natural enemies, intensive cultivation, introduction to new varieties and crops, modern agricultural practices and
accidental introduction of pests and diseases from foreign countries. Unexpected climatic conditions also cause for some pest and diseases outbreaks. Farmers incur large economic losses due to attacks from pests and diseases. Therefore, this is a major risk faced by farmers and it is needed measures to control them and mitigate the effects.

Uncertain life events such as death, divorce, theft or illness are also internal risk factors in farming.

Risk management strategies

Risk management should not concentrate on only one risk factor or only one solution. Diversification is a good strategy to reduce agricultural risk. Within the normal risk layer individual farmers are responsible and capable for managing their own business risk. Farmers adopt various strategies to manage risk affecting their production and income. These strategies depend on the characteristics of risk they face, their attitude to risk and the risk management instruments and tools available (OECD, 2009a).

There are four main types of risk management strategies available in the literature.

They are financial strategies, marketing strategies, production strategies and insurance. Other than the financial and marketing strategies, production strategies such as diversification, geographic dispersion, variety selection, timeliness, the use of cultural practices best suited to particular areas, etc. are important ways to manage risk. Diversification has been one of the more important and useful method to reduce risk and uncertainty. The chance of a large economic loss from a given hazard is reduced if there is more than one enterprise in the farm business. However, enterprises included in the business should not be subject to the same hazards or at least not to the same degree, if this strategy to be more effective in risk management (OECD, 2009b).

Agri tourism as a risk managing strategy

Agri tourism is an enterprise that can be introduced to diversify farm business successfully. While agri tourism is a mix of two major sectors- agriculture and tourism, agri tourism farms are not subject to the same hazards faced by agriculture only farms.

What is agri tourism?

Agri tourism is the practice of attracting visitors to an area used basically for agricultural purposes. It attracts tourists to rural communities for a form of relaxation that follows the growing trend of tourism that is both educational and recreational. Also it is another option for farmers wanting to diversify their farming operations that will bring more economic activities to rural areas. Generally, the image of tourism stimulates of mass-produced travel that attracts a large number of travelers. This image of mass tourism may discourage small entrepreneurs who consider tourism as an alternative option for enhancing their revenues. However, agri tourism can be viewed as small-scale, low-impact, education focused, recreational and more importantly compensating income for agri tourism operators that are mainly farmers.

Further, Agri tourism is a direct marketing activity which provides additional opportunities to farmers to reduce risks involved in farming via diversification in a competing and urbanizing economic environment. While farmers get separate income from agri tourism products that they sell to the visitors, they are more riskless than expecting income from one operation that is merely farming.

It can provide many benefits to the farmers:

- Supplementary income for the farmer apart from farming
- Continuous cash flow all around the year including the off-season
Opportunity to sell products grown and harvested in the farmer’s agricultural operation

Opportunity to sell the “experience” of farmers agricultural venue

Managing the risk in farming occurred due to uncertainties of production and marketing

**Products of agri tourism**

**Classification of Agri tourism products and services**

Agri tourism products are spreading in a wider range. Agri tourism products are not merely activities. It is included place of implementing the activities, people involved, facilities needed for tourists, something to see, something to do (activities) and something to buy the visitors/tourists and procedures.

We cannot separate agri tourism and services as tangible products and intangible services. Agri tourism products include services too. Agri tourism products and services can be classified into following categories(Sznajder and Przezbórska, 2004; Mahliyanaarachchi, 2014).

a. According to the time of availability of product or service
   - Products and services available at any time of the year
   - Products and services available on particular time of the year

b. According to the requirement of the customers
   - Tailor made products or services
   - Readymade products or services

c. According to agri tourist activities
   - Agri accommodation

- Direct marketing
- Farm tours
- Farm education programmes
- Farm festivals and cultural events
- Farm restaurant and food service

**Products and services available at any time of the year**

These are agri tourism products available throughout the year and easy to find due to their free availability. Agri tourism accommodations, farm restaurants, farm tours are available at any time of the year. The round the year availability of products or services also depends on the region or area. For instance, farm tours as banana tours, tea tours or cinnamon tours which are offered in tropical regions are available throughout the year. Farm tours as vine tours, berry farm tours, apple and pear tours are seasonal due to nature of agricultural production of these products.

Products and services available throughout the year are comparably cheaper than seasonal ones. Even in accommodation sector, there are peak times and off seasons according to the availability of tourists. During the peak time, in niche markets like agri accommodations, prices are higher than general hotel accommodation.

Farm restaurant is an agri tourism product that can be available throughout the year. However, in countries with temperate climate agri tourism products are marketable during the seasons with good weather conditions. In tropical and subtropical countries most of the agri tourism products are possible to offer to customers round the year.

**Products and services available on particular time of the year**

These are seasonal products. These products or services are available only in some seasons of the year or during specific time period. Some fruits such as mango, pears, apples, **rabutan** are available seasonally and harvesting of these...
fruits is also seasonal. Farm festivals are also available in a particular time. In south and south east Asia paddy planting and harvesting festivals are very popular and colorful in these countries. Most of these are cultural and related to religion (Buddhism, Hinduism) also. Visitors can watch these festivals and there rituals only during the available season, because these festivals cannot be demonstrated as mock festivals. While they are closely related with satisfying of gods, farmers are hesitated to make mock ones.

Due to seasonality and rareness of these seasonal products they are expensive. However in some cultures visitors can watch them in free of charge. These days even in rural area due to open economic situation, people try to make a value for everything. These are some challenges in agri tourism which is aiming to give positive impression on rural values to visitors.

**Tailor made products or services**

Agri tourism entrepreneurs can offer tailor-made products or services to the visitors according to their wishes and requirements. As an example children from cities may not have seen cooking in clay pots with firewood. They may request to the farmer of the farm stay they want to experience cooking with firewood in clay pots. Therefore, farmer can organize requested type of cooking session in his farm. It is organized according to the requirement of the visitors and can be discussed in detail their requirement before planning it. Another example is visitors from a school may request to demonstrate milking manually. Farmer can organize day session of milking and allow them to learn hand milking. These are tailor made products and organize only in request of the visitors. Further farmer can charge for this type of services or products from the visitors. Tailor made products or services are expensive because farmer has to take extra effort and spend money to customize his services or products.

**Readymade products or services**

These products or services are already available in the farm or agri tourism enterprise and may be included into the tour package. As an example, in a banana tour in Ecuadorian banana plantation, all the activities from planting to processing and packaging of banana are included to the tour package. This shows that a tour package of banana tour includes transport facilities to and from the given point of gathering of visitors (hotel, train station, bus station, airport, etc) to banana plantation, site seeing in the plantation, a tour guide service, involving in activities, Q & A session, refreshments (or lunch or both) and any other action as per schedule. When a visitor buys banana tour package it includes all above with conditions or without conditions. Most of these tour packages are with conditions apply. A farm B & B is included accommodation, breakfast, hospitality and farm tour into the package. These are readymade products and services and included into the tour package.

**Types of Agri tourism products**

Agri accommodation: Different types of farm accommodations are considered as agri tourism products. Farm accommodation is rated on the basis of standards accepted internationally and is a good business decision for owner/operators and their guests. According to the definition of a tourist, farm accommodation is the real agri tourism product. The definition of a tourist is “a person who is supposed to leave his/her hometown (permanent place) on temporary basis for the purpose of seeking new experiences, having fun & entertaining, doing sports, seeing cultural & historical places (attractions) etc, on the condition that she/he should stay no less than one day (including a night) and no longer than 12 months, make use of a tourist facility for accommodation and spend her/his own money through their holiday” (Mahaliyanaarachchi, 2014).
Direct marketing: Other Agri tourism product on the farm may include the direct marketing of farm products at the farm gate or a farmers’ market. Innovative ideas using farm-based products have the greatest potential to earn the most money. Finding the niche markets and expanding on these unique opportunities can create the most rewarding and successful business ventures.

Different events on the farm, such as bee honey collection, U pickups, and farm restaurants with out-door BBQs can be instigated as direct marketing. Marketing niches such as water gardening supplies, herbal plants and products, flowers and exotic plants and breeding exotic animals can be added as farm based markets and require careful attention to constantly changing consumer trends. These can be very profitable if developed in conjunction with other agri tourism products (Mahaliyanaarachchi, 2014).

Farm tours: Farm tours can be organized in many different fashions. There may be just one farm hosting the tour, or a group of farms in a given area may be included, providing the tourists with an overall idea about agriculture in the area. Tours may be operated individually, where a family or group of people may choose to participate in the tour on their own. Farm Tours can be operated on a large scale, if tour operators include the farm tour into their tour package in advance. A packaged tour may include a half a day or one day tour of a farm and a processing plant so that the tourists will have a fuller understanding of the food chain from nursery stage, planting and up to harvesting, processing and marketing.

There are very good examples of this type of commercial farm tours in the world. Coffee Tours in Tanzania, Banana Tours in Central America, Wine tours in France, Whisky Tours in Scotland, Orange tours in Spain, Cinnamon Tours and Tea Tours in Sri Lanka are some of them. Also joining with tour operators, farmer groups can initiate various trails or driving routes, where a number of similar enterprises can be seen along the route such as a Tea Route, Cinnamon Route and Coconut Routes (Mahaliyanaarachchi, 2014).

Use of agri tourism products in managing risk in farming

There are only few studies conducted on farmers’ perceptions of the economic benefits actually received from agri tourism and its mitigating ability of the effects of the risks faced by farmers. It has been observed that agri tourism, specifically farm-based accommodations is a “minor contributor” to the incomes of farmers in southern Germany (Oppermann, 1995; Busby and Rendle, 2000). However, past research confirms that agri tourism development in USA and rest of the world is often motivated by socially, including fulfillment of personal entrepreneurial goals, education of the public about farming, and social interactions with guests (George et al., 2011; McGehee, et al., 2007; Nickerson et al., 2001; Schilling, et al., 2012; Sharply and Vass, 2006; Weaver and Fennell, 1997). However, improving farm income is generally a primary motive behind the development of agri tourism enterprises. George et al., (2011) observe a range of net returns across different types of agri tourism attractions, concluding generally that agri tourism is a supplemental source of income for most farms.

However, Schilling et al., (2014) reveals that agri tourism farms in small farming category generate higher net cash returns per acre than their counterparts that do not engage in agri tourism. Similarly, operators of intermediate scale farms and smaller farms operated by individuals with stronger occupational ties to farming also appear to be finding success in agri tourism. Further they found that agri tourism has statistically significant and positive effects on farm profitability.

Agri tourism requires minimal additional investment and may utilize excess capacity
of labor, capital, land, and natural resources. Excess capacity may allow farmers to increase the scope of activities. Promoting agri tourism in a farm or ranch is a revenue risk management strategy. Agri tourism attracts customers to farms or ranches. A pick your-own fruits or flowers enterprise or a nursery activity will attract families. These activities provide exercise, lots of fresh air, fresh food, fresh water, relaxation and something to take home (Mahaliyanaarachchi, 2015).

For farming communities trying to diversify their economies due to less profits and high risks, agri tourism offers compensating income source that allows a large financial range for capital expenditure, depending on how much the entrepreneur wants to invest. On the other hand young people in rural areas can start an agri tourism enterprise in their farm land which will be their main income source (Mahaliyanaarachchi, 2015; Brumfield and Mafoua 2002).

Further, agri tourism will motivate and encourage farming communities to raise their crops in eco friendly approach and to conserve the biodiversity of farms which will minimize the internal risk factors of farming such as pest and disease outbreaks, soil degradation, etc.

Research studies has shown that a well developed agri tourism industry would result in a market mechanism generating additional income of US $251 to US $364 million annually in counties like Dominican Republic (Catalino and Lizardo, 2004). Agri tourism sector would improve sustainable agricultural practices by maintaining and increasing positive externalities and nonmarket services provided by agriculture. Agri tourism products and services would have the added benefit of promoting sustainable agricultural practices too.

This is a good option for farmers and planters who are willing to diversify their farming operations that will help bringing more economic activities to rural areas sustaining livelihoods of the rural people. In addition agri tourism not only allows farmers to enjoy greater economic benefits through managing risks, but also helps to remain the young generation of the farming community in the rural areas instead of migrating to urban areas for better livelihoods.

Diversification of income sources is the only alternative to stay in agriculture for farmers with small and medium sized farms due to high risks they are facing such as production or yield risk, market or price risk, regulatory risk, financial risk and personal risk. One strategy to overcome these risks adopted by some innovative farmers is adding agri tourism as an alternative business in their farms. This is another way to adding value to the crops and livestock grown on the farm or ranch. It has a potential for building and expanding successful relationships between agriculture and tourism industries. Getting back to the agricultural and rural heritage roots and nature-based recreation experiences is a major tourist attraction trend today. Many of the natural resource conservation programmes in the agri tourism farms and rural landscapes are cherished by suburban and urban tourists both local and international.

Integrating agri tourism into current agricultural crop and livestock production is a way for a crop farm or a ranch to improve its income and grow livelihoods of the rural community. This helps farmers to manage different agricultural risks they faced. A specific feature of agri tourism is with relatively little initial investment, a working farm or ranch can be converted to an agri tourism enterprise.

**Positive aspects of agri tourism in managing risk in farming**

In term of positive aspects, agri tourism through green agriculture is a main expectation of agri tourism promotion. The farmers tend to reduce agricultural inputs from outside by means of organic farming or natural farming development as tourists attractions. Hence, environmental and natural resources available in the farm will
serve as tourism resources instead of using for intensive agriculture (Ceballos, 1996). This will help to conserve available natural resources for effective management of agricultural risks faced by farmers. Farmers lose their income due to any kind of agricultural risk and it is important that any solution to manage these risks must compensate these income losses. Agri tourism is proven as a successful supplementary income source to the farmers (Schilling et al., 2014; Catalino and Lizardo, 2004).

Negative aspects of agri tourism in managing risk in farming

However, relationships between the farming and agri tourism activities may be competitive that may concern the use of agricultural resources of the farm, i.e. land, human resource, infrastructure and capital. For instance, a farmer growing commodity crops intend to develop agri tourism activity has to exclude part of the area of land from agricultural production and use it for agri tourism ( Sznajder, et al, 2009). Nevertheless, some experts suggest that even though agri tourism is associated closely with rural environment, but in the business environment, tourist farms also provide agricultural resources as accommodations and other facilities as other types of tourism business (Halfacree, 1993).

Further, in terms of tourism business model, it is a negative impact that most of agricultural resources are used for tourism and some cases the development of agri tourism activities is not an increasing factor of agricultural productivity (Brscic, 2006). Some researches reveal that the link between agri tourism and farming is getting weaker. In this view, farmers who engage in farm based tourism as an alternative source of income to mange risks in farming slowly dissociate themselves from agricultural activities (Busby and Rendle, 2000).

CONCLUSIONS

Risk management in agriculture is vital both for individual farmers and for agriculture as a sector because higher risks threaten sustainability of agriculture sector and it results in the collapse of both micro and macro economies in a country. Therefore, mitigation of effect of agricultural risks are important for progress of the sector and it is an essential need to identify appropriate risk management strategies to overcome these effects. Risk management should not concentrate on only one risk factor or only one solution. Diversification is a good strategy to reduce agricultural risk. Within the normal risk layer individual farmers are responsible and capable for managing their own business risk. Farmers adopt various strategies to manage risk affecting their production and income. Agri tourism is an enterprise that can be introduced to diversify farm business successfully.

It is observed that a range of net returns across different types of agri tourism attractions, concluding generally that agri tourism is a supplemental source of income for most farms. Therefore, we can conclude that agri tourism can be practiced as a successful risk management strategy in agriculture considering the global experiences.

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A Comparative Assessment of the Antibacterial Activity in Fruit Juice of Sri Lankan Sweet Orange Cultivars vis a vis Sour Orange


ABSTRACT

Sweet orange (Citrus sinensis), a key fruit species, is considered as a primary ingredient in herbal medical formulations against ailments such as food borne diseases. Sour orange (C. aurantium) is also very famous as a medicinal plant. There are six commonly grown sweet orange cultivars in Sri Lanka (Arogya, Bibila sweet, MKD, Sisila, BAN and MT) but the antibacterial activity present in their fruit juice is not well documented. Therefore, the present study was conducted to characterize the antibacterial activity of the fruit juice of these sweet oranges in comparison to sour orange and also to establish DNA barcodes for the tested cultivars for precise identification. Fruit juice was collected from sweet orange cultivars and sour orange and antibacterial activity was measured against three model pathogenic bacterial species, Escherichia coli, Staphylococcus aureus and methicillin-resistant S. aureus. After employing filter paper disc method, the diameter of zone of bacterial inhibition (DZI) was measured as the parameter of antibacterial activity. The genomic DNA was extracted from all the tested plants and PCR amplified using trnH–psbA primer pair and subjected to DNA sequencing, followed by alignment analysis and dendrogram construction. Arogya and MKD did not show any antibacterial activity (DZI = 0.0 mm), whereas Sisila, BAN and MT showed antibacterial activity only against E. coli and S. aureus (mean DZI of 8.2 mm and 8.4 mm respectively). Bibila sweet and sour orange showed significantly higher antibacterial activity against all E. coli, S. aureus and methicillin-resistant S. aureus (mean DZI of 10.2 mm, 10.5 mm and 7.8 mm respectively). DNA barcoding provided unique sequence identifiers for each cultivar. These antibacterial activity data in combination with DNA barcodes could help to develop new cultivars through breeding to promote the sweet orange industry in Sri Lanka.

Keywords: Sweet orange, Sour orange, Citrus sinensis, Citrus aurantium, antibacterial activity, disc diffusion method, Citrus DNA barcoding

INTRODUCTION

Sweet orange, Citrus sinensis, is one of the delicious fruits consumed by human beings and it is industrially important as one of the major fruit crops. The global Citrus cultivations cover approximately nine billion hectares, leading to 122.3 million tonnes of fresh fruit harvest (Xu et al., 2013). In Sri Lanka sweet orange industry is not so developed but there is a huge demand for sweet oranges within the country. Because of the nutraceutical values and antioxidant activities (Abeyesinghe et al., 2007), sweet orange is currently being consumed as a health promoting and disease preventing food (Okwu and Emenike, 2006). Citrus is a good source of natural bioactive compounds such as vitamin C, carotenoids (Xu et al., 2006), flavonoids (Okwu and Emenike, 2006), limonoids (Khalil et al., 2003), essential oils, acridone alkaloids (Tian-Shung et al., 1983), minerals and vitamin B (Madhuri et al., 2014). Therefore, unlike in the

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past, there is a huge attention on sweet orange in the modern society as a food for better health. There are six commonly grown sweet orange cultivars available in Sri Lanka. Their important fruit traits have been characterized and the cultivar recommendations were made for large scale planting (Herath et al., 2014). However, neither antibacterial activities of sweet orange cultivars nor the cultivar identities were established based on DNA sequences. In a large scale application of sweet orange, such as producing natural antibacterial herbal formulations, it is important to establish the identity of each sweet orange cultivar, because each cultivar is unique in its properties (Hearth et al., 2014; Youseif et al., 2014). The cultivar identity can be established using molecular methods such as DNA barcoding (Chase et al., 2005). DNA barcoding of plants is the sequencing of chloroplast or nuclear genome specific DNA loci such as the regions flanked by the primer pair trnH and psbA (Pang et al., 2012). DNA barcoding is frequently used to establish the genetic identity of many medicinally important species such as Chinese Bupleurum (Chao et al., 2014), Ginseng (Zuo et al., 2011), medicinal Labiatae plants in Chios Island (Theodoridis et al., 2012), medicinal vines (Liu et al., 2012) and medicinal orchids (Asahina et al., 2010).

Asian countries are famous for traditional medical practices such as Ayurveda, Unani, and Siddhtha. Citrus fruits are proven to possess numerous medical properties according to the ancient reports. The use of oranges in historical medical practices is also reported in India (Harborne, 1994), China (Zhou et al., 2013), Japan (Hirota et al., 2010) and Africa (Aibinu et al., 2007). Though it is not very clear that ancient people in Sri Lanka really used sweet orange or any other type of orange, the word ‘orange’ is very frequently indicated in historical reports on various medical practices (Fonseka, 1902). Plant extracts and phytochemicals with antimicrobial activity have been used as therapeutics since ancient times (Seenivasan et al., 2006). It has been reported that peel extracts of genus Citrus contain antibacterial activity on Escherichia coli, Proteus vulgaris, Bacillus subtilis, Klebsiella pneumoniae, Pseudomonas aeruginosa (Kabra et al., 2012), B. cereus (Madhuri et al., 2014) and Salmonella typhimurium (Dhanavade et al., 2011). Furthermore, there are reported antibacterial effects in Citrus fruit juices against E. coli, S. paratyphi, Shigella sonnei (Bansode and Chavan, 2012), Staphylococcus aureus, Proteus vulgaris and P. aeruginosa (Al-Ani et al., 2010). A species similar to C. sinensis known as C. aurantium, also known as sour orange because of its extreme sourness, is available in Sri Lanka. The sour orange is very popular in ayurvedic medical preparations than sweet orange. However, for fresh fruit consumption and industrial possessing of fruit juice, sour orange is not much used because of its higher acidity (Herath et al., 2014). Therefore it is important to study the medicinal values of sweet orange in comparison to sour orange. Food borne diseases caused by bacteria is a huge problem in modern day food supplies. Most of the disease causing and food poisoning bacteria are becoming resistant to available antibiotics because of the prolonged and frequent exposure to them. The plant based antibacterial compounds would be ideal to combat this problem. If natural plant extracts have antibacterial activities they are useful in controlling the bacterial diseases. According to many historical reports, orange has often been used as a popular plant material in treating ailments related to digestive tract (Fonseka, 1902). This implies that in addition to the various other biochemical properties, orange juice must have antimicrobial activities. It is important to characterize the antibacterial activity of sweet and sour oranges against the commonly found model pathogenic strains, to have an idea on the impact of the numerous food borne pathogens naturally living. However, no studies have been conducted in this regard using Sri Lankan sweet orange cultivars and sour orange. Therefore, the
present study was conducted to characterize the antibacterial activity of sweet and sour oranges in Sri Lanka against three model pathogenic strains and to assign DNA based identifiers to the tested cultivars using \textit{trnH–psbA} based DNA barcoding. This would enable the exact identification of these cultivars for large scale commercial applications in the future.

MATERIALS AND METHODS

Assessment of the Antibacterial Activity

Sample Preparation

The ripe fruits from six sweet orange cultivars (\textit{Arogya}, \textit{Bibila sweet}, \textit{MKD}, \textit{Sisila}, \textit{BAN} and \textit{MT}) and sour orange were collected from Regional Agricultural Research and Development Centers at Bandarawela and Monaragala, Sri Lanka. The juice was obtained from each fruit sample by pressing, using a household squeezer.

Antibacterial Activity

Assessment of antibacterial activity was conducted according to the completely randomized design (CRD) with three replicates. The disc diffusion method was used with three model pathogenic bacterial strains, \textit{E.coli} (JM 109) and \textit{S. aureus} (NCTC 4838) and Methicillin-resistant \textit{S. aureus} (MRSA) for the detection of antibacterial activity of the juice samples. Mueller Hinton Agar (MHA) plates were prepared by pouring 20ml of autoclaved MHA to each plate. After that, 100 µl of bacterial cell cultures were spread separately on the medium. The autoclaved 6 mm Whatman filter paper discs were placed on the medium (Figure 01) and the plates were labeled properly to identify each sample separately. The discs were moistened with 30 µl of the juice sample and the plates were incubated at 37 °C for 12 hours. Distilled water was used as the control. Finally the diameter of the zone of inhibition (DZI) was measured. In addition to the antibacterial activity, the presence of four phytochemicals in fruit juice samples was also checked.

\textbf{Tannins:} A total of 2ml of each juice sample was taken, mixed with the same amount of distilled water, and heated at 100°C for 10 min in a water bath. A total of five drops of 1% ferric chloride was added and the colour change was observed.

\textbf{Reducing Sugar:} A total of 2ml juice sample was boiled with same amount of Benedict’s solution for 10 min at 100°C. Then the colour change was observed.

\textbf{Flavonoids:} A total of 0.2 ml juice sample was added to 0.2 ml of NaOH. Then 1-2 drops of HCl was added to the solution. Finally the colour change was observed.

\textbf{Phlobatannins:} A total of 0.1 ml of HCl was added to 2 ml of juice sample. The sample was boiled in a water bath for 10 min. Finally the colour change was observed.

DNA Barcoding

Genomic DNA were extracted from young leaf samples of six sweet orange cultivars and sour orange using Dneasy® plant mini kit (QIAGEN, Solna, Sweden). PCR amplification was carried out with \textit{trnH–psbA} primer pair (Pang et al., 2012) using a thermal cycler (Takara, Japan) with initial denaturation at 94 °C for 5 min; 35 cycles of denaturation at 94 °C for 30 sec, annealing at 57 °C for 1.5 min, extension at 72 °C for 2.5 min and final extension at 72 °C for 10 min. A 15 µl PCR mixture was prepared containing 1× Gotaq® Green Master Mix (Promega Corporation, Madison, Wisconsin, USA), 0.5 µM each of forward and reverse primers and 1.5 µl of DNA template. The PCR products were sequenced using Sanger Sequencing based ABI 3500 Series Genetic Analyzer (Applied Biosystems®).

Data Analysis

The antibacterial data were analyzed using
ANOVA procedure in the statistical package SAS 9.13 (SAS Institute, Cary, NC, USA). The DNA sequence alignment analysis was carried out and a dendogram was constructed using the multiple sequence alignment package Clustal Omega (http://www.ebi.ac.uk).

RESULTS

Antibacterial Activity

The sweet orange cultivars, Arogya and MKD did not exhibit any antibacterial activity and no inhibition zone was observed in the control (Figure 01). Sour orange demonstrated the highest antibacterial activity with 10.8 mm of mean DIZ against E. coli and S. aureus and 9.3 mm of mean DZI against MRSA. The sweet orange cultivar Bibila sweet also exhibited significantly higher activity against E. coli, S. aureus and MRSA (9.5 mm, 10.2 mm and 6.3 mm respectively). Bibila sweet and sour orange exhibited more or less similar antibacterial activities. The sweet orange cultivar Sisila, BAN and MT showed significantly higher antibacterial activity against E. coli and S. aureus but they had no activity against MRSA (Table 01).

Phytochemicals

The studied phytochemicals tannins, reducing sugar, flavonoids and phlobatannins were present in all the studied sweet orange cultivars and sour orange.

Figure 01: Antibacterial activity of sweet and sour orange juices. i, ii and iii: three replicated petri dishes grown with E. coli. iv, v and vi: three replicated petri dishes grown with S. aureus. vii, viii and ix: three replicated petri dishes grown with methicillin resistant S. aureus. A to F are paper discs moistened with sweet orange juice. A: Arogya, B: Bibila sweet, C: MKD, D: Sisila, E: BAN, F: MT. G is the paper disc moistened with sour orange juice. H: control. The bacterial solution was streaked on to MHA plates and then respective paper discs were placed to examine the inhibition of bacterial growth around the disc. The ring shape zones around the disc marked the antibacterial activity and the thickness of the ring-shaped zone was measured and statistically analyzed (Table 01).
PCR amplification yielded approximately 440 bp band for all the sweet orange cultivars and sour orange (Figure 02). The DNA sequence alignment diagram (Figure 03) revealed that each DNA barcode is different from each other, making the cultivar or species is specific. Out of 442 bases aligned, 66 (15%) bases represented single nucleotide polymorphisms (SNPs). Out of the 66 SNPs, only one INDEL position was observed between sour orange and sweet oranges, possibly contributing to the species divergence. Out of the 66 SNPs, a total of 55 SNPs were observed within sweet orange cultivars in which 51 were di-allelic and four were tri-allelic.

The dendrogram resulted from DNA sequence differences at trnH–psbA region revealed clear separation of sour orange from the sweet oranges at 56% of the genetic similarity coefficient. At 68% of genetic similarity, all sweet orange cultivars were clustered together. MT and Arogya were the genetically most similar sweet orange cultivars (85%) (Figure 04).

**DISCUSSION**

*Citrus* germplasm has a huge diversity in Sri Lanka. Although sweet orange cultivars have been characterized (Herath et al., 2014) and released (DOA, 2015), their true biochemical and economic potentials have not been characterized and utilized. Being a popular and one of the tastiest fruits, sweet orange processes a huge economic value as an industrial cash crop. Historically, many orange types were heavily utilized by indigenous medicinal practices and preparations (Seenivasan et al., 2006). The ethnobotanical knowledge base is immense regarding the use of *Citrus* in indigenous medical practices (Fonseka, 1902). In many countries these ethnobotanical knowledge bases were further explored and interesting bioactive properties of *Citrus* have been identified. Antioxidant activity (Abeyesinghe et al., 2007) of sweet orange is well documented and attributed to many important phytochemicals present in *Citrus* fruits.

In the present study antibacterial activity of sweet orange cultivars and sour orange in Sri Lanka were evaluated against three model pathogenic bacterial species. In a similar study, the stem oil formulations from *Citrus aurantifolia* were tested with agar well diffusion method to identify the antibacterial effects against *S. aureus*, *E. coli* and *Salmonella paratyphi* and as the parameters of antibacterial activity, zone of inhibition and MIC were measured (Aibinu et al., 2007). Although the present study focused on fruit juice, Kabra et al., (2012) found that *Citrus medica* peels comprise antibacterial activity equivalent to 100 ppm of streptomycin, a well-known antibiotic. The methanol extracts from peels of sweet and sour

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**Table 01: Antibacterial activity of the fruit juice of sweet orange cultivars and sour orange**

<table>
<thead>
<tr>
<th>Sweet orange cultivar</th>
<th>E. coli</th>
<th>S. aureus</th>
<th>Methicillin Resistant S. aureus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arogya</td>
<td>0.0d</td>
<td>0.0d</td>
<td>0.0c</td>
</tr>
<tr>
<td>Bibila sweet</td>
<td>9.5b</td>
<td>10.2a</td>
<td>6.3b</td>
</tr>
<tr>
<td>MKD</td>
<td>0.0d</td>
<td>0.0d</td>
<td>0.0c</td>
</tr>
<tr>
<td>Sisila</td>
<td>8.2c</td>
<td>7.8c</td>
<td>0.0c</td>
</tr>
<tr>
<td>BAN</td>
<td>8.0c</td>
<td>9.7b</td>
<td>0.0c</td>
</tr>
<tr>
<td>MT</td>
<td>8.5c</td>
<td>7.8c</td>
<td>0.0c</td>
</tr>
<tr>
<td>Sour orange</td>
<td>10.8a</td>
<td>10.8a</td>
<td>9.3a</td>
</tr>
<tr>
<td>Control</td>
<td>0.0d</td>
<td>0.0d</td>
<td>0.0c</td>
</tr>
</tbody>
</table>

* Means denoted by the same letters in a column are not significantly different at $P<0.05$
orange were proven to be inhibitory against *Klebsiella pneumoniae* and *B. cereus* (Madhuri, 2014). The *Citrus* extracts were historically reported to be effective against pathogenic bacteria living in the digestive tract. Twenty five percent concentrated lemon juice was found to be effective against enteric pathogens *E. coli*, *S. paratyphi* and *Shigella sonnei* (Bansode and Chavan, 2012). Similarly, antibacterial activity of lemon peel extracts (Dhanavade et al., 2011; Dimić et al., 2012) sweet orange leaf extracts (Ekwenye and Edeha, 2010), lemon extract (Hindi and Chabuck, 2013), mandarin (Khalil et al., 2003), and *Citrus* based natural extracts against *E. coli* (Nannapaneni et al., 2008) were reported. Even the dried *Citrus* materials were proven to possess significant antibacterial effects (Samarakoon et al., 2012). Grapefruit, lemon, sweet orange and lime were proven to be very successful against pathogens in gastrointestinal tract (Srividhya et al., 2013). Therefore the antibacterial activity of sweet and sour orange detected in the present study is in line with the findings of other studies and especially highlights the important of sweet orange. In the present study it was found that certain sweet orange cultivars do not possess antibacterial activities against the model pathogens, implying the presence of bioactive compounds in different sweet orange cultivars in variable quantities. If sweet orange cultivars to be industrially used as a source of antibacterial compounds for extractions or value additions, the results of the present study would be very useful. In addition to the antibacterial activity, *Citrus* is often hailed as a genus with diverse medicinal values such as anticancerous properties. Lai et al., (2013) reported the flavonoids in *Citrus* peel can successfully suppress human prostrate xerographic tumors. They also suggested oral administration of peel extracts reduced the tumor weight by 57% to 100% and down regulate the expression of inflammatory enzymes. Lai et al., (2013) also suggested fruit and vegetable consumption as a nontoxic healing method of lethal cancers. The anticancerous activity of *Citrus* was further verified by work reported in Miller et al., (2004). Polymethoxyflavones in *Citrus* peels are related to inhibition of angiogenesis and other developmental activities of cancer (Wang et al., 2014). The alkaline extracts of *Citrus reticulata* were also found to be effective against pulmonary fibrosis (Zhou et al., 2013). Thus the antibacterial activity and other medicinal attributes such as anticancerous properties due to phytochemicals such as tannins and flavonoids clearly highlights the importance of characterizing sweet orange, sour orange and other *Citrus* germplasm further, for detailed medicinal properties.

![Figure 02: PCR amplification of trnH-psbA region in tested sweet orange cultivars and sour orange. A: Arogya, B: Bibila sweet, C: MKD, D: Sisila, E: BAN, F: MT, G: Sour orange, H: Negative control. M indicates the 100bp ladder (included twice).](image-url)
Sour orange

Figure 03: DNA sequence alignment diagram for trnH–psbA region of the chloroplast genome of sweet orange cultivars and sour orange. The alignment was obtained by using clustal omega software. The symbol ‘*’ indicates monomorphic nucleotides across the studied sweet orange cultivars and sour orange. Yellow shadings indicate the polymorphic nucleotides (i.e. SNPs). The sweet orange cultivar names or sour orange are indicated in the left and number of bases depicted in each line is marked by the number shown at the top right of each section. The DNA sequence differences shown in this illustration were used to draw the genetic dissimilarity diagram (i.e. dendrogram) shown in Figure 04.

Figure 04: Genetic similarity structure of tested sweet orange cultivars and sour orange based on the genomic DNA sequence of trnH–psbA region in the chloroplast genome. The SNPs depicted in sequence alignment diagram (Figure 03) were used to calculate the pair wise similarity of sweet orange cultivars and sour orange. A complete fruit, a cross section and a carpel of each cultivar are shown alongside. Note that sour orange is an out-group indicating the highest molecular divergence from sweet orange which is a different species. The scale bar is only relevant to fruit related images and represent 1 cm in actual size.
The sweet orange cultivars in Sri Lanka primarily consist of selections and could be traced back to the mother trees that were originally selected. Therefore, within cultivar genetic diversity is not available for these released sweet orange cultivars. In that context, DNA barcoding could be suggested to authenticate the different sweet orange cultivars and sour orange. The authentication of natural health products was reported to be essential and the required PCR based DNA sequencing primer pairs are summarized for plant and animal product DNA barcoding (reviewed in Wallace et al., 2012). SNPs markers and DNA barcoding based on the ITS region was employed to genetically identify Citrus species (Wang et al., 2012) and other medicinal plants (Techen et al., 2014). The present study could be used as an ideal model to characterize the antibacterial or any other important medicinal properties and then to authenticate the tested species and cultivars using DNA barcoding to use them in future large scale industrial applications.

CONCLUSION

The sweet orange cultivars Arogya and MKD do not have any antibacterial activity. The sweet orange cultivars Sisila, BAN and MT only have antibacterial activity against E.coli and S. aureus. Sweet orange cultivar Bibila sweet and sour orange have significantly higher antibacterial activity against E. coli, S. aureus and MRSA. Sweet orange cultivar Bibila sweet can be presented as a better variety with higher antibacterial activity in the fruit juice. The trnH–psbA based DNA barcoding resulted unique DNA sequence based identifiers for all the sweet orange cultivars and sour orange tested in the study.

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Microbial Status of Fresh Cut Cooking Banana Variety Alukesel 
(Musa acuminata × Musa balbisiana, ABB Group) as Affected by Pre-Treatments

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ABSTRACT

Fresh cut (minimally processed) cooking banana variety Alukesel was subjected to different pre-treatments, packed in polystyrene packages and stored at 5-7 °C for a week. Effect of several identified pre-treatments on Total Plate Count (TPC) and Total Yeast and Mould counts (TYM) were evaluated. Bacteria isolated from plates were identified using biochemical tests and molecular tools. In pre-treated samples, microbial counts were within safe-to-consume limits. Bacillus cereus, Enterobacter ludwigii and Bacillus thuringiensis were identified from fresh cut samples using molecular tools. Citric acid at 3% w/v effectively controlled bacteria, yeasts and moulds and this observation was significantly different from the control (p<0.05). The present study has shown that 3% citric acid was the most effective pretreatment for minimal processing of Alukesel which controlled bacteria, yeasts & moulds completely. Alukesel pretreated with 3% citric acid was free from food borne pathogens such as Salmonella, Clostridium, Yersinia, and Listeria.

Keywords: Minimal processing, Alukesel banana, pre-treatments, microbiological quality

INTRODUCTION

Minimally processed products (fresh cuts) are fruits or vegetables subjected to a process of physical alteration such as cutting, trimming, slicing while ensuring that they retain the fresh state after processing (Moretti et al., 2000). The value of fresh cuts lies in the primary characteristics of freshness and convenience. Food safety, nutrition and sensory quality in fresh cuts have to be maintained at a high level, while providing extended shelf-life and freshness (Aguilar et al., 2004).

Fresh cuts deteriorate faster than intact produce. Physical damage during preparation of fresh cuts cause an increase in respiration rates, biochemical changes and microbial spoilage, which may result in deterioration of colour, texture and flavour of the commodity of concern (Aguilar et al., 2004).

Microbiological safety is one of the key factors to be considered during the preservation of fresh cuts. The number of microorganisms found on produce can vary according to product quality, product characteristics, resident micro-flora of the product, handling, processing, storage, distribution and retail display. Further, microbial growth is influenced by the physiology of the fresh cut product, pre- and post-harvest processing treatments and packaging (Watada, 1997).

Microorganisms found in the fresh cuts can be categorized mainly into two types namely, psychrotropic and mesophilic. Some pectinolytic bacteria that can proliferate at low temperature are Bacillus spp., Xanthomonas campestris, Erwinia carotovora and Pseudomonas. Erwinia and Pseudomonas have a competitive advantage over other microorganisms that could potentially be harmful to humans (Hui et al., 2006). In terms of microbial safety, psychrotropic pathogens,
such as *Listeria monocytogenes*, *Aeromonas hydrophila* and *Yersinia enterocolitica* and mesophiles such as *Salmonella*, *Escherichia coli* and *Clostridium botulinum* are of particular concern (Goularte et al., 2004). With sufficient time, several types of yeast and moulds also may grow to levels that cause food spoilage in fresh cuts (Hui et al., 2006).

During preparation of fresh cuts, produce are immersed in pre-treatment solutions at the final stage of handling operations. Compounds such as ascorbic acid, citric acid, sodium metabisulphite, sodium chloride and calcium chloride have been utilized throughout the world as suitable preservatives to manage microbial problems and reduce browning for maintaining quality of fresh cuts such as potato, apple (Ahvenainen, 1996), bell pepper (Ediriweera et al., 2012), Pineapple plus amberella (Daranagama et al., 2012), Ela Batu (*Solanum surattense*) (Dharmabandu et al., 2007) and Jak fruit (Latifah et al., 2007).

Microbiological qualities of certain fresh cuts have been previously reported. According to Kang and Lee (1997), total aerobic plate count in fresh cut green pepper ranged from 4.6 to 7.2 log$_{10}$ CFU/g during a 6 day storage period at 5 °C. As reported by Nur Aida et al., (2007), in processed mung bean sprouts stored at 2 °C, Total plate count (TPC) ranged between 7.05-7.46 log$_{10}$ CFU/g, while Total Yeast and Mould counts (TYM) varied between 2.05-4.15 log$_{10}$ CFU/g. According to Dharmabandu et al., (2007) mean aerobic plate counts of fresh cuts of *Solanum surattense* (Ela Batu) ranged between 66 to 133 CFU/g. Microbial counts should be under the required legal limits to be considered safe to consume in any fresh cut meant for human consumption. Therefore, microbial quality is of dire significance in the preparation and storage of fresh cuts as value added products meant for consumers.

Cooking banana variety *Alukesel* belonging to family Musaceae is a hybrid derived from *Musa acuminata* and *M. balbisiana*, which contains a considerable amount of fiber, carbohydrate, vitamins and potassium (Simmonds, 1966). In Sri Lanka, variety *Alukesel* is commonly used in the preparation of savoury curries and fried chips. This commodity could be stored for longer periods intact, however undergoes browning soon after slicing. Banana thus stains the hands of the handler due to melanin formed on the cut surface. A recent investigation was carried out in our laboratory on the effect of selected pre-treatments on physicochemical / sensory properties and degree of browning of fresh cut *Alukesel* and the major findings have been published (Siriwardana et al., 2015). However, no research data are currently available on microbiological aspects of fresh cut *Alukesel*.

The objectives of current study were (1) to evaluate the microbial status of fresh cut *Alukesel* banana subjected to individual or combinations of pre-treatments of sodium chloride, calcium chloride, citric acid, ascorbic acid, and sodium metabisulphite stored at 5-7 °C for a week and (2) to identify bacteria associated with pre-treated *Alukesel* using molecular tools. If *Alukesel* banana is relatively free of hazardous food-borne pathogens and browning is managed, this type of value-added fresh cuts could be made available to local consumers for preparation of chips and curries.

**MATERIALS AND METHODS**

**Preparation of Alukesel banana**

Cooking banana variety *Alukesel* (*Musa acuminata* × *Musa balbisiana*, ABB Group) of 90 day maturity was purchased from supermarkets in Kiribathgoda, Sri Lanka and transported to the laboratory at the Department of Botany, University of Kelaniya. Banana were peeled and washed with distilled water, dipped in chilled water for 2 min. Subsequently, the produce were cut into slices (8-10 g) using...
a sharp stainless steel knife under aseptic conditions. The resulting slices were separately dipped in selected pre-treatment solutions, singly or in combination, such as 3% (w/v) ascorbic acid (T₁), 3% (w/v) citric acid (T₂), 2% (w/v) ascorbic acid (T₃), 2% (w/v) citric acid (T₄), 1.5% (w/v) citric acid + 1.5% (w/v) ascorbic acid (T₅), 2% (w/v) sodium metabisulphite (T₆), 2% (w/v) calcium chloride (T₇), 2% (w/v) sodium chloride (T₈) or distilled water (control) (T₉) for 5 min. Samples were drained and air dried for 15 min and slices (8-10) were packed in polystyrene packages of 100 g capacity and sealed by placing clip-on lids (Latifah et al., 2000). Packages were labeled and placed on plastic trays (30 cm × 40 cm) and stored in a cold room at 5-7 °C and 80-85% relative humidity. Four replicate packages were used per treatment.

Assessment of microbiological properties

Alukesel samples were subjected to the following microbiological assessments on day 0 (initial day) and after 7 days.

Total aerobic Plate Count (TPC): Twenty (20.0) g of Alukesel was homogenized with 180 mL of sterile 0.9% NaCl (Merck Specialties Private Limited, India) in a blender for 2 min and a dilution series was prepared up to 10⁻⁵. From 10⁻³, 10⁻⁴ and 10⁻⁵ dilutions, 1 mL was plated to 12 mL of molten plate count agar (PCA) (Oxoid Ltd., England). Plating was done in duplicate. Plates were incubated at 28 ± 2 °C for 72 hours and colony forming units (CFU) were determined using the equation described by SLS Part 1 (1991). Four replicate samples were used per treatment.

Total Yeast and Mould count (TYM): One mL from 10⁻³, 10⁻⁴ and 10⁻⁵ dilutions prepared under total aerobic plate count, were separately plated along with 12 mL molten yeast and mould agar (YMA). Plating was done in duplicate. Plates were incubated at 28 ± 2 °C for 72 hours and CFUs were determined (Nur Aida et al., 2007). Four replicate samples were used per treatment.

Identification of bacteria isolated from total plate count

Pure cultures of bacteria were prepared by isolating bacteria from plates prepared for TPC which were grown on nutrient agar (NA) (Oxoid Ltd., England). The plates were incubated at 28 ± 2 °C for 72 hours. Three sets of pure bacterial cultures were prepared and isolated bacteria were labeled as strain 1 (S1), strain 2 (S2), strain 3 (S3), strain 4 (S4) and strain 6 (S6). One set each of bacterial cultures was used to identify physiological and biochemical characters of bacteria through Gram’s staining, endospore staining, motility determination, growth in oxygen environment, growth in anaerobic environment, catalase activity, acid and gas production from glucose, citrate utilization, methyl red test, indole production and lactose fermentation (Benson, 2002). Based on the results of biochemical tests, bacteria were identified up to the genus level with the aid of Bergey’s Manual (Holt, 2000).

Another set of bacterial cultures was deposited in the Department of Botany, Culture collection (University of Kelaniya); a similar set of 2–day old cultures were subjected to PCR amplification and gene sequencing at Genetech Molecular Diagnostic & School of Gene Technology, Colombo 8, Sri Lanka.

DNA Extraction and PCR amplification: Total genomic DNA was isolated from colonies of S1, S2, S3, S4 and S6 (on nutrient agar) using the Invitrogen PureLink Genomic DNA mini Kit (Cat No: K1820-01) and extracted DNA was stored in 100 μL of TrisEDTA pH=8 buffer at -20 °C. DNA was then used as a template for the amplification of the 16S rRNA region by using universal primers, 27F/800R and 518F/1492R (Lane et al., 1991, Turner et al., 1999).

Agarose gel Electrophoresis and PCR product purification: The amplified PCR product was visualized on a 2% agarose gel in 0.5 X TBE containing ethidium bromide using a UV transilluminator (E-Gel imager,
Life Technologies Israel). The band size was observed using an appropriate DNA molecular weight marker. PCR products were purified using the PureLink Quick Gel Extraction and PCR Purification Combo Kit (Invitrogen USA Cat No: K2200-01).

**DNA Sequencing:** Purified PCR products were subjected to DNA sequencing using BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystem, USA, Part No. 4337455) and sequenced by programming the ABI Prism® 310 Genetic Analyzer (Applied Biosystem, USA). The 16s rRNA region sequence of the bacteria obtained, was compared with the available sequences in the National Center for Biotechnology Information (NCBI) database, using the BLAST program in order to identify bacteria to species/strain level.

**Statistical analysis**

The experimental arrangements of TPC and TYM were of completely randomized designs (CRD). Data obtained for microbiological properties were subjected to General Linear Model of ANOVA using MINITAB software. When significant effects were observed, treatment means were compared using Tukey’s multiple comparison test at p = 0.05 significance level.

**RESULTS**

**Assessment of microbiological properties**

Total aerobic Plate Count (TPC): The TPC enumerated from samples of *Alukesel* on day 0 ranged from 2.78 – 4.42 log_{10} CFU/g whereas, it ranged from zero to 4.64 log_{10} CFU/g by day 7. The TPC decreased by day 7, when compared to day 0, in most treatments except in 3% ascorbic acid, 2% ascorbic acid, 1.5% citric acid + 1.5% ascorbic acid, 2% sodium metabisulphite and control. On 7\textsuperscript{th} day after storage, the highest TPC of 4.64 log_{10} CFU/g was observed in the 2% ascorbic acid pre-treated samples. Control also indicated a high total plate count value of 4.50 log_{10} CFU/g by day 7. The lowest plate count of zero log_{10} CFU/g was observed in 3% citric acid treated samples (Figure 01). Statistically significant difference (p<0.05) was observed between day 0 and 7 for TPC of fresh cut *Alukesel* samples and the control when the effect of pre-treatment, time and interaction of pre-treatment \times time was considered (Table 01).

Total Yeast and Mould count (TYM): The TYM count enumerated from samples on day 0 ranged from zero to 4.43, which was somewhat similar to results seen on day 7 (zero to 4.10 log_{10} CFU/g). On day 0, yeasts and moulds were not observed in distilled water samples while on day 7 no colonies were observed in 3% ascorbic acid, 3% citric acid, 2% ascorbic acid, 1.5% citric acid + 1.5% ascorbic acid and 2% sodium chloride treated *Alukesel*. In most pre-treated samples, yeast and mould counts decreased by day 7, compared to day zero, except in 2% sodium metabisulphite indicating the antimicrobial activity of certain pre-treatments such as ascorbic acid, citric acid and sodium chloride (Figure 02). Control, by day 7 indicated a TYM count of 3.27 log_{10} CFU/g. There was a significant difference (p<0.05) of TYM counts of samples compared to control when the effect of pre-treatment, time and interaction effect of pre-treatment \times time were considered (Table 01).

**Identification of bacteria isolated from total plate count**

Based on biochemical tests conducted, five strains of bacteria were identified from TPC experiment (Table 02). Strains 1, 2, 3 and 6 were identified as *Bacillus sp.* and stain 4 as *Enterobacter spp.*
During molecular identification based results obtained from NCBI BLAST tool, the identity of S1, S2, S3, S4 and S6 bacterial cultures were confirmed as \textit{Bacillus cereus}, \textit{Bacillus cereus}, \textit{Bacillus cereus}, \textit{Enterobacter ludwigii} and \textit{Bacillus thuringiensis}. These results were deposited in Genebank under the accession numbers KT970720, KT970721, KT970722, KT970723 and KT970724.

Figure 01: Effect of selected pre-treatments on TPC of fresh cut Alukesel banana, in comparison to control, 0 and 7 days after storage at 5-7 °C. (T\textsubscript{1} - 3\% ascorbic acid, T\textsubscript{2} - 3\% citric acid, T\textsubscript{3} - 2\% ascorbic acid, T\textsubscript{4} - 2\% citric acid, T\textsubscript{5} - 1.5\% citric acid + 1.5\% ascorbic acid, T\textsubscript{6} - 2\% sodium metabisulphite, T\textsubscript{7} - calcium chloride, T\textsubscript{8} - sodium chloride, T\textsubscript{9} - distilled water (control)). Data points of TPC represent the mean of eight replicates ± standard error.

Table 01: ANOVA of different pre-treatments and time on TPC and TYM count of fresh cut Alukesel.

<table>
<thead>
<tr>
<th>Factor</th>
<th>d.f.</th>
<th>F value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TPC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A (pre-treatment)</td>
<td>8</td>
<td>237.78</td>
<td>0.00</td>
</tr>
<tr>
<td>B (time)</td>
<td>1</td>
<td>229.86</td>
<td>0.00</td>
</tr>
<tr>
<td>A × B</td>
<td>8</td>
<td>304.6</td>
<td>0.00</td>
</tr>
<tr>
<td>TYM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A (pre-treatment)</td>
<td>8</td>
<td>10.43</td>
<td>0.00</td>
</tr>
<tr>
<td>B (time)</td>
<td>1</td>
<td>40.15</td>
<td>0.00</td>
</tr>
<tr>
<td>A × B</td>
<td>8</td>
<td>11.25</td>
<td>0.00</td>
</tr>
</tbody>
</table>

\text{d.f.} = \text{degrees of freedom}

P<0.05 indicates a significant difference among variables and control.
Table 02: Characteristics of bacteria associated with fresh cut Alukesel.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Strain 1</th>
<th>Strain 2</th>
<th>Strain 3</th>
<th>Strain 4</th>
<th>Strain 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colony colour</td>
<td>White</td>
<td>White cream</td>
<td>White</td>
<td>Light yellow</td>
<td>White</td>
</tr>
<tr>
<td>Colony shape</td>
<td>Round with irregular margins</td>
<td>Round with irregular margins</td>
<td>Irregular and spreading</td>
<td>Round with entire margins</td>
<td>Round with lobate margins</td>
</tr>
<tr>
<td>Cell shape</td>
<td>Rod</td>
<td>Rod</td>
<td>Rod</td>
<td>Rod</td>
<td>Rod</td>
</tr>
<tr>
<td>Gram’s reaction</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Endospore formation</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Motility</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Growth under Oxygen</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Growth in Anaerobic Jar</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Acid production from Glucose</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Citrate Utilization</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Catalase test</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Indole Production</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Methyl Red Test</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Lactose</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Fermentation</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Figure 02: Effect of selected pre-treatments on TYM count of fresh cut Alukesel banana, in comparison to control, 0 and 7 days after storage at 5-7 °C. (T₁ - 3% ascorbic acid, T₂ - 3% citric acid, T₃ - 2% ascorbic acid, T₄ - 2% citric acid, T₅ - 1.5% citric acid + 1.5% ascorbic acid, T₆ - 2% sodium metabisulphite, T₇ - calcium chloride, T₈ - sodium chloride, T₉ - distilled water (control)). Data points of TYM count represent the mean of eight replicates ± standard error.
DISCUSSION

TPC provides information on the microorganisms (especially bacteria) growing aerobically at 30 °C, under the specified conditions (SLS Part 1, 1991). In Alukesel, there was a significant difference in the TPC between pre-treatments and the control, indicating the antimicrobial efficacy of pre-treatments used in this study. The most promising results in controlling TPC were displayed by Alukesel samples pre-treated with 3% citric acid, which was able to control the microbial growth completely, by day 7. It was also evident that the lower concentration of citric acid was less effective in controlling the microbial growth. According to a recent publication by our research group, it was reported that 3% citric acid pretreatment effectively controlled browning and maintained acceptable quality in fresh cut Alukesel (Siriwardana et al., 2015). Similarly, Daranagama et al., (2012) reported 4.93 log_{10} CFU/g value for TPC for fresh cut ambarella (Spondias dulcis) and pineapple together as a mixed load, after pretreating with 1% citric acid and storing at 5-7 °C for seven days. Citric acid is one of the best known chelating agents, which can chelate Cu to control browning in minimally processed fruits and vegetables (Manolopoulou and Varzakas, 2011). Further, citric acid inhibits the growth of microorganisms by lowering the pH of processed commodity (Hui et al., 2006).

According to Kumari (2009), the microbial level of all pre-treated fresh cut samples of Lasia spinosa treated with 0.5% ascorbic acid increased, during the storage period of 7 days at 5-7 °C. This is in agreement with the present data as a slight increase in TPC could be observed for samples pre-treated with 3% ascorbic acid, 2% ascorbic acid and 1.5% citric acid + 1.5% ascorbic acid by day 7, when compared to day 0. This may be possible as ascorbic acid can act as an intermediate in the metabolism and the growth rate of microorganisms, which can lead to slight increase in their proliferation. Sodium metabisulphite (at 2 %) did not display antimicrobial effect as TPC increased after 7 days of storage, although the same treatment was relatively effective in controlling browning of fresh cut Alukesel as reported by Siriwardana et al., (2015).

The mesophilic aerobic bacterial counts on finished cut vegetables ranged depending on the commodities, season of the year and growing regions. According to Heard (2000), aerobial bacterial counts on bagged salads from the retail market ranged from 4.0-9.0 log_{10} CFU/g. Allende et al., (2004) reported that initial aerobic mesophilic bacterial load on fresh cut baby spinach leaves after washing with 2.0 mM chlorine was 7.2 log_{10} CFU/g. However, it was reported that microbial populations of fresh cut baby spinach leaves increased up to 9.0 log_{10} CFU/g level, during storage of 12 days at 5 °C. According to Francis et al., (1999), the legal regulations on fresh cut vegetables established a maximum total limit of 7.7 log_{10} CFU/g for TPC. In the present study, TPC ranged from zero to 4.64 log_{10} CFU/g by day 7. It is important to highlight at this point that the TPC counts of test samples from all pre-treatments of Alukesel did not exceed the legal limit indicating that the samples processed this way are safe to consume.

The TYM counts provide a general guidance for the enumeration of viable yeasts and moulds in products at specified conditions (SLS Part 2, 1991). TYM were found to be slightly lower than TPC for Alukesel samples ranging from 0 - 4.10 log_{10} CFU/g on day 7. These results are also in agreement with those of Nur Aida et al., (2007) for minimally processed mung bean sprouts, where values ranged from 2.05 - 4.15 log_{10} CFU/g. According to Nur Aida et al., (2007) bacterial populations dominate over yeasts and moulds in vegetables. The yeasts and moulds were completely absent in the cooking banana samples pre-treated with 3% ascorbic acid, 3% citric acid, 2% ascorbic acid, 1.5% citric acid + 1.5% ascorbic acid and 2% sodium chloride. Both ascorbic and citric acid inhibit the growth of yeasts and moulds by lowering...
the pH of processed commodity. Similarly, Ediriweera et al., (2012) reported zero yeast and mould counts for fresh cut bell pepper, treated with 1% sodium chloride and stored at 5-7 °C by day 7. When sodium chloride is incorporated as a pre-treatment, high osmotic pressure results in plasmolysis of microbial cells causing dehydration and inhibition of growth of microorganisms. Further, chloride ion is toxic to most microorganisms (Hui et al., 2006). According to Nur Aida et al., (2007) the recommended limit for TYM of fresh cut produce is $5 \log_{10} \text{CFU/g}$. Since the TYM in all the pretreated samples did not exceed the recommended limit, fresh cuts of Alukesel can be considered safe to consume. In most of pre-treated Alukesel samples, yeast and mould counts on day 7 were controlled at zero level. Therefore no attempt was taken to identify yeast and moulds to genus or species level during present research.

Using molecular tools, bacteria isolated from TPC were identified as Bacillus cereus, Enterobacter ludwigii and Bacillus thuringiensis. B. cereus can readily be isolated from various food products. This species is common in nature, and may survive different stresses during food production due to chemical and heat resistant endospores. Some strains of B. cereus are harmful to humans and cause foodborne illness, while other strains can be beneficial as probiotics for humans (Doyle et al., 1989). B. thuringiensis is used worldwide as a biological insecticide due to its ability to produce crystal proteins with highly specific activity against certain insects. B. cereus and B. thuringiensis are closely related and genomic studies have proposed that they should be merged into a single species (Helgason et al., 2000). After commercialization of B. thuringiensis-based insecticides, studies have shown that B. thuringiensis like B. cereus produces enterotoxins responsible for human diarrhoea. B. thuringiensis has however, only in one case been described to be implicated in food borne disease (Jackson et al., 1995). One of the bacteria isolated and identified from Alukesel – E. ludwigii belongs to Enterobacter cloacae complex consisting six bacterial species which are widely encountered in nature. Some stains of this species are part of the commensals enteric flora of the human gastro-intestinal tract, while some strains include human pathogens and have been previously isolated in human clinical specimens (Mezzatesta et al., 2012). E. ludwigii has previously been isolated as an endophytic bacteria dwelling in strawberry fruit (De Melo Pereira et al., 2012).

This is the first report on the evaluation of microbial levels of fresh cut Alukesel, using molecular tools although related work on physicochemical/sensory properties and degree of browning of fresh cut Alukesel have been reported recently (Siriwardana et al., 2015). Although pre-treatments of ascorbic acid (3%) and citric acid + ascorbic acid (1.5% each) were better in controlling browning and sensory properties (Siriwardana et al., 2015), microbial growth was not controlled to a significant level, using the above individual or combined treatments. However, 3% citric acid pre-treatment significantly reduced microbial growth by reducing TPC and TYM counts to zero level on day 7. As indicated by our previous research, Alukesel pre-treated with 3% citric acid retained acceptable sensory properties and also managed browning to a greater extent (Siriwardana et al., 2015). Microorganisms such as Salmonella, Clostridium, Yersinia and Listeria are reported to be transmitted through contaminated food products, causing food borne diseases in humans and animals. Therefore, utmost care should be taken in identifying and quantifying microorganisms surviving on different commodities before any minimal processing technique is implemented on commercial scale in any country. However, no evidence of any of the above hazardous food-borne organisms were found during molecular identification of microorganisms from fresh cut Alukesel.
CONCLUSION
In conclusion, the present study has shown that 3% citric acid was the most effective pre-treatment for minimal processed Alukesel, which controlled bacteria, yeasts & moulds completely. Alukesel pre-treated with 3% citric acid was free from food borne pathogens such as Salmonella, Clostridium, Yersinia and Listeria.

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REFERENCES


Impact of Insurgence on the Agricultural Development in Nigeria

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ABSTRACT

Low agricultural production and productivity in Nigeria over the years compared to leading countries like Malaysia, Thailand, Indonesia, and Brazil have been largely ascribed to low fertilizer usage, low utilization of improved seed, inadequate government expenditure and the inability to compete with other sectors. The issues of environmental sustainability, capital accumulation, foreign exchange earnings ability and well-being vis-à-vis production, productivity and agricultural development are rarely considered. The study examined the impact of insurgence on the agricultural development in Nigeria using secondary time-series data collected on Nigerian agricultural share of GDP, infant mortality rate, CO₂ emission from fuel combustion and level of food production as proxies for agricultural transformation for the years, 1960-2011 while Nigerian civil war, Boko-Haram, Niger-Delta, Fulani herdsmen insurgences were used as proxies for insurgence. The data were analysed using the Vector Error Correction Model (VECM) after testing for stationarity, co-integration and lag selection using the Augmented Dickey-Fuller (ADF), Johansen and the Schwarz's Bayesian Information Criterion (SBIC) statistics respectively. The results from the VECM showed that a unit decrease in previous year food production level would increase the share of agriculture to GDP by 4.26% the following year while a shift from non-insurgence to insurgence in any year by Boko-Haram, Niger-Delta and Fulani herdsmen reduced the share of agriculture to GDP by 17.56%, 19.45% and 17.47% respectively. A similar shift from non-insurgence to insurgence in any year by Boko-Haram and Fulani herdsmen insurgences reduced food production level, on average, by 10.21 and 4.69 tonnes respectively while a shift from non-insurgence to insurgence in any year by Niger-Delta crisis and Fulani herdsmen increased CO₂ emission, on average, by about 5% and 8% respectively. It is inferred, from the results, that agricultural development should be all-embracing since its component elements have a long-run equilibrium relationship, that insurgence indirectly impact on agricultural development through its effect on the change in food production level, the share of agriculture to GDP, CO₂ emission from fuel combustion and infant mortality, and that attempt at ignoring the insurgence by any sect from any region, whether religious, cultural, or communal is also a threat to agricultural development.

Keywords: VECM, insurgence, productivity, Eigen-value, trace

INTRODUCTION

Nigeria is a populous Black African nation, blessed with population of over 160 million people, with wide geographical spread across 36 States and a Federal Capital Territory (Akhemonkhan, et al, 2012). Low agricultural production and productivity in Nigeria over the years compared to leading countries like Malaysia, Thailand, Indonesia, and Brazil have been largely ascribed to low fertilizer usage, low utilization of improved seed, inadequate government expenditure and the inability to compete with other sectors (Olatunbosun,
The Federal Government of Nigeria through the Federal Ministry of Agriculture and Rural Development (FMARD), in a bid to revamp the agriculture sector, ensure food security, diversify the economy and enhance foreign exchange earnings, embarked on Agricultural Transformation Agenda. This transformation agenda focused on the development of agricultural value chains, including the provision and availability of improved inputs, increased production and improved productivity, as well as the establishment of staple crop processing zones. Towards achieving a successful agricultural transformation, FMARD is of the opinion that policies regarding agriculture, financial services, industry, and market development need review. That could be said of without a *ceteris paribus* hypothesis when Nigeria was a *household*. Agricultural transformation goes beyond production and productivity to include environmental sustainability, capital accumulation, foreign exchange earnings ability and well-being.

Nigeria was one of the relatively secured nations in West African sub-region until recently, when the nation suddenly metamorphosed into an abode of serial bombing, hostage-taking, armed robbery, cold-blooded killings and ethno-religious conflicts traceable to militant groups with conflicting ideological, socio-economic, political and religious agenda (Akhemonkhan *et al*, 2012). Fwatshak and Larab (2004) posit that since independence, not a single decade has passed without at least one major civil crisis in Nigeria. It experienced the Western Region political crises in 1960s while the last three to four decades also witnessed some of the worst civil and sectarian crises. Cases in point include incessant military coups, and a fratricidal civil war between 1967 and 1970, the Maitasine riots, starting in Kano and spreading to most parts of Northern Nigeria in the 1980s, ethno-religious crises in Kafanchan and Zango Kataf both in Southern Kaduna in 1987 and 1992, and the June 12, 1993 post-election crises, the Niger-Delta insurgence, Bakasi Boy, O’odua People’s Congress, the current Boko-Haram and Fulani herdsmen insurgences (Darmer, 2004; Albert, 2005; and Tella, 2012). The resultant loss of lives, rising budgetary spending on security, and destruction of valuable government facilities portend devastating consequences for sustainable economic development in the country. Could low agricultural production be tied to these? What possible effects have all these on the Agricultural development in the country? This study, therefore, examined the impact of insurgency on Nigerian agricultural development.

**METHODOLOGY**

The data used for the study were mainly secondary, and covered a 56-year annual time-series data for the period 1960-2011 on annual Gross Domestic Product (GDP), Food Production level, carbon (IV) oxide (CO$_2$) emission from gaseous fuel consumption and Infant Mortality rate of Nigeria as the measures for foreign exchange earnings ability, capita formation, environmental sustainability and well-being respectively. The data were derived from Central Bank of Nigeria (CBN) statistical bulletin (CBN, 2012) and Food and Agriculture Organization (FAO) Year Book (2013) and World Development Bank indicators bulletin (2012). National insurgences, as exogenous variables were dummied for the different insurgence in the country since 1960. Such insurgences, included in the study, were the Nigerian civil war, Ethno-religious crisis, Niger-Delta, Boko-Haram sect insecurity challenge and the Fulani herdsmen of national concern. The insurgences were dummied with value 1 for the years they occurred and 0 otherwise. The Augmented Dickey-Fuller (ADF) unit-root test was used to test for non-stationarity, Co-integration techniques were used to establish valid relationship among the endogenous variables while the relationship was tested.
using Johansen co-integration test (Hai et al., 2004) while number of lags was selected using the Schwarz’s Bayesian Information Criterion (SBIC). The dynamic model underlying the equation was written, in generic form, as a Vector Error Correction Model (VECM), with four equations, one for each of the endogenous variables as:

$$\Delta Z_t = \sum_{j=1}^{p-1} \phi_j \Delta Z_{t-j} + \gamma' Z_{t-1} + x_t + \mu_t$$ \[1\]

where is a column vector of four variables and represents the exogenous variables The $\phi_j = [j=1, \ldots, (p-1)]$ are a set of $(4 \times 4)$ matrices of parameters on the dynamic terms of the model, where the preset lag-length of the model is $p$. Attention was focused on the long-run part of the VECM, where $\gamma'$ is the co-integrating vectors respectively, and $\gamma$ is $nxr$ matrix to reflect the reduced rank of the system, where it was implicitly assumed that there are $r < n$ co-integrating vectors in the model, $\mu_t$ as a vector of white-noise error terms, with $\mu_t \sim N(0, \sigma)$ and Where $\phi = -\sum_{j=1}^{p} A_j$ and $-\gamma' = I - \sum_{j=1}^{p} A_j$, $j=1,2,\ldots,p-1$ from the corresponding VAR process of finite order $p$, given as $z_t=\sum_{j=1}^{p} A_j z_{t-j} + x_t + \mu_t$. The Vector Error Correction Model was used because the time series were not stationary in their levels but in their first difference, and co-integrated. The VECM was explicitly written as:

$$\Delta GDP = \Delta GDP_{t-1} + \Delta GDP_{t-2} + \Delta GDP_{t-3} + \gamma' GDP_{t-2} + \gamma' GDP_{t-3} + \gamma' GDP_{t-4} + \gamma' GDP_{t-5} + \gamma' GDP_{t-6}$$
$$+ C_{t-1} + C_{t-2} + C_{t-3} + C_{t-4} + C_{t-5} + C_{t-6} + W_{t-1} + W_{t-2} + W_{t-3} + W_{t-4} + W_{t-5} + W_{t-6}$$

RESULTS AND DISCUSSION

The ADF statistics for the variables at level form and first difference are shown in Table 01. The ADF statistic values for the variables at their level form were lower than the critical values at 1%, 5% and 10%, so that the null hypothesis that they have unit root at level form is rejected. However, the ADF statistic values for the variables at their first difference were greater than the critical values at 1%, 5% and 10%, so that the null hypothesis that they have unit root at first difference is not rejected. Augmented Dickey-Fuller (ADF) test for the variables indicate that all variables are non-stationary at level form but stationary at first difference. This indicates that the variables are integrated, at least, of order one $I(1)$ and any attempt to specify the equation in the level form of the series will be inappropriate and may lead to the problem of spurious regression. In particular, the results of econometric analysis at the level of the series may not be suitable for policy making.

The Johansen trace statistic test and maximum likelihood test is presented in Table 02. The results showed that the trace values at both none and at most one of 55.48 and 30.21 respectively were higher than their corresponding critical values at 5% level of significance. This implies that the null hypothesis of no co-integrating relationship can be rejected at both the 5% and 1% levels of significance for GDP, Infant mortality, CO$_2$ emission and food production level in Nigeria. Trace test indicated two co-integrating equations at the 5% level. Similarly, the Johansen maximum Eigen statistics at both none and at most 1 of 25.27 and 22.81 respectively were, higher than their corresponding critical values at 5% level of significance. Maximum Eigen-value test also indicated two co-integrating equations at the 5% level. This also implied that the null hypothesis of no co-integrating relationship can be rejected at both the 5% and 1% levels of significance for GDP, Infant mortality, CO$_2$ emission and food production in Nigeria, implying that a form of long-run equilibrium relationship exist among GDP, Infant mortality, CO$_2$ emission and food production in Nigeria, and are integrated at order $I(2)$. This also implies that there exists an error-correction model that describes the short-run dynamics consistently with the long-run relationship.
Table 01: Augmented-Dickey-Fuller (ADF) Unit Root Test

<table>
<thead>
<tr>
<th>Variable</th>
<th>Augmented-Dickey-Fuller</th>
<th>Augmented-Dickey-Fuller</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>With Intercept</td>
<td>With Intercept and Trend</td>
</tr>
<tr>
<td></td>
<td>Level</td>
<td>First Difference</td>
</tr>
<tr>
<td>GDP</td>
<td>0.925</td>
<td>-6.907*</td>
</tr>
<tr>
<td>CO₂</td>
<td>-1.679</td>
<td>-7.082*</td>
</tr>
<tr>
<td>Infant Mortality</td>
<td>0.486</td>
<td>-4.311*</td>
</tr>
<tr>
<td>Food Production</td>
<td>2.388</td>
<td>-7.723*</td>
</tr>
<tr>
<td>Niger-Delta Crisis</td>
<td>-2.313</td>
<td>-9.628*</td>
</tr>
<tr>
<td>War</td>
<td>-2.773</td>
<td>-3.016*</td>
</tr>
</tbody>
</table>

*That the null hypotheses that the series contain a unit root are rejected at 1% significance level.

Table 02: Unrestricted Co-integration Rank Test (Trace and Maximum Eigen-value)

<table>
<thead>
<tr>
<th>Hypothesized No. of CE(s)</th>
<th>Eigenvalue</th>
<th>Trace Statistic</th>
<th>0.05 Critical Value</th>
<th>Prob.**</th>
<th>Max-Eigen Statistic</th>
<th>0.05 Critical Value</th>
<th>Prob.**</th>
</tr>
</thead>
<tbody>
<tr>
<td>None *</td>
<td>0.415892</td>
<td>55.48356</td>
<td>40.17493</td>
<td>0.0008</td>
<td>25.27045</td>
<td>24.15921</td>
<td>0.0353</td>
</tr>
<tr>
<td>At most 1 *</td>
<td>0.384473</td>
<td>30.21311</td>
<td>24.27596</td>
<td>0.0080</td>
<td>22.80803</td>
<td>17.79730</td>
<td>0.0081</td>
</tr>
<tr>
<td>At most 2</td>
<td>0.140940</td>
<td>7.405081</td>
<td>12.32090</td>
<td>0.2866</td>
<td>7.140083</td>
<td>11.22480</td>
<td>0.2376</td>
</tr>
<tr>
<td>At most 3</td>
<td>0.005622</td>
<td>0.264998</td>
<td>4.129906</td>
<td>0.6667</td>
<td>0.264998</td>
<td>4.129906</td>
<td>0.6667</td>
</tr>
</tbody>
</table>

* denotes rejection of the hypothesis at the 0.05 level, **MacKinnon-Haug-Michelis (1999) p-values

The Vector Error Correction Model parameters and their associated standard errors and t-statistics are presented in Table 03. The result showed that in co-integrating equation 1, only Infant mortality and food production were statistically significant at 1% and 5% levels respectively while in co-integrating equation 2, infant mortality, previous year food production level and CO₂ were statistically significant but all at 5% level. The negative sign of the coefficient 0.005603 of food production level in the first co-integrating equation implies a tendency to reduce (correct) large differences in the food production level through an upward adjustment, leading to increasing short-run food production by about 0.006 tonne but a decline in the long-run food production level. Contrary to food production level, the positive sign of the coefficient 0.001384 of infant mortality in first co-integrating equation implies a tendency to correct large differences in infant mortality through a downward adjustment, leading to decreasing short-run infant mortality by about 1 in every 1000 birth but an increase in the long-run. However, the reverse is the case in the second co-integrating equation for food production level and infant mortality with downward and upward adjustment respectively, leading to respective decreasing and increasing short-run in food production level and infant mortality by 0.024 tonne and about four in every 100 birth but increasing and decreasing in the long-run. For CO₂ in the second co-integrating equation, the positive sign of the coefficient 0.017681 implies a tendency to correct large differences in infant mortality through a downward adjustment, leading to decreasing short-run CO₂ by about 0.02% but an increase in the long-run. In the GDP equation, previous year food production level and insurgence from Boko-Haram, Niger-Delta and Fulani herdsmen with respective coefficients of -4.26, -17.56, -19.45 and -17.47 statistically affected the share of agriculture to GDP at 1%, 5%, 1% and 1% levels of significance respectively.
Table 03: Estimates of the Co-integration Equation and Vector Error Correction Model

<table>
<thead>
<tr>
<th></th>
<th>D(GDP)</th>
<th>D(INFMORT)</th>
<th>D(CO2)</th>
<th>D(FOODPR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CointEq1</td>
<td>-0.033752</td>
<td>0.001384***</td>
<td>0.001096</td>
<td>-0.005603**</td>
</tr>
<tr>
<td></td>
<td>(0.04538)</td>
<td>(0.00040)</td>
<td>(0.00168)</td>
<td>(0.00254)</td>
</tr>
<tr>
<td></td>
<td>[-0.74382]</td>
<td>[3.48965]</td>
<td>[0.65162]</td>
<td>[-2.39017]</td>
</tr>
<tr>
<td>CointEq2</td>
<td>0.244287</td>
<td>-0.004418**</td>
<td>-0.01681**</td>
<td>0.024010**</td>
</tr>
<tr>
<td></td>
<td>(0.20987)</td>
<td>(0.00183)</td>
<td>(0.00778)</td>
<td>(0.01084)</td>
</tr>
<tr>
<td></td>
<td>[1.16400]</td>
<td>[-2.40898]</td>
<td>[-2.27290]</td>
<td>[2.21447]</td>
</tr>
<tr>
<td>D(GDP(-1))</td>
<td>0.280047</td>
<td>-0.003546*</td>
<td>0.015879**</td>
<td>0.019950</td>
</tr>
<tr>
<td></td>
<td>(0.23255)</td>
<td>(0.00203)</td>
<td>(0.00862)</td>
<td>(0.01201)</td>
</tr>
<tr>
<td></td>
<td>[1.20426]</td>
<td>[-1.74536]</td>
<td>[1.84221]</td>
<td>[1.66060]</td>
</tr>
<tr>
<td>D(INFMORT(-1))</td>
<td>-9.381258</td>
<td>0.650522***</td>
<td>1.577901</td>
<td>0.463514</td>
</tr>
<tr>
<td></td>
<td>(13.6453)</td>
<td>(0.11923)</td>
<td>(0.05780)</td>
<td>(0.70494)</td>
</tr>
<tr>
<td></td>
<td>[-0.68751]</td>
<td>[5.52310]</td>
<td>[0.31197]</td>
<td>[0.65752]</td>
</tr>
<tr>
<td>D(CO2(-1))</td>
<td>-3.782723</td>
<td>0.019830</td>
<td>0.245333</td>
<td>-0.064705</td>
</tr>
<tr>
<td></td>
<td>(4.14399)</td>
<td>(0.03621)</td>
<td>(0.15360)</td>
<td>(0.21409)</td>
</tr>
<tr>
<td></td>
<td>[-0.91282]</td>
<td>[0.54765]</td>
<td>[1.59791]</td>
<td>[-0.30224]</td>
</tr>
<tr>
<td>D(FOODPR(-1))</td>
<td>-4.262452***</td>
<td>-0.013699</td>
<td>0.053301</td>
<td>-0.374688**</td>
</tr>
<tr>
<td></td>
<td>(1.0034)</td>
<td>(0.07209)</td>
<td>(0.11492)</td>
<td>(0.10107)</td>
</tr>
<tr>
<td></td>
<td>[-3.8756]</td>
<td>[-0.50548]</td>
<td>[0.46382]</td>
<td>[2.33914]</td>
</tr>
<tr>
<td>ETHRE</td>
<td>58.62064</td>
<td>0.250388</td>
<td>-0.746030</td>
<td>1.817173</td>
</tr>
<tr>
<td></td>
<td>(43.1091)</td>
<td>(0.37668)</td>
<td>(1.59790)</td>
<td>(2.22789)</td>
</tr>
<tr>
<td></td>
<td>[1.35982]</td>
<td>[0.66473]</td>
<td>[0.46688]</td>
<td>[0.81394]</td>
</tr>
<tr>
<td>BOKHARAM</td>
<td>-17.5648**</td>
<td>1.677113*</td>
<td>1.035623</td>
<td>-10.20569*</td>
</tr>
<tr>
<td></td>
<td>(8.413)</td>
<td>(0.88613)</td>
<td>(7.75903)</td>
<td>(5.29199)</td>
</tr>
<tr>
<td></td>
<td>[-2.0878]</td>
<td>[-1.89262]</td>
<td>[-0.25500]</td>
<td>[1.94795]</td>
</tr>
<tr>
<td>ND</td>
<td>-19.44645**</td>
<td>1.483331**</td>
<td>4.568898*</td>
<td>-2.964529</td>
</tr>
<tr>
<td></td>
<td>(6.3263)</td>
<td>(0.57081)</td>
<td>(2.42141)</td>
<td>(3.37487)</td>
</tr>
<tr>
<td></td>
<td>[-3.0379]</td>
<td>[2.59865]</td>
<td>[-1.88688]</td>
<td>[-0.87841]</td>
</tr>
<tr>
<td>FHERD</td>
<td>-17.4688***</td>
<td>0.763116</td>
<td>8.148013**</td>
<td>-4.691009***</td>
</tr>
<tr>
<td></td>
<td>(4.8579)</td>
<td>(0.78516)</td>
<td>(3.33070)</td>
<td>(1.64221)</td>
</tr>
<tr>
<td></td>
<td>[-3.5960]</td>
<td>[0.97192]</td>
<td>[-2.44634]</td>
<td>[-2.85652]</td>
</tr>
<tr>
<td>WAR</td>
<td>23.62436</td>
<td>0.478650</td>
<td>-0.435803</td>
<td>-1.037762</td>
</tr>
<tr>
<td></td>
<td>(54.3434)</td>
<td>(0.47565)</td>
<td>(2.01772)</td>
<td>(2.81222)</td>
</tr>
<tr>
<td></td>
<td>[0.43399]</td>
<td>[1.00631]</td>
<td>[-0.21599]</td>
<td>[-0.36900]</td>
</tr>
</tbody>
</table>

|                | 0.274678   | 0.897439   | 0.402158 | 0.222024 |
|                | 0.073200   | 0.054830   | 0.112147 | 0.073919 |
|                | 355871.9   | 25.64363   | 461.4586 | 896.4185 |
|                | 96.59076   | 0.843992   | 3.580265 | 4.900441 |
|                | 1.363314   | 31.50098   | 2.421662 | 1.027390 |
|                | -275.2570  | -52.45258  | -120.3699| -135.9742|
|                | 12.18030   | 2.700110   | 5.590207 | 6.254222 |
|                | 12.61331   | 3.133123   | 6.023220 | 6.687335 |
|                | 21.03915   | -3.387234  | 0.409396 | 1.396596 |
|                | 100.3327   | 2.331413   | 4.096325 | 5.004876 |

|                | -580.7175  | 26.92415   | 28.97112 |

*R-significant at 10% level, **signficant at 5% level, ***significant at 1% level, standard Error in parenthesis and t-Statistics in brackets; GDP is Gross Domestic Product; CO₂ is carbon (IV) oxide; FOODPR is Food Production Level; INFMORT is Infant-mortality; ETHRE is Ethno-religion; BOKHARAM is Boko-Haram; ND is Niger-Delta; FHERD is Fulani Herdsmen. D in front of each of the independent variables implies that the variable is differenced once before becoming stationary.
This means that a unit change in previous year food production level would reduce the share of agriculture to GDP the following year by 4.26% while a shift from non-insurgence to insurgency in any year by Boko-Haram, Niger-Delta and Fulani herdsmen would reduce the share of agriculture to GDP the following year by 17.56%, 19.45% and 17.47% respectively in the short-run. The effect of the change in previous year food production level on share of agriculture on GDP could be attributed to the reduction on food production by insurgency the year before. In the equation explaining the change in infant mortality, unit change in the immediate past year GDP and infant mortality as endogenous variables significantly affected infant mortality while Boko-Haram and the Niger-Delta insurgency significantly affected infant mortality. The coefficient of the immediate past year GDP and infant mortality were -0.004 and 0.659 respectively while Boko-Haram and Niger-Delta insurgency had coefficients of -1.677 and 0.571 respectively. These mean that 1000 unit decrease in immediate past year GDP would increase infant mortality by four in every 1000 children under the age of five years while 10 units increase in the immediate past year infant mortality would increase mortality of children under the age of five years by seven in every 1000 birth in the short-run. Also a shift from non-insurgence to insurgency of Boko-Haram in the current year would increase mortality of children under the age of five years by 2% on average while a similar move from non-insurgence to insurgency of Niger-Delta in the current year would increase it by 1% on the average in the short-run. In the equation explaining the CO$_2$ emission from fuel consumption, only Niger-Delta and Fulani herdsmen insurrections significantly affected the CO$_2$ emission from fuel consumption.

The coefficient of the current year Niger-Delta and Fulani herdsmen insurrections were -4.569 and 8.148 respectively. These mean that a shift from non-insurgence to insurgency of Niger-Delta in the current year would reduce CO$_2$ emission by 4.57% on the average while a similar move from non-insurgence to insurgency of Fulani herdsmen in the current year would increase it by 8.15% on the average in the short-run. In the equation explaining food production, only Boko-Haram and Fulani herdsmen insurrections, among the insecurity challenges statistically had significant effect on the change in food production. The coefficients of the current year Boko-Haram and Fulani herdsmen insurrections were -10.206 and -4.691 respectively. These mean that a shift from non-insurgence to insurgency of Boko-Haram and Fulani herdsmen in the current year would reduce food production by 10.21 and 4.69 tonnes on average respectively in the short-run. There was no statistical evidence that the endogenous variables adjust to any deviation from long-run equilibrium with respect to the war, so that it could be treated as weakly exogenous.

**CONCLUSION**

The study examined the effect of insurgence on the agricultural development in Nigeria using Vector Error Correction Model (VECM) on Nigerian GDP, infant mortality rate, CO$_2$ emission and food production as proxies for agricultural transformation on Boko-Haram, Niger-Delta, Fulani herdsmen insurrections and the prominent Nigerian civil war as proxies for insurgence. It is inferred, from the results, that agricultural development should be all-embracing since it component elements have a long-run equilibrium relationship. Insurgence indirectly impact on agricultural development through its effect on the change in food production level, the share of agriculture to GDP, CO$_2$ emission from fuel combustion and infant mortality, and that attempt at ignoring the insurgence by any sect from any region, whether religious, cultural, or communal is a threat to agricultural development.
REFERENCES


Vegetative Growth and Yield Associated Flowering Time Variation in Sri Lankan Rice “Hondarawala”


Received : 09th June 2015 / Accepted : 21st Septeber 2015

ABSTRACT

Breeding new varieties adaptable for changing climate is an essential need in sustainable rice production. Around 2000 Sri Lankan rice accessions at Plant Genetic Resources Centre (PGRC), Sri Lanka have not been fully characterized for the yield potential and sensitivity to mild photoperiodic differences for days to flowering (DF). DF is a candidate key determinant in yield components in rice and understanding the physiological and molecular nature for DF is important to manipulate crop yield through breeding programmes. The objective of this study was to assess the genetic diversity of 15 Hondarawala accessions from PGRC using selected 37 morphological characters and DF. DF varied from 58-189 days while accession number 3988 did not flower until 200th day of seed germination. Principal Component Analysis (PCA) revealed that four principle components (PA) explained 86.5% of total observed variation. Variation of DF positively associated with most of morphological characters of vegetative growth while a few characters were negatively associated. In the dendogram, 10 clusters formed at rescale distance of 5. Widely variable DF accessions distributed among clusters.

Keywords: Days to flowering, genetic diversity, morphological characters, Sri Lankan rice

INTRODUCTION

Rice is the most important food crop in Sri Lanka contributing to more than 40% of daily calorie requirement. There is a wide variation in DF among Asian origin rice which is distributed in a range of agro-ecological zones in the region (Lu and Chang 1980). DF had affected the ecological adaptation of rice (Izawa, 2007). Sri Lankan traditional rice germplasm which is conserved at PGRC, Sri Lanka, consists of around 2000 accessions. Sri Lankan traditional rice germplasm is relatively a large genetic resource in a geographically small island of Sri Lanka. There is a genetic diversity among accessions as they exhibit a wide variation in morphology and DF. Reasonable information on genetic and molecular mechanism of DF in rice is available (Yano and Izawa, 2005). However, Sri Lankan rice germplasm has rarely been used in flowering time studies except for the work by Chandrarathna (1964) mainly.

Increased and stable rice production is required for increasing population during the era of climate change. New adaptation strategies are needed to develop new varieties to meet these challenges. The genetic potential of Sri Lankan traditional rice has not been completely characterized and exploited for its potential contribution in breeding for yield increment. DF in rice is controlled by both genetic factors and environmental signals. Photoperiod and temperature are the two main environmental signals that determine the flowering time in rice (Songet al, 2012).
Molecular basis for DF had been explained through many quantitative trait loci (QTLs): *Hd1* (Yano et al., 2000), *Hd3a* (Kojima et al., 2002), *Ehd1* (Doi et al., 2004), *Ghd7* (Xue et al., 2008), *RFT1* (Komiya et al., 2002). Matsuzaki et al. (2015) have demonstrated that the circadian clock is a regulatory network of multiple genes that retains accurate physical time of day by integrating the perturbations on individual genes under fluctuating environments in the field.

It is evident that different temperatures (of low country and hill country) in different ecological regions in Sri Lanka affect DF of rice. Effect of temperature at different phases of rice growth has not been completely studied. Among the known flowering time genes of rice, *Ehd1* and *Hd1* together control panicle development (Endo-Higashi and Izawa, 2011). *DTH8*, a QTL for DF is located on chromosome 8, also regulate yield potential and plant height (Wei et al., 2010). However, it is not known how these interact with each other on the crop yield potential. When three Sri Lankan traditional rice genotypes were grown under long day (LD), day neutral (DN) and short day (SD) photoperiods, days to flowering were increased under LD while there was an inverse relationship between enhanced vegetative growth and yield (Geekiyanage, 2012). The above traditional rice genotypes may represent the general response of Sri Lankan traditional rice germplasm on relationship between DF and, vegetative growth and yield. To test the relationship between DF and, vegetative growth and yield components of a Sri Lankan traditional rice in which several accessions are listed for one variety at PGRC, we selected *Hondarawala* variety which consisted of 15 accessions. As *Hondarawala* had been identified as suitable for low phosphorus field conditions, it had been exploited in breeding programmes for new improved varieties claiming desired plant type and yield. Currently, the maximum yield potential has been achieved in improved rice while there are needs for improvement of the rice quality. Therefore genetic resources of traditional rice must be utilized for further yield increase strategies for both yield and quality enhancement. We used the accessions of same variety *Hondarawala* with different DF for the experiment. The objectives of the experiment were to analyze the variation of DF, vegetative growth and yield among accessions and relationships between DF and vegetative growth and yield.

**MATERIALS AND METHODS**

**Rice Accessions**

Fifteen accessions of Sri Lankan traditional rice variety *Hondarawala* (Accession numbers: 3906, 6198, 4070, 4071, 3988, 3678, 3850, 3977, 6199, 6690, 6428, 4243, 3521, 3528, 6200) were obtained from Plant Genetic Resources Center (PGRC), Sri Lanka.

**Field Experiment**

Rice accessions were grown at Rice Research and Development Institute, Batalagoda, Sri Lanka (in IL1 agro ecological zone, latitude 7° 29’ 12” N and longitude 80° 21’ 53” E with a height above MSL 137m). Average ambient temperature during the cropping season was 30 °C and the soil was Dark brown earth (DBE). Each replicate consisted of 3 m long 3 row-plots with 9 plants: 20 cm x 20 cm within and between rows and 40 cm between plots in a Completely Randomized Design (CRD) with 4 replicates. The experiment was carried out in wet season during north eastern monsoon (*Maha*), 2012/2013 during which day length varies 11-11.5 hours in Sri Lanka. The seeds were sown in the upland nursery bed in December, 2012 with 15cm spacing in between each accession. Seedlings were transplanted by 21-days. Fertilizer application, pest and disease...
management and weed control were according to the recommendation by Department of Agriculture, Sri Lanka. The basal dressing of urea, TSP and MOP (as in the ratio of N: P: K) was applied during land preparation. Top dressing of 25, 50 and 50 kg/ac of urea were applied at 2 weeks, 5 weeks and 7 weeks of planting respectively. Manual weeding was done at regular intervals and the competition from weeds was kept minimal. Approximately permanent standing water level of 5 cm was maintained throughout the experiment.

**Evaluation of morphological traits**

Thirty seven morphological characters; 15 quantitative and 21 qualitative characters and DF were recorded (Table: 01). With respective to each character in a given accession, average value of replicates was considered for analysis. Data were collected at heading and at harvest of each variety. Measurement techniques were based on descriptors of rice published by the Team of NRC research project 12-129 (2014), International Rice Research Institute (1980 and 2007) and PGRC (2006).

**Statistical analysis**

Data were analyzed using PCA with correlation matrix through SPSS software (version 20), IBM, USA to define the patterns of variation between all explanatory variables. Grouping of variables into PCs was noted and thereby the dimension of the data set reduced. The 14 accessions which flowered during the experimental period of 200 days were clustered using Hierarchical Cluster analysis through SPSS software (version 20); IBM, USA which grouped and sorted the closely related accessions into clusters, using the first four PC scores of varieties. Measure of dissimilarity was the Euclidean distance and the clustering method was Ward’s linkage. The number of clusters was determined at the rescaled distance of 5.

**RESULTS AND DISCUSSION**

**Variation in DF among Hondarawala accessions**

DF and quantitative morphological characters of vegetative growth and yield components were largely varied among all Hondarawala accessions (Table: 02 and 03). DF among 15 accessions varied from 58-189 day while accession number 3988 did not flower until 200th day of seed germination. Seeds had been germinated in late January, 2013 which was the end of mild short day season in Sri Lanka under natural field conditions during which the photoperiod changes from short day to long day. As a result, the inductive photoperiod durations may have changed over time. Vegetative growth phase, reproductive phase and ripening phase are the 3 main phases of rice growth (Vergara and Chang, 1985). In vegetative growth phase, there are 2 sub-phases as basic vegetative phase (BVP) which is the photoperiod insensitive vegetative phase and photoperiod sensitive phase (PSP). BVP is a highly variable time duration which may be genetically controlled (Chandrarathna, 1964). In Hondarawala accessions DF varied from 58 to 200+ days indicating that juvenile period of BVP is variable among them. Additionally, effect of temperature variation during the period may have played a role in determining the DF. Duration from panicle initiation (PI) to flowering is also affected by photoperiod (Coolhaas and Wormer, 1953; Best, 1961; Janardhan and Murty, 1967). Based on results in a rice cultivar, Collinson et al., (1992) found that PI occurred when about 80% of the PSP had elapsed in a rice cultivar. These findings suggest that wide variation of DF among accessions and non-flowering at non-inductive photoperiod must be highly determined by the genetic factors of each accession.
Table 01: The quantitative and qualitative characters measured during the experiment

<table>
<thead>
<tr>
<th>Character</th>
<th>Abbreviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plant height at maturity</td>
<td>PH</td>
</tr>
<tr>
<td>Culm length at maturity</td>
<td>CL</td>
</tr>
<tr>
<td>Culm number at maturity</td>
<td>CN</td>
</tr>
<tr>
<td>Culm diameter at maturity</td>
<td>CD</td>
</tr>
<tr>
<td>Panicle length at maturity</td>
<td>PL</td>
</tr>
<tr>
<td>Grain length at maturity</td>
<td>GL</td>
</tr>
<tr>
<td>Grain width at maturity</td>
<td>GW</td>
</tr>
<tr>
<td>Leaf number at maturity</td>
<td>LN</td>
</tr>
<tr>
<td>Leaf length at maturity</td>
<td>LL</td>
</tr>
<tr>
<td>Leaf width at maturity</td>
<td>LW</td>
</tr>
<tr>
<td>Ligule length at maturity</td>
<td>LiL</td>
</tr>
<tr>
<td>Root length at maturity</td>
<td>RL</td>
</tr>
<tr>
<td>Shoot weight at maturity</td>
<td>SW</td>
</tr>
<tr>
<td>Panicle weight at maturity</td>
<td>PW</td>
</tr>
<tr>
<td>Plant height at vegetative stage</td>
<td>PHV</td>
</tr>
<tr>
<td>Culm number at vegetative stage</td>
<td>CNV</td>
</tr>
<tr>
<td>Leaf angle</td>
<td>LA</td>
</tr>
<tr>
<td>Flag leaf angle</td>
<td>FLA</td>
</tr>
<tr>
<td>Leaf blade pubescence</td>
<td>LBP</td>
</tr>
<tr>
<td>Leaf blade Colour</td>
<td>LBC</td>
</tr>
<tr>
<td>Leaf senescence</td>
<td>LS</td>
</tr>
<tr>
<td>Ligule colour</td>
<td>LiC</td>
</tr>
<tr>
<td>Ligule shape</td>
<td>LiS</td>
</tr>
<tr>
<td>Internode color</td>
<td>IC</td>
</tr>
<tr>
<td>Culm angle</td>
<td>CA</td>
</tr>
<tr>
<td>Culm strength</td>
<td>CS</td>
</tr>
<tr>
<td>Panicle type</td>
<td>PT</td>
</tr>
<tr>
<td>Panicle exertion</td>
<td>PE</td>
</tr>
<tr>
<td>Panicle axis</td>
<td>PA</td>
</tr>
<tr>
<td>Secondary branching</td>
<td>SB</td>
</tr>
<tr>
<td>Awning after full heading</td>
<td>AP</td>
</tr>
<tr>
<td>Awn colour</td>
<td>AC</td>
</tr>
<tr>
<td>Apiculus colour</td>
<td>ApC</td>
</tr>
<tr>
<td>Lemma and palea colour</td>
<td>LPC</td>
</tr>
<tr>
<td>Lemma and palea pubescence</td>
<td>LPP</td>
</tr>
<tr>
<td>Pericarp colour</td>
<td>PCC</td>
</tr>
<tr>
<td>Sterile lemma colour</td>
<td>SLC</td>
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</table>
Table 02: Descriptive statistical explanation of the variation of quantitative characters among the accessions of Sri Lankan traditional rice variety *Hondarawala*.

<table>
<thead>
<tr>
<th>Character</th>
<th>Unit</th>
<th>Range</th>
<th>Average</th>
<th>Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>DF</td>
<td>Days</td>
<td>58 – 200+</td>
<td>111.50</td>
<td>39.32</td>
</tr>
<tr>
<td>PH</td>
<td>cm</td>
<td>119.68 – 198.25</td>
<td>163.14</td>
<td>20.16</td>
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<tr>
<td>CL</td>
<td>cm</td>
<td>99.10 – 155.00</td>
<td>119.83</td>
<td>14.16</td>
</tr>
<tr>
<td>CN</td>
<td>Number</td>
<td>10 – 42</td>
<td>18.48</td>
<td>8.57</td>
</tr>
<tr>
<td>CD</td>
<td>mm</td>
<td>4.5 – 6.5</td>
<td>5.80</td>
<td>0.99</td>
</tr>
<tr>
<td>PL</td>
<td>cm</td>
<td>5.70 – 28.50</td>
<td>23.19</td>
<td>5.82</td>
</tr>
<tr>
<td>GL</td>
<td>mm</td>
<td>6.8 – 14.8</td>
<td>7.50</td>
<td>0.64</td>
</tr>
<tr>
<td>GW</td>
<td>mm</td>
<td>2.5 – 4.0</td>
<td>3.10</td>
<td>0.44</td>
</tr>
<tr>
<td>LN</td>
<td>Number</td>
<td>30 – 148</td>
<td>69.52</td>
<td>20.79</td>
</tr>
<tr>
<td>LL</td>
<td>cm</td>
<td>42.46 – 75.90</td>
<td>62.17</td>
<td>9.41</td>
</tr>
<tr>
<td>LW</td>
<td>mm</td>
<td>9.4 – 14.4</td>
<td>11.77</td>
<td>1.30</td>
</tr>
<tr>
<td>LiL</td>
<td>cm</td>
<td>0.70 - 2.68</td>
<td>1.77</td>
<td>0.59</td>
</tr>
<tr>
<td>RL</td>
<td>cm</td>
<td>11.5 – 30.0</td>
<td>20.09</td>
<td>5.42</td>
</tr>
<tr>
<td>SW</td>
<td>g</td>
<td>93.33 – 415.93</td>
<td>166.62</td>
<td>65.50</td>
</tr>
<tr>
<td>PW</td>
<td>G</td>
<td>2.48 – 44.90</td>
<td>14.17</td>
<td>11.75</td>
</tr>
<tr>
<td>PHV</td>
<td>Cm</td>
<td>121.00 – 157.20</td>
<td>142.89</td>
<td>11.75</td>
</tr>
<tr>
<td>CNV</td>
<td>Number</td>
<td>11 – 23</td>
<td>16.81</td>
<td>2.94</td>
</tr>
</tbody>
</table>

Table 03: Variation of quantitative characters of Sri Lankan traditional rice variety *Hondarawala* within clusters derived through Ward’s linkage method.

<table>
<thead>
<tr>
<th>Cluster Number</th>
<th>PGRC accession number</th>
<th>Quantitative Character</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DF (Days) PH (cm) CN LN LL (cm) LiL (cm) LW (mm) PW (g)</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>3977 68 129.3 14 71 50.4 0.7 12.2 15.1</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>6199 96 156.3 13 55 64.7 2.1 13.4 10.0</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>6428 58 119.7 17 67 42.5 1.7 11.0 23.1</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>3850 131 180.5 19 57 66.9 2.1 11.2 6.8</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>3521 189 172.7 23 91 67.6 1.6 11.2 9.9</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>6690 119 175.8 29 114 68.4 2.3 12.0 7.9</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>3906 79 160.0 16 30 66.8 1.2 12.8 18.2</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>3528 186 172.0 42 39 75.9 0.7 11.2 13.2</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>4243 99 149.0 24 81 56.9 2.1 11.4 44.9</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>6200 82 173.3 10 59 50.5 1.8 14.4 30.3</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>6198 141 198.3 16 78 73.8 2.5 9.4 2.5</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>4070 104 165.5 13 63 58.5 1.9 10.0 6.8</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>4071 103 165.8 13 63 64.1 1.6 12.0 4.9</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>3678 106 166.0 13 72 63.3 2.7 12.6 4.9</td>
<td></td>
</tr>
<tr>
<td>*</td>
<td>3988 200+ 160.3 15 39 63.4 0.9 11.2 -</td>
<td></td>
</tr>
</tbody>
</table>

*Accession number 3988 of which days to flowering exceeded the experimental period of 200 days was not included in the Cluster Analysis.
Relationship between days to flowering on variation in qualitative morphological characters

Except for the colour of ligule, all other 20 qualitative morphological characters were varied among accessions (Table: 04). Occurrence of heavy secondary branching in the panicle was observed in the longest DF accessions of 3528 and 3521.

Several workers of Yu et al., (1995), Yoshimura et al. (2001), Rutger and Mackill (2001), Rutger and Tai (2005) had reported that genes for hairs on rice leaf and hull are linked. According to Hu et al., (2013) all glabrous leafy rice produced glabrous hulls while hairy leafy rice produced hairy hulls. However, all Hondarawala accessions produced pubescent hulls and glabrous or pubescent leaves irrespective of DF variation indicating a genetic diversity of Hondarawala for leaf and hull pubescence. Further, a variety of pericarp colours and lemma and palea colours was observed indicating the allelic richness among accessions.

There were awns in all seeds only in one accession while others were without awns or partly awned. DF did not have a relationship with the presence of awns (Table:04).

Relationship between days to flowering on quantitative morphological characters

The correlation analysis revealed that there were strong significant positive correlations between DF and morphological characters of PH, CL, LL, RL, SW, PHV, LN and CNV while LW and PW were negatively correlated with DF (Table: 05). DF among accessions was a major determinant in quantitative morphological characters and yield components. Delayed flowering time increased the vegetative growth and reduced the panicle weight. Similarly, in our preliminary studies with Sri Lankan traditional rice varieties of Devaraddili and Kohu Ma wee under SD, LD and DN conditions, Deveraddlili and Kohu Ma wee did not flower by 200th at under LD condition and tiller number was significantly increased. Under DN condition, DF delayed in contrast to SD and yield was reduced (Geekiyanage et al, 2012). Tehrim et al (2012) had reported that DF of different rice genotypes was positively associated with days to maturity and straw yield per plant while DF negatively associated with seed setting percentage and harvest index.

In an experiment on photoperiodic effect fewer than four combinations of genetic compositions of Hdl and Ehd1, Hdl expression in non-functional hdl and ehd1 background under LD increased the DF and tiller number with reduced spikelet number (Endo-Higashi and Izawa, 2011). Although genetic factors of the accessions have not been revealed, our observation during this experiment supports the above report as DF negatively correlated with total panicle weight. Although Endo-Higashi and Izawa (2011) reports that Ehd1 and Hdl together suppress number of panicles and number of spikelets under SD with the shortest DF, in Hondarawala accessions, short DF increased the panicle weight (Table:03) indicating a different genetic control in flowering time.

Principal Component Analysis and Cluster Analysis

First four PCs explained 86.5% of total observed variation. (Table: 06). In PCA, most of the morphological characters of vegetative growth (PH, CN, LN, LL, RL and LL) which were affected by DF, included in simultaneous PCs with complex structures. Four PCs were rearranged removing the complex structures. Variation in DF among accessions was clearly exhibited within each cluster.

In the Hierarchical Cluster Analysis, ten clusters were formed at rescale distance of 5 (Figure: 01). DF varied within each cluster (Table: 03). At rescaled distance less than 5 even, there were 3 clusters with more than one accession in each (Figure: 01): Accessions 3977 and 6199 are grouped in one such cluster, except for LW, quantitative characters of DF, PH, LN, LL and
PW, and qualitative characters of LBP, PT, and PA were different from each other (Table: 03 and 04). Accession numbers 3850 and 3521 in third cluster were different to each other by quantitative characters of DF, PW and LN and qualitative characters of AC, AP, LPC, and PCC. Accession numbers 4070 and 4071 in 9th cluster were almost similar to each other while accession 6198 was different in most of qualitative and quantitative characters including DF.

Table 04: Variation of qualitative characters within clusters derived through Ward’s linkage method.

<table>
<thead>
<tr>
<th>Cluster number</th>
<th>PCRC code number</th>
<th>LBP 1</th>
<th>LBP 2</th>
<th>LBP 3</th>
<th>LBP 4</th>
<th>LBP 5</th>
<th>LBP 6</th>
<th>LBP 7</th>
<th>LBP 8</th>
<th>LBP 9</th>
<th>LBP 10</th>
<th>Variation of qualitative characters</th>
</tr>
</thead>
</table>
| 1              | 3977            | 05    | 05    | 05    | 05    | 05    | 05    | 05    | 05    | 05    | 05    | Moderate, strong, 
|                |                 |       |       |       |       |       |       |       |       |       | 
| 2              | 6600            | 06    | 06    | 06    | 06    | 06    | 06    | 06    | 06    | 06    | 06    | Moderate, strong, 
|                |                 |       |       |       |       |       |       |       |       |       | 
| 3              | 3850            | 07    | 07    | 07    | 07    | 07    | 07    | 07    | 07    | 07    | 07    | Moderate, strong, 
|                |                 |       |       |       |       |       |       |       |       |       | 
| 4              | 3521            | 08    | 08    | 08    | 08    | 08    | 08    | 08    | 08    | 08    | 08    | Moderate, strong, 
|                |                 |       |       |       |       |       |       |       |       |       | 
| 5              | 4090            | 09    | 09    | 09    | 09    | 09    | 09    | 09    | 09    | 09    | 09    | Moderate, strong, 
|                |                 |       |       |       |       |       |       |       |       |       | 
| 6              | 4080            | 10    | 10    | 10    | 10    | 10    | 10    | 10    | 10    | 10    | 10    | Moderate, strong, 
|                |                 |       |       |       |       |       |       |       |       |       | 
| 7              | 4070            | 11    | 11    | 11    | 11    | 11    | 11    | 11    | 11    | 11    | 11    | Moderate, strong, 
|                |                 |       |       |       |       |       |       |       |       |       | 
| 8              | 4071            | 12    | 12    | 12    | 12    | 12    | 12    | 12    | 12    | 12    | 12    | Moderate, strong, 
|                |                 |       |       |       |       |       |       |       |       |       | 
| 9              | 4091            | 13    | 13    | 13    | 13    | 13    | 13    | 13    | 13    | 13    | 13    | Moderate, strong, 
|                |                 |       |       |       |       |       |       |       |       |       | 
| 10             | 4072            | 14    | 14    | 14    | 14    | 14    | 14    | 14    | 14    | 14    | 14    | Moderate, strong, 
|                |                 |       |       |       |       |       |       |       |       |       | 

Interm: - Intermediate

*Accession number 3988 of which days to flowering exceeded the experimental period of 200 days was not included in the Cluster Analysis

Table 05: Correlation among the quantitative characters within accessions of Sri Lankan traditional rice variety Hondarawala.

<table>
<thead>
<tr>
<th>Character</th>
<th>DF</th>
<th>PH</th>
<th>CL</th>
<th>CN</th>
<th>CD</th>
<th>PL</th>
<th>GL</th>
<th>GW</th>
<th>LN</th>
<th>LL</th>
<th>LW</th>
<th>LiL</th>
<th>RL</th>
<th>SW</th>
<th>PW</th>
<th>PHV</th>
<th>CNV</th>
</tr>
</thead>
<tbody>
<tr>
<td>DF</td>
<td>1.00</td>
<td>.65*</td>
<td>.42*</td>
<td>.66*</td>
<td>.266</td>
<td>-.961</td>
<td>-1.216</td>
<td>.145</td>
<td>.772*</td>
<td>-.493*</td>
<td>-.023</td>
<td>.606*</td>
<td>.365*</td>
<td>.454*</td>
<td>.345*</td>
<td>.456*</td>
<td></td>
</tr>
<tr>
<td>PH</td>
<td>1.00</td>
<td>.39*</td>
<td>.153</td>
<td>.318</td>
<td>.057</td>
<td>.167</td>
<td>-.590*</td>
<td>.106</td>
<td>.779*</td>
<td>-.192</td>
<td>.426*</td>
<td>.447*</td>
<td>.342*</td>
<td>.457*</td>
<td>.608*</td>
<td>.010*</td>
<td></td>
</tr>
<tr>
<td>CL</td>
<td>1.00</td>
<td>.506*</td>
<td>.131</td>
<td>.318*</td>
<td>.057</td>
<td>.167</td>
<td>-.590*</td>
<td>.106</td>
<td>.779*</td>
<td>-.192</td>
<td>.426*</td>
<td>.447*</td>
<td>.342*</td>
<td>.457*</td>
<td>.608*</td>
<td>.010*</td>
<td></td>
</tr>
<tr>
<td>CN</td>
<td>1.00</td>
<td>.316*</td>
<td>.139</td>
<td>-.346*</td>
<td>.028</td>
<td>.085</td>
<td>.490*</td>
<td>-.276</td>
<td>-.330*</td>
<td>.172</td>
<td>.768*</td>
<td>.070</td>
<td>.081</td>
<td>.608*</td>
<td>.010*</td>
<td></td>
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<tr>
<td>CD</td>
<td>1.00</td>
<td>.316*</td>
<td>.139</td>
<td>.403*</td>
<td>.201</td>
<td>.323*</td>
<td>-.043</td>
<td>.023</td>
<td>-.216</td>
<td>-.088</td>
<td>.262</td>
<td>.195</td>
<td>.177</td>
<td>.268</td>
<td>.010*</td>
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<tr>
<td>PL</td>
<td>1.00</td>
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<td>.139</td>
<td>.403*</td>
<td>.201</td>
<td>.323*</td>
<td>-.043</td>
<td>.023</td>
<td>-.216</td>
<td>-.088</td>
<td>.262</td>
<td>.195</td>
<td>.177</td>
<td>.268</td>
<td>.010*</td>
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<tr>
<td>GL</td>
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<td>.121</td>
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<tr>
<td>GW</td>
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<td>.097</td>
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<td>.113</td>
<td>.084</td>
<td>-.387*</td>
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<td>.608*</td>
<td>.280</td>
<td>.011</td>
<td>.010*</td>
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</tr>
<tr>
<td>LN</td>
<td>1.00</td>
<td>.097</td>
<td>-.367*</td>
<td>.113</td>
<td>.084</td>
<td>-.387*</td>
<td>.100</td>
<td>.608*</td>
<td>.280</td>
<td>.011</td>
<td>.010*</td>
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<td></td>
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<td>.608*</td>
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<td>.023</td>
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<td>.177</td>
<td>.268</td>
<td>.010*</td>
<td></td>
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* > 0.3 , significant correlation (+) positive, (-) negative
Table 06: First four PCs on variation among accessions of Sri Lankan traditional rice variety *Hondarawala*

<table>
<thead>
<tr>
<th>PC</th>
<th>PC 1</th>
<th>PC 2</th>
<th>PC 3</th>
<th>PC 4</th>
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<tbody>
<tr>
<td>Contribution to variation</td>
<td>31.7 %</td>
<td>23.8 %</td>
<td>17.8 %</td>
<td>13.2 %</td>
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<tr>
<td>Composition of characters</td>
<td>PW (0.787)</td>
<td>CNV (0.930)</td>
<td>PHV (0.837)</td>
<td>CL (0.945)</td>
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<tr>
<td></td>
<td>GW (0.857)</td>
<td>SW (0.942)</td>
<td>GL (0.869)</td>
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</tr>
<tr>
<td></td>
<td>PL (0.857)</td>
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</table>

Figure 01: Dendogram of *Hondarawala* accessions derived through Ward’s linkage method of Cluster Analysis based on 17 vegetative and yield characters.

Based on the cluster analysis, accessions with distinct flowering times and related morphological characters can be identified. Genes responsible for DF may associate with genes for morphological characters or DF genes are pleiotropic. In our attempt to identify the relationship between DF in Sri Lankan rice accessions of *Hondarawala* and morphological characters of vegetative and yield could be useful in future breeding programmes for yield increment.

CONCLUSION

DF and quantitative morphological characters of PH, CL, CN, CD, PL, GL, GW, LN, LL, LW, LiL, RL, SW, PW, PHV and CNV varied among 15 accessions.

Qualitative morphological characters of LA, FLA, LBP, LBC, LS, LiS, IC, CA, CS, PT, PE, PA, SB, AP, AC, ApC, LPC, LPP, PCC and SLC were varied among accessions. Vegetative
morphological characters of PH, CL, LL, RL, SW, PHV, LN and CNV were positively correlated with DF while LW and PW (a yield component) were negatively correlated. First four Principal components (PA) explained 86.5% of total observed variation among accessions.

ACKNOWLEDGEMENTS

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Development and Quality Evaluation of Ready to Bake Vegetarian Cake Mix

R. M. N. A. Wijewardana1*, S. B. Nawarathne2 and I. Wickramasinghe2

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ABSTRACT

Three different formulas of ready to bake vegetarian cake mixes were prepared and quality and storability were analyzed. Proximate composition, beta carotene, colour (Lightness, redness and yellowness) of flour mix in terms of L*, a* and b* and total plate count were determined (n=3). The results were analyzed by completely randomized design using ANOVA by SAS statistical package and mean separation was done by using Least Significant Difference (LSD) at α= 0.05. The initial moisture content of cake mix 1, 2 and 3 were 5.23%, 6.41% and 5.61% and it was increased up to 5.63%, 6.79% and 5.84% respectively. High percentage of fat was recorded in Mix-1 (T1) and the least in Mix-3 (T3) and least in Mix 2 (T2) . All three mixes were not exceeded the safe limit of water activity for microorganisms (aw= 0.6) and resulted low microbial load (1.8*103). Addition of pumpkin flour and bale powder proportionately increased the yellowness of flour mixes and the L*, a* and b* colour values were significantly (p<0.05). The prepared cakes were analyzed for physico-chemical properties and consumer acceptability using 5 point Hedonic scale. Mix- 3 (T3) that containing wheat flour, rice flour, bael powder and pumpkin flour 10%, 17%, 3%, and 15% respectively was identified as the most suitable combination of ready to bake vegetarian cake mix (dry mix) and could be stored for 90 days without noticeable quality deterioration. According to the results obtained, the vegetarian ready to bake cake mix 3 was identified as the most preferable formula with the highest consumer acceptability.

Keywords: Pumpkin flour, Dehydration, ready to bake, vegetarian cake mix, bael

INTRODUCTION

In Sri Lanka, postharvest losses in fruits and vegetables estimated to be 30-40%, due to climatic and seasonal variations. Sri Lanka is an agricultural country because majority of rural population is still engaged in agricultural sector. The vegetable sub-sector is considered as the second most important sub-sector. Vidanapathirana, 2008 reported that vegetable are produced on a year round basis and a large number of farmers are involved in the production process in Sri Lanka. The fruit can be stored up to 6 months hence it can play an important role providing nutrition to the consumers even the dry spells (Nisha and Veerarrangavathatham, 2014). Pumpkin (Cucurbita moschata: Cucurbitaceae) is a known vegetable in many tropical and tropical countries due to high content of vitamin A, the color of pumpkin pulp is orange. The main nutrients are lutein and both alpha and beta carotene, the latter of which generates vitamin A in human body (Ahamad et al., 2011). Carotenoids are the primary source of vitamin A for most of the people in the developing countries (Boileau et al., 1999) where vitamin A deficiency is still common (Chakravarty,2000)

Pumpkin takes a prominent place among other vegetables because; it’s high productivity, nutritional value, good storability, and long period of availability and better transport qualities. Fresh pumpkin is very susceptible for microbial spoilage after opening even at refrigerator conditions. But it has a possibility
to use as an ingredient for food preparation because it is rich in carotenoids, minerals, vitamins pectin and dietary fiber. Pumpkin flour is the most convenient way of preservation because of its stability. The flour as an ingredient for food application due to desirable flavor and attractive deep yellow color profiles. It has been reported that utilization of pumpkin flour as a supplement for bakery products snacks, cakes, cookies, bread etc. (Ptitchkina et al., 1998).

In recent years, the development and evolution of functional foods targeting the consumer demand has been increased considerably. Further, pumpkin is rich in nutrients and use as cooked vegetable and as a thickening agent (Usha et al., 2010). The study was conducted to develop a ready to bake vegetarian cake mixture by replacing wheat flour with dry flour of rice, pumpkin and bael.

### METHODS AND MATERIALS

#### Sample preparation

Pumpkins (Cucurbita maxima) were harvested from a farmer field at North Central province in Sri Lanka at the proper maturity stage. Ash strips were appeared on the fruit surface. The diseased and damaged fruits were rejected. Bael (Aegle marmelos) fruit powder was prepared by drying at 50°C in vacuum drier (SELECTA, Spain) for 12 hrs and the pulp followed by grinding and sifted to get fine particials less than 150 µm. Soursop (Annona muricata) and pumpkins (Cucurbita maxima) prepared for dehydration by dipping in 1.5g/l sodium metabisuphyte solution after slicing followed by blanching for 3 minutes in hot water (60°C) and dried in vacuum drier at 50 °C. Powder was prepared by drying followed by grinding and particle size is 150 µm. Ginger powder was prepared by fresh, clean, washed rhizomes drying in vacuum drier at 50 °C and sifted to get fine particles (150 µm). Rice flour was sifted and get particles (200 µm). Wheat flour, baking powder, vanilla, sugar, non-fat milk powder, glecerol monostarate (mould inhibitor) was used as other ingredients, and purchased from the local market.

#### Product formulation: Ready to bake vegetarian cake mix

Three different cake mixes (treatments) were prepared by mixing wheat flour, rice flour, dehydrated bale (Aegle marmelos) powder, Sour sop (Annona muricata) powder and pumpkin powder in different proportions. Cakes were prepared by adding 40 ml of refined soya oil to 250g of prepared cake mixture and mixed well by adding 120ml of water in a stainless steel bowl till smooth batter was formed. The batter was then placed in a paper lined tray and baked in a pre-heated oven at 180 °C for 45 minutes. The cakes prepared from three formulations were tested as shown in Table 01.

#### Determination of physicochemical parameters

Moisture, fat, protein, total ash, fibre, β-Carotene, a_w, colour and microbial quality (Total Plate Count) were determined in cake mixtures.

**Moisture content:** Moisture content was determined using the method described in AOAC, 2005. 10 g sample was dried in hot air oven at 105°C ±1°C in pre-weight dishes till constant weight. The dried sample was transferred to desicicators with dishes and cooled to room temperature. The dish was then weighed and moisture content in per cent was calculated from loss of weight.

**Ash:** 5g of ground sample was taken in a pre-weighed silica crucible and charred over the heater to make it smoke free. The crucible with the sample was ignited at 600°C for 3 hours in a muffle furnace. When muffle furnace was slightly cooled, the crucible with ash was taken out, kept in desiccators to cool and constant weight was taken. The difference between the weight of the silica crucible as empty and with ash was the amount of total ash. The percent ash was calculated (AOAC , 2005).
**Table 01: Different formulas of ready to bake vegetarian cake mix**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Composition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T1</td>
</tr>
<tr>
<td>Wheat flour</td>
<td>8</td>
</tr>
<tr>
<td>Rice flour</td>
<td>20</td>
</tr>
<tr>
<td>Sour sop flour</td>
<td>2</td>
</tr>
<tr>
<td>Pumpkin flour</td>
<td>15</td>
</tr>
<tr>
<td>Bael powder</td>
<td>-</td>
</tr>
<tr>
<td>Baking powder</td>
<td>5.6</td>
</tr>
<tr>
<td>Vanilla powder</td>
<td>0.2</td>
</tr>
<tr>
<td>Sugar</td>
<td>40</td>
</tr>
<tr>
<td>Milk powder</td>
<td>8</td>
</tr>
<tr>
<td>GMS</td>
<td>0.8</td>
</tr>
<tr>
<td>Ginger powder</td>
<td>0.4</td>
</tr>
</tbody>
</table>

GMS- Glycerol monostearate

**Crude fat and crude protein:** Crude fat and crude protein were determined according to the method given in AOAC (2005).

**Crude fiber:** Two grams of sample was put into 250 mL conical flask and 1.25% Sulfuric acid solution was added. The sample was heated about 30 min and was filtered using vacuum filter and washed until traces of acid was undetected using pH paper. The Whatman paper 5B which pore size 125 micrometer was placed in the Buchner flask. After that the acid extracted was transferred into 250 mL conical flask and 1.25% NaOH solution was added. Digest the contents for half an hour, filter and wash free of alkali using hot distilled water. The residue was transferred to crucibles, weighed, dried in oven overnight at 105ºC, and then placed in a muffle-furnace at 600 ºC for 3 hrs. The loss in weight after ignition represents the crude fiber in the sample (AOAC 2005).

**β -Carotene:** β- Carotene content was determined using High Performance Liquid Chromatography (C-R6A, Shimadzu, Japan). Five grams of sample was saponified with 20 ml of 95% ethanol and 5ml of 100% KOH and refluxed for 30 min at 85ºC. The mixture was extracted with hexane until the sample become colorless. The extracted sample was then filtered through a 0.45µm. nylon membrane filter and analyzed using revised-phase high performance liquid chromatography .The test solution was injected under isocratic conditions into the μBondpack C\textsubscript{18} column (300nm x 3.9mm, 125A, 10µm) with a ternary mixture of acetonitrile-methanol-ethyl acetate (88:10:2 v/v) as mobile phase with the flow rate of 1.0 ml/minute. Detection was performed at 436nm. The results expressed as µg/100g in dry weight (Tee and Lim 1991).

**Preparation of Standard β -Carotene:** Standard of β -Carotene solutions were prepared by taking 10mg in 100 ml n-Hexane. The standard solutions were prepared as 20, 40, 60, 80ppm dilutions.

**Total phenolic content (TPC):** Total phenolic content was determined according to the method described by Singleton et al., 1999 with some modifications.

**Colour :** Colour in terms of CIE L*,a*,b* values was measured with colour difference meter (Konica Minolta TR 400). In which L* value represents lightness, a* value shows greenness-redness and b* value indicates blueness-yellowness, of the samples. The colour
variation of cake mix during storage (90 days) and prepared product were evaluated.

**Evaluation of microbial quality:** The total plate count was conducted throughout the storage period to evaluate the microbial content of the products as described in AOAC (1990). One gram of test sample was taken and diluted in 9 ml of distilled water. Added 1 ml of this test sample to 9 ml of diluents (water) using separate sterile pipets, prepare decimal dilutions up to $10^{-4}$. All plates were incubated at $35 \pm 0.5 \degree C$ for 48 hours.

Number of counts (N) was taken per milliliter or per gram of product using the following equation.

$$N = \frac{\Sigma C}{(n_1 + 0.1 n_2) d}$$

Where,

- $\Sigma C$ the sum of colonies counted on all the dishes retained.
- $n_1$ the number of dishes retained in the 1st dilution
- $n_2$ the number of dishes retained in the second dilution; and
- $d$ the dilution factor corresponding to the first dilution

**Quality evaluation of prepared cakes**

Crude protein, moisture content, colour (internal cut surface) and external (outer appearance), total phenolic contents and $\beta$ carotene of the product were evaluated.

**Evaluation of organoleptic properties of prepared cakes:**

Three different recipes/formulas (Table 01) were subjected to evaluate sensory properties. Thus, Prepared cakes were served for 15 trained panelists and evaluated for external appearance, internal appearance, colour, aroma, taste, texture and overall acceptability using 5 point Hedonic scale (5-like extremely, 4-like moderately, 3-neither like nor dislike, 2-dislike moderately, 1-dislike extremely). The selected best formula was tested for physicochemical characteristics and its storability as dry instant cake mix.

**Statistical analysis**

Parametric data obtained from the study pertaining to the completely randomized design were analyzed using ANOVA SAS statistical package. Mean separation was done by using Least Significant Difference (LSD) at $\alpha=0.05$. The results with respect to sensory evaluation were analyzed using Friedman test of Minitab statistical package and treatment means were compared at $p<0.05$ using multiple comparison procedure.

**RESULTS AND DISCUSSION**

**Chemical analysis of ready to bake vegetarian cake mixes:**

The data obtained from the proximate analysis of three cake mixtures (treatments) are given in the Table 02. Slight increase in moisture content was observed in all treatments. Moisture contents of different cake mixes were in the range of 5.21-6.41 % and lowest moisture level was recorded by mixture (T3). The increase in moisture content might be due to hygroscopic nature of pumpkin powder and wheat flour and the higher water absorption capacity in the composite flour compared to wheat flour (Bhat and Bhat, 2013). Higher fat percentage was recorded in Mix-1 (T1) and Mix-3 (T3) and least % recorded in Mix 2 (T2). The reason may be due to incorporation of higher percentage of pumpkin flour in which high percentage of fat comparatively to wheat and rice flour. Higher percentage of protein was recorded in Mix 3 (T3) and Mix 2 (T2). Total ash content was ranged from 4.5-5.0 % and the higher fiber content (2.5%) was recorded by Mix 2 (T2) and
Mix 3(T3). The higher β carotene content (0.12 mg/100g) was recorded in Mix 3 and the lowest (0.05 mg/100g) was recorded by mix 2 (T2). The higher β carotene composition of Mix 3 (T3) may be due to addition of dehydrated bael powder together with dehydrated pumpkin flour affect to develop yellow color of the product.

Moisture, water activity, colour and total plate count of ready to bake vegetarian cake mixture with addition of different composition of pumpkin flour during storage is shown in Table03. The initial moisture content of cake mix 1, 2 and 3 were 5.23%, 6.41% and 5.61% and it was increased up to 5.63%, 6.79% and 5.84% respectively. The moisture content was higher in Mix 1 and it was the lowest in Mix 3 (T3). The increase in moisture content due to hygroscopic nature of dehydrated fruit and vegetable powder, wheat flour and rice flour collectively and those substances were added in high proportions resulted in higher water absorption capacity in the composite flour mixes. The results were in conformation with Sunday and Dikson, 1992; Eke et al., 2009 in banana cake; See et al., 2007 in bread. Kulkarni and Joshi 2013 reported that the effect of replacement of refined wheat flour with pumpkin powder on the textural and sensory qualities of biscuit and the replacement level at 2.5% (w/w) of refined wheat flour with pumpkin powder was found to be optimum for the preparation of carotene enriched biscuits. Microbiological quality is a common criterion used to determine the acceptability and shelf life of dehydrated plant based products. Microbial count of the dehydrated foods depends on handling quality during the period of processing and storage (Jay, 2000). There was no significant difference observed for water activity in 3 cake mixes beginning from initial storage period to 60 days and slight increase was recorded thereafter. The all three mixes tested were not exceeded the safe limit of water activity for all microorganisms (0.6 a_w). Therefore lower microbial load were resulted. That may be due to maintenance of water activity below 0.62 in all samples during storage (Table 03). Addition of pumpkin flour proportionately increased the yellowness of flour mixes and the L*, a* and b* colour values were significantly (p<0.05) higher in the mixes having higher percentage content of pumpkin powder. Mix 1(T1) recorded the lower L* value (86.12) and the higher value (89.14) was recorded by Mix 3 (T3) at the end of storage. A reduction of colour intensity was observed due to destruction of β carotene during storage.

**Evaluation of Organoleptic properties of prepared cakes:**

A sensory evaluation was conducted to determine the consumer acceptability aof the product and to select the best formula. Among the quality attributes evaluated, external appearance and taste were not shown any significant difference (p<0.05) among treatments (Figure 01). All treatments tested were scored higher estimated median (5-Like extremely) for its external appearance. Mix 2 (T_2) and Mix 3 (T_3) given a highest score of estimated median (4-like moderately) for internal appearance, colour and aroma and it was significantly different from T_1. Treatment three scored 4 (like moderately) in its all attributes which were tested and it was significantly different from other treatments. It was identified that the estimated median of overall acceptability in mix 1(T_1) and Mix 2 (T_2) were scored 2- dislike moderately and 3-neither like nor dislike respectively. Formula 3 (T_3) was identified as a most preferable formulabased on sensory analysis. Findings are conformation with Pongjanta et al., 2006, reported that 20% pumpkin flour incorporated butter cake and chiffon cake were scored higher estimated median values and it was significantly different from other treatment tested. The similar observations were recorded that Ravi et al., 2010, pupkin flour incorporated dhola mix had significant impact on chemical, physical and sensory properties.
Table 02: Proximate composition of ready to bake vegetarian cake mix

<table>
<thead>
<tr>
<th>Composition</th>
<th>Mix-1 (T1)</th>
<th>Mix-2 (T2)</th>
<th>Mix-3 (T3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture (%)</td>
<td>5.61±0.01&lt;sub&gt;b&lt;/sub&gt;</td>
<td>6.41±0.03&lt;sub&gt;a&lt;/sub&gt;</td>
<td>5.21±0.01&lt;sub&gt;c&lt;/sub&gt;</td>
</tr>
<tr>
<td>Fat (%)</td>
<td>1.8±0.02&lt;sub&gt;b&lt;/sub&gt;</td>
<td>1.4±0.02&lt;sub&gt;b&lt;/sub&gt;</td>
<td>1.8±0.02&lt;sub&gt;a&lt;/sub&gt;</td>
</tr>
<tr>
<td>Protein (%)</td>
<td>32.7±0.03&lt;sub&gt;b&lt;/sub&gt;</td>
<td>36.2±0.01&lt;sub&gt;b&lt;/sub&gt;</td>
<td>36.8±0.03&lt;sub&gt;a&lt;/sub&gt;</td>
</tr>
<tr>
<td>Total ash (%)</td>
<td>3.0±0.01&lt;sub&gt;b&lt;/sub&gt;</td>
<td>3.4±0.01&lt;sub&gt;b&lt;/sub&gt;</td>
<td>4.5±0.01&lt;sub&gt;a&lt;/sub&gt;</td>
</tr>
<tr>
<td>Fiber (%)</td>
<td>2.4±0.01&lt;sub&gt;b&lt;/sub&gt;</td>
<td>2.5±0.02&lt;sub&gt;a&lt;/sub&gt;</td>
<td>2.5±0.02&lt;sub&gt;a&lt;/sub&gt;</td>
</tr>
<tr>
<td>β carotene (mg/100g)</td>
<td>0.07±0.02&lt;sub&gt;b&lt;/sub&gt;</td>
<td>0.05±0.03&lt;sub&gt;a&lt;/sub&gt;</td>
<td>0.12±0.01&lt;sub&gt;a&lt;/sub&gt;</td>
</tr>
</tbody>
</table>

Means with the same letters on the same row are not significantly different at α=0.05

Table 03: Physico-chemical and microbial analysis of ready to bake vegetarian cake mix during storage

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Days of storage</th>
<th>Treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mix 1 (T1)</td>
<td>Mix 2 (T2)</td>
</tr>
<tr>
<td>Moisture (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>5.61±0.02&lt;sub&gt;b&lt;/sub&gt;</td>
<td>6.41±0.01&lt;sub&gt;b&lt;/sub&gt;</td>
</tr>
<tr>
<td>30</td>
<td>5.62±0.01&lt;sub&gt;b&lt;/sub&gt;</td>
<td>6.47±0.03&lt;sub&gt;b&lt;/sub&gt;</td>
</tr>
<tr>
<td>60</td>
<td>5.73±0.02&lt;sub&gt;c&lt;/sub&gt;</td>
<td>6.63±0.03&lt;sub&gt;c&lt;/sub&gt;</td>
</tr>
<tr>
<td>90</td>
<td>5.84±0.01&lt;sub&gt;b&lt;/sub&gt;</td>
<td>6.79±0.01&lt;sub&gt;b&lt;/sub&gt;</td>
</tr>
<tr>
<td>L*</td>
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</tr>
<tr>
<td>0</td>
<td>16.70±0.03&lt;sub&gt;d&lt;/sub&gt;</td>
<td>16.76±0.03&lt;sub&gt;c&lt;/sub&gt;</td>
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<tr>
<td>30</td>
<td>16.45±0.02&lt;sub&gt;c&lt;/sub&gt;</td>
<td>14.64±0.02&lt;sub&gt;c&lt;/sub&gt;</td>
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<tr>
<td>60</td>
<td>14.73±0.01&lt;sub&gt;c&lt;/sub&gt;</td>
<td>14.55±0.03&lt;sub&gt;c&lt;/sub&gt;</td>
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<tr>
<td>90</td>
<td>13.64±0.02&lt;sub&gt;c&lt;/sub&gt;</td>
<td>13.24±0.02&lt;sub&gt;c&lt;/sub&gt;</td>
</tr>
<tr>
<td>Total plate count (CFU/g)</td>
<td>88.21±0.02&lt;sub&gt;c&lt;/sub&gt;</td>
<td>92.43±0.01&lt;sub&gt;c&lt;/sub&gt;</td>
</tr>
<tr>
<td>0</td>
<td>1.1*10&lt;sup&gt;5&lt;/sup&gt;±0.02&lt;sub&gt;c&lt;/sub&gt;</td>
<td>5.3*10&lt;sup&gt;5&lt;/sup&gt;±0.03&lt;sub&gt;c&lt;/sub&gt;</td>
</tr>
<tr>
<td>30</td>
<td>2.0*10&lt;sup&gt;5&lt;/sup&gt;±0.01&lt;sub&gt;c&lt;/sub&gt;</td>
<td>2.1*10&lt;sup&gt;5&lt;/sup&gt;±0.03&lt;sub&gt;c&lt;/sub&gt;</td>
</tr>
<tr>
<td>60</td>
<td>1.7*10&lt;sup&gt;5&lt;/sup&gt;±0.01&lt;sub&gt;c&lt;/sub&gt;</td>
<td>2.3*10&lt;sup&gt;5&lt;/sup&gt;±0.02&lt;sub&gt;c&lt;/sub&gt;</td>
</tr>
<tr>
<td>90</td>
<td>2.3*10&lt;sup&gt;5&lt;/sup&gt;±0.01&lt;sub&gt;c&lt;/sub&gt;</td>
<td>2.4*10&lt;sup&gt;5&lt;/sup&gt;±0.01&lt;sub&gt;c&lt;/sub&gt;</td>
</tr>
</tbody>
</table>

Means in each treatments followed by the same letter within each parameter are not significantly different alone the same columns and rows p<0.05
Figure 01: Sensory attributes of vegetarian cake

Physico chemical properties of prepared cakes

The physicochemical properties of cake prepared by adding different treatments are given in Table 04. Moisture contents of cakes were between 18.6% - 22.30%. The intensity of internal colour was increased while the percentage of pumpkin flour was increased and addition of dehydrated bael powder. Maximum L* value is in mix 3 (50.69), and mix 1, (48.77). The b value in mix 3 (41.24) followed by mix 1(39.41) respectively. According to the studies of Pongjanta et al., (2006) pumpkin flour incorporated butter cake, chiffon cake and sweet bread were reported that average b* values of all pumpkin powder substitute samples were higher than the control. However, water activity and texture was not significantly different (p<0.05) for all levels of pumpkin flour substituted butter cake, chiffon cake and sweet bread. The protein content was 3.50% to 4.16% and higher protein content was recorded from the cake prepared by mix 3 (T3). Ravi et al., (2010) reported that incorporation of pumpkin flour in instant Dhokla mix resulted significant increase of nutrients such as protein, fiber and β carotene.

Table 04: Physico chemical properties of vegetarian cake

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mix 1 (T1)</th>
<th>Mix 2 (T2)</th>
<th>Mix 3 (T3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture %</td>
<td>18.6±0.02</td>
<td>22.3±1.32</td>
<td>19.5±1.46</td>
</tr>
<tr>
<td>Colour (external)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a*</td>
<td>11.59±0.02</td>
<td>10.58±0.12</td>
<td>10.90±1.23</td>
</tr>
<tr>
<td>b*</td>
<td>14.4±0.01</td>
<td>10.26±0.02</td>
<td>12.26±1.37</td>
</tr>
<tr>
<td>L*</td>
<td>32.24±0.02</td>
<td>24.90±1.34</td>
<td>31.68±1.01</td>
</tr>
<tr>
<td>Colour (internal)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a*</td>
<td>8.32±0.01</td>
<td>11.23±0.02</td>
<td>9.2±1.32</td>
</tr>
<tr>
<td>b*</td>
<td>37.61±1.32</td>
<td>39.41±1.12</td>
<td>41.24±0.12</td>
</tr>
<tr>
<td>L*</td>
<td>46.18±0.23</td>
<td>48.77±0.12</td>
<td>50.69±0.22</td>
</tr>
<tr>
<td>Protein (%)</td>
<td>4.08±0.12</td>
<td>3.50±1.32</td>
<td>4.16±1.46</td>
</tr>
<tr>
<td>β carotene (mg/100g)</td>
<td>0.03±0.21</td>
<td>0.02±0.27</td>
<td>0.07±0.13</td>
</tr>
<tr>
<td>Total phenolic content (mgGA /g)</td>
<td>3.05±0.24</td>
<td>3.21±1.23</td>
<td>3.40±0.11</td>
</tr>
</tbody>
</table>

Means in each treatments respect to different parameters followed by the same letter alone the columns were not significantly different at p<0.05
CONCLUSIONS

In conclusion, cake mixture $T_3$ was the best formula as a vegetarian cake mix. The composition was wheat flour (10%), rice flour (17%), bael powder (3%) and pumpkin flour (15%).

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