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Carbon Footprint across the Coffee Supply Chain: The Case of Costa Rican Coffee

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Abstract: The issue of carbon emissions has been on the corporate sustainability agenda for some years. For those working in agricultural supply chains, the challenges remain significant, given the diverse direct and indirect emissions occurring throughout the value chain. This study determines the carbon footprint of the supply chain of Costa Rican coffee exported to Europe, using best practice methodology to calculate greenhouse gas emissions. Overall, it was found that the total carbon footprint across the entire supply chain is 4.82 kg CO_{2e} kg^{-1} green coffee. The carbon footprint of the processes in Costa Rica to produce 1 km of green coffee is 1.77 kg CO_{2e}. The processes within Europe generate 3.05 kg CO_{2e} kg^{-1} green coffee. This carbon footprint is considered as "very high intensity". This paper also identifies the sources of the most intense emission and discusses mitigation possibilities on which efforts must be focused.

Key words: Climate change, carbon footprint, coffee supply chain, Costa Rica.

1. Introduction

Climate change is a known and largely accepted reality, and the world's climate will continue to change as long as greenhouse gas levels keep rising [1]. The effects of climate change are clearly perceivable, and impacts are being felt worldwide. This is especially so for communities dependent on climate for their livelihoods, namely farmers. Human activity in industry and agriculture has much responsibility in this regard; agriculture directly contributes to approximately 10%-12% of global greenhouse gas (GHG) emissions, according to the latest IPCC report [2].

The growing public concern about climate change has given rise to responses from government and industry. The corporate world has responded by starting to evaluate the global warming potential of their products. For those working in agricultural supply chains, the challenges remain significant, given the diverse direct and indirect emissions occurring throughout the value chain.

In terms of GHG emissions, agriculture is a complex process that results in many direct non-carbon dioxide emissions in addition to direct carbon dioxide and indirect GHG emissions [3]. This complexity is particularly significant in coffee supply chains, since coffee beans change hands dozens of times on the journey from producers to consumers [4].

Over the last 20 years, with growing demand, there has been a move to greater intensification of coffee growing and heavy use of agrochemicals [5], which led to an increase in environmental impacts at farm level. In the next stage of the coffee supply chain, a common practice for processing coffee is the wet milling process. Coffee produced through this method is regarded as being of better quality [5], but inherent in this method lays the significant challenge of properly managing the resulting effluent.

"Carbon footprint" has become a widely used term

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and concept to define responsibility and abatement action against the threat of global climate change [6]. A carbon footprint is obtained by quantifying GHG emissions produced during a defined period of time, which is then expressed in carbon dioxide equivalent.

To date, there is little information in scientific literature about carbon emissions in the coffee sector. Given this lack of information, this study is an attempt to understand coffee's carbon footprint and to identify a response that helps to reduce impacts over time.

The main purpose of this study has been to determine the carbon footprint of a Costa Rican coffee supply chain using best practice methodology to calculate greenhouse gas emissions. Its purpose was also to develop a tool to calculate GHG emissions in the coffee supply chain, to enable replication in other coffee supply chains as necessary. Additionally, the study sought to identify "hot spots" of GHG emissions in the coffee supply chain, in order to determine where mitigation efforts should be focused, and to evaluate alternatives of mitigation efforts and their impact on the carbon footprint.

To meet these objectives, the study focused on different stages of the coffee supply chain: at farm level, in the central mill, and during the process of exportation. In order to assess the carbon footprint of the entire coffee supply chain, results of processes undertaken outside Costa Rica and within Europe were drawn from an existing study that evaluates the carbon footprint of coffee exported to Germany [7].

Finally, it is worth noting that sustainability measures and carbon reductions are still largely optional practices within supply chains. However, as consumers, NGOs and governments increasingly demand more of it, companies and stakeholders involved in the coffee business will have to meet these expectations through greater efforts on sustainability practices and through lower carbon emissions. The adaptability of the results of the present study and the calculation tool developed will be extremely valuable in evaluating carbon footprint in other regions.

2. Literature Review

The current section synthesizes published information related to carbon footprints. It summarizes public knowledge on greenhouse gas emissions, the impact of coffee in terms of carbon emissions, the definition of carbon footprint and carbon footprint methodologies as well as the theoretical base and understanding of the topic.

2.1 Greenhouse Gas Emissions

The effects of climate change are clearly perceivable and accelerating. Whereas all of these changes cannot be attributed to human activities only, it has to be acknowledged that the accelerated concentration of carbon dioxide (CO_2) particles in the atmosphere which reached 389 ppm in September 2011 [8] and the implications of altering natural lifecycles, have not occurred randomly.

The United Nations Framework Convention on Climate Change (UNFCCC) acknowledges in its definition of climate change that the change of climate is attributed directly and indirectly to human activity, which alters the composition of the global atmosphere [9]. Levels of all key greenhouse gases are rising as a direct result of human activities [1].

Of the greenhouse gases, CO_2 is of greatest concern because it contributes the most to enhanced greenhouse effect and climate change [10]. Currently, carbon dioxide is responsible for over 60% of the enhanced greenhouse effect, mostly from the burning of fossil fuels [1]. Deforestation is the second largest source of carbon dioxide, when forests are cleared for agriculture or development. The production of lime to make cement accounts for 3% of CO_2 emission from industrial sources [11].

Methane is the second most abundant GHG after carbon dioxide [12]. Domesticated animals (cattle) emit methane, which is produced by enteric fermentation of food by bacteria and other microbes in the animals' digestive tracts. The decomposition of manure also releases methane. Other sources of

methane include wetland rice farming by the decomposition of organic matter in the flooded soil, disposal and treatment of garbage and human wastes by anaerobic decomposition [1].

Nitrous oxide is an important anthropogenic GHG and agriculture represents its largest source [13]. Part of that nitrous oxide is produced by the use of fertilizers and manures. The nitrogen contained in those products enhances the natural process of nitrification and denitrification. Bacteria and other microbes in the soil carry out this process to convert part of the nitrogen into nitrous oxide [14].

Chlorofluorocarbons (CFCS), hydro chlorofluorocarbons (HCFCs), hydro fluorocarbons (HFCs), per fluorocarbons (PFCs) and sulfur hexafluoride (SF_6) are long-lived and potent greenhouse gases; very small emissions of these gases relative to CO_2 can have a large climate impact [15].

Agriculture directly contributes to approximately 10%-12% of global greenhouse gas emissions, according to the latest IPCC report [2]. Agricultural practices generate the greenhouses gases from carbon dioxide (CO_2) linked to land conversion, soil management and energy use, nitrous oxide (N_2O) connected to the use of fertilizers, and methane (CH_4) which is mainly related to waste management of the product [16]. Globally, agricultural methane (CH_4) and nitrous oxide (N_2O) emissions increased by nearly 17% from 1990 to 2005 [2].

According to the Carbon Dioxide Information Analysis Center (CDIAC), Costa Rica emitted about 2,000 thousand metric tones of carbon during 2010 and an average of 0.5 metric tones of carbon per capita [17]. Greenhouse gas emissions from agriculture represent approximately 39% of the Costa Rican emissions, according to the national inventory of GHG emissions carried out in 2005 [18].

2.2 Impact of Coffee in terms of Carbon Emissions

Coffee is the world's most widely traded tropical agricultural commodity [19]. In the world economy, the coffee trade was worth approximately US$16.5 billion by 2010 [20]. It is a major source of revenue for more than 40 tropical countries, and it generates more than 120 million jobs [21]. Around 125 million people worldwide depend on coffee for their livelihoods [4], and people are involved in the sector from farm level through to processing and sale [5].

According to CIRAD, coffee is grown on more than 10 million hectares worldwide [21]. The world production for 2011/2012 was estimated at 131.4 million bags [22], and the USDA has forecasted a record 148 millions bags of coffee worldwide for the 2012/2013 harvest [23].

Coffee is particularly important to the Costa Rican export portfolio. In 2010 dry green coffee[1] exports were ranked 9th in terms of importance and represented 12.1% of the total value of agricultural exports and 2.8% of the total exportation of the country [24]. During the coffee harvest season 2010/2011, Costa Rica was the 14th largest coffee producing country, producing 1.19% of the worldwide coffee production, according to the International Coffee Organization [25].

As a result of production on such a large scale, the coffee supply chain is an important contributor to global GHG emissions [26].

A study carried out in Costa Rica and Nicaragua during 2011 at farm level (which evaluated greenhouse gas emissions in coffee grown with differing input levels under conventional and organic management) found that the carbon footprint for 1 kg of fresh coffee cherries were between 0.26 and 0.67 kg CO_{2e} for conventional and 0.12 and 0.52 kg CO_{2e} for organic management systems. According to this study, it can be deduced that main contributors to GHG emissions were the inputs of organic and inorganic nitrogen [26].

In terms of footprint throughout the whole coffee value chain from bean to cup, the full carbon footprint including these various different processes reaches 59.12 g CO_{2e} per cup of coffee [7].

[1]Green coffee is the coffee in the naked bean before roasting.

2.3 Defining Carbon Footprint

The growing public concern about climate change has aroused the interest of industries to evaluate the global warming impact of their products across their supply chain. Carbon accounting in today's globalised world is becoming complex and difficult, because value chains are growing longer and even more complex [27]. In agricultural commodities like coffee (the unit of analysis for this study) the value chain starts from cultivation and end at the disposal after consumption [28].

The carbon footprint is recognized as a valuable indicator of GHG emissions [29]. The United States Environmental Protection Agency points out that a carbon footprint represents the total amount of greenhouse gases that are emitted into the atmosphere each year by a person, family or company [30]. Department for Environment Food and Rural Affairs (DEFRA) suggested that the carbon footprint should be used as a tool to identify main sources of emissions for all types of goods and services [3].

Wiedmann et al. [6] proposed a definition of carbon footprint exclusively related to the total amount of carbon dioxide emissions that is directly and indirectly caused by an activity or product. Wright et al. [31] suggested that as data collection for CO_2 and CH_4 emissions is relatively straightforward, these two carbon-based gases should be used in the determination of carbon footprint. They propose the term "climate footprint" for the inclusion of other GHG (non carbon-based gases) for full life cycle assessments [31].

For the purpose of this study, the concept of carbon footprint includes the emissions of GHG involved in the assessed activity. Taking into account that no greenhouse gas affects the atmosphere to the same extent, that each GHG has different global warming potential, and each GHG is normalized against CO_2 using a global warming factor, the carbon footprint is therefore expressed as CO_2 equivalent (CO_{2e}) [32].

2.4 Carbon Footprint Methodologies

In recent years, voluntary initiatives to mitigate climate change and overall sustainability have increased. Worldwide standards and methodological frameworks have been developed in the context of carbon footprint. These standards aim to identify, measure, reduce, mitigate and even neutralize the emissions of products, events, companies or territories. Both private stakeholders and public-private partnerships have been implemented and are working on these initiatives [33].

The European Union is leading this field. More specifically, the United Kingdom and France are the world leaders in the development of strategies and tools for the determination and assessment of the carbon footprint [34].

The British government, through its DEFRA and the Carbon Trust, teamed up with the British Standard Institute (BSI) to create a methodology for calculating GHG emissions embedded in goods and services by developing a Publicly Available Standard 2050 (PAS 2050), it was one of first public product carbon methodologies to be published [35].

The French Agency for Environment and Energy Management (ADEME) created Bilan Carbone, a GHG emission assessment tool. It is widely used in France and has influence in neighboring countries. The main aim of Bilan Carbone is to audit and set the GHG emissions according to weight, within a given scope of study, so that practical conclusions and areas of improvement can be put forward [36].

In 2008, Germany created the Project Carbon Footprint of Products (PCF Projekt), a practical tool for the estimation of the climate impact of individual products and processes [37].

International standards of carbon accounting include the Greenhouse Gas Protocol, which is an accounting tool to understand, quantify, and manage greenhouse gas emissions [38]. Finally, ISO 14067, a carbon footprint standard for products, is currently under development by the International Organization

for Standardization [39]; it is considered a fully international-based standard for the quantification and communication of GHG emissions of products and services [33].

3. Materials and Methods

Coffee goes through several stages on its journey from the grower to consumer; multiple sites and multiple companies are involved in this supply chain, which makes it complex. Traceability is difficult; data in the different process is in many cases not available, especially at farm level. This study extends its analysis to the whole coffee supply chain, emphasizing the collection of high quality data of its life cycle, and backtracking to their origin.

The methodology is structured in three sections: scope of the study, carbon footprint calculation tool and the process of data collection (farm level, central mill, exportation, and processes within Europe).

3.1 Scope of the Study

This study was conducted in Costa Rica and evaluates the different processes involved in the supply chain of coffee exported to Europe. The information used is drawn from the 2009/2010 coffee production period.

The study covers three different stages of the coffee supply chain in Costa Rica: farm level, milling and the process of exportation (Fig. 1).

In order to take a broader view of carbon emissions across the coffee value chain, other stages such as final processing (roasting), distribution and preparation related to the final country destination were integrated but not directly counted; information at these stages was taken from a previous coffee carbon footprint study (Fig. 2).

The scope for this study was defined using PAS 2050:2011 a carbon standard development by the British Department for DEFRA and the British Standards Institution (BSI) [3].

The main scopes defined for the stages directly evaluated are presented in Fig. 3.

Defining the functional unit

According to PAS 2050, the functional unit defines the function of the product that is being assessed and the quantity of product to which all of the data collected will relate, so the carbon footprint must be defined in terms of a functional unit [3].

The functional unit defined for this study was 1 kg of green coffee. Therefore, the results of the carbon footprint are presented as kilograms of carbon dioxide (CO_{2e}) per 1 kg of green coffee (kg CO_{2e} kg^{-1} green coffee).

Exclusion of process from the analyzed system

In order to simplify the process PAS 2050 allows the exclusion of some elements of the carbon footprint.

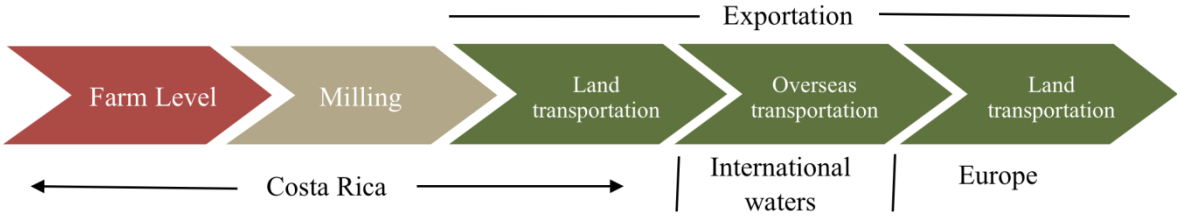

Fig. 1 Stages of the coffee supply chain evaluated.

Fig. 2 Stages of the coffee supply chain within Europe.

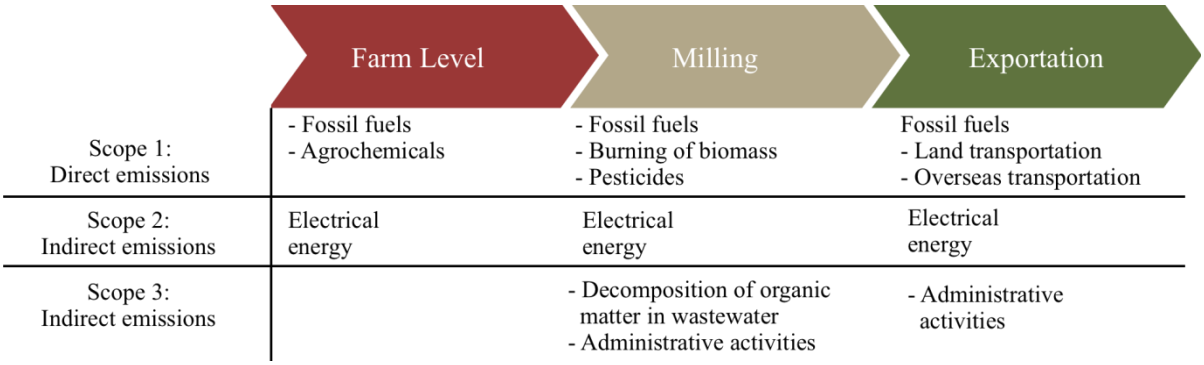

Fig. 3 Scopes defined for the stages of the coffee supply chain carried out in Costa Rica.

At least 95% of the total emissions have to be assessed, but materials that contribute less than 1% of the footprint can be excluded.

When land use change occurred more than 20 years prior to assessment, no land use change emissions should be included [3]. The land under coffee production in Costa Rica during 1990 to 2002 has been maintained at a constant level, registering reduction of the production area by 2008 [40]. Because the land destined to produce coffee has been in agricultural production for more than 20 years, no emissions from land-use change have been included. Carbon storage from shade trees and perennial crop are also excluded from the PAS 2050 method.

Other things not included are: human energy inputs to process and preprocess, transport of employees to and from their normal place of work.

3.2 The Carbon Footprint Calculation Tool

Before collecting primary data from the field, a methodology was developed to quantify the GHG emissions. As guidance, PAS 2050:2011 [3] were used, as well as the IPCC guidelines for National Greenhouse Gas Inventories [41].

Conversion factors provided by the IPCC and DEFRA were used to determine the footprint of each emission factor. Because of variation in factors caused by the sources of inputs (e.g., electricity) from country to country, specific Costa Rican conversion factors on electricity and fossil fuels were used from the National Climate Change Strategy (NCCS) [42].

To measure the carbon footprint, an Excel calculation tool was created, into which all data and emission factors were inputted. The model is structured in three different steps, as is explained in the following section (Fig. 4).

Step 1: Coffee production

First, the amount of coffee produced or processed at every stage was determined, in order to have a reference for which the emissions of each stage can be divided to obtain the carbon footprint of a specific source of emission. The information on coffee is presented as green coffee;

Step 2: Calculating carbon emissions

To calculate the emissions of each source, every activity data (e.g. amount of fossil fuels) is multiplied by its specific emission factor, as explained in Eq. 1 [3];

(Eq. 1) CO_2 emissions = source of emission or activity data × emission factor

Eq. 1 was used mostly to calculate the emissions caused by the consumption of fossil fuels, electricity, aerial transportation for marketing purposes, oversea transportation and administrative activities.

Different conversion sources were used to calculate the emissions as follows: Fossil fuel emissions and the electricity were calculated using the national average fossil fuels and energy emission factors for Costa Rica, provided by ENCC [42, 43]. The emission factors from the use of goods and services by the administrative department in Costa Rica, aerial transportation, the overseas transportation, and the land transport in Europe from port to warehouse were obtained from DEFRA.

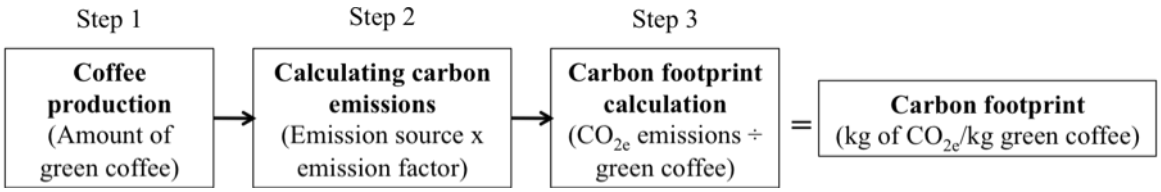

Fig. 4 Steps followed to calculate coffee carbon footprint.

Carbon emissions from the use of fertilizers, the decomposition of organic matter in wastewater and from burning biomass were calculated with the following specific equations.

Emissions from fertilizers

Agrochemicals encompass the production of chemicals, transportation, and direct and indirect N_2O emissions from soil for the application of fertilizers. The emission factors for producing fertilizers and pesticides were obtained from DEFRA [44]. The N_2O emissions were estimated using equations introduced by IPCC guidelines [45].

Eq. 2 was used to calculate the direct emissions by the application of nitrogen from synthetic fertilizers.

(Eq. 2) $CO_{2e} = (F_{SN} \times FE_1) \times (44/28) \times (GWP\ N_2O/1000)$

CO_{2e} = equivalent CO_2 emissions;

F_{SN} = annual amount of synthetic fertilizer N applied to soils, kg N yr^{-1};

FE_1 = emission factor for N_2O emissions from N inputs, kg N_2O–N (kg N input)$^{-1}$;

F_{SN}*FE_1 = annual direct N_2O–N emissions from N inputs to managed soils, kg N_2O–N yr^{-1};

44/28 = conversion of N_2O–N emissions to N_2O emissions;

GWP N_2O = Global Warming Potential of N_2O, t CO_{2e};

The indirect emissions, by the application of nitrogen from synthetic fertilizers, were calculated using Eq. 3 to calculate volatilization of N_2O, and Eq. 4 to calculate the leaching of N_2O.

(Eq. 3) Volatilization $CO_{2e} = ((F_{SN} \times Frac_{GASF}) \times EF_4) \times (44/28) \times (GWP\ N_2O/1000)$

CO_{2e} = equivalent CO_2 emissions;

F_{SN} = annual amount of synthetic fertilizer N applied to soils, kg N yr^{-1};

$Frac_{GASF}$ = fraction of synthetic fertilizer N that volatilizes as NH_3 and NO_x, kg N volatilized (kg of $N_{applied}$)$^{-1}$;

FE_4 = emission factor for N_2O emissions from atmospheric deposition of N on soils and water surfaces, [kg N–N_2O (kg NH_3 – N + NO_x–N volatilized)$^{-1}$];

(F_{SN} × $Frac_{GASF}$) × EF_4 = annual amount of N_2O–N produced from atmospheric deposition of N volatilized from managed soils, kg N_2O–N yr-1;

44/28 = conversion of N_2O–N emissions to N_2O emissions;

GWP N_2O = Global Warming Potential of N_2O, t CO_{2e}.

(Eq. 4) Leaching $CO_{2e} = ((F_{SN} \times Frac_{LEACH\text{-}(H)}) \times EF_5) \times (44/28) \times (GWP\ N_2O/1,000)$

CO_{2e} = equivalent CO_2 emissions;

F_{SN} = annual amount of synthetic fertilizer N applied to soils in regions where leaching/runoff occurs, kg N yr^{-1};

$Frac_{LEACH}$ = fraction of all N added to/mineralized in managed soils in regions where leaching/runoff occurs that is lost through leaching and runoff, kg N (kg of N additions)$^{-1}$;

FE_5 = emission factor for N_2O emissions from N leaching and runoff, kg N_2O–N (kg N leached and runoff)$^{-1}$;

((F_{SN} × $Frac_{LEACH\text{-}(H)}$) × EF_5) = annual amount of N_2O–N produced from leaching and runoff of N additions to managed soils in regions where leaching/runoff occurs, kg N_2O-N yr^{-1};

44/28 = conversion of N_2O–N emissions to N_2O emissions

GWP N_2O = Global Warming Potential of N_2O, t CO_{2e}.

Emissions from decomposition of organic matter in wastewater

The emissions of methane (CH_4) produced by the decomposition of organic matter in wastewater were estimated using equations obtained from the waste section of the IPCC guidelines [46].

The emissions by the decomposition of organic matter in wastewater were calculated as follows: to obtain the amount of organic degradable material the Eq. 5 and Eq. 6 were used to determine the emission factor for treatment systems, and net methane emissions were calculated with Eq. 7. Finally, CO_{2e} released by burning CH_4 in the dryers was calculated using Eq. 8.

(Eq. 5)	Total organic degradable material in wastewater for each industry sector	=	Total industry product × Wastewater generated × Chemical Oxygen Demand
(Eq. 6)	Emission factor	=	Maximum Methane Producing Capacity × Methane Correction Factor for the Treatment

(Eq. 7)	Net methane emissions	=	((Total organic degradable material in wastewater – Sludge removed) × (Emission factor for treatment system)) – Recovered CH_4
(Eq. 8)	CO_{2e}	=	(Net methane emissions)*(44/16)

44 = Molecular weight of CO_2; 16 = Molecular weight of CH_4.

Emissions from burning biomass

The emissions caused by burning biomass, for drying coffee, were calculated with equations obtained from the energy section of the IPCC guidelines [47].

The biomass consumed was calculated with Eq. 9. From the burning of biomass different GHG are emitted, such as CO_2, CH_4 and N_2O, the emissions of these gases are calculated with Eq. 10.

To convert the emissions of CH_4 and N_2O to CO_{2e}, the emissions of each gas were multiplied by its specific global warming potential, and the results were totaled to obtain the emissions expressed in CO_{2e} by burning biomass (Eq. 11).

(Eq. 9)	Consumption (TJ)	=	Consumption (mass, volume or energy unit) * Conversion factor (TJ/unit)
(Eq. 10)	Emission of CO_2	=	Consumption (TJ) * Emission factor (kg CO_2/ TJ) * Efficiency factor (0.98)
	Emission of CH_4	=	Consumption (TJ) * Emission factor (kg CH_4/ TJ) * Efficiency factor (0.98)
	Emission of N_2O	=	Consumption (TJ) * Emission factor (kg N_2O/ TJ) * Efficiency factor (0.98)
(Eq. 11)	CO_{2e}	=	Emission of CO_2 × 1(GWP) + Emission of CH_4 × 25(GWP) + Emission of N_2O × 298(GWP)

Step 3: Carbon footprint calculation

The emissions of each stage are totaled and standardized in kg of CO_{2e}. These emissions are divided into the total amount of coffee produced or processed in each stage. The result of this division is the carbon footprint of each stage; it is expressed in kg CO_{2e} kg^{-1} green coffee (Eq. 12).

(Eq. 12)	Carbon footprint = emissions/green coffee
	kg CO_2 kg^{-1} green coffee = kg CO_2 emitted/kg green coffee produced or processed

3.3 Data Collection

With established scopes for the study and the tools with which to calculate the emissions, the primary data was obtained at each stage of the coffee supply chain evaluated, as described below.

Farm level

Costa Rican coffee production is largely concentrated in smallholder systems, about 92% of them produce less than 26 ton of cherry coffee per year, and their production represents 41% of national production [48].

In order to assess the CO_{2e} emissions for the farm level, a range of farms in the Costa Rican Central Valley coffee cluster were selected for the study.

The farms were visited to collect data from the producers using a questionnaire; records of the farms were also reviewed to understand the usage of fossil fuels in different farm activities, agrochemicals and fertilizer, and electricity consumed during this period.

The principal sources of emissions identified at farm level are presented in Fig. 5.

It is important to note that the farms evaluated produce coffee under shade in a poly-culture system. Coffee plants and shade trees are CO_2-fixing; plants absorb CO_2 from the atmosphere though photosynthesis and use light energy to run enzyme-catalyzed reactions; in this process, plants produce sugars and other organic compounds for growth and metabolism [49]. The absorbed carbon goes to form above-ground biomass, as well as roots.

Wasmman et al. [50] indicated that there is an equilibrium point when no more carbon is stored. That is when new carbon fixation is cancelled out by attrition of trees. This carbon will eventually return to the atmosphere if and when the trees are liquidated. According to Hester et al. [51] carbon accumulated in leaves comes back to the atmosphere after a relatively short period of time, when the fallen leaves decompose. Carbon in wood is stored for years; the time depends on the tree species, growing condition, and on various uncertain occurrences such as fire or diseases. According to this information, fixation and emissions of carbon through the decomposition of organic matter in an established coffee-producing system are in a constant balance; leaves and wood

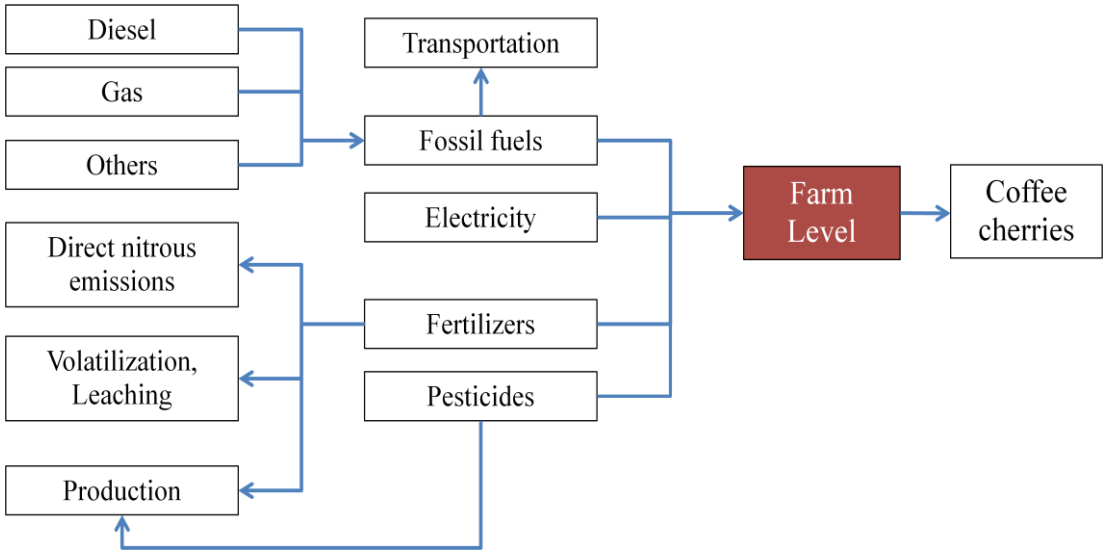

Fig. 5 Overview of the sources of emissions identified at farm level.

from pruning practices eventually decompose and carbon stored is released into the atmosphere. In Costa Rica, the pruning system on coffee varies depending on the technical criteria; the total pruning is done above 40 cm to 50 cm, and renovation of coffee plantation varies between 15 and 20 years [52].

Since PAS 2050 excludes carbon stored in living organisms, such as trees or perennial crops [26], the carbon stored in the coffee stem and shade trees were not considered in this study for the carbon inventory.

Central mill

After the harvest, the producers bring their coffee from the farm to the central mill, where the coffee cherries are concentrated and processed as parchment, and then it is converted into green coffee. This study evaluates two different milling facilities with these characteristics. The mills are located in the Central Valley of Costa Rica.

The milling process used in Costa Rica is the wet process, a common practice in Central America. The wet milling process is the practice used to convert the cherry coffee into green coffee at the central mill [53]. This process consists in selection, washing, natural fermentation, de-pulping and drying. From washing to de-pulping, a considerable amount of water is used. After wet processing, the water contains coffee mucilage[2]; this wastewater was sampled and a lab carried out COD[3] analyses; these results were used in the calculations (specifically in Eq. 7) to obtain the emissions of methane through the decomposition of organic matter in wastewater.

In addition, the records and information of fossil fuels, electricity, administrative activities, and the amount of biomass burned to dry coffee were collected for both mills. The sources of emissions identified for the milling process are presented in Fig. 6.

Exportation

According to ICAFE, 18% of coffee production of Costa Rica is sold in the local market and 82% is exported [48]. The United States is the principal market destination, representing 56% of the total exportation, and 39% is exported to Europe: Belgium, Luxemburg, Germany, Italy, the Netherlands and Portugal are the main buyers in Europe [24].

This study evaluated coffee exported to Europe. In this stage, a number of actors are involved in the transporting process from the central mill to its final destination in a warehouse in Europe, as explained below.

[2]The mucilage contains 50% sugars, 33% protein and pectin, and 17% dashes [54].
[3]Chemical Oxygen Demand.

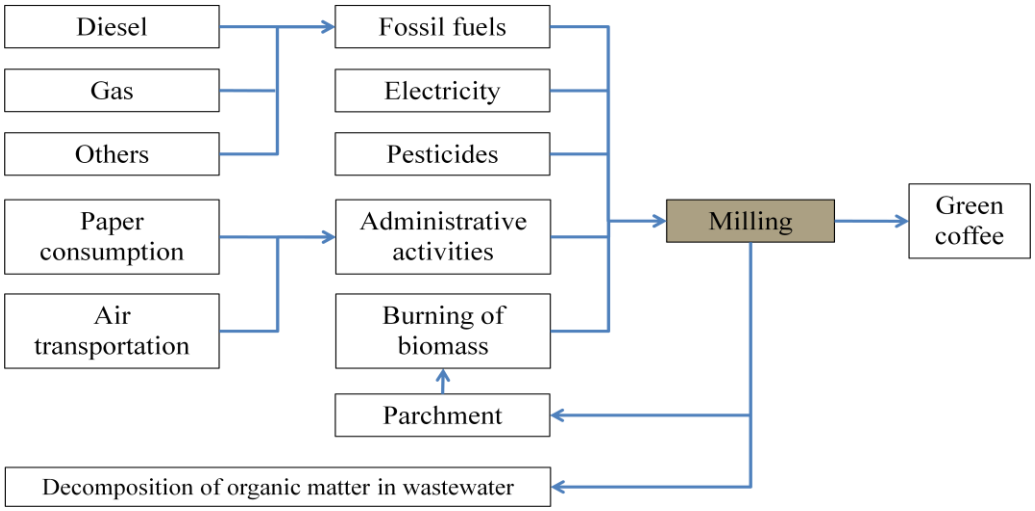

Fig. 6 Overview of the source of emissions identified for the milling process.

The information collected at this point is related toland transportation from the mill to port in Costa Rica: records of fossil fuels consumed were obtained. With regard to overseas transportation, information was obtained on both the amount of containers, the weight in tons of coffee exported, as well the distance in kilometers (7,869 km) from Costa Rica to Europe. In the absence of data from a particular carrier of the land transport in Europe from port to warehouse, an average of 600 km was used as the distance from port to the final destination. The sources of emissions identified for the process of exportation are detailed in Fig. 7.

The processes in Europe

In order to assess the remaining carbon footprint of the coffee value chain, findings of an existing study were used. This information was obtained from a case study that evaluates the carbon footprint of coffee processed in Germany [7]. Originally, this information was given in g CO_{2e} per cup, but for standardizing the functional unit defined in this study, it was converted into kg of CO_{2e} per kg of green coffee. PAS 2050 permit the use of secondary data from a published study or other source to calculate the impact of downstream life cycle stages [3].

The information of processes within Europe included the following stages: roasting, packaging, distribution, grinding and purchasing, consumption and disposal. The modeling of these stages is detailed in Fig. 8.

Electric energy is relevant in the roasting process; the general German electricity network provides this service. Besides electric energy, natural gas is also used in the roasting phase, and nitrogen gas is applied injected into the package to preserve the beans. The direct emissions of CO_2 from roasting coffee beans are excluded, since PAS 2050 exclude biogenic carbon sources from the assessment.

The roasted coffee is then packaged and distributed to retailers. Packaging includes primary and secondary packaging for the handling and delivery of the coffee as well as consumer packaging. The packaging used by end consumers includes a bag and a clip per 500 g of ground coffee. Electricity used at this stage is also significant in terms of emissions.

During the distribution stage, the roasted coffee is transported from the roasting plant to the coffee shop stores. From the roasting plant in Hamburg, the roasted coffee beans are delivered to the centre (Gallin) by lorries. From here, the coffee is distributed to three different distribution points: Bremen, Gerhnsheim and Neumarkt. From these distribution centers, the coffee is transported to affiliated shops.

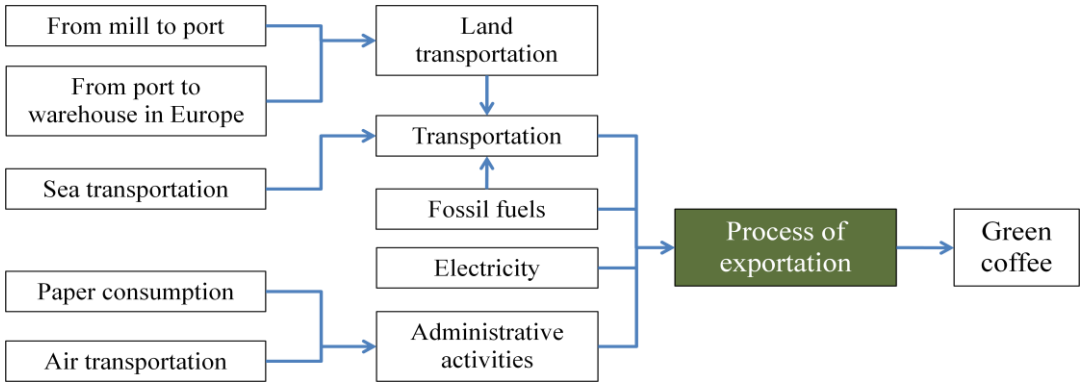

Fig. 7 Overview of the source of emissions identified for the process of exportation.

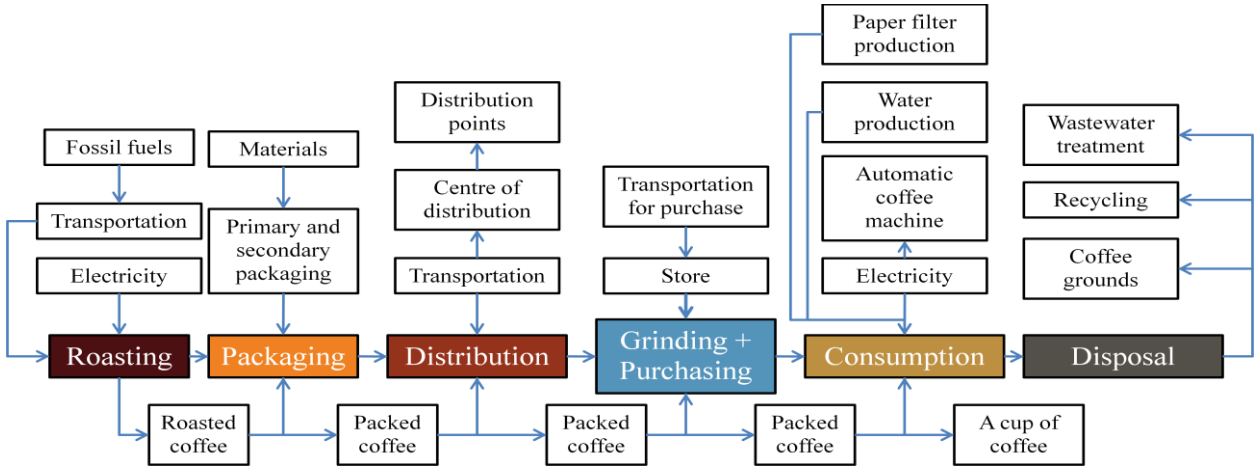

Fig. 8 Overview of the source of emissions identified for the process in Europe.

At the point of purchase, it has been assumed that not only one package of 500 g of coffee is purchased but also a whole basket of commodities with an overall weight of 20 kg. It is also assumed that the products come with a shopping bag made from low-density polyethylene, as secondary packaging. The purchase is done by car in an average distance of 5 km.

Consumers use different methods to prepare coffee: French press, filter drip, and automatic coffee machine. To prepare a cup of coffee using a French press, 125 g of water is needed, together with 0.0141 kw h of electricity. For filter drip coffee, 0.0125 kw h, and for an automatic coffee machine 0.085 kw h. Data drawn from the combination of these preparation methods is used.

The end of life phase took into account the disposal of primary and secondary packaging and coffee grounds. The coffee skin from the roasting plant is used to generate thermal energy and as a substitute for wood pallets and natural gas.

4. Results and Discussion

The following section addresses the potential carbon footprint of Costa Rican coffee. Additionally is presented a case study of the contribution of mitigation measures implemented at the stage of the milling process.

4.1 The Processes in Costa Rica

The carbon footprint calculated for the Costa Rican coffee, from farm level to a European warehouse is 1.77 kg of CO_{2e} per kilogram of green coffee (Fig. 9).

The emissions at farm level are the greatest (58%), followed by the central mill (27%), and finally the process of exportation to Europe (15%). PAS 2050 classifies as “high intensity” emissions in a range of

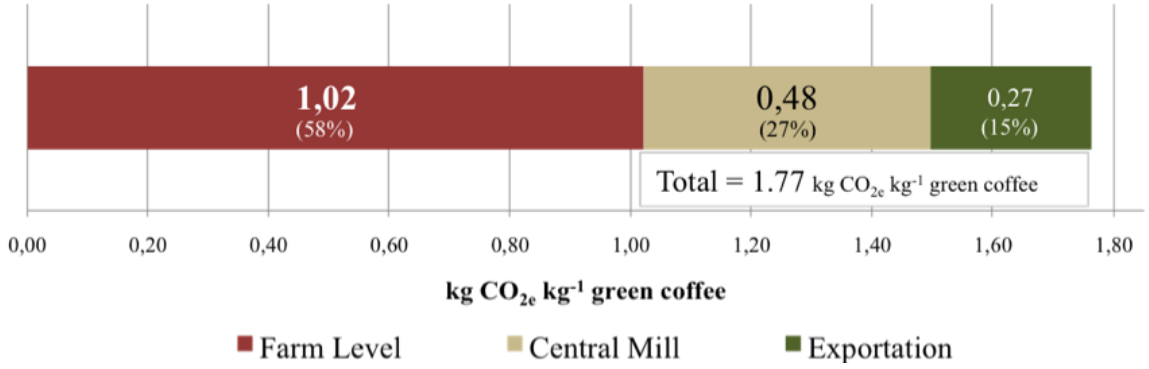

Fig. 9 Carbon footprint of three stages of the coffee supply chain.

1-3 kg CO_2 per kg. Products in this category include: greenhouse crops, rice and dairy [3]. According to this Classification, this coffee carbon footprint is technically considered a high intensity source of emissions.

The following section describes in detail the contribution of the respective processes in the value chain.

Farm level

This stage represents the most carbon intensive of the processes in Costa Rica. The farm level is responsible for 58% (Fig. 9) of total carbon footprint calculated for the processes in Costa Rica, or 1.02 kg CO_{2e} kg^{-1} green coffee (Table 1).

Fertilizers represent the highest inputs on the farm, both from the production of chemical fertilizers and due to N-fertilization: N_2O emissions of leaching and volatilization. 94% of the emissions at this stage come from fertilizers (Table 1). In contrast, the emissions from pesticides represent just 1%. Emissions from fossil fuels total 3%, mostly for the transportation of coffee cherries to the gathering centers. Electricity represents 2% of the emissions at the farm level.

Central mill

The central mill contributes 27% (Fig. 9) of emissions in Costa Rica, which represent 0.48 kg CO_{2e} kg^{-1} green coffee (Table 2).

The process of wet milling requires substantial amounts of water. After the wet processing, the remaining wastewater retains large amounts of solids and decomposing sugars. When this wastewater is not treated, it represents a source of pollution mainly if it is dumped directly into local water bodies. Additionally, the process releases gases such as methane (CH_4), which has a much higher global warming potential than CO_2. The emissions from untreated wastewater account for 79% of the total emissions at this stage (Table 2).

Table 1 Carbon footprint at farm level.

Emission source	CO_{2e} emission (kg CO_{2e} kg^{-1}) green coffee	(%)
Fertilizers	0.96	94
Fossil fuels: diesel, gas, others	0.03	3
Electricity	0.02	2
Pesticides	0.01	1
Total	1.02	100

Table 2 Carbon footprint at central mill.

Emission source	CO_{2e} emission (kg CO_{2e} kg^{-1}) green coffee	(%)
Decomposition of organic matter in wastewater	0.374	79
Fossil fuels: diesel, gas, others	0.076	16
Administrative activities	0.024	5
Biomass burning	0.001	0.3
Total	0.48	100

Exportation stage

Exporting 1 kg of green coffee from Costa Rica to Europe produces 0.27 kg CO_{2e} kg^{-1} green coffee (Table 3) and represents 15% of the emissions in Costa Rica (Fig. 9).

The overseas transportation is the main factor in terms of CO_{2e} emissions at this stage (70%). The distance from Costa Rica to Europe explains the large percentage of emissions for this phase.

Table 3 Carbon footprint of the exportation stage.

Emission source	CO_{2e} emission (kg CO_{2e} kg^{-1}) green coffee	%
Sea transportation	0.185	70
Transportation by land from port to storage destination	0.041	15
Transportation by land from mill to port	0.033	12
Administrative activities	0.006	2
Total	0.27	100

In order to obtain the carbon footprint of the processes within Europe (from roasting processes to disposal of the waste generated), results from existing literature were used. These results are presented in the following section.

4.2 Processes in Europe at Destination

The carbon footprint related to the processes in Europe is 3.05 kg CO_{2e} kg^{-1} green coffee (Table 4), which represents 63% of total emissions (Fig. 10).

Table 4 indicates that emissions are released in the roasting process (6%), packaging (4%), distribution (5%), grinding and purchasing (9%); the emission by consumption are the greatest (71%), and from the end of phase (disposal) (5%).

In the roasting stage, emissions are mainly driven by both electricity supply and provision of thermal energy. According to PAS 2050, the direct CO_{2e} emissions of the roasting process are not included as they originate from biogenic source [7].

The consumption stage is the most intensive source of emission and has a big impact on the overall carbon footprint; emissions at this stage come from the high demand of energy required for the preparation of coffee with an automatic coffee machine. The carbon footprint at this point is 2.15 kg CO_{2e} kg^{-1} green coffee, higher than the sum of the emissions from all other stages in Europe.

In the following section, the results of the carbon footprint in Costa Rica were combined with the results of the processes within Europe in order to obtain the total carbon footprint of the Costa Rican coffee supply chain.

Table 4 Carbon footprint of the processes in Europe.

Stage	CO_{2e} Emission kg CO_{2e} kg^{-1} green coffee	%
Roasting	0.19	6
Packaging	0.13	4
Distribution	0.15	5
Grinding + purchasing	0.29	9
Consumption	2.15	71
Disposal	0.14	5
Total	3.05	100

PCF Pilotprojekt Deutschland [7].

4.3 Overall Results

The total carbon footprint calculated for Costa Rican coffee across its full supply chain is 4.82 kg of CO_{2e} per kilogram of green coffee. The carbon footprint covered all processes conducted in Costa Rica and Europe. Farm level to a European warehouse produced 1.77 kg CO_{2e} kg^{-1} green coffee, and processes in Europe produced a carbon footprint equal to 3.05 kg CO_{2e} kg^{-1} green coffee (Fig. 10).

The main carbon emissions in the coffee supply chain are released at farm level (21%), the central mill (10%), and the process of consumption (45%); the carbon footprint related to consumption is 2.15 kg CO_{2e} kg^{-1} green coffee, higher than the total emissions released by the process carried out in Costa Rica (1.77 kg CO_{2e} kg^{-1} green coffee) (Fig. 10).

PAS 2050 classifies as "very high intensity" emissions in a range of > 5 kg CO_2 per kg. Products in this category include some concentrated foodstuffs [3]. According to this classification, the carbon footprint of Costa Rican coffee is technically considered a very high intensity source of emissions.

However, comparing the results of this study with other carbon studies on coffee, the level of emissions produced by Costa Rican coffee is lower (4.82 kg CO_{2e} kg^{-1} green coffee) than the total carbon footprint of a study of coffee exported to Germany, which showed emissions equivalent to 7.15 kg CO_{2e} kg^{-1} green coffe[4] [7]. Differences are mainly concentrated at farm level by the use of fertilizers.

[4]Information originally given in g CO_{2e} per cup, and converted into kg of CO_{2e} per kg of green coffee.

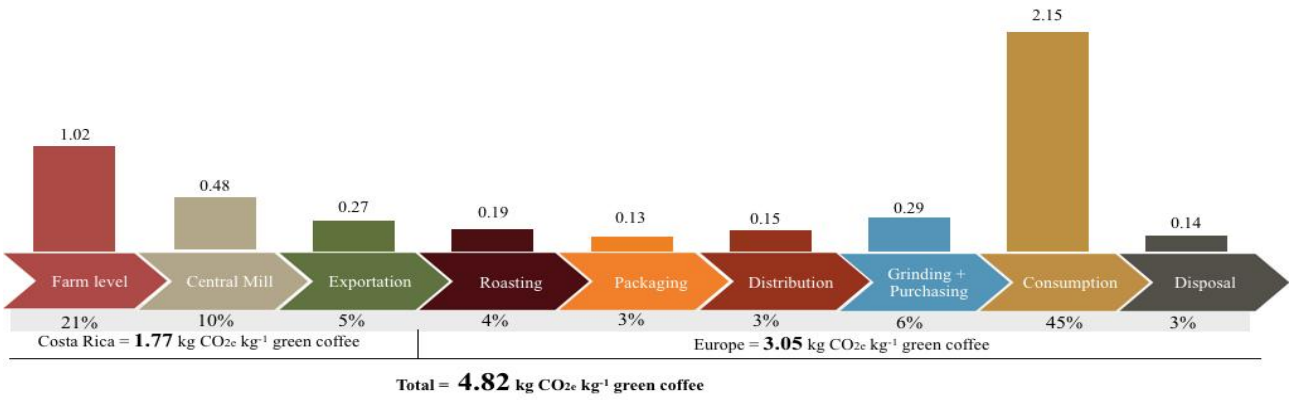

Fig. 10 Carbon footprint of Costa Rican coffee supply chain.

The following section describes in detail, the contribution of the respective processes in the supply chain to the resulting carbon footprint.

4.4 Hot Spots

The hot spots identified by this study are: fertilizers applied at farm, wastewater as a result of the wet milling process, and the electricity used for the preparation of coffee consumption using an automatic coffee machine. These emissions are collectively responsible for 73% of total emissions in the supply chain evaluated.

Fig. 11 shows in detail the contribution of each emission source in the potential carbon footprint of the Costa Rican coffee.

These results show the prominence of specific emissions variables for each component in the coffee supply chain. This can help to guide and establish mitigation strategies that can form the basis for action and reduce the impact of these activities on the environment.

The following section presents a specific mitigation strategy implemented at the milling stage; it includes the resulting implications of this strategy on the reduction of emissions.

Mitigation Possibility at Milling Stage

This section reveals the results of mitigation practices implemented in the central mill evaluated by this study. This mitigation effort is specifically focused on treating the wastewater generated after the milling process. The data for potential emissions is linked to the information presented in section 4.3 (overall results). The result of mitigation practices at this stage makes a substantial difference to resulting emissions (Fig. 12).

In terms of carbon footprint, the mitigation efforts carried out in the central mill represent a reduction of 7% or 0.34 kg CO_{2e} per kilogram of green coffee. This means that producing 1 kg of green coffee under these conditions reduces the potential emissions equal from 4.82 kg CO_{2e} to 4.48 kg CO_{2e} (Fig. 12).

Mitigation was achieved in the following way: one bio-digester or anaerobic reactor in each mill reprocesses the remaining wastewater. The decomposition of sugars and solids (contained in the coffee mucilage) in an anaerobic environment break down this organic matter into biogas (methane CH_4). The biogas obtained is burned in the coffee dryers. (The equivalent in CO_2 from burning this gas is much less than if the gas were emitted as methane[5] or if the wastewater were not treated).

Based on the assumption that most countries have regulations to restrict dumping of untreated wastewater, it can be inferred that most mills in the region have some type of wastewater treatment system in order to operate legally. These measures could be considered as part of a mitigation effort, though the treatment systems would need to be assessed in order

[5]The Global-warming potential of methane is 25 times more than carbon dioxide [55].

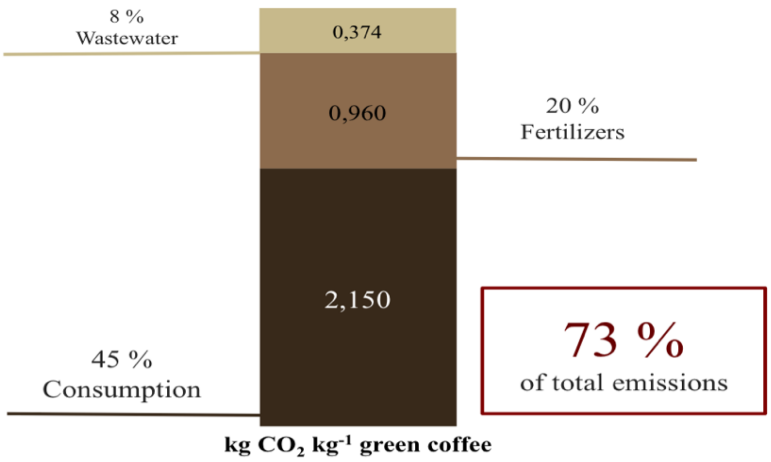

Fig. 11 Hot spots identified.

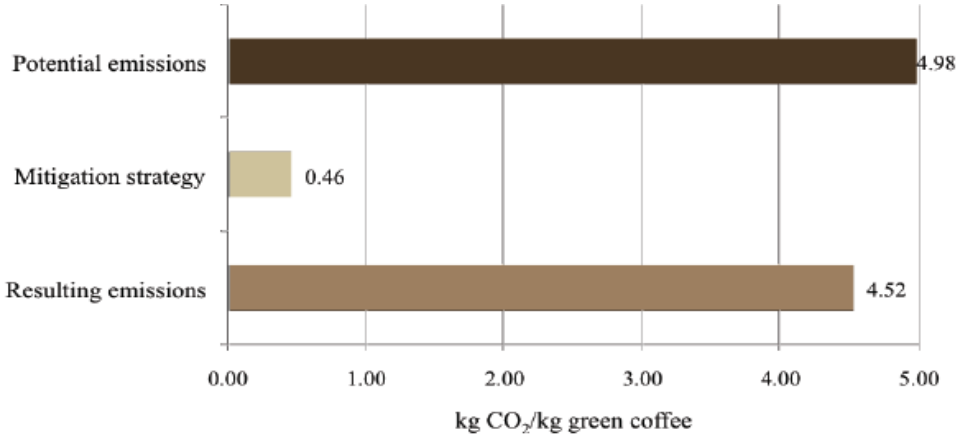

Fig. 12 Results of mitigation strategy implemented in the central mill.

to establish their real impacts on emissions and the potential financial cost of implementation and that they could represent.

4.5 Implications

In order to reduce the carbon footprint of coffee during its life cycle, the multiple actors implicated in the supply chain need to establish concrete actions or strategies to address the principal sources of emissions. Emissions vary across each stage of the chain, hence, it is reasonable to focus first on managing the key hot spots identified.

Large companies such as roasters and retailers could engage their suppliers in order to manage their GHG emissions in a more integrated and collaborative way, with a common plan and focused efforts to optimize efficiency.

It is also important to consider the promotion of technical upgrades at producer level, for example, improving their management practices through training programs in order that they optimize the use of inputs on the farm, specifically the use of fertilizers. These actions can reduce the carbon footprint at farm level.

Efforts should also be focused on the milling process, specifically proper management of wastewater. This study has given an example of how biogas can be produced from wastewater and the use of that gas used for the drying process of coffee. This effort reduced the carbon footprint significantly. Nevertheless, a cost benefit analysis of the implementation and operation of the anaerobic reactors would be needed in order to understand its

financial viability.

With regards to overseas transportation, companies involved at exportation stage could proactively seek to work with shipping companies that are actively working on reducing their own footprints.

Stakeholders involved in the coffee value chain have to take into account that consumers are now more aware about environmental issues including their own consumption. Increasingly, they are asking companies to provide information on emissions of products and services that they purchase and seeking to reduce their own footprints.

Aside from the potential cost savings to be made in reduction of carbon in the supply chain (e.g., through energy, fertilizers or transport costs), the proper management of emissions is also an opportunity for companies to develop competitive advantages in the marketing of their products or services. Some are already actively doing so. Despite the fact that sustainable practices and reduction of carbon emissions are still largely voluntary in most countries, there is a growing move towards regulation and carbon credit schemes that seek to incentivize and reward business for adopting carbon reduction strategies. For this reason it is increasingly important to invest in reduce or even neutralizing the carbon footprint in the supply chain. Australia, by way of example, is facing an emerging new business landscape in this respect; the transition to a low-carbon economy has begun [56, 57], and with the Clean Energy Act 2011 that came into effect in 2012, government has introduced a price on carbon to entities with greater emissions such as energy; even though agricultural emissions are not yet covered, it will face indirect effects through the increase of costs of electricity, amongst other utilities.

Compared with other agricultural products such as banana or pineapple that can be consumed as fresh products, the consumption of coffee requires a considerable amount of CO_{2e}, as was evidenced in this study, largely due to the highly energy demand from automatic coffee machines. Consumers also therefore play a critical role in the life cycle of coffee; as the most significant contributor to the overall footprint, they are directly part of the problem and should take the responsibility to minimize their own impact. Interesting work could also be done in improving the energy efficiency of coffee machines in this regard. Some companies (that manufacture products such as shampoo, with a similar consumer-heavy footprint) have embarked on consumer-focused campaigns to raise awareness and reduce water and energy usage at point of use.

The effecting of a range of policies and tools can reduce net carbon emissions from the supply chain too. According to the World Bank the carbon market has demonstrated that it is an effective tool in reducing GHG emissions [58]. Based on the principle that polluters pay, Bowen [59] suggested that a uniform global carbon price delivered by carbon taxes or carbon trading would be an ideal tool to reduce GHG emissions in a cost-effective way. In Europe for example, the carbon price in the market varies between US$18.8 ton^{-1} (€13.5 ton^{-1}) and US$ 12.9 ton^{-1} (€9.2 ton^{-1}) [60], which can be translated to US$0.019 and US$0.013 per kilogram of CO_{2e} emitted. Therefore, if the externality cost associated to the carbon footprint calculated were applied on coffee, it would vary between US$0.09 and US$0.06 per kilogram of coffee. This cost should be shared out amongst the key actors involved and thereby it would be reflected in the "social cost"[6] of coffee.

5. Conclusions

Coffee has considerable impact on the environment; the carbon footprint of the coffee supply chain calculated in this study is classified as a product with very high intensity emissions. Most emissions come from a few sources, which account for most of the impact generated per unit produced. In this sense

[6]The social cost includes the private costs plus the externalities costs [61].

focused mitigation efforts should be easier to implement. The hot spots identified produce about 72% of total emissions across the coffee supply chain evaluated, these are: fertilizers applied at farm level, wastewater as a result of the wet milling process, and the preparation of coffee using an automatic coffee machine due to the consumption of coffee in Europe.

A greater understanding of the topic and lessons learned by other business can be beneficial in helping to manage the carbon footprint generated. For instance, this study presented a mitigation strategy implemented in the milling process for managing wastewater, the result of which significantly reduced the carbon emissions.

Complementary studies are necessary to determine the real impact of the poly-culture system in the fixing and storing of carbon in order to establish the potential compensation of GHG emissions, mostly in the early growing stages of the plants.

For those involved in the coffee supply chain; this carbon footprint study reveals a useful perspective on carbon emissions through the life cycle of the product. The concern over GHG emissions and climate change is growing, so an effective management of carbon generated can only imply long-term benefits to both business and the environment.

Finally, as consumers are also directly and significantly part of the story on the coffee carbon footprint, they must be involved in the task of reducing its impact and be part of the solution.

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Econometric Analysis of Risk Preference Patterns among Smallholder Organic Producers in South Africa

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Abstract: The article assesses the determinants of farmers' decisions to participate in organic farming, eliciting farmers risk preferences and empirically analysing farmer's sources of risk and risk management strategies. The ordered probit results indicate that older farmers, who are less risk averse and reside in the sub-ward Ogagwini, Ezigani and Hwayi were more likely to be certified organic. Similarly, the propensity to adopt organic farming is positively correlated to household size, livestock ownership, asset base and tenure security. At higher pay-offs, farmers were intermediate to moderately risk-averse, with little variation according to personal characteristics. In general, price, production and financial risks were perceived as important sources of risk. Seven principal components, explaining 66.13% of the variation were extracted. Socio-economic factors having a significant effect on the various sources of risk were age, gender, education, location, information access and risk taking ability. The dimensions of risk strategies were named as diversification, precautionary savings and social networks. Results provide practical insights for policy changes relevant in motivating the adoption of organic practices, increasing smallholder farmers' capacity to manage risk and driving growth in the organic food market.

Key words: Organic farming, ordered probit, principal components, risk preferences.

1. Introduction

Modern agricultural methods have resulted in spectacular increases in productivity [1]. However, the majority of the chronically hungry are small farmers in developing countries who produce much of what is eaten, are often too poor to purchase inputs and are marginalized from product markets [2]. Scialabba [3] argued that 75% of the world's 1.2 billion poor live in rural areas of developing countries. They suffer from problems associated with subsistence production in isolated and marginal locations with low levels of technology. These subsistence and smallholder's livelihood systems are prone to the risks of drought and floods, crop and animal diseases and market shocks. Notwithstanding, they also possess important resilience factors associated with the use of family labour, livelihood diversity and indigenous knowledge that allow them to exploit risky environmental niches and to cope with crises. Pro-poor policies and technology options based on efficiency in production and employment generation associated with family farms can be expected to improve these household conditions.

Due to the prevailing constraints and conditions for numerous small scale farmers to succeed, the practice of organic agriculture has been identified as a pathway to sustainable development and to enhance food security [4, 5]. Organic farming is one of the sustainable approaches to farming that can contribute to food and nutritional security [6]. Driven by increasing demand globally, organic agriculture has grown rapidly in the past decade [7]. Policy makers at the primary end of the food chains must wrestle with the dual objective of reducing poverty and increasing

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the flow of ecosystem services from rural areas occupied by small scale farmers and/or family farms [8].

The identification of organic agriculture as a development pathway, leading to improved livelihoods, is based on a central assumption that decreased use of external inputs, combined with price premiums for products will provide economic gain which can improve aspects of the farmer's livelihood, for example, food access, health, or education [9]. Pannell et al. [10] stated that the adoption of an innovation such as a conservation practice is principally influenced by the characteristics and circumstances of the farmer and the characteristics of the practice, especially, its relative advantage over existing practices and landholder's ability to try the practice. Farmers adopt an innovation if they expect that the practice will help them achieve their goals, which may include economic, social and environmental goals. Within this conceptual framework, there is compelling evidence that adoption is strongly affected by risk related issues [11-13].

Several studies have reviewed and summarized the factors that influence adoption decisions in agriculture example [14, 15]. However, there appears to be a paucity of empirical studies which address the practical implications of risk, specifically, in relation to the adoption of organic farming practices by smallholder farmers. Evenmore, there are no readily accessible and contextualized studies that combine quantitative investigations of the influence of motivations and risk perceptions on the adoption of organic practices in South Africa. As farmers are the ultimate decision makers in the adoption process, understanding their perceptions of a given technology are important in the adoption process and critical in designing information dissemination and support programmes.

This study seeks to: (1) establish the determinants of farmers' decision to participate in organic farming distinguishing among the fully-certified organic, partially-certified organic and non-organic farmers; (2) elicit farmers risk preferences and empirically analyse farmers sources of risk and risk management strategies; (3) make policy recommendations that have an implication on technology adoption, increase smallholders capacity to bear risk and enable government and other role players have a clear understanding of producers' production decisions.

2. Materials and Methods

The study was carried out among 200 smallholder farmers in the rural Umbumbulu Magisterial District, uMgungundlovu District Municipality, within the province of KwaZulu-Natal, South Africa. The survey farmers were stratified into three groups of fully-certified organic, partially-certified organic and non-organic farmers. The farmers were selected through a census survey of 151 organic farmers (purposively selecting 48 fully-certified organic and 103 partially-certified organic farmers). Another sample of 49 non-organic farmers was randomly selected within the same region from a sample frame constructed from each of the five neighbouring wards. Producer and household structured questionnaires were used to record household activities, socio-economic and institutional data as well as household demographics through personal interviews with the principal decision maker.

The farmers were further asked to give their perceptions of the main sources of risk affecting their farming activity by ranking 20 potential sources of risks on likert-type scales ranging from 1 (no problem) to 3 (severe problem). These sources of risk were developed from findings of the research survey and from past research on the sources of risk in agriculture and challenges that smallholder farmers face in trying to access formal supply chains. The Arrow Pratt Absolute Risk Aversion (APARA) coefficient was used to measure the farmer's degree of risk aversion and the experimental gambling approach to establish the risk classification.

The ordered probit model is used to determine the factors that influence a farmer's organic farming status. Based on the review of literature, the model is estimated as follows:

(1) Organic farming status = f (age, gender, education, household size, farm size, farm income, off farm income, input costs, land tenure, location, land tenure, livestock, chicken ownership, risk attitudes and assets) (1)

The organic farming status is modelled using the ordered probit model with the model outcomes as:

$S_i = 3$ (fully-certified organic);

$S_i = 2$ (partially-certified organic) and

$S_i = 1$ (non-organic).

The farmer's decision on their organic farming status is unobserved and is denoted by the latent variable s_i^*. The latent equation below models how s_i^* varies with personal characteristics and is represented as:

$$s_i^* = X_i'\alpha + \varepsilon_i \quad (2)$$

where, the latent variable s_i^* measures the difference in utility derived by individual i from either being fully-certified organic, partially-certified organic or non-organic.

(i = 1, 2, 3…n) n represents the total number of respondents. Each individual i belongs to one of the three groups.

X_i is a vector of exogenous variables,

α is a conformable parameter vector, and the error term ε_i is independent and identically distributed as standard normal, that is ε_i-NID (0, 1).

The method of Principal Components Analysis (PCA) was applied to the scores of the sources of risk to analyse further underlying dimensions of the variation among the sources of risk. The decision about which Principal Components (PCs) to retain depends on the percentage of the variance accounted for by the variable, the absolute variance accounted for by each PC, and whether the PC can be meaningfully interpreted.

The PCs estimated as linear functions of the original sources of risk are mathematically presented as:

$$PC_1 = a_{11}X_1 + a_{12}X_2 + \ldots + a_{1k}X_k$$
$$PC_2 = a_{21}X_1 + a_{22}X_2 + \ldots + a_{2k}X_k$$
$$PC_k = a_{k1}X_1 + a_{k2}X_2 + \ldots + a_{kk}X_k \quad (3)$$

where,

k = 1…20;

$a_{i1}…a_{ik}$ = the component loadings; and

$X_1…X_k$ = the sources of risk.

The coefficients a_{i1}, $a_{i2}…a_{ik}$ were chosen such that the first PC (PC_1) will have as large a variance as possible, the second PC (PC_2) was chosen to be uncorrelated with the first, and to have as large variance as possible, etc.. The PCs thus provide measures of the amount of common variation as well as magnitudes and nature of divergences in the farmers' scores for their perceptions of sources of risk.

The relationships between the perceptions of risk sources against farm and farmer socioeconomic characteristics were explored using factor analysis and multivariate regression methods. In regression analysis, the standard factor scores achieved from the factor analyses of the sources of risk were regressed on farms' and farmers' socioeconomic characteristics to identify the impact of these characteristics on the farmers' perceptions of risk sources. Specifically, the regression models can be represented as:

$$FSR_{it} = \beta_0 + \beta_1\text{Age} + \beta_2\text{Gender} + \beta_3\text{Education} + \beta_4\text{Geography} + \beta_5\text{Landsize} + \beta_6\text{Information} + \beta_7\text{access} + \beta_8\text{Household size} + \beta_9\text{Household Income} + \beta_{10}\text{Risk taking} \quad (4)$$

where, FSR_{it} = standardized factor scores for sources of risk factors (I = 1, 2, 3,. . . 7), achieved from the factor analyses of sources of risk;

Age, Gender, Education, Geography, Land size, Information, access, Household size, Household Income, Risk taking = Explanatory variables;

ε_t = Error term.

All of the regression models were tested for possible violations of the basic assumptions of a linear regression model. Specifically, a simple correlation matrix, and collinearity diagnostics (tolerance and

variance inflation factor, VIF) was inspected to detect any potential multicollinearity. The first order autocorrelation problem was checked using the Durbin-Watson statistics.

A Herfindahl index is used to calculate enterprise diversification and represent the specialization variable. Although, this index is mainly used in the marketing industry to analyze market concentration, it has also been used to represent crop diversification [16, 17]. The Herfindhal index is bound by zero (complete diversification) to one (complete specialization).

$$\text{Herfindhal index (DH)} = \sum_{i=1}^{N} S_i^2 \quad (5)$$

where,

N is the number of enterprises and is the value share of each *i-th* farm enterprise in the farm's output;

$S_i = \frac{S_i}{\sum_1 S_i}$ is the proportion of the *i-th* activity in acreage/income.

3. Results and Discussion

3.1 Determinants of Organic Farming Adoption

The ordered probit model successfully estimated the significant variables associated with the farmer adoption decisions. The results are presented in Table 1. The following variables were found to be significant determinants in the organic farming adoption decision by smallholder farmers in the study area: age, household size, land size, locational setting of the farmer depicted by the sub-wards Ogagwini, Ezigani, and Hwayi, farmer's risk attitude, livestock ownership (chicken and goat ownership), land tenure security as depicted by the rights, the farmer can exercise on his/her own cropland to build structures and asset ownership.

The study established that older farmers with large household sizes were more likely to be certified organic. Farming in the study area and many rural areas of South Africa is undertaken by older farmers, with the average age of the farmers in the study area being over 50 years old. Farming in many instances is also considered as an alternative option to retirement from wage employment, as the younger members of the household migrate to urban areas in search for jobs. Similar findings have been recorded by several authors [18-20]. Higher subsistence pressure leads to greater adoption of new agricultural technology aimed at improving food access among large households [20]. Large family sizes are also an indication of availability of labour and provide the opportunity for the farm to develop the technical know-how required for certified organic farming. The potential to meet peak labour demand also highlights the importance of the availability of family labour.

The significant and negative correlation between land size and adoption implies that smaller farms appear to have greater propensity for adoption of certified organic farming. This finding is supported by several studies reviewed in the literature that allude to

Table 1 Adoption of organic farming among smallholder farmers: ordered probit model results.

Variables	Parameter	Robust std error	*P*-values
Age	0.0194072	0.0079204	0.014***
Gender	0.3796234	0.2707705	0.161
Household size	0.0504668	0.027152	0.063*
Land size	-0.2352607	0.1083583	0.030**
Off farm income	-0.0001223	0.0001129	0.279
Location (sub-ward)			
Location (1 = ogagwini)	20.894311	0.6380815	0.000***
Location (1 = ezigani)	4.191274	0.7234394	0.000***
Location (1 = hwayi)	5.158803	0.8495047	0.000***
Risk attitudes	-0.7595078	0.3773067	0.044**
Fertility (manure)			
Chicken ownership	0.0424046	0.0148472	0.004***
Cattle ownership	-0.0418692	0.0431078	0.331
Goat ownership	-0.1005212	0.0569375	0.077*
Land tenure rights			
Land tenure (1= build structures)	0.4803418	0.2372247	0.043**
Land tenure (1= plant trees)	0.0235946	0.3023182	0.938
Land tenure (1= bequeath)	0.1335225	0.2619669	0.610
Land tenure (1= lease out)	-0.3840883	0.2593139	0.139
Land tenure (1= sell land)	0.0829177	0.2978485	0.781
Asset ownership	0.5853967	0.205389	0.004***

Significance levels: ***$P < 0.01$: **$P < 0.05$: *$P < 0.1$

the fact that organic farms tend to be smaller than conventional farms. The significance of the locational setting implies that farmers who reside in the sub-wards Ogagwini, Ezigani, and Hwayi were more likely to be certified organic. This suggests the presence of local synergies in adoption which raises the question about the extent to which ignoring these influences biases policy conclusions. The closer a farmer is to the nearest adopter, the higher the frequency of contact, the more likely the farmer will receive valuable information, thus increasing their skill and decreasing their uncertainty [21].

The results further show that fully-certified organic farmers are more likely to take risks compared to the partially-certified and non-organic farmers. Similar studies[22, 23] found that organic farmers are less risk averse than their conventional counterparts. The significance of livestock is explained by the importance of manure for organic farming. Chicken manure is commonly used in the study area as other animal manure for soil fertilization. The results showed that when farmers have security of land tenure, the propensity to adopt certified organic farming is higher. Land ownership is customary and farmers have permission to occupy. Informal arrangements based on traditional social capital resources assure affordable and flexible access to land for most people. The propensity to adopt was also positively influenced by asset index which is a proxy for wealth.Wealthier farmers are better able to bear risk and, therefore more likely to try new technologies. Similarly, they may be better able to finance the adoption of technologies and appropriate farming practices.

3.2 Risk and Risk Management among Smallholder Farmers

3.2.1 Risk Aversion Classification

Table 2 presents the distribution of risk aversion preferences for each prospect for the fully-certified organic, partially-certified organic and non-organic farmers. It can be noted that on average, the majority of the respondents revealed their preference for prospects representing intermediate and moderate risk aversion alternatives across the three farmer groups. Table 2 further shows that non-organic farmers were the most risk averse of the farmer groups. This may explain why they have not adopted certified organic farming despite organic certification being introduced in the area since 2000. These results conform to a priori expectations regarding the risk preference patterns of smallholder farmers.

3.2.2 Farmers' Perception of Risk Sources

It is evident from the rankings in Table 3 that some of the sources of risk were common across the farmer groups. In general price, production and financial risks were perceived as the most important sources of risk. These were identified across the farmer groups as: uncertain climate, lack of cash and credit to finance inputs; tractor unavailability, delays in payment for produce sent to pack house and livestock damage to crops. There is a clear indication that labour and access to crop land are not a constraining factor with the South African Government having made great strides through land reform programmes to ensure access to land for small emerging black farmers.

3.2.3 Principal Component Analysis of Farmers' Perceived Sources of Risk

PCs that explained 66.13% of the variance in the original scores were extracted from the covariance matrix using STATA 11 as reported in Table 4. PCs that meet Kaiser's criterion (have Eigen values ≥ 1, have estimated component coefficients > 0.3, and can be meaningfully interpreted are retained [24, 25]). The Eigen values for the seven PCs are all above one. The factor loadings 1 to 7 can be described as "financial and incentives index", "input-output index", "crop production index", "labour bottleneck index", "production information index", "market opportunity index" and "input availability index", respectively.

PC_1 (Financial and incentive index) explained 18.37% of the variance with all six estimated coefficients above 0.3 being positive. This index suggests that

Table 2 Distribution of smallholder farmers according to risk preference patterns.

Farmer group	Risk aversion classification					
	Extreme	Severe	Intermediate	Moderate	Slight to neutral	Neutral to preferring
Fully certified organic (n = 55)	7.3	5.5	30.9	40	7.3	9.1
Partially certified organic (n = 95)	4.2	8.3	44.8	29.2	5.2	7.3
Non-organic (n = 46)	20.4	8.2	30.6	30.6	0	4.1
Pooled data (n = 196)	9.0	7.5	37.5	32.5	4.5	7.0

Source: Field data.

Table 3 Identification of risk sources and rank.

Constraint	Fully certified organic n = 48		Partially certified organic n = 103		Non-organic n =49	
	Mean	Std dev.	Mean	Std dev.	Mean	Std dev.
Livestock damage crops	2.56	0.744	2.82	0.488	2.80	0.539
Uncertain climate	2.96	0.189	2.83	0.409	2.82	0.486
Uncertain prices for products sold to pack house	2.21	0.793	2.13	0.591	-	-
Uncertain prices for products sold to other markets	1.94	0.811	2.02	0.595	2.17	0.761
More work than the family can handle	2.58	0.599	2.32	0.688	2.53	0.649
Lack of cash and credit to finance inputs	2.78	0.567	2.58	0.615	2.78	0.468
Lack of information about producing organic crops	2.02	0.687	2.20	0.632	2.16	0.717
Lack of information about alternative markets	2.38	0.623	2.29	0.602	-	-
Lack of proper storage facilities	2.56	0.660	2.46	0.543	2.41	0.643
Lack of affordable transport for products	2.72	0.492	2.42	0.560	2.06	0.852
Lack of telephones to negotiate sales	2.69	0.509	2.55	0.633	2.22	0.771
Inputs not available at affordable prices	2.52	0.642	2.80	0.447	2.51	0.545
Tractor is not available when I need it	2.76	0.501	2.89	0.416	2.46	0.713
Cannot find manure to purchase	1.92	0.778	2.56	0.660	2.20	0.645
Cannot find labour to hire	1.73	0.764	1.76	0.816	2.00	0.764
Cannot access more cropland	1.95	0.753	1.98	0.805	1.92	0.794
Delays in payment for products sent to pack house	2.22	0.723	2.89	0.315	-	-
Lack of bargaining power over product prices at the pack house	2.16	0.672	2.20	0.704	-	-
Lack of information about consumer preferences for our organic products	2.23	0.654	2.44	0.604	-	-
Pack house does not reward me fully for my own product	1.86	0.780	2.02	0.866	-	-

Mean score (1 (no problem) to 3 (severe problem) and Rank is in ascending order; 1 means most important and 20 least important.

farmers who are concerned with uncertain prices for the formal and informal market options are also faced with the risk of labour unavailability as well as lack of bargaining power. These farmers are also concerned about the lack of information on consumer preferences and the ability of the pack house to give farmers incentives for production. PC_2 (Input-output index) accounted for 12.74% of the variance and shows that fully-certified and partially-certified farmers, who rank lack of cash and credit to finance inputs as a source of risk, are also concerned with the lack of proper storage facilities to store their crops. These farmers also experience challenges to purchase manure for organic farming, and delays in payment for products that have been sent to the pack house.

PC_3 (Crop production index) accounted for 8.94% of the variation and shows that farmers who strongly perceive livestock damage to crops as a major source of risk are also concerned about inputs not being available at affordable prices. Across the three farmer groups, lack of cash and credit to finance inputs was identified as a source of risk. However, these farmers did not perceive lack of affordable transport for products as a major risk. The latter can be attributed to the fact that the produce is collected at the farm gate and transport costs are limited to produce sold in the local market or surrounding farms.

PC_4 (Labour bottleneck index) explained 7.66% of

Table 4 Estimated principal components for the sources of risk variables.

Proportion	PC1	PC2	PC3	PC4	PC5	PC6	PC7
	18.37	12.74	8.94	7.66	7.43	5.77	5.21
Eigen values	3.6748	2.5483	1.7874	1.5325	1.4866	1.1538	1.0417
Sources of risk	Factor loadings						
Livestock damage crops	0.1100	-0.1156	0.3452	0.2196	0.2857	-0.0013	-0.2347
Uncertain climate	0.0757	0.0462	0.0187	-0.2487	-0.4786	-0.1421	0.2498
Uncertain prices for products sold to pack house	0.3281	-0.0683	-0.0500	0.0549	-0.3858	-0.0258	0.2812
Uncertain prices for products sold to other markets	0.3690	-0.1476	-0.0176	-0.0476	-0.0498	0.1235	-0.1389
More work than the family can handle	0.1083	0.0648	0.2948	0.5425	0.0253	0.1286	-0.0136
Lack of cash and credit to finance inputs	0.0279	0.3881	0.3753	-0.0694	0.1017	0.1417	0.0874
Lack of information about organic farming	0.1746	-0.0545	-0.0123	0.0754	0.3494	-0.1293	0.1272
Lack of information about alternative markets	0.2371	0.0901	0.1686	0.1849	0.0141	0.5791	-0.1677
Lack of proper storage facilities	-0.0776	0.3881	-0.2332	-0.0969	0.2711	-0.1649	-0.0234
Lack of affordable transport for products	0.0498	0.1455	-0.4236	0.2461	0.2707	0.1866	0.2077
Lack of telephones to negotiate sales	0.2397	-0.1594	0.0795	-0.2056	0.2309	0.3997	0.2935
Inputs not available at affordable prices	0.0256	0.2961	0.4164	0.1253	-0.1322	0.1380	0.3008
Tractor is not available when I need it	0.0195	0.2949	0.0251	-0.2040	0.2671	-0.2627	0.4099
Can not find manure to purchase	0.0410	0.4545	-0.0444	0.0499	-0.2645	0.1226	-0.2108
Can not find labour to hire	0.3307	-0.0497	0.2221	0.0955	-0.0049	-0.3651	-0.1058
Can not access more cropland	0.1567	0.1187	0.2744	-0.5214	0.1259	0.0288	-0.1877
Delays in payment for products sent to pack-house	0.1748	0.4314	-0.1998	0.2250	-0.1263	-0.0296	-0.2235
Lack of bargaining power over product prices at the pack-house	0.3734	0.0006	-0.0859	-0.1015	0.0098	-0.1224	-0.2903
Lack of information about consumer preferences for our organic products	0.3706	0.0829	-0.0977	-0.0456	0.1177	-0.3165	-0.0481
Pack-house does not reward me fully for my own product	0.3594	-0.0640	-0.1541	0.1723	-0.0063	0.0119	0.3410

the variance and implied a labour bottleneck risk. More work than the household can handle was identified as a major risk. However, lack of crop land was not perceived as a risk. The latter is due to the fact that land in the area is not a constraining factor and expansion of cropland is possible upon request to the local headman. On the other hand, organic farming is a labour intensive technology and would require more labour than conventional farming, however, the returns may be higher if farmers access the niche markets as is currently the case with the fully-certified and partially-certified smallholder farmers who are supplying an market food retail store in KwaZulu-Natal province. PC_5 (Production information index) displays a variation of 7.43% in the farmers' rankings, and captures a lack of production information risk. This risk is closely linked to weak support for extension services and advice to enable smallholder farmers to produce more food and reap greater benefits from their organic farming and harvest. The South Africa Government is in the process of revitalizing extension services to ensure access to information and improved agricultural practices among smallholder farmers especially in rural areas.

PC_6 (Market opportunity index) is a lack of information about alternative markets risk and accounted for 5.77% of the variation in the farmers' scores for the sources of risk. What both established and emerging black smallholdershave in commons is subsistence farming with surplus production being rare in this rural context. Moreover, the accidental but limited excess farming output is usually sold in local markets. The PC_7 is an input availability risk and accounts for 5.21% of the variation. The farmers perceived lack of inputs at affordable prices, tractor not available when needed and little or no reward

from the pack house as major risk sources. Lack of access to inputs and incentives is a deterrent to the development and growth of smallholder farming.

3.2.4 Relationship between Perceptions of Risk Sources against Farm and Farmer Socio-economic Characteristics

Table 5 shows the relationships between the farmer's perceptions of sources of risk and the farm and farmer socioeconomic variables. Multicollinearity was not found to be a problem as correlations were low and nonlinear principal components analysis drawing on studies [26] that focused on socioeconomic variables did not show strong relationships. The variance inflation factors as defined by Hair [27] had all values around 1. The equations for "financial and incentive", "input-output" and "labour bottlenecks" are statistically significant at a 1%, 1% and 5% level of significance, respectively. The equations for "crop production" and "input availability" are significant at less than 20%. All Durbin-Watson statistics for the six regression models ranged from 1.5 to 2.5, suggesting that autocorrelation is not a problem for these models. The goodness of fit is fairly low as it is the case for discrete choice models [28].

The socio-economic factors, age, gender, education, location, information access and risk taking ability had a significant effect on the various sources of risk: older farmers were concerned about the availability of labour while female farmers considered input-output risk and crop production risks as significant and relevant. Farmers residing in the non-organic areas of Hwayi and Numgwane sub-wards were more concerned about financial and incentive risk as well as input availability. These farmers have limited access to financial resources and incentives for production while farmers residing in the pioneer organic areas of Ogagwini and Ezigoleni considered input-output risk as less relevant. Farmers with access to information perceived input-output risk and crop production risks as less relevant but financial and incentive risk are significant and more relevant. Farmers who were more likely to take risk perceived labour bottleneck risks as much less relevant.

3.2.5 Risk Management Strategies Used by Farmers

The most important traditional risk management strategies used by the surveyed farmers in rural KwaZulu-Natal and presented in Table 6 are identified as crop diversification, precautionary savings and participating in social network.

Enterprise diversification is a self-insuring strategy used by farmers to protect against risk [17]. The overall Herfindahl index of crop diversification is estimated at 0.61 which indicates that the cropping system is relatively diverse. These results confirm previous findings by Rahman [29] who obtained an estimated DH of 0.49-0.69 among smallholder farmers in three regions in Bangladesh. As shown in Table 6, non-organic farmers practiced more crop diversification with a DH index of 0.23 compared to organic farmers with a DH index of 0.72. Further, crop diversification was practised by 69.1% of fully-certified farmers, 81.2% of the partially certified farmers and 96.8% of the non-organic farmers.

Cunha [30] indicated that the quantitative significance of precautionary saving depends on how much risk consumers face. The current level of saving in the study area was low with savings ranging from less than R500 to over R5000 per month. The level of savings was low across all farmers. There were two main categories of social networks identified were farmers association and most notably burial clubs and stockvels. The findings are consistent with economic theory which postulates that in the absence of insurance markets, poor farm households tend to be risk averse and are reluctant to participate in farm investment decisions that are uncertain or involve high risk. The farmers association is used as a vehicle by the organic farmers to gain access to markets for their organic produce while the burial clubs and stockvels are sources of access to credit and/or loans. In the latter instance, farmers do not have to produce

collateral. The burial clubs and stockvels are common in most rural areas and are a source of mitigating liquidity and financial risk where possible.

4. Conclusions

This study sought to identify among others, independent variables that explain the adoption of organic farming and thereby facilitate policy prescriptions to augment adoption especially in developing countries. The technology adoption analysis of the independent variables used in the ordered probit analysis revealed some underlying patterns of influence. Given the limited prospect of identifying such variables through further research, it is concluded that efforts to promote organic farming will have to be tailored to reflect the particular conditions of individual locales. The propensity of adoption decisions by neighbourhoods to affect others

Table 5 Results of multiple regressions for sources of risk against socio-economic variables.

Independent variables	Description of variable	Sources of risk						
		Financial and incentive	Input-output	Crop production	Labour bottlenecks	Production information	Market opportunity	Input availability
Constant		-1.35**	-0.362	-0.674	-1.202*	0.291	-0.638	0.1
Age	Years	-0.004	0.008	-0.009	0.017**	-0.001	0.007	-0.01
Gender	Male = 0	-0.321	0.626***	0.52**	-0.127	-0.019	0.024	-0.194
Education	Years	-0.013	0.065***	0.002	-0.046*	0.022	0.02	-0.026
Location	1 = Ogagwini; 2 = Ezigani; 3 = Hwayi; 4 = Numgwane	0.243***	-0.114*	0.074	0.073	-0.049	0.004	0.18**
Land Size	Hectares	0.101	-0.084	0.086	-0.208**	-0.028	-0.079	-0.115
Information	Hours	0.089***	-0.051***	0.021	0.03	-0.05**	-0.007	-0.008
Household size	Number	0.032	0.029	0.028	-0.007	0.02	-0.012	-0.017
Household Income	Rands/year	0.045	0.005	0.013	-0.004	-0.001	0.035	-0.008
Risk taking	1 = less likely to take risk 2 = more likely to take risk	0.05	-0.135	0.057	0.191*	0.064	0.002	0.117
Adj. R^2		0.223***	0.188***	0.048	0.12**	0.003	-0.070	0.028*
Durbin Watson statistics		1.464	1.785	1.632	1.642	2.147	2.477	1.779

***, **, * represent significance at 1%, 5% and 10%, respectively.

Table 6 Risk management strategies used by the different farmer groups.

Risk management strategy	Fully certified organic $n = 48$	Partially certified organic $n = 103$	Non-organic $n = 49$
Enterprise diversification index (DH)	0.7220	0.8962	0.2303
Practice crop diversification (% of respondents)	69.1	81.2	96.8
Savings (% of respondents)			
Savings bank account	60.9	48.9	46.8
Current level of savings[1] (% of respondents)			
less than R500	27.27	37.84	35.29
R501-R1000	45.45	29.73	41.18
R1001-R5000	21.21	29.73	17.65
More than 5000	6.07	2.70	5.88
Social networks (% of respondents)			
Membership of EFO	100	100	10
Others (burial clubs, stockvel[2])	33	25	25

[1]Currency exchange is U$ 1 ≈ R6.78 as at September 2011.
[2]*A Stokvel* is a club serving as a rotating credit union in South Africa where members contribute fixed sums of money to a central fund on a weekly, fortnightly or monthly basis.

must be given due importance, for price marketing, extension delivery and development purposes, while delineating target domains for introducing new technologies especially where resources are limited. Identified sources of risk faced by smallholder farmers provide useful insights for policy makers, advisers, developers and sellers of risk management strategies. This information can yield substantial payouts in terms of the development of quality farm management and education programs as well as the design of more effective government policies. New technologies and rural development programs need to be tailored to the risk attitudes of a particular group of farmers if they are going to be effective. Due to the risk-averse nature of smallholder farmers, policy makers need to develop strategies that enable them better manage and reduce risk while mitigating the identified sources of risk.

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Effect of Self-fertilization on Performance, Breeding and Germplasm Management of Four Local Faba Bean Cultivars

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Abstract: Faba bean is self- and cross-fertilized species. The consequences of self-fertilization are important factors determining the germplasm management in such species with levels of heterogeneity and heterozygsity. Effects of self-fertilization on floral, yield and yield components characters were evaluated by comparing two levels of selfing, produced in bee-proof cages, in open-pollination at two locations in Sudan. Selfing process results in no significant differences in yield and yield components within each cultivar. Autofertile lines with reduced partial dependence on insects for seed set could be produced. Spatial isolation should be used to maintain the genetic purity of such lines. An alternate strategy for entries multiplication should focus on increasing heterozygosity and the maintenance of cross-fertilization inside the entries to prevent contamination with foreign pollens. Our results follow the previous knowledge on faba bean genetic resources conservation and management.

Key words: Faba bean, germplasm, insect pollinators, line cultivar, self-fertilization, management.

1. Introduction

Faba bean (*Vicia faba* L.) is one of the earliest domesticated food legumes. The crop is used as a source of edible protein for food in developing countries (Asia, Central America and Africa) and feed in Europe. As a biological N_2-fixer crop, it is used to save the farmer the cost of artificial nitrogen fertilizer for the subsequent cereal crops, therefore, the crop is especially appreciated and esteemed in organic system of agriculture.

In Sudan, faba bean is grown as winter crop in the Northern part of the country along the River Nile banks (relatively cooler and with longer winter season than other part of the country) and in Darfur on the upper Terraces of Marra Mountain where the climatic conditions are suitable for its production. The production of the crop is also extend to nontraditional areas at latitudes lower than 14 °N, where the growing season is restricted to a short period during which high temperature prevails at the beginning and end of the winter season.

Faba bean is not indigenous to Sudan but was very early introduced from either Egypt or Ethiopia. Since the start of modern breeding work on faba bean in Sudan at the Agricultural Research Corporation (ARC) and as a result of the imported genetic material from different countries (e.g., Europe, Egypt, former USSR, Ethiopia and ICARDA), a number of cultivars with good seed yield, stability and quality have been released for both traditional and new areas [1]. In addition to these cultivars, local landraces (from early introductions) were grown by the farmers. The main

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genetic variations which exist among theses material include: mode of flowering and maturity, seed yields and its yield components, seed size, disease resistance or tolerance to powdery mildew, pest resistance or tolerance to leaf minor and aphis, testa and hilum color and degree of autofertility [2]. The presence of such germplasm in a country like Sudan (semi-arid climate) constitutes a genetic resource that needs to be maintained and exploited. Conservation and management of such genetic material could make the country one of the most important resources of faba bean germplasm for semi-arid tropics, especially after the extension of Plant Genetic Resources (PGR) unit at the ARC in 1995 to hold the mandate to conserving and enhancing utilization of crop genetic resources in Sudan. Focus is on collection, multiplication and characterization of germplasm, to make such germplasm available to users within ARC and other research centers. For an appropriate strategy to multiply, regenerate and conserve of the diversity in a crop species, its mode of reproduction is of crucial importance [3].

Faba bean is partially allogamous species [4]. Insects carry out both self- and cross pollination when visiting other flowers. Under Sudan conditions, the degree of cross-fertilization ranges from 12% to 18% [1, 5, 6] and the autofertility reached up to 88% [6]. In Europe, and as a result of its partial allogamy, faba germplasm are maintained as population and inbred cultivars through process of self-fertilization [7]. The consequences of self-fertilization were found to be different when comparing species entries; inbreeding effects was low in self-pollinated cultivars compared to the open-pollinated ones [8, 9]. Selfing process results in reduction in: plant height and 100-seed weight [9], number of seeds/pod [10] and yield [11]. Therefore, for a curator, plant breeder and gene bank manager, in addition to the loss of diversity due to random genetic drift, the effect of self-fertilization is one of the issues that must face when multiplying and regenerating seeds. In order to conserve our present *Vicia faba* germplasm, information on the effect of selfing process is of great importance. Therefore, the objectives of the present study was to investigate the effects of changes related to selfing on perfornace, breeding and germplasm management of our faba bean.

2. Materials and Methods

2.1 Planting Material

The planting material consisted of four local, open-pollinated cultivars (Hudeiba/93, Ed-damar, Bassabier and Selaim), normally grown in the Northern part of the Sudan (traditional areas of production). To evaluate the effect of self-fertilization, selfed seeds of two levels of inbreeding (S_1 and S_2) were produced from the original (open-pollinated) seed (S_o) using bee proof cages. In self pollination conditions, for each cultivar, S_1 was produced from S_0 and S_2 from S_1. As a result, 12 genotypes; namely, Hudeiba/93-S_0, Hudeiba/93-S_1, Hudeiba/93-S_2, Ed-Damar-S_0, Ed-Damer-S_1, Ed-Damer-S_2; Bassbier-S_0, Bassbier-S_1, Bassbier-S_2; Selaim-S_0, Selaim-S_1, Selaim-S_2 were produced. These genotypes were assessed for floral, yield and yield components characters in field experiments for two consecutive years in 2008/2009 and 2009/2010 seasons and at two locations; namely, the Demonstration Farm of the Faculty of Agriculture, Shambat (latitude 15°39′N, longitude 32°31′E and altitude 230 m) and Hudeiba Research Station (Lat. 17°35′N and Long. 33°57′E and altitude 350 m). Each genotype in a replicate was planted in an experimental unit consisting of three rows of 2 m long. The inter-raw and intra-raw spacing were 70 and 20 cm, respectively; the planting was in both sides of the ridge. The layout of the experiment was in a complete randomized block design with two replicates. The experimental unit was irrigated at an average interval of 10 days, with a total of 8 irrigations during both seasons. At the two locations and in both years, weeding was carried out and insecticides were applied as required. The soil of the

experimental sites is clay with alkaline pH. The climate of the two locations is semi-arid with mean annual rainfall of 100-200 mm (in autumn) and maximum temperature of about 42 °C in summer and 28 °C in winter (early and late winter).

2.2 Data Collection

Data were collected from 10 random plants, in each experimental unit. Characters examined were 1) floral characters: number of days from sowing to flowering, number of flowering nodes, number of flowers per inflorescence; 2) yield and yield components characters: number of pods per podded node, number of pods per plant, number of seeds per pod, number of seeds per plant, 100-seed weight in gram, and yield in kg ha^{-1}.

2.3 Statistical Analysis

The computer program Plabstat [12] was used for statistical analysis of the collected data. The data were analyzed corresponding to the completely randomized block design.

3. Results

3.1 Floral Characters

Analysis of variance for individual characters showed no significant differences for the effect of selfing within each cultivar (Table 1). Means of nine traits of S_0, S_1 and S_2 of each of the four cultivars were of nearly similar values (Table 2). However, the significant difference occurred among the cultivars, except days to 50% flowering which revealed no significant differences. Moreover, these traits responded significantly for the effect of year and year × location interaction (Table 3). In the second year, the cultivars flowered late in the season compared with the first one; as a result, the number of flowering nodes and number of flowers per inflorescences were increased in the second season (Table 3).

3.2 Yield and Yield Components

Analysis of variance revealed no significant differences in yield and yield components among S_0, S_1, and S_2 for each cultivar. However, significant differences were among the cultivars and the levels of selfing among the cultivars (Tables 1 and 2). With the exception of the number of pods per plant and number of seeds per pod, other components were affected significantly by location. For the effect of year on the cultivars, number of pods per plant, seeds per pod and seed weight showed non significant differences, other traits showed significant differences. Most of the traits were higher expressed in the second year than in the first one and at the two locations (Table 3).

On the other hand, all studied traits exhibited no response to the effect of location × year interaction. The effect of genotype × location interaction was only significant for pods per podded node, number of pods

Table 1 Mean squares for floral characters, yield and yield components of three generations of four faba bean cultivars.

	DF	DTF	FN	FPI	PPN	PPP	SPP	SP	SWT	Yield (kg ha^{-1})
Location (L)	1	12.04	1.08	14.72**	4.64*	9.62	0.01	15.12*	0.55**	156,767.35**
Year (Y)	1	88.1**	6.72**	10.40**	2.19**	045	1.45**	0.61	0.01	7,250.42*
L × Y	1	77.04**	12. 8**	0.84**	0.11	1.92	0.02	0.23	0.03	1,911.64
cultivar (C)	3	0.97	23.67*	0.32**	0.24*	33.87**	0.21**	1,007.64**	2,895.20**	15,0823.88**
C × L	3	0.63 *	4.41**	0.02	0.18*	3.88**	0.01	0.66	0.01	4,664.14*
Selfing within C	9	0.66	0.23	0.04	0.04	0.15	0.04	004	0.07	17,875.75
Error	76	0.92	0.75	0.10	0.07	0.8 7	0.03	0.33	0.27	1,735.61

Source of variation, floral characters, yield and yield characters;
DF = degrees of freedom; DTF = number of days to flowering; FN = number of flowering nodes; FPI = number of flowers per inflorescence; PPN = number of pods per podded nodes; PPP = number of pods per plant; SPP = number of seeds per pod; SP = Number of seeds per plant; SWT = 100-seed weight (g);
*significant at 5% level;
**significat at 1% level.

Table 2 Mean of floral characters yield and yield characters of three levels of selfing (S_0, S_1 and S_2) in four faba bean cultivars.

Cultivar/generation	Floral characters			Field and yield characters					
	DTF	NF	FPI	PPN	PPP	SPP	SP	SWT	Yield ha^{-1}
Hudeiba/93-S_0	38.00	14.70	3.16	1.94	16.27	2.38	36.58	43.48	2,736.82
Hudeiba/93-S_1	38.50	14.13	3.35	1.78	16.11	2.26	36.39	43.52	2,725.89
Hudeiba/93-S_2	38.00	14.05	3.24	1.94	15.69	2.34	36.35	43.65	2,718.07
Mean	38.17	14.29	3.25	1.88	16.02	2.33	36.44	43.55	2,726.93
LSD	1.13	0.7	0.34	0.34	1.03	0.16	0.5	0.54	21.56
Ed-Damar-S_0	38.50	14.41	3.29	1.81	16.42	2.52	35.61	45.33	2,772.70
Ed-Damar-S_1	38.50	13.70	3.22	1.85	16.25	2.26	35.58	45.16	2,754.02
Ed-Damar-S_2	38.63	13.51	3.19	1.83	16.08	2.36	35.67	45.24	2,763.44
Mean	38.54	13.88	3.23	1.83	16.25	2.38	35.62	45.24	2,763.43
LSD	1.03	0.89	0.34	0.31	1.15	0.14	0.57	0.52	34.77
Bassabier-S_0	37.50	14.07	3.08	2.13	15.65	2.45	35.53	43.70	2,655.53
Bassabier-S_1	38.50	13.82	3.14	1.90	14.88	2.47	35.45	43.59	2,648.84
Bassabier-S_2	38.25	13.30	3.16	1.84	15.00	2.35	35.25	43.72	2,638.69
Mean	38.05	13.73	3.13	1.95	15.18	2.42	35.41	43.67	2,647.69
LSD	1.01	0.89	0.39	0.29	0.83	0.19	0.48	0.48	28.16
Selaim-S_0	38.38	12.20	2.99	1.78	13.88	2.19	22.91	66.28	2,591.14
Selaim-S_1	37.88	11.82	2.85	1.76	13.50	2.25	22.89	66.00	2,586.19
Selaim-S_2	38.38	11.76	3.10	1.66	13.50	2.30	22.90	65.92	2,587.89
Mean	38.21	11.93	2.98	1.73	13.63	2.25	22.90	66.07	2,588.41
LSD	0.96	0.72	0.28	0.15	0.9	0.18	0.64	0.52	27.1
Overall mean	38.25	13.46	3.15	1.85	15.27	2.34	32.59	49.63	2,681.61
LSD	0.55	0.52	0.18	0.17	0.53	0.11	0.34	0.30	24.21

DTF = number of days to flowering; FN = number of flowering nodes; FPI = number of flowers per inflorescence; PPN = number of pods per podded nodes; PPP = number of pods per plant; SPP = number of seeds per pod; SP = Number of seeds per plant; SWT = 100-seed weight (g).

per plant and yield/ha. Highest yield/ha was produced at the Hudeiba location for all cultivars.

4. Discussion

Generally, the higher performance of genotypes of the most of the traits in the second season than in the first one (in the two locations) may due to the optimum daily temperature during the second growing season (November to March). The highest yield obtained at Hudeiba could be attributed to the fact that Hudeiba lies north of Shambat (north of latitude 15°N), where there is relatively cooler and longer winter, which resulted in increasing number of pods per podded nodes and number of pods per plant, hence high yield. Therefore, the effect of cultivar × location interaction was only significant for pods per podded node, number of pods per plant and yield/ha.

The non significant changes in the floral characters, yield and yield components among the levels of selfing do not corrborate findings [7, 8] who found self-fertilization to reduce faba bean performance significantly (i.e., showed the effect of inbreeding depression), especially for seeds per plant. However, no inbred lines of yield capacity as its cross-bred offspring has bean reported in faba bean, but it could be possible that the S_0 sown seeds were smaller than the S_1 and S_2 sown seeds, thus counterbalancing any small inbreeding depression.

Studies on local Sudanese lines and cultivars revealed high degree of autofertility [6, 13]. Therefore,

autofertile lines with reduced partial dependence on insects for proper set of (selfed) seed (so-called tripping) could be produced. However, for high yielding faba bean cultivars, breeding programs should be based on the utilization of heterosis for the production of synthetic cultivars [14]. Lines with high general combining ability and high degree of cross-fertilization are the prerequiste for production of these cultivars [15]. Such lines could be selected from the present material, but attention should be given to insure adequate bee polllination in the multiplication fields. Here a suitable environment for pollinators acitivitiy to increase the degree of cross-fertilization is of siginificant importance [7, 16].

The consequences of self-fertilization should be an important factor in determining the germplasm management in species like faba bean. This is because faba bean populations consist of a mixture of individuals differing in their inbreeding coefficient as well as in the heterozygosity [16, 17]. In such crop species, a given genotype can not be properly maintained, because even stocks with an equal coefficient of inbreeding will produce progenies with differing coefficient of inbreeding [17]. This creates differences between productivity of these progenies which are found at homozygous level, thus decreasing the validity of the results. In addition, the contamination with pollen from whole field reduces the genetic variation between the genotypes, and subsequently the heritability and the gain from selection [18].

In faba bean germplasm management, usually there are difficulties associated with heterogeneous nature of its accessions as well as the danger of cross-pollination among them [7]. In the light of the

Table 3 Effect of genotype, environment and year on three levels of selfing (S_0, S_1 and S_2) in four faba bean cultivars

Genotype/Location	Year	Floral characters			Yield and yield characters					
		DTF	NF	FPI	PPN	PPP	SPP	SP	SWT	YIELD (kg ha^{-1})
Hudeiba/93										
Shambat Farm	1	36.33	14.77	2.50	1.53	16.75	2.17	36.63	43.43	2,731.93
	2	40.33	14.12	3.18	1.88	15.65	2.47	35.75	43.47	2,673.98
Hudeiba Station	1	37.83	13.55	3.35	1.78	15.58	2.20	36.52	43.70	2,748.07
	2	38.17	14.73	3.78	2.33	16.12	2.47	36.82	43.60	2,753.72
Grand mean		38.17	14.29	3.25	1.18	16.02	2.33	36.44	43.55	2,726.93
LSD		0.93	0.57	0.28	0.28	0.84	0.13	0.41	0.44	17.61
Ed-Damar										
Shambat Farm	1	37.17	13.43	2.40	1.52	15.85	2.18	34.48	45.03	2,661.61
	2	41.50	14.67	3.18	1.85	15.20	2.55	35.45	45.33	2,750.76
Hudeiba Station	1	37.67	13.15	3.42	1.97	16.92	2.25	36.40	45.07	2,819.73
	2	37.83	14.25	3.93	1.98	17.03	2.55	36.15	45.53	2,821.61
Grand mean		38.54	13.88	3.23	1.83	16.25	2.38	35.62	45.24	2,763.43
LSD		0.84	0.73	0.27	0.26	0.94	0.12	0.47	0..43	28.39
Bassabier										
Shambat Farm	1	37.17	14.47	2.40	1.52	15.20	2.38	35.57	43.77	2,668.45
	2	39.83	13.52	3.02	1.85	15.12	2.57	35.53	43.42	2,567.58
Hudeiba Station	1	37.67	13.43	3.43	1.93	15.37	2.42	35.97	43.92	2,707.57
	2	37.67	13.52	3.65	2.52	15.02	2.33	35.57	53.58	2,657.17
Grand mean		38.08	13.75	3.13	1.95	15.18	2.43	35.41	43.67	2,647.69
LSD		0.83	0.72	0.32	0.24	0.68	0.16	0.39	0.39	23.00
Seleim										
Shambat Farm	1	36.33	11.25	2.12	1.42	13.00	2.15	22.55	66.10	2,250.56
	2	40.17	11.42	3.07	1.68	13.15	2.25	22.55	65.90	2,531.50
Hudeiba Station	1	38.17	11.62	2.92	1.85	14.33	2.20	23.22	66.13	2,631.26
	2	38.17	13.43	3.82	1.98	14.02	2.38	23.28	66.15	2,640.30
Grand mean		38.21	11.93	2.98	1.73	13.63	2.25	22.90	66.07	2,588.41
LSD		0.78	0.59	0.23	0.12	0.73	0.15	0.29	0.43	22.72

DTF = number of days to flowering; FN = Number of flowering nodes; FPI = Number of flowers per inflorescence; PPN = Number of pods per podded nodes; PPP = Number of pods per plant; SPP = Number of seeds per pod; SP = Number of seeds per plant; SWT = 100-seed weight.

present results and in order to improve our faba bean, agronomic and genetic features of cultivars should be maintained. In Sudan, collections of faba bean genetic materials are small and breeders have accumulated their own stock of genetic diversity in some traits like mode of reproduction and maturity, seed yield and yield components, pests and diseases resistance or tolerance and seed size and colour [2]. Screening such lines for these traits, the diversity and the ranges of genetic variability could be of value when expanding faba bean cultivation into non-traditional areas or for unseen future circumstances. Among the procedures used for faba bean germplasm management, pure line is practiced. However, for the adaptation of the present cultivars to local environment, an alternate strategy for entries multiplication should focus on increasing heterozygosity in faba bean entries. In this case, attention should be given to prevent genetic erosion through contamination with foreign pollens from other bean entries, but the maintenance of the level of cross-fertilization inside the entries is of crucial important as reported by Breese [3]. To prevent gene flow, barriers of the same or other crop species [18, 19], spatial isolation [20] have been reported to maintain the genetic purity of cultivars. In faba bean, it is difficult to have a uniform recommendation on the isolation requirement to prevent such gene flow. Hence, it is necessary to determine the extent of the degree of natural cross-fertilization in a multiplication field to formulate a suitable maintenance programs.

5. Conclusions

On the basis of the present investigation, and as a result of non significant effect of selfing process on yield and yield components within each of our cultivar, lines with high degree of autofertility could be produced from the present material. Therfore, for the effective conservation and maintenance of such genetic material, attention should be given to prevent genetic erosion through contamination with foreign pollens from other bean entries taking in consideration the importance the maintenance of the level of cross-fertilization inside the entries. Moreover, if collection effort is made for local land races present at Marra Mountain as well as those from North Sudan together with conservation of cultivars that showed good adaptation, Sudan can build a germplasm stock for a crop basically introduced to those semi tropical regions.

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A Quantitative Approach to Analyse Rural Population and Development in Some African and Southern-Central American Countries over 10 Years

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Abstract: In developing countries, the emigration from rural territories to urban areas has brought about some negative impacts strictly associated to a lack of services a drop of ecological sustainability and environment protection with the consequence to worsen the marginalization of these territories. In the world, more than 50% of poverty is located in rural areas and the most incidence of it is in Sub-Saharan African countries; in Latin American nations, instead, the most percentage of poverty is located in urban areas. The aim of this research was to estimate, by a multiple regression model, in 46 countries of Africa and in 23 nations of Souther-Central America, which socio-economic variables were able to play a fundamental role on the rural population and on the development of rural areas in 2000 and 2010 using some statistical data published in the FAO Statistic book. In analysed African countries there has been an increase of people living in the rural space and a growth by 21% of agricultural Gross Domestic Product (GDP). In Southern and Central American nations, there has been a meaningful emigration from rural territories due to an expansion of commercial flows and per capita income in rich areas, thus people have decided to move from the rural territories to the urban territories, worsening the poverty and living conditions in the countryside.

Key words: Rural areas, developing countries, emigration, social capital, multiple regression model, agricultural gross domestic product.

1. Introduction

1.1 A New Model of Agriculture in Developed and Developing Countries

In the last 60 years, specifically in developed countries, there have been many changes in the primary sector, which has completely modified its production model and its agricultural scenario. In fact, the agricultural sector is shifted from a productivism paradigm, over the 1950s and 1980s, to a post productivism model, with the effect to develop a territorial and productive specialization in many rural areas in the world [1].

The aim of productivist paradigm was both to increase the level of production in agriculture by a territorial concentration and specialization of agrarian productions and also to guarantee to farmers high prices of their commodities. The direct effect was to produce surpluses, which placed on the market depressed prices of agricultural commodities in the world and particularly in the third world countries. In the nations located in developing world, the productivism model in the primary sector, by assessed market price, has had some negative economic impacts as, for instance, a drop of the international price of commodities.

Nowadays, some African nations have to deal with two contrasting processes: a deactivation of agriculture, with a strong impact on rural development and on environment [2], and the grabbing of cultivated

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land by other developed states or big companies, with the aim to use these cultivated surfaces to produce raw material in terms of renewable energy sources and to ensure also a stability in food supplies against exogenous upset events. The first and foremost impacts of these choices have been not to respect the Millennium Development Goals (MDOs), signed by many rich nations to halve hunger and malnutrition, to reduce HIV/AIDS and poverty within 2015; in addition, the leading effect has been to impoverish developing countries of Africa and South America, depriving them of land for agricultural purposes.

It is harsh to find out a description to detail accurately rural areas in the world, because, up to now, an international common and complete definition of them does not exist. One of the most common and useful definition of rural areas is that proposed by Organisation for Economic Cooperation and Development (OECD), based on the population living in a delimited surface. Hence, rural areas are considered territories with a population lower than 150 inhabitants per km^2. More than 80% of population in the world lives in rural areas, where there are a lot of problems closely associated with hunger, poverty and malnutrition.

The abandonment of poor rural areas both in Africa and also in South America has fostered an uninterrupted problem of emigration from the rural space to urban areas, with the negative impact of assembling poor people in slums located near peripheral urban areas and building shantytowns without any sewage, running water and other public services as schools, hospitals and public transports. In many cases, rural areas in developing countries are tightly linked to the poverty, hunger and malnutrition, this is a lack of quality and quantity of food both in terms of vitamins and minerals and also in terms of deficiency in ingested calories per day. The main reason of dichotomy among rural areas and urban territories is caused by a lower level of services, social capital and infrastructures in rural areas than in urban zones. Poor living conditions in rural areas and poverty are pivotal explanations of emigration from rural territories to big cities and towns, which are considered a good and quick way of escape from the great poverty, with the drawback to aggravate inadequate and poor living conditions in peripheral urban areas, due to a lack of houses and to a lack of public and fundamental services as water, transport, school and hospital. The migration from rural territories to urban areas has had some negative impacts strictly connected to a drop of environment sustainability in developing countries, technological deterioration of rural space and land fragmentation. Thus, the foremost consequence of rural poverty has been to develop and to cause an autopoietic effect of rural emigration from the countryside, worsening the marginalization of rural territories located in developing nations [3].

The accessibility to knowledge, education, water and social capital are important factors to reduce the poverty in rural areas [4]; in fact, 75% of 1.2 billion of people in the developing world, who lives in poverty, is located in rural territories, where there are no opportunities to access to knowledge with the effect to enhance culture divide and socio-economic discrimination among urban areas and rural territories. Another variable which can act on the poverty and social marginalization in rural areas is an high level of feminization; this implies that agricultural productions and livestock are coped by women, often adolescents without any rights and without any opportunities to study and to be involved in political decisions, in government choices about the agricultural production and in other socio-economic activities. The consequence of this socio-political exclusion is an upsurge in emigration towards urban areas increasing vulnerability and instability of rural territories. In general way, the attention about problems of feminization and the rural woman division of labour in developing countries, which is typical in some African small farms, has been pointed out only during

some conflicts, that were able to create a significant impoverishment and a sharp decreasing of rural households, due to the absence of men in productive and agronomical management. In fact, man and children are often engaged in the war or in other conflicts, with the negative result to increase the social differentiation and exclusion and the emigration from rural areas to urban territories [5].

1.2 Poverty in African and South and Central American Rural Areas

The causes of poverty in rural areas are very hard to explain because of a complexity and multi-dimension of the problem and of the socio-economic and political variables involved in rural poverty. In general, an improvement in infrastructures, both in physical terms (transport, school, public administration) and also in social terms (skills, knowledge, education, social and human capital) are very important to solve the poverty and the shortage of proper living conditions in rural areas.

In the world, 63% of worldwide poverty is made up by rural poverty with a high percentage of poverty incidence in Sub-Saharan African countries (65%-90%); instead, in Latin American nations, the most percentage of poverty is located in urban areas [6]. An element able to increase the vulnerability of rural areas is HIV/AIDS, that is concentrated for 95% in rural territories, which are not able to deal with this illness; the first and foremost consequence of HIV/AIDS diffusion is to reduce labour force in the primary sector to cultivate fields, to breed livestock and to improve living conditions in the countryside. The United Nation and the Food and Agriculture Organization (FAO) have decided, to ameliorate the general living conditions of population in rural territories, throughout some measures to better agricultural productions and to halve HIV/AIDS, hunger and poverty through different specific actions defined in the MDOs.

The agrarian reform in developing countries of South-America was a powerful leverage to guarantee a correct and shared development of rural areas; for instance, some political actions have attempted to reduce agricultural crises expropriating land from large landowners and giving it to peasants. However, in some cases, the effects have been negative because the soil given to the farmers was chemically deficient and agronomical inferior, with the consequence to increase violence and poverty in the countryside and to widen new flows of emigration from rural territories to urban areas [7].

The variables rural poverty and environmental protection in developing countries are closely linked each other because a degradation of rural resources, with the aim to improve the production in cultivated soils, has had some negative economic impacts in terms of drop in the level of income in rural areas [8]. This implies a pivotal role of institutions to reduce the poverty in rural territories placing farmers in good conditions to produce not only commodities but agricultural products with a high level of added value in the food business chain. The direct effect of this action is to reduce the migration from poor rural areas, to protect the environment and to minimize the depletion of natural resources. In fact, the role of agriculture shall be to guarantee multifunctionality throughout the production of positive externalities. The primary sector shall be considered as a public good, recognising to the farmers, the role and function of an ecological guard. A correct management of rural areas in developing nations is important to prevent and to reduce the negative impact of natural disasters; an excessive productive specialization can have many negative impacts to guarantee a correct and balanced development in rural space; a deep ecological and environmental footprint, has had the consequence to create a negative agronomical context of production sensitive to pests. The deepening of farm activities [9] is a good solution to improve income in rural areas of developing countries as a result of an increase of added value in their agricultural products by fair trade

and organic food markets.

Recent studies about advantages and disadvantages of globalisation towards rural areas have pointed out as the globalisation is an opportunity for rural areas to reduce poverty but, without any rules, negative impacts can overwhelmed advantages with the consequence of a growth of the exodus from the countryside to urban areas, worsening living conditions in rural territories. In particular, this is true in some developing countries, where people live below the international value of poverty equal to 1.08 dollars per day, and the emigration has left in the countryside are old people and women only, who have none possibilities to emigrate or to set up new commercial activities with the effect to reduce the improvement of living conditions in rural areas [10]. The growth and the development in other economic sectors, as industry and service sector, drains workforce, people, human capital and social capital from rural areas; this is particularly true in developing nations, where there is an abundance of many resources, with the negative effect of increasing inequality, hunger, poverty and emigration towards peri-urban areas. Hence, it is important to underline the pivotal role of agriculture and rural areas in reducing poverty and in lowering the socio-economic disparity and impoverishment of rural space by national and international subsides and supports with the aim to help poor areas and to improve the integration among urban and rural areas [11]. The absence in rural areas of a mechanism of incentives and rewards, also in terms of social capital, good school system, job opportunities, houses, general services, is a constraint that implies a growth of emigration towards rich areas, generating the urban trap, due to an urban based system, lessening the socio-economic development in rural areas and worsening other economic opportunities to improve good standard living conditions in developing nations [12].

1.3 The Objective of the Research

The aim of this research was to define, using a quantitative approach, throughout a multiple regression model, the socio-economic variables able to play a fundamental role on the development of rural areas, in terms of people live in rural territories, and to reduce the emigration from rural areas.

2. Materials and Methods

2.1 Object of Research

The quantitative model, using a multiple regression model, has put in some statistical data relationship, published by Food and Agriculture Organization in the FAO Statistic book; it has estimated the most important parameters able to effect on rural population in developing world in two different years 2000 and 2010. The multi-regression model was applied in 46 countries of Africa and in 23 nations of Southern and Central America (Table 1). The main goal of this research was to analyse, in two different period of time, which socio-economic independent variables have had an effect on the dependent variable in terms of people living in rural areas (Table 2) and to value the effect of some political-economic choices in two analysed groups of nations to diminish the marginalization of rural territories located in developing countries.

2.2 Methods

The main part of this research was to define a quantitative paradigm using a multiple regression model and to find out which independent and dependent variables have been correlated to the socio-economic development of rural areas in terms of people live in rural territories. In fact, the dependent variable rural population is a proxy variable of living conditions because adverse economic conditions can act on a growth of migration from rural areas. This quantitative model of multiple regression has allowed to verify in developing countries, located in Africa and in South and Centre America, if the independent variables GDP in constant price in the primary sector, the number of undernourished people, the life

Table 1 List of countries analysed over 10 years

Southern and Central America	Africa	
Antigua and Barbuda	Algeria	Malawi
Argentina	Angola	Mali
Bahamas	Benin	Mauritania
Barbados	Botswana	Mauritius
Bolivia	Burkina Faso	Morocco
Brazil	Burundi	Mozambique
Chile	Cameroon	Namibia
Colombia	Cape Verde	Niger
Costa Rica	Central African Republic	Nigeria
Cuba	Chad	Rwanda
Dominican Republic	Congo	Sao Tome and Principe
Ecuador	Congo, Democratic Rep. of the	Senegal
El Salvador	Côte d'Ivoire	Sierra Leone
Guatemala	Eritrea	Somalia
Guinea	Ethiopia	South Africa
Haiti	Gabon	Sudan
Honduras	Gambia	Swaziland
Nicaragua	Ghana	Tanzania, United Republic of
Panama	Guinea	Togo
Paraguay	Guinea-Bissau	Tunisia
Peru	Liberia	Uganda
Uruguay	Libyan Arab Jamahiriya	Zambia
Venezuela	Madagascar	Zimbabwe

Table 2 Definition of socio-economic variables used in the multi-regression model.

Variable	Definition of variable	Value/Measure
	Dependent variable	
RPOP	Rural population	1,000 people
	Independent variable	
AGDP	Agricultural Gross Domestic Product (GDP)	Million of US dollar in constant price year 2000
UNDP	Undernourished people	Million of people
LE	Life expectancy	Years
NTT	Net total trade	Million of US dollar

expectancy and the net income from trade export, in terms of Net Total Trade, have had some effects on rural areas in terms of rural population.

The model of multiple regression, in which it has been included and estimated all analysed social and economic variables, in its algebraic form of matrix, can be represented in this explicit form [13]:

$$y = X\beta + u \quad (1)$$

where y and u are vectors with n-dimensions and X has dimension $n \times k$.

In analytical terms, the model of multiple regression in its general formulation can be written in this way:

$$y = \alpha_0 + \alpha x_1 + \beta x_2 + \gamma x_3 + \delta x_4 + u_{jt} \quad (2)$$

α_0: constant term;

x_1, x_2, x_3, x_4 independent variables;

$\alpha, \beta, \gamma, \delta$ estimated indicators of the model;

u_{jt} term of statistic error.

Basis assumptions, to use a multiple regression model, are:

Statistic error u_i has conditional average zero that is $E(u_i|X_i) = 0$;

(X_i, Y_i), $i = 1...n$ are extracted as distributed independently and identically from their combined distribution;

X_i, u_i have no fourth moment equal to zero;

There is not correlation among regressors and random noise so that the value between β expected and β estimated is the same.

To analyze if there is heteroschedasticity on standard errors, it has used White's Test on the error terms [14].

3. Results and Discussion

In Southern and Central American countries, over 10 years of study, it was possible to observe a drop in rural population, associated to an increase by 25% in ingested calories per day, even if in some countries Haiti, Bolivia, Colombia, Dominican Republic, Ecuador and Paraguay, there has been a growth of malnourished people for economic problems and natural disasters due to climate change effects. In Southern and Central American countries there has been an increase of life expectancy that in average has shifted from 71 to 73 years (Table 3).

In analysed African countries, there has been, during the time of study, a significant increase of people living in the rural space and in the same time there has been a meaningful growth by 21% of

Table 3 Descriptive statistics of analysed variable in African countries (Source: elaboration on data FAO Statistics Division, 2011).

Variable	Mean 2000	Mean 2010
Dependent variable		
Rural population (1,000 unit)	9,309.41	10,940.88
Independent variables		
Agricultural GDP (million of US dollar in constant price year 2000)	1,280.19	2,015.95
Undernourished people (million of people)	4.54	5.00
Life expectancy (year)	55	57
Net Total Trade (million of US dollar)	378	2,197

Table 4 Descriptive statistics of analysed variable in Southern and Central American countries (Source: elaboration on data FAO Statistics Division 2011).

Variable	Mean 2000	Mean 2010
Dependent variable		
Rural population (1,000 unit)	4,509.44	4,268.92
Independent variables		
Agricultural GDP (million of US dollar in constant price year 2000)	3,907.88	6,174.87
Undernourished people (million of people)	2.04	1.80
Life expectancy (year)	71	73
Net Total Trade (million of US dollar)	-575	55

agricultural GDP. Per capita calories per day are risen by 4.7% with an increase of the percentage of malnourished people and a growth of life expectancy, which in average has shifted from 55 to 57, even if, in 12 African countries out of 46 in 2010, the life expectancy was under 50 year. The analysis of descriptive statistics tables in African countries has pointed out a sharply growth of agricultural GDP and a strong increase of net total trade. In Southern and Central American nations, the descriptive statistics table has underlined a decrease of rural population and a growth both in terms of agricultural GDP and also in terms of net total trade (Table 4). Comparing the descriptive statistics table in African nations to Southern and Central American states, main results have pointed out a significant reduction of undernourished people and in particular in few African countries, there has been a positive impact of the MDGs to solve some problems of hunger and child mortality. Civil wars, riots and other conflicts have been the main cause of the growth of poverty in some African states instead in many Southern and Central American countries, an improvement of social conditions has improved the welfare level and also living conditions in poor rural areas [15, 16].

The climate change has had some effects on developing countries because in many analysed nations, there has been significant rainfall but unfortunately, it has not had positive effects on crop yields and other agricultural activities. In Sub-Saharan and equatorial African countries, there has been a significant percentage of incidence about malnourished people [17]; for example, in Angola in 2010, there was an increase of agricultural production of 52%, compared to the same source of data in the 2000, but this has not lessened the percentage of undernourished people, that has been above 35%. According to the FAO Statistics division, in the world, there has been an expansion of permanent crops and an unimportant reduction of pastures and arable lands, linked to a rise of capital stock, in US constant dollar at 1995 price, used in land (55%) and livestock (24%). The average value of Human Development Index (HDI), which is a composite index made by life expectancy, literature ratio and GDP, in all countries of the world over ten years of study was equal to 63 but only 17 countries out of 18, have been characterised by a low HDI, and they are located in Africa, where there has been a significant diffusion of local riots, national conflicts and ethnic wars [18]. Poverty and deprivation in rural areas located in analysed developing countries are the consequence of emigration and marginalization as it has been investigated in some European nations even if, other socio-economic aspects such as the amount of public funds used to better living condition in rural poor areas are pivotal to explain poverty in these countries [19]. A fair allocation of public funds may be a good chance to improve economic growth and social welfare in African countries and in rural poor areas with positive and meaningful outcomes in lowering the level of deprivation [20]. During 2000 in African rural areas, the statistical model pointed out a

direct correlation among the independent variables gross domestic product in the primary sector and undernourished people and the dependent variable people in rural areas. The parameters of the multiple regression model have underlined an indirect correlation between the dependent variable rural population and the independent variable net total trade (Table 5). This has supported the idea about people who live in rural spaces are more sensitive to the poverty and they have suffered from malnutrition. In poor countries, the rural areas have been fundamental to contribute to development of GDP made by primary sector. The coefficient of determination R^2 and the adjusted R^2 have pointed out a value of 0.95 and 0.94, which means as the multi-regression model fits well the statistical data and the adjusted R^2 has demonstrated too that the quantitative approach is not biased and it is able to be a good prediction and a good explanation of the regression model on the total variation. The results of White's test in African countries in 2000 showed a value of χ^2 (14 degree of freedom) equal to 22.04 which has implied that there was not heteroscedasticity both at 5% of critical Chi-square and also at 1% of critical Chi-square. The final expression of the multiple regression model used during the year 2000 is calculated as:

$$RDOP = \alpha_0 + \alpha AGDP + \beta UNDP + \gamma NTT + u_{jt} \quad (3)$$

where, RDOP stands for rural population; α_0: constant term; AGDP represents agricultural gross domestic product; UNDP stands for undernourished people; NTT represents net total trade; α, β, γ are estimated indicators of the multiple regression model; u_{jt} term of statistic error.

In the year 2010, the parameters of the multiple regression model have pointed out as independent variables undernourished people and agricultural GDP have been directly correlated with the dependent variable people living in rural areas (Table 6). The results during the year 2010, using the multi-regression model, have underlined as the growth of agricultural production has had a positive effect on the people in African rural areas even if the growth of agricultural productions was able to improve the general living conditions in terms of reduction of malnutrition. The coefficients of determination R^2 and the adjusted R^2 have pointed out a value of 0.85 and 0.81, which implies as the multiple regression model is a good prediction and a good explanation of the regression model on the total variation. The results of White's test in African countries in 2010 showed a value of χ^2 (14 degree of freedom) equal to 16.16, which has implied as there is not heteroscedasticity both at 5% of critical Chi-square and also at 1% of critical Chi-square. The final expression of the multiple regression model used during the year 2010 is:

$$RPOP = \alpha_0 + \alpha AGDP + \beta UNDP + u_{jt} \quad (4)$$

RPOP stands for rural population; α_0: constant term; AGDP represents the agricultural gross domestic product; UNDP stands for the undernourished people;

Table 5 Main results in the multi-regression model in African countries in the year 2000 (Source: our elaboration on FAO Statistics Division 2011).

Dependent variable	Rural population (RPOP)
Independent variable	
Agricultural Gross Domestic Product (AGDP)	1.84 (5.35)***
Undernourished people (UNDP)	1,229.68 (21.76)***
Life expectancy (LE)	n.s.
Net total trade (NTT)	-1.52 (2.86)***
Constant	7,735.22 (1.88)*
R^2	0.95
Adjusted R^2	0.94

n.s. means not significant; *denotes significance at 10%; ***denotes significance at 1%.

Table 6 Main results in the multi-regression model in African countries over 2010 (Source: our elaboration on FAO Statistics Division 2011).

Dependent variable	Rural population (RPOP)
Independent variable	
Agricultural Gross Domestic Product (AGDP)	2.12 (2.34)**
Undernourished people (UNDP)	1,166.66 (3.33)***
Life expectancy (LE)	n.s.
Net total trade (NTT)	n.s.
Constant	n.s.
R^2	0.85
Adjusted R^2	0.81

n.s., means not significant; **denotes significance at 5% ***denotes significance at 1%.

α, β estimated indicators of the multiple regression model; u_{jt} term of statistic error.

In 2000, in Southern and Central American rural territories, the quantitative approach has underlined a direct correlation among the dependent variable people living in the rural areas and the independent variables agricultural GDP and the variable undernourished people. This means as in rural space people have suffered a lot of malnourished problems, even if, the level of per capita calories has been above 1,800 per day. The multiple regression model has pointed out during the year 2000 an indirect relationship among the dependent variable rural population and independent variables life expectancy and Net Total Trade; in fact, in Southern and Central American rural areas there has been a growth of life expectancy and an expansion of commercial flows with the consequence to increase the migration flow from the countryside to urban areas (Table 7).

The emigration from rural poor areas in developing countries is able to produce and to sustain insecurity and poverty lowering the level of social capital and development; in this paper, as well as other studies in Africa, arid rural zones has pointed out a high meaningfulness among poverty, rural emigration, geographical aspects and weather conditions [16]. The results in this paper have beard out a direct correlation between rural development in terms of people living in rural areas and poverty in Africa rural zones but not in South and Centre American nations. In fact, during recent years other quantitative analysis have investigated as in Latin American countries, there have been a drop in poverty in poor rural areas compared to urban territories where there is the highest rate of poverty instead, in African nations, there has been an increase of population in cities and other urban aggregations exacerbating the marginalization in poor rural areas and the poverty in these territories [21].

The coefficient of determination R^2 and the adjusted R^2 have pointed out a value of 0.97 and 0.96, that

Table 7 Main results in the multi-regression model in Southern and Central American countries in 2000 (Source: our elaboration on FAO Statistics Division 2011).

Dependent variable	Rural population (RPOP)
Independent variable	
Agricultural Gross Domestic Product (AGDP)	0.38 (3.85)***
Undernourished people (UNDP)	1,170.25 (6.35)***
Life expectancy (LE)	-130.86 (2.79)**
Net total trade (NTT)	-0.21 (2.42)**
Constant	9,962.11 (2.98)***
R^2	0.97
Adjusted R^2	0.96

**Denotes significance at 5%; ***Denotes significance at 1%.

suggests as the regression model fits well the statistical data and the adjusted R^2 demonstrated also as the model was not biased upwards. The results of White's test in South and Centre American rural areas in 2000 showed a χ^2 test (14 degree of freedom) equal to 19.22 which has implied that there is not heteroscedasticity. The final expression of the multiple regression model during the year 2000 is calculated as:

$$RPOP = \alpha_0 + \alpha AGDP + \beta UNDP + \gamma LE + \delta NTT + u_{jt} \quad (5)$$

where, RPOP stands for rural population; α_0: constant term; AGDP represents the agricultural gross domestic product; UNDP stands for the undernourished people; LE stands for life expectancy; NTT represents Net Total Trade; α, β, γ, δ estimated indicators of the multiple regression model; u_{jt} term of statistic error.

During the year 2010, the statistical model of multiple regression has pointed out as there was a direct correlation among the dependent variable people living in rural areas and the independent variables agricultural GDP and undernourished people. The statistical model has underlined as there has been an increase of net total trade, able to reduce the number of people living in the rural territories (Table 8). The coefficient of determination R^2 and the adjusted R^2 have pointed out a value of 0.95 and 0.94 that has implied as the multi-regression model fits well the statistical data; the adjusted R^2 has demonstrated also as the model has

Table 8 main results in the multi-regression model in Southern and Central American countries in 2010 (Source: our elaboration on FAO Statistics Division 2011).

Dependent variable	Rural population (RPOP)
Independent variable	
Agricultural Gross Domestic Product (AGDP)	0.18 (4.46)***
Undernourished people (UNDP)	1,523.07 (9.84)***
Life expectancy (LE)	ns
Net total trade (NTT)	-0.06 (3.49)***
Constant	ns
R^2	0.95
Adjusted R^2	0.94

ns means not significant; ***denotes significance at 1%.

been a good prediction and a good explanation of the regression on the total variation. The results of White's test in Southern and Central American rural areas in 2010 showed χ^2 (14 degree of freedom) equal to 18.53 that has implied as there has not been heteroscedasticity both at 5% of critical Chi-square and also at 1% of critical Chi-square. The final expression of the multiple regression model in 2010 is calculated as:

$$RPOP = \alpha AGDP + \beta UNDP + \gamma NTT + u_{jt} \quad (6)$$

where, RPOP: rural population; AGDP represents the agricultural gross domestic product; UNDP stands for the undernourished people; NTT represents net total trade; α, β, γ estimated indicators of the multiple regression model; u_{jt} term of statistic error.

4. Conclusions

The analysis has pointed out as African rural areas are poorer than Southern and Central American ones due to a different level of economic growth with many consequences and impacts on the development of rural territories. To solve the poverty in rural areas and to put into action some priorities defined in the MDGs it is important to give responsibility to different communities, increasing the level of social capital and strengthening the network of social relationships among all stakeholders. This analysis has pointed out that there is a strong dualism among rural areas located in Africa and rural territories of South and Centre America. In fact, there was a significant growth of people living in African rural territories, where there has been a drop of export both in agricultural sector and in other economic sectors with negative impact on living conditions in rural areas due to civil wars and other conflicts. To solve the problem of poverty in rural areas, it is important to increase the added value of agricultural by a growth in agricultural gross domestic product or in other sources of income by expanding export which is able to improve the quality of living conditions lessening poverty and food insecurity in poor rural areas [22]. Strategies to tackle and to reduce poverty in rural areas of developing countries, in Africa and in South and Centre America, have to take into account the role and effects of government decentralisation in small local councils, able to use taxes to improve living conditions and to arise education and training in rural areas, with the positive consequence to give to local authorities more opportunities to reduce by an endogenous way the poverty in these areas [23], throughout a new bottom-up approach, in which every stakeholder can propose solutions and actions to solve contingent problems. New opportunities of micro-finance and micro-credit to create farms may be a positive tool to reduce the marginalization of rural areas, to lessen the emigration from rural territories and to create a safety net to prevent natural disasters due to an excessive use of soil in the countryside [24, 25].

To sum up rural areas in developing countries have to better the living conditions in the countryside with the aim to improve the life expectation which is directly correlated with the rural population who has been sensitive to an improvement of living conditions over the time of observation.

African countries have had more issues than Southern and Central American nations due to a lot of civil wars and domestic conflicts that have led and relegated in poverty and socio-economic marginalization the people in the poor rural areas. Local authorities and regional governments should

intervene to better hygienic conditions in the rural areas to prevent any propagation of diseases, in particular towards undernourished people, with the consequence to increase the rural population in the countryside and their standard living conditions.

In Latin America countries, a lower level of malnourished people can be explained by a low percentage of population living in rural areas. In Africa, in contrast to what has been observed in other analyzed countries, the quantitative approach has pointed out as to reduce significantly the poverty and the level of undernourished people the local governance have to direct the export of agricultural products mainly to feed local rural communities and after solving the undernourished issues using part of them to send agrarian goods to other countries for sale.

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Productivity of Soil Fertilised with Fermented Calliandra, Gliricidia and Leucaena Browses and Maize Forage

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Abstract: Fermented Callliandra, Gliricidia and Leucaena browses and maize material (milk stage) were applied to the soil to determine their effect on soil productivity. Hopi Red Dye Amaranthus (*Amaranthus cruentus*) was used as the test crop. Its DM yield was determined. The browse materials had higher total N and narrower C:N ratio than the maize material. Calliandra material had higher levels of insoluble fiber (ADF), fiber bound N (ADFN) and lignin. Application of the browse and maize materials raised C, N, and C:N ratio of the soil in which they were applied compared to the control soil. The treated soils maintained higher levels of C and N and a narrower C:N ratio up to the third crop. Amaranthus DM yield was highest ($P \leq 0.05$) with browse treatments. Treatment with maize material did not have DM yield advantage over the control soil. Treatments with browse materials could have given higher yields because the materials had more N and fermentation could not only have increased the proportion of soluble N, but also degradability of the materials, thus making the N and other nutrients in the fermented materials available for plant growth. DM yield was highest ($P \leq 0.05$) with the first and third crops but lowest ($P \leq 0.05$) with the second crop. Plant growth in the second crop could have coincided with high demand for the N by the soil micro-organisms decomposing the added materials. During the third crop, decomposition of the readily degradable components of the added materials could have been complete and the N became available for plant growth. DM yield dropped ($P \leq 0.05$) with the fourth crop as the available N could have been depleted from the soil for plant growth.

Key words: Fermented, Calliandra, Gliricidia, Leucaena, Amaranthus, soil productivity.

1. Introduction

Use of agro-forestry technologies such as green manuring and alley farming with leguminous shrubs have potential to alleviate soil deterioration and providing fodder for livestock [1-3]. According to Drechsel and Reck [4] as reported by Habamenshi et al. [3] regular pruning of agro-forestry species planted on contours, hedgerows or field boundaries could provide an additional fresh leafy biomass of up to 8 tones per year which could be sufficient as green manure for 0.45 ha. Attah-Krah and Reynolds [5] as reported by Topps [1] reported a productivity of over 20 tones of dry matter (DM) per ha per year from a mixture of *L. leucocephala* and *G. sepium* with *P. maximum* under humid zone conditions. However, according to Kang et al. [6] as reported by Topps [1], the diverse botanical origin and complex chemical composition of legume shrubs and trees used in alley farming has to be recognized as a challenging task. According to Bareeba and Aluma [7], the browses namely, Calliandra and Leucaena have substantial levels of tannins and lignin. The tannins and lignin bind protein and protect it from degradation in the rumen [8, 9]. Kabi and Bareeba [10] in a rumen degradation experiment found that although *Calliandra calothyrsus* was superior to mulberry (*Morus alba*) in annual herbage biomass production

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and digestible rumen undegradable protein, it had lower rumen degradable protein than *M. alba.* Such binding of protein could also limit its degradation in soil and affect availability of the protein nitrogen in the soil for plant growth. Costa and Gunasen [11] buried air dried leaf and stem prunnings of *Calliandra calothyrsus*, *Cassia spectabilis*, *Eupatorium inulaefolium*, *Fleminga congesta*, *Gliricidia sepium* and *Tithonia diversifolia* in the soil at a depth of 5 cm. for 2, 5, 8 and 12 weeks and found that whereas immobilization of nitrogen occurred only in the first 2 weeks after incubation, phosphorus immobilization persisted over a longer period and green manure from Calliandra provided the total seasonal nitrogen requirement of 60 kg N ha^{-1} per season for maize. Habamenshi et al. [3] evaluated the potential of *Calliandra calothyrsus* and *Alnus acuminate* green manures as sources of nitrogen (N) and phosphorus (P) for maize production and found that their effect on soil N, available phosphorus, organic matter (OM) and pH were not significant and neither did the manure influence maize yields. Ensiling as a means of storing green fodder by acidification resulting from anaerobic fermentation of the stored material has profound effects on the chemical and nutrient composition of the stored feed material [12-15]. Kato [16] found that fermentation produced high levels of soluble N in the form of non protein nitrogen (NPN) in maize and *Gliricidia sepium*, but not in *Calliandra calothyrsus* and *Leucaena leucocephala.* Fermentation also increased rumen degradation of DM, OM, N and conversion of N into microbial protein in Gliricidia, Leucaena and maize, but not in Calliandra. The purpose of this study was to investigate the effect of applying fermented Calliandra, Gliricidia, Leucaena and maize plant material on soil carbon (C), N and DM yield of Amaranthus.

2. Materials and Methods

The study investigated Amarathus growth response to application to the soil of fermented Calliandra, Gliricidia and Leucaena browse and maize materials. This was a pot experiment carried out in a screen house at the Department of Agriculture, Kyambogo University during the period 2007-2008.

The fermented materials were air dried and ground in a laboratory mill to pass through a 2 mm sieve before application to the soil. The soil used in the experiment was collected from a crop field. The soil was spread out in a screen house to dry after which it was ground to pass through a 2 mm sieve and mixed thoroughly. A sample of the soil was taken for laboratory analysis.

Treatments were made in four replicates. Four kilograms of soil were used per pot. The ground fermented materials were applied at the rate of 5 g kg^{-1} of soil which, was equivalent to the rate of manure application of 10 t ha^{-1} for 3 years for Uganda, each hectare being equivalent to a plough share of 2,000,000 kg of soil [17, 18]. The volume of the materials applied to the soil was also determined.

Four successive plantings were made without changing the soil or treatments in the pots. Each planting cycle lasted four weeks. Planting was by seed broadcast in the pots and the seeds were covered thinly with soil. Adequate moisture for crop growth was maintained with tap water. The pot soils were sampled at the beginning and after the first, second and third harvests for chemical analysis. Five plants from each pot were harvested at flower bud stage by cutting the plants at collar level, weighed and fresh weights recorded. Whole plant materials for the five plants from each pot were packed in paper bags and dried in the oven at 60 °C for 72 h to determine dry matter (DM) content (%) and yield (kg).

The soil samples were analyzed for soil OM and C according to Walkley and Black [19] and N by the Kjeldah method [20]. Samples of the fermented materials were analyzed for total N by the Kjeldah method [20], NPN by the trichloro acetic acid method [21], neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) by the

Van Soest and Robertson procedures [22]. Neutral detergent fiber nitrogen (NDFN) and acid detergent fiber nitrogen (ADFN) were obtained by determining N in the NDF and ADF residues, respectively.

The data obtained was subjected to statistical analysis by Genstat Release 12.2 and differences between the means were separated using the least significant difference (LSD) method at probability level of 5%.

3. Results and Discussion

The chemical composition of the fermented materials applied to the soils is shown in Table 1. The browse materials had higher levels of N than the maize material but the maize material had a much higher C:N ratio. The browse materials had much less soluble N (NPN), particularly Calliandra compared to the maize material. Also, Calliandra material had higher levels of ADF, fiber bound N (ADFN) and lignin. Application of the browse materials would introduce more N in the soil than the maize material. However, their low levels of soluble N would limit their decomposition in the soil and availability of their nutrients for plant growth. The maize material had less N and a wider C:N ratio, but much more soluble N, which would make it more decomposable in the soil and make its nutrients available for plant growth.

The initial levels of C, N and C:N ratios in the soils and after the third harvest are shown in Table 2. The initial levels indicate that the treated soils attained higher ($P \leq 0.05$) levels of C and N as a result of the materials added compared to the control soil. Soils treated with Gliricidia and maize materials had higher ($P \leq 0.05$) levels of C because of their low density and therefore the higher volume of the materials applied. The treated soils had higher levels of N, though not significant ($P \geq 0.05$), than the control. The values after the third harvest indicate that the soils treated with browses maintained higher ($P \leq 0.05$) levels of C than soil treated with maize material and the control soil. Soil treated with Calliandra material had the highest ($P \leq 0.05$) level of *C. Calliandra* material had higher content of insoluble fiber, fiber bound N and lignin compared to the other browses and could have resisted degradation. Maize material had higher content of soluble N, less insoluble fiber, less fiber bound N and less lignin and could have therefore, been readily degraded. All browse treated soils maintained higher levels of N than the maize treated soil and control soil. However, the C:N ratio was similar for all treatments and lower than that of the control soil. The fact that the browse treated soils maintained higher levels of C and N indicate that the treatments had effect on the C and N content of the soil. The C:N ratio was similar for all treatments as it is possible that irrespective of rate of decomposition of the added organic matter to the soil, the C:N ratio subsequently settles to the constant soil ratio of about 10:1 [23, 24].

Table 1 Chemical composition (% DM) of the fermented browses and maize materials applied to the soil.

Fermented browses and maize materials				
Composition	Calliandra	Gliricidia	Leuceana	Maize
Carbon	47.37	45.84	46.58	46.79
Nitrogen	3.07	3.74	4.05	1.12
C:N Ratio	15.43	12.26	11.50	41.78
NPN (% Total N)	11.15	31.97	15.24	37.96
NDF	77.00	55.70	75.55	70.51
ADF	66.08	32.74	45.01	32.95
NDFN (% Total N)	78.16	59.74	79.92	42.47
ADFN (% Total N)	68.08	13.47	42.59	14.24
ADL	34.46	12.15	27.06	4.40

The mean DM yield (kg) of Amaranthus according to soil treatments is shown in Table 2. Amaranthus grown on soils treated with browse material had higher ($P \leq 0.05$) DM yield than that grown on soils treated with maize material or the control soil, except in the case of Leucaena treatment which had similar ($P \geq 0.05$) yield with the control soil. Soil treatment with maize material had lower ($P \leq 0.05$) yield than the control soil and therefore had no yield advantage over the control soil. Treatment of soils with the browse material could have given higher yields as the materials had more N and therefore provided more N

Table 2 Mean level (%) of carbon, nitrogen and C:N ration and Amaranthus DM yield (kg) on soils, fertilized with fermented Calliandra (Call.), Glricidia (Glir.), Leucaena (Leuc.) and Maize material.

	Soil Treatment					
Composition	Control	Call.	Glir.	Leuc.	Maize	LSD
Materials						
kg	0	20	20	20	20	
Volume (cc)	0	45	55	41	70	
Initial						
Carbon	0.97^{e}	1.06^{c}	1.15^{b}	1.01^{d}	1.25^{a}	0.04
Nitrogen	0.11^{b}	0.12^{b}	0.13^{ab}	0.14^{ab}	0.13^{ab}	0.02
C:N	8.82	8.83	8.85	7.21	9.62	
Mean values after three planting cycles						
Carbon	1.00^{c}	1.12^{a}	1.06^{b}	1.06^{b}	1.01^{c}	0.04
Nitrogen	0.09^{c}	0.15^{a}	0.15^{a}	0.14^{ab}	0.13^{b}	0.02
C:N	11.11	7.47	7.07	7.57	7.77	
DM yield (kg)	0.229^{bc}	0.376^{a}	0.343^{ab}	0.299^{abc}	0.183^{c}	0.136

^{abcde}Values having different superscripts in a row are significantly different ($P \geq 0.05$).

for plant growth. In contrast, Habamenshi et al. [3] found no significant influence of *Calliandra calothyrsus* and *Alnus acumuniata* green manures on soil N and maize yield. It is therefore, possible that fermentation of the browses in this study made the browse N available for plant growth. Fermentation not only increases the proportion of soluble N or NPN but also degradability of the fermented material [16], thus, making the N and other nutrients in the fermented material available. Hence, the higher DM yields obtained with soils treated with fermented browses, Callliandra, Gliricidia and Leucaena.

The effect of planting cycles on DM yield is shown in Table 3. Mean DM yield, as well as for each treatment including the control, was lowest ($P \geq 0.05$) for planting cycle 2 and highest for planting cycle 3. It is possible plant growth in cycle 2 coincided with high demand for N by the soil microorganisms decomposing the added treatment materials. In cycle 3, decomposition of the readily degradable components of the added materials could have been complete and the N became available for plant growth. DM yield dropped ($P \geq 0.05$) in cycle 4. It is possible that in cycle 4 DM, yield dropped as a result of depleted available N in the soil for plant growth. Habamenshi et al. [3] found no significant influence on soil N and maize yield by Calliandra and Alnus green manures, however, his study was not carried further than the first season.

Table 3 Effect of planting cycles on DM yield (kg) of Amaranthus grown on soils fertilized with fermented browses and maize material.

	Planting Cycles			
Treatment	1	2	3	4
Control	0.551^{a}	0.090^{c}	0.037^{c}	0.237^{b}
Calliandia	0.535^{a}	0.225^{b}	0.544^{a}	0.199^{b}
Cliricidia	0.400^{b}	0.266^{c}	0.506^{a}	0.201^{d}
Leucaena	0.419^{a}	0.157^{b}	0.472^{a}	0.148^{b}
Maize	0.227^{b}	0.046^{d}	0.333^{a}	0.125^{c}
Mean	0.426^{a}	0.157^{b}	0.378^{a}	0.182^{b}

^{abcd}Values having different superscripts in a row are significantly different ($P \geq 0.05$). LSD: 0.061.

4. Conclusions

The results show that fermented browse, Calliandra, Glicidia and Leucaena unlike fermented maize or grass forage would improve soil productivity. It is possible enriching compost with browses and composting by anaerobic fermentation as in silage making would improve compost quality. The results obtained in this study are indicative results that need to be tested further under field conditions.

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More Benefit from Less Land: A New Rice-Pea-Rice Cropping Pattern for Resource-Poor Farmers of Bangladesh

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Abstract: The experiments were conducted at the Pulses Research Centre, Ishurdi, Pabna, Bangladesh during the 2005-2006 and 2006-2007 crop seasons to determine the economic viability of planting legumes for both vegetable and forage purposes in the fallow period between monsoon-rice and spring-rice. The objectives were to ensure better land utilization, break up the mono cropping, improve soil health, and generate extra-income for small and resource-poor farmers of Bangladesh. Crop compositions used in the experiments were monsoon-rice (cv. BRRIdhan-32, BRRIdhan-39 and BINAdhan-4) followed by pulses (grasspea, chickpea and field pea) followed by spring-rice (cv. BRRIdhan-28, BRRIdhan-29 and BINAdhan-6). Based on the data from two years in a pooled analyses, it was observed that monsoon-rice variety BINAdhan-4, followed by field pea (as vegetable & forage) and spring-rice variety BINAdhan-6 produced the highest yields of 5.0 t ha^{-1} rice grain, 3.25 t ha^{-1} (green vegetable) + 18.1 t ha^{-1} (forage) legumes and 7.8 t ha^{-1} rice grain, respectively. The cropping pattern of monsoon-rice (BINAdhan-4)-field pea (as vegetable + forage)-spring-rice (BINAdhan-6) gave the highest net return of USD$1,705 ha^{-1} $year^{-1}$ compared to other patterns with different rice varieties and chickpea and grasspea. This is a new finding, and is being practiced by farmers of Bangladesh who have enhanced their farm income substantially. This has also generated job opportunities for rural women to pick the green vegetable of field pea.

Key words: Benefit, land, rice, pea, cropping pattern.

1. Introduction

Bangladesh is a densely populated country, where population density and per capita cultivable lands are, 964/sq.km. and 0.05 ha, respectively [1]. Every year cultivable lands are utilized for housing, office building, roads and others construction work for blooming populations. Day by day, the population per unit area increases but cultivable land decreases which is alarming for growing economy of the country. In this endeavor, researchers, extentionist and farmers are trying to increase cropping intensity through the highest utilization of lands to fulfill the requirements of blooming population. Whereas, after harvesting of monsoon rice (July-December) some lands are used for short duration mustard cultivation before transplantation of spring rice. Simultaneously grasspea (*Lathyrus sativus* L.) and blackgram (*Vigna mungo*) can be grown as relay crop in the existing monsoon rice field as fodder crop before spring rice transplantation. However, about 20% of total cultivable lands (0.829 million ha) remains fallow which can be utilized to cultivate winter pulses like grasspea, chickpea (*Cicer arietinum*) and field pea (*Pisum sativum*) for vegetable and green forage [2]. Apical part of the soft shoot (7.0-7.5 cm) is used as

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vegetable which is choused by the consumers due to it is good taste and high market price. Growing pulses act as a catch crop between two rices and provides an extra income and employment to the farmers. In case of chickpea, similar findings also reported [3]. In winter season there is a high scarcity of fodder. Whereas, after picking of growing under shoots, rest of the part of grasspea, chickpea and field pea can be used as nutritious fodder for feeding the animals.

Beside this, to meet high demand of food against her blooming population, the highest emphasis has been given to cereal production, as the population suffers from protein malnutrition. The soil condition is also poor in nutrients and water content. However, growing of legumes helps in improving the soil condition and pulses as a food legume has a role in human food, animal feed and sustainable agriculture [4]. Pulses also act as ameliorative crops and thus, improve the soil health. Soil aggregation, soil structure, permeability, fertility and infiltration rate improved with the inclusion of pulses in the system [5]. A legume cans fix 20-60 kg residual N ha^{-1} for the succeeding crop [6]. To get higher productivity and income from a cropping system, the constituent crops are to be considered with respect to duration, productivity, economic and physical feasibility, and effects on soil and subsequent crops which remains to be properly understood, therefore, the present investigation was undertaken.

2. Materials and Methods

A field experiment was conducted for two consecutive years of 2004-2005 and 2005-2006 on calcareous gray food plain soils at Pulses Research Centre, Ishurdi, Pabna, Bangladesh. The experimental soil was clay loam in texture having pH 7.5, containing organic matter 1.2%, total N (%) 0.063, K 12 μg mL^{-1}, S 15 μg mL^{-1} and Zn 1.9 μg mL^{-1}. Season wise, experiment was laid out in RCB design with five replications. Second crop, relay pulses, were sown in the monsoon rice plots in such a way, that every pulses were placed in every rice varieties' plot. After the harvest of pulses for vegetable and forage, third crop of spring rice was placed in the pulses plot in the same way. First crop, transplant (T) aman rice, i.e., monsoon rice (var. BR32, BR39 and BINAdhan-4), second crop, winter pulses (Lathyrus var. BARI Khesary-1, Chickpea var. BARI-chhola-5 and Field pea var. Norail local) and third crop, Boro rice, i.e., spring rice (var. BR-28, BR-29 and BINAdhan-6) were used in the experiment. The unit plot size was 5 m × 4 m. Soil samples were collected during start and compilation of the two years cycle experiment and chemically analysed.

2.1 1st Crop: Monsoon Rice (T. aman Rice)

Thirty day's old seedlings of *T. aman* rice were transplanted on 10 July in 2004 and 17 July in 2005 at 25 cm × 15 cm spacing. Fertilizers were used @ 60-40-40-20-10 kg ha^{-1} of N-P_2O_5-K_2O-S-Zn in the forms of urea, triple supper phosphate, muriate of potash, gypsum and zinc sulphate, respectively. Except N, all fertilizers were used at final land preparation. Nitrogen fertilizer was used as top dress in 3 equal splits at 15, 30 and 45 days. After Transplanting (DAT) Weeding was done at 20 and 40 DAT. Depending upon crop duration, rice varieties, BR-39, BR-32 and Binadhan-4 were harvested on 6th, 17th & 12th November in 2004 and 19th, 27th & 23rd October during 2005, respectively. Data on yield contributing characters were recorded from 10 randomly selected plants from each plot and grain yield (t ha^{-1}) and straw weight (t ha^{-1}) were recorded from whole plot at harvest. The recorded data were statistically analysed.

2.2 2nd Crop: Winter Pulses

Winter pulses, i.e., grasspea (var. BARI Khesary-1), chickpea (var. BARI chhola-5) and field pea (var. Norail local) were sown in the existing rice field as relay crop on 4 November in 2004 and 26 October in 2005. The crop was fertilized with 40 and 20 kg ha^{-1}

P_2O_5 and K_2O, respectively before 2 days of sowing. Later on, 40 kg N ha^{-1} was top dressed in 3 equal splits at 20, 40 and 60 day. After Emergence (DAE), the tender twigs of the pulses were clipped for vegetable. Shoot picking for vegetable was started on 52 DAE in grasspea, 56 DAE in chickpea and 52 DAE in field pea in 2004-2005. Similarly, in 2005-2006, it was started on 54 DAE in grasspea, 59 DAE in chickpea and 54 DAE in field pea. Last harvest of vegetable was on 102 DAE in grasspea, 100 DAE in chickpea and 102 DAE in field pea in 2004-2005. Similarly, in 2005-2006, it was on 100 DAE in grasspea, 95 DAE in chickpea and 100 DAE in field pea. After the last pickup for vegetable, pulses were harvested for fodder. The recorded data were statistically analyzed.

2.3 3rd Crop: Boro Rice

After fodder harvesting, 45 days aged seedlings of BR-28 and 55 days aged seedlings of BR-29 and BINAdhan-6 were transplanted on 7 February in 2005 and 8 February in 2006, respectively. Different aged seedlings of different varieties were used for adjusting harvesting time to avoid natural calamities. Fertilizer management and weeding was similar to monsoon rice. The variety BR-28, BR-29 and Binadhan-6 were harvested on 3, 13 and 21 May in 2005 and 2, 12 and 20 May in 2006, respectively. Data on yield contributing characters were recorded from 10 randomly selected plants from each plot and grain yield (t ha^{-1}) and straw yield (t ha^{-1}) were recorded from whole plot at harvest. The recorded data were statistically analyzed. All types of production costs were recorded to find out the net return and benefit cost ratio.

3. Results and Discussion

3.1 1st Crop: Monsoon Rice (T. aman Rice)

The two years pooled results of yield and yield contributing characters of different *T. aman* rice varieties are presented in Table 1. Significant difference was observed among the three varieties in case of filled grains/panicle, 1,000 seeds weight, grain and straw yield but others characters failed to produce any significant difference. Numerically, the highest plant height 108.50 cm was recorded in BINAdhan-4. The lowest plant height 98.85 cm was found in BR-39. Numerically the highest effective panicles/hill 8 in BR-32 & BINAdhan-4 and the lowest 7 was in BR-39. Significantly the highest filled grains/panicle 108 was obtained by BINAdhan-4 and the lowest 83 was in BR-39. Significant difference was observed in 1000 seeds weight and it was the highest 22.84 g was in BINAdhan-4 and the lowest 21.22 g was in BR-32. The highest grain yield 5.00 t ha^{-1} was obtained from BINAdhan-4 which might be due to cumulative influence of increased plant height, number of effective panicle/hill, number of filled grains/panicle, 1,000 seeds weight. Similar results also reported by Bangladesh Institute of Nuclear Agriculture [7]. The lowest grain yield 3.94 t ha^{-1} was obtained from BR-39, it might be due to less production of yield contributing characters. Significantly the highest straw yield 5.90 t ha^{-1} was obtained by BINAdhan-4 which might be due to cumulative influence of the highest plant height as well as increased yield contributing characters. The lowest straw yield 4.44 t ha^{-1} was obtained from BR-39. There was not much difference in crop duration but numerically the highest duration, 129 days was recorded in BR-32 and the lowest crop duration 123 days was in BR-39.

Earlier, farmers grew long duration and less yield potential varieties or other varieties which were not fit in cropping pattern but after these finding farmers are growing BINAdhan-4 due to its high yield potential and fitting in the mentioned cropping pattern.

3.2 2nd Crop: Winter Pulses

The two years pooled results with figures of vegetable and fodder yield of three pulses are presented in Table 2. Significantly, the highest duration of first harvest of vegetable 57 days after

Table 1 Performance of different *T. aman* rice varieties on yield and yield contributing characters (pooled over two years).

Treatment	Plant height (cm)	No. of effective panicle/hill	No. of filled grain/ panicle	1,000-seeds weight (g)	Grain yield (t ha^{-1})	Straw yield (t ha^{-1})	Duration (days)
BR-32	104.63	8	98b	21.22c	4.44b	5.12b	129
BR-39	98.85	7	83c	22.14b	3.94c	4.44c	123
BINAdhan-4	108.50	8	108a	22.84a	5.00a	5.90a	124
CV (%)	5.23	6.40	4.05	2.20	11.50	12.80	2.10
LSD (0.05)	10.90	4.20	6.80	0.32	0..45	0.45	7.20

Means with different letters within the same block are significantly different.

Table 2 Performance of different relay pulses as vegetable and fodder (pooled over two years).

Treatment	1st harvest of vegetable (DAE)	Last harvest of vegetable (days)	Vegetable harvesting duration (days)	Frequency of vegetable harvesting	Total vegetable wt. (t ha^{-1})	Total fodder wt. (t ha^{-1})
Lathyrus	53.0b	101.0	48	6.5ab	2.45b	16.75b
Chickpea	57.5a	98.0	40	5.5b	1.50c	6.50c
Field pea	53.0b	101.0	48	7.5a	3.25a	18.10a
CV (%)	4.08	3.95	6.70	7.48	12.65	13.40
LSD (0.05)	2.30	5.90	8.00	1.20	0.30	1.10

Means with different letters within the same block are significantly different.

emergence (DAE) was observed in chickpea. The lowest duration of first harvest of vegetable 53 DAE was found in lathyrus and filed pea. Differences on last harvest of vegetable had no significant effect among the different pulses but numerically the longest duration of last harvest of vegetable 101 DAE was observed in lathyrus and field pea. The lowest duration of last harvest of vegetable 98 DAE was observed in chickpea. Total duration of vegetable harvesting was not significant difference but numerically the longest duration of vegetable harvesting 48 days was observed in lathyrus and field pea. The lowest duration of vegetable harvesting 40 days was found in chickpea**.** Significant difference was observed in the frequency of vegetable harvesting and it was the highest 7.5 was in field pea which was identical to lathyrus and the lowest 5.5 was in chickpea. Significantly the highest vegetable 3.25 t ha^{-1} was obtained from field pea which might be due to cumulative influence of early start of vegetable harvesting, longest duration of vegetable harvesting and significant increased of vegetable harvesting frequency. The lowest vegetable production 1.5 t ha^{-1} was observed in chickpea due to its later start vegetable harvesting and shortest harvesting duration and the lowest frequency of vegetable harvesting. The highest fodder yield of 18.10 t ha^{-1} was obtained from field pea and the lowest 6.5 t ha^{-1} was in chickpea, it might be due to less crop growth. The highest fodder weight in field pea might be due to its characteristically vigorous plants and resulting in higher number of branches. It was also reported that clipping of the young shoots during vegetative growth caused increase in auxiliary branches which resulted in higher by-product yields in chickpea [8, 9].

Before the development of this technology, maximum lands remain fallow within the window of monsoon rice-spring rice but at present, these windows are using by the relay cropping of field pea/grasspea as vegetable + fodder. As a result, fallow lands are using through cropping, i.e., same lands are using more times which ensure more benefit, especially resource poor farmers of Bangladesh, because, vegetable harvesting is a laborious job which is only possible by the poorer section.

3.3 3rd Crop: Spring Rice (Boro Rice)

The two years pooled results of yield and yield contributing characters of different spring rice varieties are presented in Table 3. It appears that, the

Table 3 Performance of different boro rice varieties on yield and yield contributing characters (pooled over two years).

Treatment	Plant eight (cm)	No of effective panicle/hill	No. of filled grain/panicle	1,000-seeds weight (g)	Grain yield (t ha^{-1})	Straw yield (t ha^{-1})	Duration (days)
BR-28	89.25	12	149.00c	21.18c	5.20c	6.40c	128c
BR-29	100.90	13	178.50b	21.95b	6.65b	7.38b	149b
BINAdhan-6	102.65	14	234.00a	23.29a	7.80a	8.39a	159a
CV (%)	9.20	4.90	5.10	2.90	11.50	13.90	4.65
LSD (0.05)	15.20	3.20	18.00	0.15	0.30	0.30	6.60

Means with different letters within the same block are significantly different.

plant height was not significantly difference among them but numerically the highest plant height 102.65 cm was obtained from BINAdhan-6. The lowest plant height 89.25 cm was found in BR-28. There was no significant effect of varieties on number of effective panicles/hill, however, the highest number of effective panicles/hill 14 in BINAdhan-6 and the lowest 12 was in BR-28. Significantly the highest number of filled grain/panicles, 234 was observed in BINAdhan-6 and the lowest 149 was found in BR-28. Significant difference was observed in 1,000-seeds weight and it was the highest 23.29 g in BINAdhan-6 and the lowest 21.18 g was in BR-28. The highest grain yield 7.8 t ha^{-1} was obtained from BINAdhan-6. The highest grain yield in BINAdhan-6 might be due to the cumulative influence of significant increase of number of filled grain/panicles, 1,000-seeds weight and numerical increase of number of effective panicles/hill. This result is an agreement with the findings of Bangladesh Institute of Nuclear Agriculture [10]. The lowest grain yield 5.2 t ha^{-1} was found in BR-28 which might be due to less yield contributing characters.. Significantly the highest straw yield 8.39 t ha^{-1} was found in BINAdhan-6, it might be due to cumulative influence of the highest plant height and increased yield attributes, and the lowest 6.40 t ha^{-1} was found in BR-28 due to less yield contributing characters. Significantly, the longest duration 159 days was observed in BINAdhan-6 and the lowest duration 128 days was observed in BR-28. Although, the longest duration was observed in BINAdhan-6 but it was harvested before succeeding crop plantation and also given higher yield. Considering the above findings, maximum farmers are cultivating this variety in the spring season due to its high yield and suitability in the mentioned cropping pattern.

3.4 Economics

Agroeconomic performance of rice, pulses as vegetable + fodder, rice cropping pattern under this study is presented in Tables 4-6. Among the different monsoon rice varieties, the highest gross return of Tk. 55,900 ha^{-1}, net return of Tk. 40,900 ha^{-1} and Benefit Cost Ratio (BCR) 3.73 were found in BINAdhan-4. The lowest gross of Tk. 43,840 ha^{-1}, net return of Tk. 29,390 ha^{-1} and BCR 3.03 was found in BR-39 (Table 4).

Among the different relay pulses as vegetable + fodder, field pea produced the highest gross return of Tk. 46,075 ha^{-1}, net return of Tk. 34,648 ha^{-1} and BCR 4.03 were obtained by field pea. The lowest gross return of Tk. 21,250 ha^{-1}, net return of Tk. 13,417 ha^{-1} and BCR 2.70 were found in chickpea (Table 5).

In the different spring rice varieties, the highest gross return of Tk. 86,390 ha^{-1}, net return of Tk. 66,000 ha^{-1} and BCR 4.24 were found in BINAdhan-6. The lowest gross return of Tk. 58,400 ha^{-1}; net return of Tk. 38,650 ha^{-1} and BCR 2.96 were found in BR-28 (Table 6).

Perceptible changes in soil chemical properties occurred through the inclusion of pulses in the rice based cropping pattern (Table 7). After completion of two years cycle, the pH of the soil was decreasing trend but the organic mater, N, P, S and Zn content of the soil was increasing and K remained unchanged irrespective of different pulses. It might be due to inclusion of pulses in the cropping systems. Similar findings also reported by Ali [11].

Table 4 Agro-economic performance of different *T. aman* rice varieties (pooled over two years).

Treatment	Grain yield (t ha^{-1})	Straw yield (t ha^{-1})	Cost of production (Tk ha^{-1})	Gross return (Tk ha^{-1})	Net return (Tk ha^{-1})	Benefit cost ratio (BCR)
BR-32	4.44	5.12	14,675	49,520	34,845	3.37
BR-39	3.94	4.44	14,450	43,840	29,390	3.03
BINAdhan-4	5.00	5.90	15,000	55,900	40,900	3.73

Price: ploughing = Tk. 750 ha^{-1} plough^{-1};
Paddy = Tk. 10,000 ton^{-1}; Human labour = Tk. 70 head^{-1} day^{-1};
Straw = Tk. 1,000 ton^{-1}; Urea = Tk. 6.0 kg^{-1}; TSP = Tk.18.0 kg^{-1};
MoP = Tk. 15.0 kg^{-1}.

Table 5 Agro-economic performance of different relay pulses as vegetable and fodder (pooled over two years).

Treatment	Vegetable wt. (t ha^{-1})	Fodder wt. (t ha^{-1})	Cost of production (Tk ha^{-1})	Gross return (Tk ha^{-1})	Net return (Tk ha^{-1})	BCR
Lathyrus	2.45	16.70	10,092	36,300	262,008	3.60
Chickpea	1.50	6.50	7,833	21,250	13,417	2.70
Field pea	3.25	18.10	11,427	46,075	34,648	4.03

Price :

Pulses	Seeds	Vegetable	Fodder
Lathyrus	Tk. 30.00 kg^{-1}	Tk. 8.00 kg^{-1}	Tk. 1.00 kg^{-1}
Chickpea	Tk. 50.00 kg^{-1}	Tk. 12.00 kg^{-1}	Tk. 0.50 kg^{-1}
Field pea	Tk. 40.00 kg^{-1}	Tk. 10.00 kg^{-1}	Tk. 0.75 kg^{-1}

Table 6 Agro-economic performance of different Boro rice varieties (pooled over two years).

Treatment	Grain yield (t ha^{-1})	Straw Yield (t ha^{-1})	Cost of production (Tk ha^{-1})	Gross return (Tk ha^{-1})	Net return (Tk ha^{-1})	BCR
BR-28	5.20	6.40	19750	58400	38650	2.96
BR-29	6.65	7.38	20050	73800	53750	3.68
BINAdhan-6	7.80	8.39	20390	86390	66000	4.24

Price: Ploughing = Tk. 750 ha^{-1} plough^{-1};
Paddy = Tk. 10,000 ton^{-1}; Human labour = Tk. 70 head^{-1} day^{-1};
Straw = Tk. 1,000 ton^{-1}; Urea = Tk. 6.0 kg^{-1}; TSP= Tk.18.0 kg^{-1};
MoP = Tk. 15.0 kg^{-1}.

Table 7 Soil fertility status of Initial and after two years cropping cycle (final).

Soil status	Treatment	pH	OM (%)	Total N (%)	P (µg mL^{-1})	K (µg mL^{-1})	S (µg mL^{-1})	Zn (µg mL^{-1})
Initial	All	7.5	1.20	0.063	12	0.17	15	1.9
	Lathyrus	7.4	1.23	0.067	14	0.17	16.0	2.0
Final	Chickpea	7.4	1.23	0.068	15	0.17	17.0	2.0
	Filedpea	7.4	1.23	0.067	14	0.17	16.0	2.0

4. Conclusions

In this study, BINAdhan-4-field pea (as vegetable + fodder)-BINAdhan-6, cropping pattern combindly (3 crops) produced the highest net return of Tk. 141,548 ha^{-1} year^{-1} equivalent to $1,705 ha^{-1} year^{-1} (1$ = Tk. 83.00). Thus, the cropping system consisting of monsoon rice (BINAdhan-4) followed by relay crop of field pea (for vegetable & fodder) and spring rice (BINAdhan-6) appears to be the most appropriate in terms of food, forage and vegetable production and net returns for resource poor-farmers in Bangladesh. This technology has also generated job opportunities for rural women to pick the green vegetable of pea. The poor farmers of Bangladesh are using this technology; as a result, fallow lands are bringing under cultivation without hamper of existing crops and farmers are getting more benefit from less land.

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Nutritional Properties of Commercial Flavored Milk

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Abstract: Consumers are becoming increasingly health conscious, and food product choices have expanded. Flavored milk increases milk consumption in children and providing adequate calcium intake as well as associated nutrients. About 40% of the sugar in flavored milk is naturally occurring lactose beverages. The goal of this study was to test the truthfulness of the labeling concerning protein, fat and sugar content of the flavored milk. Moreover, the compliance with the Gulf standard was tested. Ten different brands of flavored milk were used in this study. As per labeled on the package, this study found that: one company did not mention clearly that their products are nonfat milk. We also found that there were variations in the percentage of fat between the ten types of flavored milk. For calcium content, all companies indicated clearly the calcium content except company eight. Concerning laboratory analysis, one company considered as mislabeled since they stated on the package that the percentage of fat 1.1-1.2 and the lab analysis showed it contained 3.1%. All companies were truthful about protein content. Moreover, for calcium, all companies have low calcium content than what has stated on the package except one company.

Key words: Milk, nutrition, biochemical, flavors.

1. Introduction

Milk is the number one source of calcium, magnesium, potassium and phosphorus in children's diets [1]. Flavored milk provides the same nine essential nutrients as unflavored milk, including three of the five nutrients identified as "nutrients of concern" for children in the 2005 Dietary Guidelines for Americans—calcium, magnesium and potassium [2]. Children who drink flavored milk are more likely to meet daily calcium recommendations compared to their peers who do not drink flavored milk. They also tend to drink fewer nutrient-void sodas and sugary fruit drinks. Moreover, they have no higher intakes of added sugars compared to children who do not drink flavored milk [3]. Murphy et al. [1] found that flavored milk provides only a small fraction (< 2%) of the total added sugars consumed. In another study, Frary et al. [4] concluded that children who consume sweetened dairy products (including flavored milks) get more calcium, consume less added sugars and saturated fat and are more likely to meet recommendations for calcium, folate and iron compared to those who consume sugar-sweetened beverages such as sodas and fruit drinks. As students get older, they may start choosing sodas and other empty-calorie beverages in place of milk. Studies suggest that flavored milk can be an effective strategy to avoid this switch [5]. Several studies also suggested that offering flavored milk will encourages increased milk consumption and adequate calcium intake as well as association with adequate milk consumption is associated with healthier weights in children [6-8].

The amount of added sugars in flavored milk is significantly less than the amount in soft drinks. About 40% of the sugar in flavored milk is naturally occurring lactose (10 grams per 8 ounces), yet, all of the sugars in nutrient-poor sodas are added sugars. A study reported that children who regularly drank sodas and other low-nutrient, sugar-sweetened beverages were more likely to become overweight or obese compared to children who do not drink these beverages [9].

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Another advantage of consumed flavored milk is that it may be more easily digested by those who are sensitive to lactose [10, 11]. Suarez et al. [12] found that many individuals with lactose intolerance could comfortably drink two cups of milk a day when consumed in small servings with meals. Lactose-reduced or lactose-free milks also are widely available.

The American Academy of Pediatrics [13] and American Heart Association, American Stroke Association [14] recommended restricting the sale of sweetened drinks in schools to help prevent some of the health problems associated with too many sodas and sweetened beverages. The Academy [13] recommends replacing sweetened drinks in school vending machines with real fruit juices, water and low fat white or flavored milk. They also stated that as sweetened drink consumption rises, milk consumption declines and milk is the primary source of calcium in the diets of children and adolescents. Moreover, a clinical report from the American Academy of Pediatrics [13] suggested flavored milks (reduced fat or fat-free) with modest amounts of added sweeteners are “generally recommended” to help optimize the bone health and calcium intakes of children and adolescents [15].

Connors et al. [16] conducted a study of factors influencing milk drinking behaviors; they found that milk flavor and packaging were frequently cited as important factors influencing milk consumption. They also stated that the availability of different types and flavors of milk increases the opportunity for children to practice decision making and create a sense of personal control. In another study conducted in St. Louis area, in 300 schools with approximately 165,000 students, the newly added flavors of milk and colorful packaging increased the average sale of flavored milk in the first 10 weeks, with an average of almost 20% per school and overall milk sales increased as much as 14% per school [5].

This study was conducted to estimate the biochemical and nutritional analysis of flavored milk and to compare the information on the package with the laboratory analysis and to discuss the compliance of the vendor with the Gulf Standards.

2. Materials and Methods

2.1 Milk Samples

Ten types of flavored milk were used in this study. Milk samples were collected from the local market. Triplicate of each of the ten containers were sent for analysis.

2.2 Biochemical and Nutritional Analysis

Protein nitrogen and fat contents of milk was determined by Kjeldahl and Soxhlet Methods according to AOAC [17]. Protein is precipitated from milk by trichloroacetic acid (TCA) solution in Kjeldahl flask. Final concentration of TCA in mixture is ca 12%. The TCA solution is separated from protein precipitate by filtration. Nitrogen content of protein precipitate was determined as total nitrogen in Milk-Kjeldahl Methods [18]. Glucose was determined using glucose oxidase reagent (CAS-50-99-7), sucrose was determined using invertase reagent (CAS-57-50-1) as described [19] and food coloring was determined according to Puttemans et al. [20].

3. Results and Discussion

Ten types of flavored milk were used in this study. These include five products from one company, ranging from full fat, strawberry, banana, vanilla, and chocolate milk. The other five were from five different companies and were chocolate low fat, chocolate caramel, and three brands of chocolate milk. The ten types of the flavored milk content, as per labeled on the container (per 100 mL) and analyzed are presented in Table 1.

As per labeled on the container, the percentage of total fats ranged from 1.1% for company 1 to 3.08 for company 9. According to the Gulf Standards (GSO, 785), the flavored milk is divided according to the

Table 1 Biochemical analysis of milk products under study.

Code	Characteristics	Total fat (%)		Calcium (mg 100 mL^{-1})		Protein content (g 100 mL^{-1})		Sucrose content (%)		Carbohydrate content (g 100 mL^{-1})		Non fat solids (g 100 mL^{-1})	
		A	L	A	L	A	L	A	L	A	L	A	L
Company 1	Regular milk	3.1	1.10	36.0	100	3.04	3.1	0	NA	4.50	4.7	7.3	8.5
Company 2	Chocolate	1.4	2.00	31.0	96	3.00	3.7	6.6	NA	10.60	9.0	8.4	NA
Company 3	Strawberry	2.1	2.00	33.2	110	3.11	2.8	6.0	NA	10.00	10.2	6.7	NA
Company 4	Banana	1.9	2.00	38.1	110	3.05	2.8	6.0	NA	9.40	10.2	6.7	NA
Company 5	Vanilla	1.8	2.00	27.8	110	3.70	2.8	6.2	NA	9.70	10.2	7.3	NA
Company 6	Chocolate	1.9	2.00	25.5	105	3.20	3.0	7.4	NA	11.20	11.0	8.3	NA
Company 7	Chocolate	3.1	3.00	23.1	96	3.70	2.8	5.5	NA	8.50	9.4	7.0	NA
Company 8	Chocolate caramel	2.1	3.30	27.8	NA	3.79	3.3	8.0	5.2	12.12	18.3	8.7	9.0
Company 9	Chocolate	3.0	3.08	29.7	121	3.70	3.3	7.6	11.3	11.40	11.5	8.3	NA
Company 10	Chocolate	3.1	3.20	28.7	110	3.20	2.9	6.2	NA	10.00	11.6	8.7	8.5

NA: not analyzed; A: Analyzed; L: Labeled.

percentage of fat in the milk into full fat milk if it contains at least 3% fat, low fat if it contains less than 3% and more than 0.5% fat and no fat milk if it contains less than 0.5% fat. Accordingly, as per labeled on the package, company 1 is low fat milk and, however, it did not mention that clearly. As per labeled, company 7-10 are considered as full fat milk since it stated that it contains 3% fats. A study on 22 brands of UK retail milk they found that: (1) highly significant variations in fat composition between summer and winter milk; (2) organic milk are different in composition from conventional milk; (3) considerable variation existed in milk fat composition between brands [21].

Concerning food coloring, only company 8 stated that a carotenoid was added, however, the rest of the company did not mention any information about food colorings. According to the Gulf standards, coloring is allowed within the limit (18 PPM for tetrazene to 150 PPM for Carmel). The calcium content as per 100 mL of milk and as stated on the package is indicated in Table 1. The calcium content ranged from 12 mg 100 mL^{-1} of milk for company 2 to 121 mg 100 mL^{-1} belongs to company 9. Only company 8 did not mention any information on the package regarding calcium content.

As for protein content, as per 100 mL of milk was around 3 g 100 mL^{-1} and ranged from 2.9 for companies (3-5) to 3.7 for company 2. Only two companies mentioned sucrose content on the package, company 8 and company 9 where these were 5.2% and 11.3%, respectively. According to the Gulf Standards, natural sugars are allowed to be added to the milk, however, the allowed amounts are not stated in the Standards. Total carbohydrate content ranged from 4.7 g 100 mL^{-1} for company 1 to 18.3 g for company 8. For non-fats solids, only three companies stated the content as grams per 100 mL of milk, these were 8.5% for companies 1 and 10 while it was 9 g 100 mL^{-1} for company 8. According to the Gulf Standards, the total non-fats solids should not below 8.5%. According to the package labeled, these three companies are within the limit.

Generally, the Gulf Standards stating that several information have to be stated clearly on the package. This information includes: type of milk products (flavored milk, low fat flavored milk, and non-fats flavored milk), type of flavor added, heat treatment, name of added materials and their numbers. Similarly, in the USA, the Nutrition Labeling and Education Act (NLEA), requires most foods to bear specific nutrition and ingredient labeling and requires food, beverage, and dietary supplement labels that bear nutrient content claims and certain health messages to comply

with specific requirements of U.S. FDA Food Labeling.

Only company 2 complied with the regulations since they mentioned clearly that they have 1.5% fats and stated clearly as “low fat milk”. However, company 1, has low fat but did not mention that clearly on the package. A survey was conducted on 40 different food categories on sale in New South Wales in 2001 to assess levels of compliance by comparing the claims on the label and data in the nutrition information panel with requirements of the Foods Standards Code and the Code of Practice on Nutrient Claims. 12.9% of the nutrient claims did not comply with current regulations, especially those in the voluntary Code of Practice. Adoption of mandatory requirements for all claims within the Food Standards Code may improve the levels of compliance. Implications for the regulation of nutrition and related claims are discussed. The impact of nutrition claims on consumer purchasing and consumption behavior deserves further study [22]. Donnell [23] investigated nutritional differences in fatty acid (FA) composition of conventional milk with milk labeled as recombinant bST (rbST)-free or organic. They used 292 milk samples obtained from the 48 contiguous states of the United States represented the consumer supply of pasteurized, homogenized milk of 3 milk types: conventionally produced milk with no specialty labeling, milk labeled rbST-free, and milk labeled organic. The results stated that there were no meaningful differences that would affect public health.

The laboratory tests were performed to check the truthfulness of the label on the package. The percentages of total fats for the 10 companies are presented in Table 1. Accordingly, companies 2-6 were found to be within the range of the low fat milk, i.e., both laboratory analysis and package information is identical. Similarly, companies 7-10 are within full fat milk, i.e., the percentage of fat is 3% or more in both laboratory data and package information. However, company 1 stated that the percentage of fat is 1.1 and does not exceed 1.2, while laboratory data showed that the fat is 3.1%. Similarly, company 8 stated on the package that the fat content is 2.1% and laboratory data showed that the fat is slightly higher, 3.3%, which means that it should be labeled as full fat milk instead of low fat milk.

For calcium content, laboratory analysis showed that the calcium content is lower than stated on the package except for company 2 which stated that it contains 96 mg 100 mL^{-1} of milk and was found to have 31 mg 100 mL^{-1} of milk. Companies 1, 3-7 were lower in calcium since they stated on the package to have from 96 to 110 mg 100 mL^{-1} of milk, however, the laboratory data ranged from 23 to 38 mg 100 mL^{-1} of milk.

For protein content, both laboratory analysis and package information were similar (around 3 g/100 mL of milk) and none of the companies has mislabeling concerning protein content.

As stated earlier, only companies 7 and 8 have information about sucrose contents on the package and it was 5.2% and 11.3%, respectively. However, the laboratory analysis showed that company 7 has 8.0% sugars and company 9 has 7.6%. In general, the sugar analysis show percentage of sucrose ranged from zero for company 1% to 8.0% for company 8. Laboratory analysis was similar to that stated on the package for companies 1, 3-6, 9 and 10. Company 2 has more sugars (10.6 g 100 mL^{-1} of milk) than stated on the package (6.0 g 100 mL^{-1} of milk). In contrast, company 8 has fewer carbohydrates (12.12 g 100 mL^{-1} of milk) than was stated on the package (18.3 g 100 mL^{-1} of milk).

None fat solid ranged from 6.7% for companies 3 and 4 to 8.7 for company 8. Only three companies stated none fat solids, interestingly, these companies have similar results of the laboratory analysis and what is stated on the package. The Gulf Standards stating that none fat solid should not be lower than 8.5%.

Finally, protein content in milk products ranged from 3% to 3.79% while fat content ranger from 1.1% to 3.3% and calcium from 23.1 to 38.1 mg 100 mL^{-1}, which are within ranges of international standards.

4. Conclusions

It could be concluded that sector of milk products processing in Saudi Arabia needs a special attention to be paid towards quality control to be met with Gulf, International Standards and FDA's oversight.

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Above-ground Biomass Allocation in a Planted Forest in a Semi-arid Region of Northern Mongolia

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Abstract: Investigation of the above-ground biomass allocation patterns on Scots pine plantations is critical for quantifying the productivity and carbon cycle of forest ecosystems. We estimated above-ground biomass and net primary production of a 25-year-old *Pinus sylvestris* L. (Scots pine) plantation, in a semi-arid region of Mongolia. The above-ground biomass of sample trees was divided into stem wood, stem bark, live branches, dead branches and needles. Total biomass for the stand was only 18.03 Mg ha^{-1}, of which 47.6% was found in stem wood, 25.8% in live branches and 14.8% in needles. The growth rate of the Scots pine plantation in the study region was relatively low compared with other regions. In the study area, it was observed that the rate of biomass accumulation in the plantation was very slow; this can be explained by very limited growing conditions and intensive crown closure. The results from this study indicate that it may be necessary to carry out thinning to increase biomass production by reducing competition between trees in the Scotch pine plantation.

Key words: Biomass allocation, dry biomass, Scots pine plantation, productivity, forest ecosystems.

1. Introduction

Global warming has led to an increasing interest in global carbon storage and carbon balance [1]. The role of Scots pine plantations in the global carbon budget and their response to climatic change requires a detailed understanding of the underlying ecosystem processes [2]. Mongolian coniferous forests are primarily composed of *Pinus sylvestris* L. (Scots pines), with their natural range limited to small areas of the Khentii mountains, covering around 5% of the total forest area in Mongolia. The harsh continental climate is characterized by sunny days, extended dry and cool periods, short growing seasons, low precipitation and large annual, seasonal, monthly and diurnal fluctuations in air temperature [3]. The mean annual temperature in the study area is 1.0 °C, and the mean annual precipitation is 273 mm.

Reforestation activities in Mongolia started in the 1970's. Since then, 98,000 ha have been reforested and restored, 50% being replanted with seedlings [4]. Our investigation was carried out in the Tujiin nars forest in Northern Mongolia. Tujiin Nars National Park covers 45 thousand ha, with Scots pine as the dominant forest species. According to the statistics, over 19,000 ha of these degraded forests were restored between 1971 and 2011 [5].

Tree biomass can be divided into different components according to physiological functions; these most commonly include stems, stem bark, live and dead branches, needles and roots [6, 7].

The objective of this study was to obtain basic data including the parameters noted above of each biomass component of a 25-year-old Scots pine plantation. In order to achieve this objective, we made dimensional measurements of individual Scots pines and determined the relative distribution of above-ground biomass at the stand level (Fig. 1).

This is the first attempt to estimate above-ground biomass allocation and production of a Scots pine plantation in a semi-arid region in Northern Mongolia.

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The same initial planting design, soil preparation and planting techniques have been used since 1971 for the establishment of Scots pine plantations in Tujiin nars forest. The rehabilitation of this forest has been recognized by the Mongolian government as playing a leading role in the conservation of forest soil, biodiversity and forest ecosystems in Mongolia.

Fig. 1 Location of the study area in Mongolia.

2.1 Materials

This study was carried out in a 25-year-old, even-aged Scots pine plantation, covering a 35 ha portion of the coniferous forest in Tujiin nars National Park, Selenge province, northern Mongolia (50°14′N, 106°38′E). The plantation, growing in a semi-arid, cool-temperate climate zone, is located on a flat area of sandy soil at approximately 690 m elevation. The main purpose in establishing the plantation was to protect the soil from erosion after forest fire and clear-cutting. The plantation was established, in 1987, from two-year old Scots pine seedlings raised in the Khond forest nursery in Selenge province. To prepare the land, a plough-mounted tractor was used to dig trenches 70 cm wide and 20-30 cm deep, 3-5 m apart. Initial planting density was 2,500 seedlings per hectare. A permanent sample plot covering an area of 2 ha was established at the site in September 2010. A total of 1,776 trees were used in the current study.

2.2 Methods

The plot was divided into 50 m × 100 m subplots. In each subplot tree height, and stem diameter at breast height (DBH) of all trees were measured using calipers, tapes and clinometers. Additionally, in each subplot, the mean crown diameter, height upto first live branches and crown height of planted trees were determined. The selected sample trees were harvested at ground level and the crown was separated from the main stem. For each sample tree, as well as DBH, measurements of stem diameter were taken from the base to the top at 0.5 m intervals. Sample tree stem volume was calculated by summing the volume of the 0.5 m sections. Stem volume per hactare was calculated by multiplying stem volume by stem density.

The above-ground biomass was defined as the sum of dry mass of stem wood, stem bark, live and dead branches and needles of all sampled trees. We selected three trees, whose DBH were closest to the mean of Scots pine trees in each subplot. Branch biomass and needle biomass were estimated using the following procedure: first, the total fresh mass of the entire tree crown was determined in situ for each harvested tree. Then, for each tree, four branches were selected randomly from upper, middle and bottom positions in the crown (10-12 branches per tree). The fresh mass was weighed in the field, and samples were transferred to the laboratory in plastic bags. Dry biomass was calculated by estimating the measured mass lost during drying (2 days at 105 °C).

2.3 Data Analysis

Differences in biomass allocation and water content of planted trees were studied by considering five biomass components including stem wood, stem bark, needles, live and dead branches. Analyses of variance (ANOVA) was used to determine differences between the relative water content and proportion of each biomass component. The analytical estimates of height and diameter generally refer to predominant height and arithmetic mean diameter. To describe a tree's morphometric characteristics, the mean and

standard error of the mean values were obtained from measured and derived variables.

3. Results and Discussion

Stand characteristics of the Scots pine plantation are shown in Table 1. Results of this investigation varied among subplots (Table 1), but no significant difference was observed between tree height, DBH, density and basal area. The mean DBH of the stand was 8.91 ± 0.46 cm, mean height 6.50 ± 0.26 m, mean volume 23.21 m^3 ha^{-1} and mean basal area was 5.63 m^2 ha^{-1}. The number of trees growing in each subplot varied. For example, there were 903 trees ha^{-1} in subplot III, while in subplot IV there were 872 trees. The crown height averaged 4.47 ± 0.22 m (Table 1).

Differences in biomass allocation between tree components on this 25-year-old Scots pine plantation are presented in Table 2. Results of the investigation are that most of the trees above-ground biomass was allocated to stem wood (47.6%), followed by live branches (25.8%), while the smallest proportions of total living biomass were stem bark (8.1%) and dead branches (3.7%) (Table 3). Above-ground biomass per tree totaled 20,304 kg and the total above-ground biomass was 18.03 Mg ha^{-1}.

The distribution of total above-ground biomass shows dry biomass of stem wood (8.58 ± 0.71), stem bark (1.46 ± 0.11 Mg ha^{-1}), needles (2.67 ± 0.34 Mg ha^{-1}) and live branches (4.65 ± 0.23 Mg ha^{-1}) while, compared with live branches, dead-branch biomass is (0.67 ± 0.21 Mg ha^{-1}) (Table 3). The proportion of water in the above-ground fresh biomass was around 50% (Fig. 2).

Although biomass of live branches was significantly higher than dead branches, the total biomass of all branches made-up only 29.5% of stand biomass.

This is the first study to our knowledge that provides data on above-ground biomass allocation in a Scots pine plantation in Northern Mongolia. Compared with a number of earlier studies [8-10], a higher proportion of biomass was found in stem wood and live branches. Indeed, the larger amount of needles per unit sapwood area in the less fertile stands might be related to lower physical activity in the needles, relative to the transport capacity of the sapwood, with this being due to poor nutrient conditions consequent upon water deficit. The accumulation of nutrients in the ground surface is limited by an extremely slow rate of litter over the time period [11, 12], leading to decreasing nutrient concentrations. Additionally, intensive crown closure in the Scots pine plantation has to be considered as another limiting factor on crown development. Studies

Table 1 Stand characteristics of subplots.

Sample plots	Age (years)	DBH (cm)	Height (m)	Density (tree ha^{-1})	Volume (m^3 ha^{-1})	Basal area (m^2 ha^{-1})	Crown height (m)
I	25	8.69 ± 0.42	6.14 ± 0.25	888	21.8	5.27	4.33 ± 0.24
II	25	8.75 ± 0.38	6.23 ± 0.29	891	25.1	6.42	4.51 ± 0.21
III	25	8.62 ± 0.45	6.03 ± 0.18	903	23.05	5.35	4.26 ± 0.18
IV	25	9.62 ± 0.59	7.63 ± 0.32	872	22.9	5.48	4.69 ± 0.25
Mean		8.91 ± 0.46	6.50 ± 0.26	888	23.21	5.63	4.47 ± 0.22

Table 2 Stand characteristics of Scots pine plantation.

Items	Height (m)	DBH (cm)	Crown height (m)	Crown diameter (m)	Largest crown diameter (m)
Mean	6.34	8.05	4.33	2.00	2.33
Standard error	0.26	0.46	0.22	0.10	0.11
Standard deviation	2.33	3.99	1.89	0.88	0.93
Sample variance	5.43	15.92	3.58	0.78	0.87
Range (maximum, minimum)	8.24-4.36	11.21-5.42	6.25-2.58	2.91-1.41	2.93
Confidence level	0.53	0.91	0.42	0.20	0.21

Table 3 Mean value of fresh and dry mass of above-ground biomass per hectare.

Parts of above-ground biomass	Fresh biomass (g)	Dry biomass (g)	Proportion of total dry biomass (%)
Stem wood	16.8	8.58 ± 0.71	47.6
Stem bark	2.94	1.46 ± 0.11	8.1
Live branches	9.35	4.65 ± 0.23	25.8
Dead branches	1.32	0.67 ± 0.21	3.7
Needles	5.29	2.67 ± 0.34	14.8
Total above-ground biomass	35.7	18.03	100

Value in parentheses is ± SE.

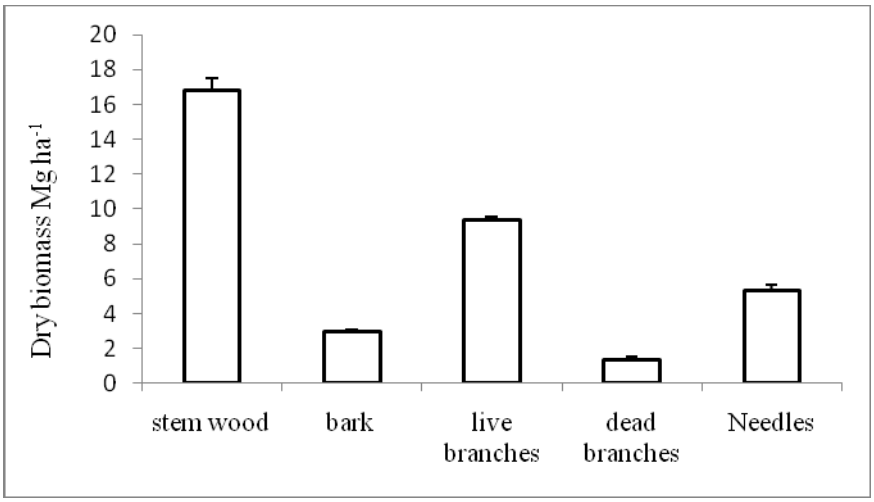

Fig. 2 Biomass allocation of above-ground biomass in Scots pine plantation.

of tree response to initial spacing have previously focused on growth traits such as height, DBH, stem volume and above-ground biomass [13-15]. In the study area, the planting was designed with an intended spacing of 4 m between rows with trees planted at 1 m intervals, but in practice, trees were unevenly spaced. Our results, showing that when spacing between trees is reduced biomass allocated to branches and foliage usually decreases to the benefit of the stem, as reported by Fang et al. [12], Lahcen Benover et al. [13], Pinkard and Neilson [14]. A previous study found that crown closure begins after 12 years and the mean height of the first live branches above the ground surface gradually increases with age [5].

Crown architecture is known to play an important role in forest productivity [16] and previous studies have shown that there is a strong relationship between crown diameter and DBH in Scots pine forests [17, 18]. Investigations of crowns in Scots pine plantations have shown that the average crown diameter differs between plantation, and that within-row crown measurements are much less than between-row crown measurements. In addition, it is known that crown volume and spatial distribution of the needles are important in carbon gain at the crown level and that they directly affect the availability and efficient use of light within the crown [19, 20].

4. Conclusions

We found a very slow rate of biomass accumulation in the Scots pine plantation over the last 25-year period, with the main part of stand biomass located in stem wood (47.6%) and live branches (25.8%). According to our data, the dry biomass of needles was only 14.8% of the total dry biomass of the stand. This low value can be explained by the very limited growing conditions in our study area. The initial planting design negatively influenced the timing of crown closure. In this case, crown closure began during an early stage of development, with some

variation due to uneven tree distribution. The results from this study indicate that it may be necessary to implement thinning to increase biomass production and reduce competition between trees in this plantation. We recommend further investigations and experiments focusing on improving biomass production in the Scots pine plantations of the region. Work on biomass allocation and the productivity of Scots pine plantations could lead to improved carbon budget models for similar forest ecosystems elsewhere.

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Immobilization of *Lactobacillus rhamnosus* TISTR108 on Crude Pectin of Krung Kha Mao Leaves (*Cissampelos pareira* L.) to Produce Lactic Acid in Longan Juice

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Abstract: L-(+)-lactic acid production was studied by immobilized *Lactobacillus rhamnosus* TISTR108 on crude pectin from Krung Kha Mao Leaves. Central composite design was employed to determine the maximum lactic acid production of 42.88 g L^{-1} in predicted model with the factors at 4.11 g L^{-1} of pectin, 6.05 mL L^{-1} inoculum and 1.09 mm of bead diameter. Statistical analyses demonstrated very high significance for the regression model, since the *F*-value computed 116.09 was much higher than the tabulated *F*-value 2.08 for the lactic acid production at 5% level for linear and quadratic polynomial regression models. The highest experimental lactic acid production was 43.57 g L^{-1} at 96 h of fermentation, 1.58% higher than the predicted value.

Key words: L-(+)-lactic acid, *Lactobacillus rhammosus*, Krung Kha Mao leaves (*Cissampelos pareira* L.), longan juice, response surface methodology.

1. Introduction

Lactic acid (2-hydroxypropionic acid or 2-hydroxypropanoic acid, $CH_3CHOCOOH$) is the most widely carboxylic acids with application in food preservation, flavor enhancement, pharmaceutical, cosmetic, leather and textile industries, biodegradable plastics and chemical feed stock and many other chemicals [1-4]. Lactic acid represents approximately 85% of the demand for food and food-related applications, whereas for non food industrial applications represent only 15% of the demand [1]. Lactic acid, a highly hygroscopic and syrupy liquid is commercially available at different grades (qualities): 20%-80% technical grade, 85% food-grade, 90% (< 0.1% ash) pharmacopoeia-grade and the lowest ash content (< 0.01%) plastic-grade [5].

A lactic acid bacterium (LAB) produce lactic acid as a primary metabolite and its production is strictly dependent on the final cell growth [4]. The most widely homofermentative bacterial strains used in such fermentation are *Lactobacillus bulgaricus*, *L. leichmanii*, *L. delbrueckii*, *L. amylophillus*, *L. amylovorus*, etc. [2, 6]. *L. rhamnosus* is one of the most important LAB to produce lactic acid for industrial biotechnology objectives in renewable raw materials such as cassava, potato residues media, hydrolyzed a corn starch, persimmon juice, wheat bran hydrolysate etc. [6-9]. Longan (*Euphora longana* Lam.) is a subtropical fruit which grown in China and Southeast Asia including Thailand. The oversupply of longan in Thailand makes their price lower. Longan juice contains total sugar (sucrose (142.1 g L^{-1}), glucose (27.7 g L^{-1}) and fructose (39.1 g L^{-1})), organic acid, amino acid, essential oils, volatiles, vitamins and minerals (iron, magnesium, phosphorus and potassium) which are suitable for growth of LAB to produce lactic acid [10].

Immobilization technology has several advantages which permits higher cell densities in bioreactors,

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improves stability, makes reutilization and continuous operation possible, and precludes the need to separate the cell from the substrate products following processing [11]. Pectin gel entrapment is the one method of immobilization of whole cells [11-12]. Crude pectin was extracted from Krung Kho Mao (KKM) leaves (*Cissampelos pareira* L.) by distilled water at 25-28 °C and 0.2% w/v of solvent [13]. The extracted pectin consist mainly of galacturonic acid (70%-75%) which is the primary structure feature of pectin (a linear chain of poly-α-(1→4)-D-galacturonic acid) [12, 14]. Crude pectin from KKM is cheaper than commercially gel entrapment to produce lactic acid, and pectin beads are efficiency as strong acid protection in comparison with commercial citrus pectin, commercial apple pectin and sodium alginate [13].

The lactic acid yield will depend on the condition of immobilization, high concentrations of sugars and other fermentable compounds. This paper studies a novel immobilization of cells with crude pectin from KKM to optimize the highest lactic acid production by response surface methodology (RSM) in longan juice. The model of lactic acid production was predicted and compared with the observed values.

2. Materials and Methods

2.1 Materials

2.1.1 Preparation and Extraction of Pectin from KKM Leaves

KKM leaves were cleaned to immerse in water, and dried at 50 °C for 3 h. The dried leaves had stored in vacuum at room temperature. The ratio of solid:distilled water is 1:50 to extract crude pectin at 80 °C for 60 min. Supernatant was filtrated in a vacuum filter to concentrate with a rotary evaporator, and precipitated the crude pectin by 95% ethanol. The pectin was dried in a vacuum oven to powder storage at room temperature [12-14].

2.1.2 Preparation of Longan Juice

The longans were craved a seed and a fruit peel to extract carbon source, vitamin and mineral in distilled water by disintegration method. The longan juice was filtrated particles and colloids by muflin filter to pasteurized at 70 °C for 15 min, and preserved at 4 °C [10, 15].

2.2 Microorganism

The homofermentation of lactic acid bacterial *Lactobacillus rhammosus* TISTR108 were maintained at Thailand Institute of Scientific and Technological Research (TISTR) to produce well the L-(+)-lactic acid in anaerobic condition. The culture was refreshed fortnightly activity and stored in deMann Rogosa Sharpe (MRS) agar at 4 °C [13, 16].

2.3 Fermentation

The stock cultures were transferred to 50 mL of sterile MRS broth in a 250 mL Erlenmeyer flask, and then incubated for 24 h at 37 °C and 100 rpm on a rotary shaker. The active cells were harvested sedimentation to immobilized potassium pectate entrapment from de-esterification of crude pectin with catalyzed 0.2 M KOH. The mixture of initial inoculums and 20 mL of potassium pectate were flowed through a peristatic pump to form bead gel with silicone tube in 150 mL of 0.2 M $CaCl_2$, and then washed spherical beads with distilled water to remove excess of calcium ions and unentrapped cells [13, 16]. The fresh beads were incubated for 12 h at 4 °C to fermented lactic acid in sterile longan juice which consisted 10, 20 and 120 g L^{-1} of the yeast extract, $CaCO_3$ and the total sugar, respectively. The cultural medium was carried out in 250 mL Erlenmeyer flasks containing 100 mL of total medium to incubate at 37 °C for 96 h under static conditions [10].

2.4 Analytical Methods

Lactic acid concentration was analyzed using a high performance liquid chromatography (HPLC) system (Shimadzu Co., Tokyo, Japan) with an Inertsil C8-3 column (1.6 × 250 mm) and a UV detector adjusted to 210 nm. The operation of mobile phase was

maintained flow rate at 1 mL min^{-1} and room temperature using 20 mM KH_2PO_4 (pH 3). The fermentative media were measured pH using a digital pH meter (Denver Instrument Co., Model 215) to harvest bead cells, and then filtrated supernatant with a 0.45 μm cellulose acetate filter. The filtrates were determined the total sugar by Lane and Eynon assays [17]. The one gram of bead cells was broken to dissolving with 20 mL of 5% w/v EDTA at 100 rpm on a rotary shaker for 30 min. The numbers of active cells were determined between 30 and 300 CFU g^{-1} using available plate counting method [18].

2.5 Response Surface Method

The central composite design (CCD) for three independent variables each at tree levels with eight factorial points, six star points and six replicates at the center points was utilized to develop a second order polynomial model which determined the optimal values of variables for lactic acid production (Y_a) [19]. The three independent variables have assigned concentrated pectin (X_1), inoculation of *L. rhammosus* (X_2) and inner diameters of silicone tube were immobilized cell with gelatinous entrapment (X_3).

The variables of the experiments were coded according to the following equation:

$$X_i = \frac{(X_j - X_{cp})}{\Delta X_j} \quad (1)$$

where, X_i is the coded value of an independent variable, X_j is the real value of an independent variable, X_{cp} is the real value of an independent variable at the center point, and ΔX_j is the step change value.

The prediction of this model was explained by the following quadratic equation:

$$Y_a = b_0 + \sum b_i X_i + \sum b_{ii} X_i^2 + \sum b_{ij} X_i X_j \quad (2)$$

where, Y_a is the predicted response, b_0 is the offset term, b_i is the linear effect, b_{ii} is the squared effect, and b_{ij} is the interaction effect, X_i is the ith independent variable. Table 1 shows the range and the levels of the variables investigated in this experiment. The CCD method was designed a total 20 experiments with the variety of independent variable.

Table 1 Real values of variables used in central composite design.

Variables		Range and levels				
		-1.682	-1	0	1	1.682
Pectin (g L^{-1})	X_1	2.318	3	4	5	5.682
Inoculation (ml L^{-1})	X_2	1.636	3	5	7	8.364
Bead diameter (mm)	X_3	0.659	1	1.5	2	2.341

2.6 Statistical Data Analysis

The response surface and contour plots were generated to understand the interaction of different variables by Statistica version 7.0 of Statsoft Inc. (USA). Data and quadratic model building were analyzed by SPSS for Window version 11.5.

3. Results and Discussion

3.1 Optimization for Lactic Acid Production is Immobilized Cells Longan Juice by KKM Pectin

The experimental design of the three variables in real and coded units is assigned conditional cultivation to present in Table 2 with the observed and predicted values. The application of linear regression analysis method yielded the following regression Eq. 3 to the experimental data.

$$Y_b = 40.87 + 1.32X_1 + 5.28X_2 - 1.66X_3 - 0.73X_1X_2 + 0.50X_1X_3 - 0.04X_2X_3 - 6.00X_1^2 - 4.33X_2^2 - 1.49X_3^2 \quad (3)$$

where, Y_b is the predicted response that is the lactic acid concentration and X_1, X_2 and X_3 are the coded values of the independent variables. The interception of polynomial equation is to look alike the average of experimental center points that the difference is 0.03 g L^{-1} of lactic acid production. In addition, the quantitative inoculation of *L. rhamnosus* is attributed high variation of lactic acid production that the linear coefficiency of the concentrated pectin, quantitative inoculation and bead size are demonstrated 1.32, 5.28 and 1.66, respectively. Indicating the inoculation is high relatively lactic acid production when the other factors are fixed in Table 2. The quadratic coefficiency of the concentrated pectin is also high, which resulted in 6.00, 4.33 and 1.49 to analyze the

Table 2 Operational conditions assays for experimental and mathematically predicted values for the production of lactic acid.

Runs	Independent variables						Lactic acid (g L^{-1})	
	Real values			Coded values			Experimental values	Predicted values
	X_1	X_2	X_3	X_1	X_2	X_3		
CCD design								
1	3.000	3.000	1.000	-1.000	-1.000	-1.000	26.08	23.83
2	3.000	3.000	2.000	-1.000	-1.000	1.000	22.38	19.60
3	3.000	7.000	1.000	-1.000	1.000	-1.000	37.19	35.94
4	3.000	7.000	2.000	-1.000	1.000	1.000	31.12	31.56
5	5.000	3.000	1.000	1.000	-1.000	-1.000	28.09	26.95
6	5.000	3.000	2.000	1.000	-1.000	1.000	24.17	24.70
7	5.000	7.000	1.000	1.000	1.000	-1.000	34.06	36.13
8	5.000	7.000	2.000	1.000	1.000	1.000	32.18	33.72
9	2.318	5.000	1.500	-1.682	0.000	0.000	18.55	21.68
10	5.682	5.000	1.500	1.682	0.000	0.000	28.25	26.13
11	4.000	1.636	1.500	0.000	-1.682	0.000	16.73	19.74
12	4.000	8.364	1.500	0.000	1.682	0.000	39.52	37.51
13	4.000	5.000	0.659	0.000	0.000	-1.682	38.25	39.44
14	4.000	5.000	2.341	0.000	0.000	1.682	34.05	33.86
15	4.000	5.000	1.500	0.000	0.000	0.000	40.51	40.87
16	4.000	5.000	1.500	0.000	0.000	0.000	41.21	40.87
17	4.000	5.000	1.500	0.000	0.000	0.000	40.30	40.87
18	4.000	5.000	1.500	0.000	0.000	0.000	41.15	40.87
19	4.000	5.000	1.500	0.000	0.000	0.000	41.34	40.87
20	4.000	5.000	1.500	0.000	0.000	0.000	40.88	40.87
Instead of variables in the Eq. 3								
21	4.110	6.050	0.980	0.110	0.525	-1.040	-	42.56
22	4.110	6.050	1.090	0.110	0.525	-0.820	-	42.81
23	4.110	6.470	0.980	0.110	0.735	-1.040	-	42.51
24	4.110	6.470	1.090	0.110	0.735	-0.820	-	42.76

concentrated pectin, quantitative inoculation and bead size, respectively, in linear regression method. And the interaction coefficiencies of the independent variables are lower than another to effect 0.73, 0.50 and 0.04 in relatively between concentrated pectin versus quantitative inoculation, concentrated pectin versus bead size and quantitative inoculation versus bead size, respectively.

The student's *t*-distribution and the probability values were checked the significance of each of the coefficients, which in turn may represent the pattern of the interaction between the test variables in Table 3. A larger magnitude of the *t*-test and smaller *P*-value denote greater significance of the corresponding coefficient [20]. Almost the coefficients are the significant first and second order unless the interactive factors were not effected the calculated response in the X_1X_2, X_1X_3 and X_2X_3 terms. The linear coefficients of X_1 and X_2 had a positive shift on the response, as an

Table 3 Model coefficients estimated by linear regression.

Factors	Coefficients	Standard error	Computed *t*-value	*P*-value
b_0	40.870	0.432	94.510	< 0.001
b_1	1.321	0.287	4.605	< 0.001
b_2	5.283	0.287	18.415	< 0.001
b_3	-1.657	0.287	-5.776	< 0.001
b_1b_2	-0.732	0.375	-1.953	0.056
b_1b_3	0.495	0.375	1.322	0.192
b_2b_3	-0.040	0.375	-0.106	0.916
b_{11}	-5.997	0.279	-21.474	< 0.001
b_{22}	-4.328	0.279	-15.498	< 0.001
b_{33}	-1.491	0.297	-5.340	< 0.001

increase in their concentration led to an increased yield. The same is observed with the quadratic coefficients of X_1^2 and X_2^2 that the strong negative shift revealed a reduction in lactic acid production when their concentrations were increased in the second order of the system. The first and second orders of X_3 and X_3^2 coefficients are effected negative shift on the yield when the bead size is increased in cell entrapment.

The mathematically model was performed in the analysis of variance (ANOVA) and the results were summarized in Table 4. The model was checked statistical significance by the Fisher's *F*-test that is the highly significant of the ratio of mean square regression to mean square residual is 116.090 and has a very low probability value at 95% of confident. The goodness of fit of the model was checked by adjusted determination coefficient (adjusted R^2) that is 0.9461 to attribute variation of the independent variables for lactic acid production, and only 5.39% of the total variation is assigned to the another factors. The high value of correlation (R = 0.9769) demonstrates a similar agreement between the experimental observations and predicted values [19]. This relation is also approved by the plot graph of predicted and actual experimental values of lactic acid concentration in Fig. 1A that all of the points were clustered around the diagonal line. Indicating, the model could well predict the experiments. The internally studentized residuals and the predicted response are presented in Fig. 1B. The small residuals less than 3% that the model could adequately describe the response of lactic acid production out of the experimental ranges studies [21]. The statistically significant lack of fit *F*-statistic implies that the terms in the model do not capture all of the assignable-cause variation of the response variable [22]. The terms of X_1X_2, X_1X_3 and X_2X_3 were not highly significant within the range of this study that agreed dismissal these parameters [20]. Therefore, the model was modified to describe the lactic acid production by Eq. 4.

$$Y_c = 40.87 + 1.32\,X_1 + 5.28\,X_2 - 1.66\,X_3 - 6.00\,X_1^2 - 4.33\,X_2^2 - 1.49\,X_3^2 \quad (4)$$

The response surface and the contour plots are the graphical representations of the regression equation and one plotted to understand the interaction of the

Table 4 Analysis of variance for the quadratic model and the express terms.

Sources	Degree of freedom	Sum of square	Mean square	*F*-value	*P*-value
Model	9	3,523.699	391.522	116.090	< 0.001
Error	50	168.629	3.373		
Lack of fit	4	26.084	6.521	21.416	< 0.001
Pure error	45	13.702	0.304		
Total	59	3,692.329			
X_1	3	1,519.946	506.649	623.984	< 0.001
X_2	3	1,942.427	647.476	797.426	< 0.001
X_3	3	218.865	72.955	89.851	< 0.001
Error	49	39.786	0.812		
X_1X_2	1	12.863	12.863	0.220	0.641
X_1X_3	1	5.891	5.891	0.101	0.752
X_2X_3	1	0.038	0.038	0.001	0.980
Error	55	3,213.560	58.428		
X_1^2	1	1,377.600	1,377.600	50.261	< 0.001
X_2^2	1	734.403	734.403	26.794	< 0.001
X_3^2	1	101.531	101.531	3.704	0.059
Error	55	1,507.489	27.409		
Total	59	3,692.329			

R^2 = 0.9543; adjust R^2 = 0.9461; R = 0.9769.

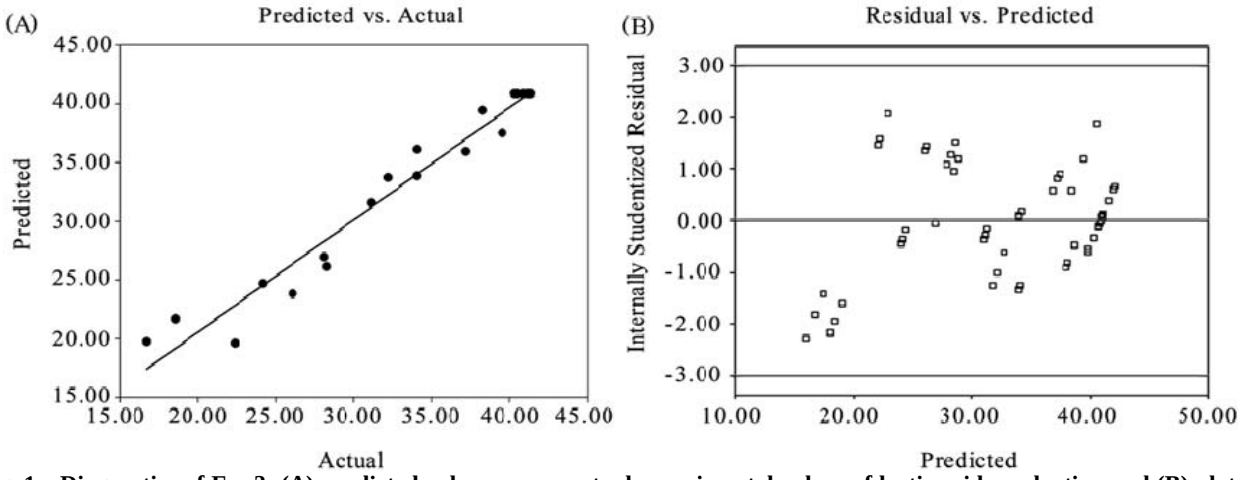

Fig. 1 Diagnostics of Eq. 3: (A) predicted values versus actual experimental values of lactic acid production and (B) plot of the residuals versus predicted values of lactic acid production.

variables and to locate the optimum level of each variable for maximum response of lactic acid production. If the surfaces are rather symmetric and flat near the optimum, the optimized values may not vary widely from the single variable conditions [19]. There are three pairs of response surface and contour plots in Figs. 2-4. The first convex graph was plotted the maximum response of lactic acid production 41.31 g L^{-1} when the concentrated pectin and quantitative inoculation are 4.11 g L^{-1} and 6.05 mL L^{-1}, respectively to describe by Eq. 5 and Fig. 2. The interactive variables of pectin and inoculum size are described by Eq. 6 and Fig. 3 to determine 38.01 g/L of lactic acid production when the conditional cultivation of concentrated pectin and bead size are 4.11 g L^{-1} and 1.09 mm, respectively. And the interaction between the inoculation and bead size were represented the convex point 38.61 g L^{-1} of lactic acid production when the quantitative inoculation and bead diameter are 6.47 mL L^{-1} and 0.98 mm, respectively in Eq. 5 and Fig. 4.

$$Y_d = -105.86 + 49.94X_1 + 14.55X_2 - 0.37X_1X_2 - 5.85X_1^2 - 1.04X_2^2 \quad (5)$$

$$Y_e = -55.65 + 44.37X_1 + 5.45X_3 + 0.99X_1X_3 - 5.57X_1^2 - 4.24X_3^2 \quad (6)$$

$$Y_f = -3.95 + 12.03X_2 + 7.62X_3 + 0.04X_2X_3 - 0.93X_2^2 - 3.58X_3^2 \quad (7)$$

The maximum of predicted response was 42.81 (Y_b) when X_1, X_2 and X_3 were 4.11, 6.05 and 1.09, respectively which were calculated from the polynomial equation. Thus the maximum concentration of lactic acid was 42.81 g L^{-1} when the concentrated pectin, quantitative inoculation and bead size were 4.11 g L^{-1}, 6.05 mL L^{-1} and 1.09 mm, respectively. The identically maximum response is predicted 42.88 g L^{-1} (Y_a) of lactic acid production to calculate by the fit model.

3.2 The Growth Rate of Lactic Acid Production in Longan Juice

The optimal condition is consisted of the concentrated pectin, qualitative inoculation and bead size to ferment at 4.11 g L^{-1}, 6.05 mL L^{-1} and 1.09 mm, respectively in Fig. 5. The rate of lactic acid production was considered high for the first log phase for 12 h while the rate of reducing sugar was advantaged slowly. After 12 h of usefully reducing sugar is been highly for 48 h as long as it was decreased since 60 h after fermentation. The supernatant is determined fast reduced pH at the initial 6.40 unstill the fermentation was produced for 36 h to slowly decrease pH. The relation of pH and lactic acid production are ensured negative interaction that the rate of production was reduced after 12 h of fermentation. Because, the external pH was declined by production, the acid is protonized as soon as it is

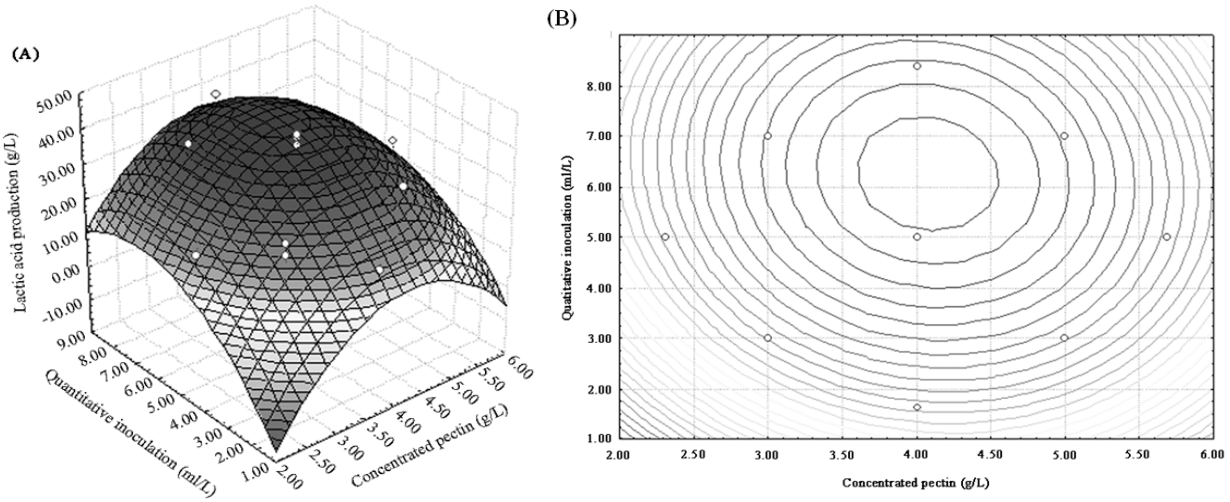

Fig. 2 Response surface (A) and contour (B) plots had been showing the interaction of the concentrated pectin and quantitative inoculation on lactic acid production.

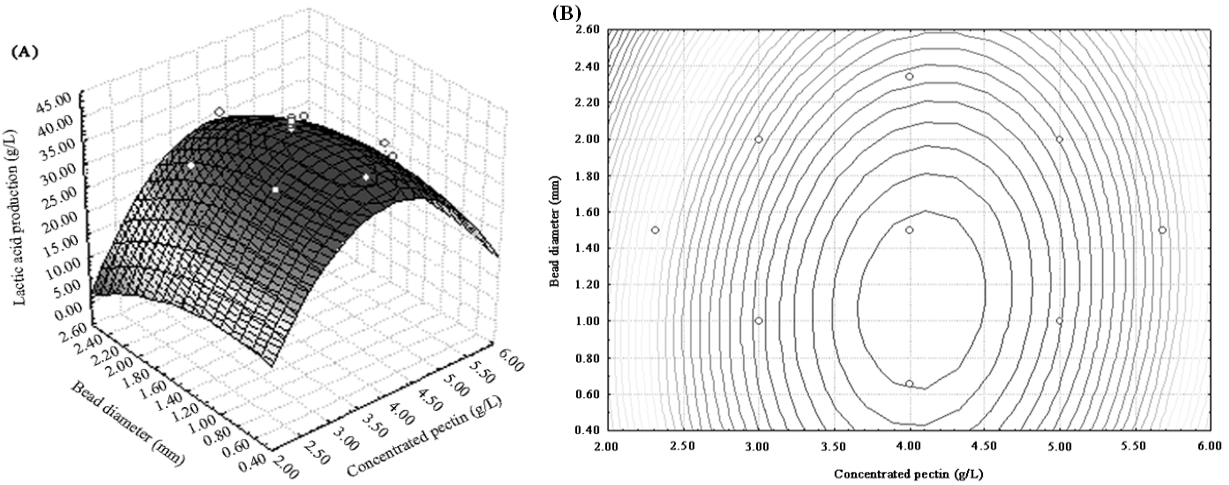

Fig. 3 Response surface (A) and contour (B) plots had been showing the interaction of the concentrated pectin and bead diameter on lactic acid production.

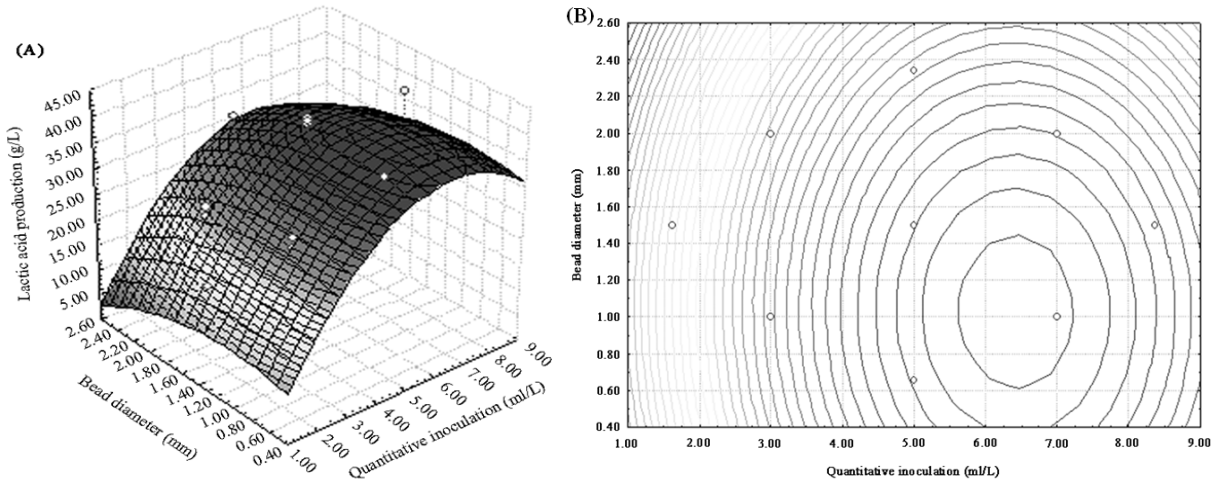

Fig. 4 Response surface (A) and contour (B) plots had been showing the interaction of the quantitative inoculation and bead diameter on lactic acid production.

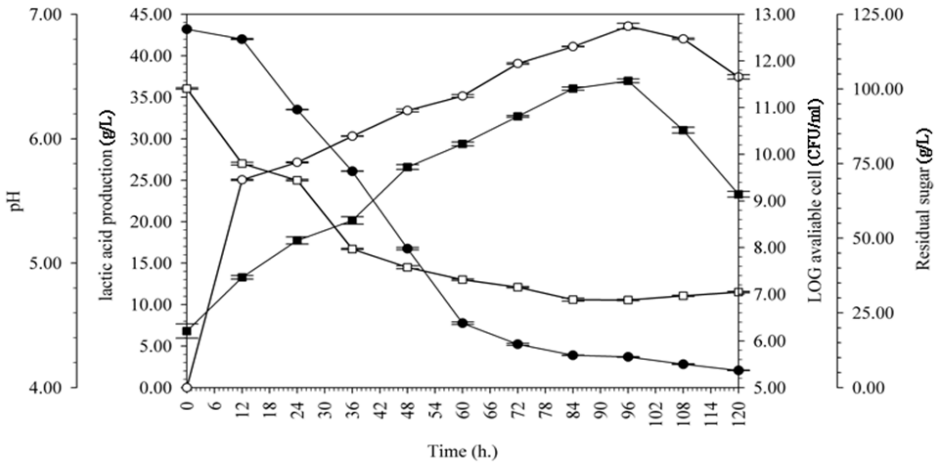

Fig. 5 Time course of the available cell density (■), abundantly residual sugar (●) and supernatant pH (□) were analyzed fermentation of immobilized *L. rhamnosus* TISTR108 for lactic acid production (○).

Table 5 Comparative carbon sources were reported for lactic acid production.

Microorganism	Cell immobilization	Carbon sources	Lactic acid production (g L^{-1})	Productivity (g L^{-1} h)	Lactic acid yield (g g^{-1})	References
L. rhamnosus + *L. brevis*	Free cell	Corn stove	14.80	0.40	0.73	Cui et al. [23]
L. rhamnosus	Free cell	Apple pomace	32.46	5.41	0.88	Gullon et al. [24]
L. rhamnosus	Free cell	Longan juice	41.38	0.86	0.40	Choojun and Suttisuwan [10]
Lactococus lactis + *L. casei*	Alginate entrapment	Whey	29.89	1.24	0.83	Choojun and Suttisuwan [25]
L. casei	Pectate entrapment	Whey	32.95	1.09	0.35	Panesar et al. [11]
L. rhamnosus	Pectate entrapment	Whey(flask)	38.76	0.40	0.99	Choojun and Orathai [13]
L. rhamnosus	Pectate entrapment	Longan juice(flask)	43.57	0.45	0.40	This study

exported out of the bacteria. Uncharged, it diffuses back into the cell and dissociates due to the higher intercellular pH. The cell has to use ATP to pump out protons and energy is eventually depleted, causing growth to stop and the bacteria to die [20]. The primary metabolite of lactic acid production is detected interactive growth and production that the highest production is valued 43.57 g L^{-1} at 96 h. The comparison of the highest experimental and predicted values are resulted the actual more valuable than prediction 1.58% of experimental value.

The highest efficiency of lactic acid production was compared for carbon sources to summarize in Table 5. Choojun and Suttisuwan [10] and this study are produced more qualitative lactic acid in longan juice than corn stove, apple pomace and whey. The longan juice was fermented to increase lactic acid production of 41.38 g L^{-1} and 43.57 g L^{-1} under the free cell and pectate entrapment, respectively. But the other carbon sources are produced 14.80-32.46 g L^{-1} and 29.89-38.8 g L^{-1} under the free cell and immobilization, respectively [10-11, 13, 23-25]. The longan juice is been the most suitable substrate for lactic acid production compared with the other carbon sources and the modified synthetic medium [10].

4. Conclusions

The central composite design method and response surface analysis could estimate lactic acid production in longan juice. *L. rhamnosus* TISTR 108 was inoculated in optimal conditions of the concentrated pectin, qualitative inoculation and bead diameter are valued 4.11 g L^{-1}, 6.05 mL L^{-1} and 1.09 mm, respectively that the maximal lactic acid production was predicted 42.88 g L^{-1}. The best experimental production is valued 43.57 g L^{-1} for 96 h after fermentative time.

Acknowledgments

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Comparative Evaluation of Peptidases Produced by *Penicillium corylophilum* and *Penicillium waksmanii* in Solid State Fermentation Using Agro-industrial Residues

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Abstract: In this present work, the best conditions for production of peptidases under solid state fermentation by the fungi *Penicillium corylophilum* and *Penicillium waksmanii*, partial purification using Sephadex G-75 gel filtration column, as well as the biochemical characterization of the partial purified enzymes were investigated. *P. corylophilum* showed the best production in medium containing wheat bran, agro-industrial residue, without additives (egg albumin or casein), in which peptidase activity reached 520 U mL^{-1} and the enzyme displayed the optimum activity between pH range from 7 to 8 and 60 °C. It also showed high stability in wide pH range and temperature until 45 °C for 60 min of incubation. On the other hand, *P. waksmanii*, the best production was noted in a medium containing wheat bran (95%) and casein (5%), reaching 545 U mL^{-1}, with proteolytic optimum activity at pH 7.5 and 55 °C. The enzyme was mainly stable in pH range from 8 to 9 and at temperatures until 45 °C for 60 min of incubation. The peptidases secreted by both fungi were inhibited in the presence of phenylmethane sulfonyl fluoride, showing that they belong to the subclass of serine peptidases.

Key words: Serine peptidase, biochemical characterization, enzymatic extract, fungi.

1. Introduction

Endopeptidases (EC 3.4.21-25) are enzymes that break internal peptide bonds in proteins and peptides. They are considered as the largest group of industrial enzymes with increasing demand in many business sectors and basic research [1, 2].

Microorganisms are interesting sources of enzymes and used in fermentation processes because of their biochemical diversity, facility of growth, adaptation to different environments and also the possibility of genetic manipulation [3]. The microbial production of enzymes is a result of the biochemical diversity of each species and the medium used for fermentation, and also environmental conditions [1, 4].

Microbial products (enzymes, food, feed, pharmaceutical agents) can be obtained by bioprocess using different substrate. Among the systems used to metabolites production, it is possible to emphasize the technology of solid state fermentation (SSF), in which interesting advantages are to point out as lower energy requirements, produce lesser wastewater and are environmental-friendly [1, 3].

Peptidases production, in special, enables the use of agro-industrial residues as substrate in fermentative process. According to other studies, wheat bran has been highlighted among other agro-industrial residues

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as rice husk, rice bran, spent brewing grain, and palm kernel cake for production of peptidases in SSF [1].

Studies on the production of peptidases by *Penicillium* species have shown wide diversity in the secretion of these enzymes, such as aspartic peptidase [5, 6], metallopeptidase [7, 8] and serine peptidase [9, 10]. This diversity justifies the intense study of enzymes synthesized by species of this genus in search of better production conditions and industrial applications.

Fermentation parameter variations such as composition of the culture medium, inoculum concentration, growth temperature, and incubation time, are key factors that limit the production of enzymes [11, 12], and determine the kind and the yield of enzyme in each fermentation. Thus, the aim of this study was to investigate the best conditions for peptidase production in solid state fermentation (SSF) by *Penicillium corylophilum* and *Penicillium waksmanii* and establish comparative parameters of biochemical characteristics of the partially purified peptidases.

2. Materials and Methods

2.1 Isolation, Cultivation of Microorganisms and Preparation of Inoculum

Penicillium corylophilum and *Penicillium waksmanii* were obtained from the mycology collection of the Laboratory of Enzymatic Technology, FCFRP, USP. They were cultivated in potato dextrose agar for 120 h at 30 °C. Spore suspension was realized using saline containing 0.1% $(NH_4)_2SO_4$, $MgSO_4 \cdot 7H_2O$ and NH_4NO_3 (w/v) and was used as inoculum in SSF.

2.2 Solid State Fermentation (SSF)

The effect of composition of the culture medium in the production of peptidases was studied initially. In 250 mL Erlenmeyer flasks, 5 g of medium composed of wheat bran alone or its combination with the inducers egg albumin or casein in percentages of 5, 10 and 20 of each protein supplement, were added. The medium was moistened (66%) with 10 mL of saline containing 0.1% (w/v) of the following salts: $(NH_4)_2SO_4$, $MgSO_4 \cdot 7H_2O$ and NH_4NO_3. All material was autoclaved for 40 min at 121 °C [13].

Other parameters such as incubation temperature (35, 40 and 45 °C), inoculum size, (1×10^6, 5×10^6 and 2.5×10^6 spores) and incubation time (24 to 168 h) were also studied. Fermentation was carried out for 168 h and after every 24 h, one flask of each trial was removed and it was added 40 mL of deionized water at 4 °C. The solubilized material was centrifuged at 5,000 g for 20 min at 4 °C. The crude enzymatic extract supernatant was then used for quantitation of proteolytic activity.

2.3 Enzymatic Activity Assays

The enzymatic activity assay was performed using casein as substrate [14]. The assay was performed with 100 μL of crude enzymatic extract, 200 μL of monobasic sodium phosphate buffer (NaH_2PO_4) 50 mM (pH 6.5), and 1,000 μL of casein solution 1%. The reaction was conducted at 37 °C and was stopped by adding 600 μL of trichloroacetic acid (TCA) 10% after 45 min. Reaction and blank tubes were centrifuged at 10,000 g for 15 min at 25 °C and the absorbance of the supernatant was determined by spectrophotometry at 280 nm against each respective blank.

The proteolytic activity was expressed as U mL^{-1}, defined as the amount of enzyme needed to release 1 μmol of tyrosine per min in the tested conditions [15].

2.4 Partial Purification

The crude enzymatic extract was precipitated with ethanol in the proportion of 1:2 (enzymatic extract: ethanol) at 4 °C for 24 h and then centrifuged at 10,000 g for 20 min at 4 °C. The precipitates were resuspended in sodium phosphate monobasic (NaH_2PO_4) buffer 50 mM (pH 6.5), and then submitted to chromatography using Sephadex G-75

gel filtration column (4 × 100 cm). The eluted fractions were concentrated by ultrafiltration membrane of 10 kDa and used for partial biochemical characterization.

2.5 Biochemical Characterization of the Peptidases

The biochemical characterization of the peptidases produced by each fungus was performed using azocasein 1% as substrate, according to the protocol described by Ducros et al. [16], with modifications. The reaction mixture consisted of 100 μL of partially purified enzymatic extract, 100 μL of Hepes buffer 50 mM (pH 7.0), and 200 μL of azocasein 1%. The reaction was carried out for 15 min at 37 °C and stopped by adding 800 μL of TCA 10%. Reaction and blank tubes were centrifuged at 10,000 g for 10 min at 25 °C, then 800 μL of the supernatant were transferred to a test-tube and added 933 μL of 1 M NaOH to it. The absorbance was measured in a spectrophotometer at 440 nm against each respective blank.

The optimum pH of the peptidase was determined using the following buffers, all at 50 mM: Acetate (pH 4.5 and 5.0), Mes (pH 5.5, 6.0 and 6.5), Hepes (pH 7.0, 7.5 and 8.0), Bicine (pH 8.5 and 9.0) and Caps (pH 9.5, 10.0 and 10.5). The reaction was conducted at 37 °C for 5 min. The pH stability was studied by incubating the enzyme for 1 h at 25 °C at different pH values and then the enzymatic reaction was carried out at optimum pH in the presence of azocasein 1% at 37 °C for 5 min.

Once the optimum pH had been defined, optimum temperature was determined by performing the same reactions in the temperature range from 25 to 70 °C, with 5 °C increases. The thermal stability was studied by incubating the enzyme at temperatures from 40 to 55 °C, for 5, 15, 30, 45 and 60 min, which the reaction was conducted in the optimum temperature and pH of the enzyme.

The subclass of the peptidases was studied according to the response of peptidase to the following enzyme inhibitors: iodoacetic acid (IAA), ethylenediamine tetraacetic acid (EDTA), phenylmethanesulfonyl fluoride (PMSF) and pepstatin, at 10 mM, according to the protocol described by Dunn [17], with modifications. The enzymatic reaction was carried out at optimum pH in the presence of azocasein 1% at 37 °C for 5 min.

3. Results and Discussion

3.1 Effect of Composition of Medium on the Production of Peptidase

The composition of the medium is a determining factor in the production of peptidase. Species of the genus *Penicillium* have a great capacity of growing in various environmental conditions and to use different substrates as nutrient [18, 19]. Therefore, different compositions medium were tested using wheat bran or in combination with casein and egg albumin as the substrate, using an initial inoculum of 5×10^6 spores (5 g substrate) at 30 °C.

Penicillium corylophilum showed the best peptidase production in SSF with a medium containing wheat bran without protein supplement, egg albumin (Fig. 1a) or casein (Fig. 1b), which reached proteolytic activity of 520 U mL^{-1} within 96 h. Wheat bran has been highlighted among other agro-industrial residues for production of peptidases in SSF [1, 20], as it is rich in protein composition, and also has texture and porosity that facilitate mycelial dispersion [21, 22]. Peptidase production using wheat bran as substrate by *Penicillium* species was also demonstrated by Raja et al. [19] and Shimakage et al. [8].

However, *Penicillium waksmanii*, showed different results, peak production occurred in a medium containing wheat bran (95%) and casein (5%) at 72 h with 545 U mL^{-1} of proteolytic activity (Fig. 1d), in which results were better than medium containing wheat bran and egg albumin (Fig. 1c). It is noted that between the studied protein supplements, casein presented better induction in peptidase production for both fungi, when comparing to egg albumin supplement. It can be considered that casein becomes

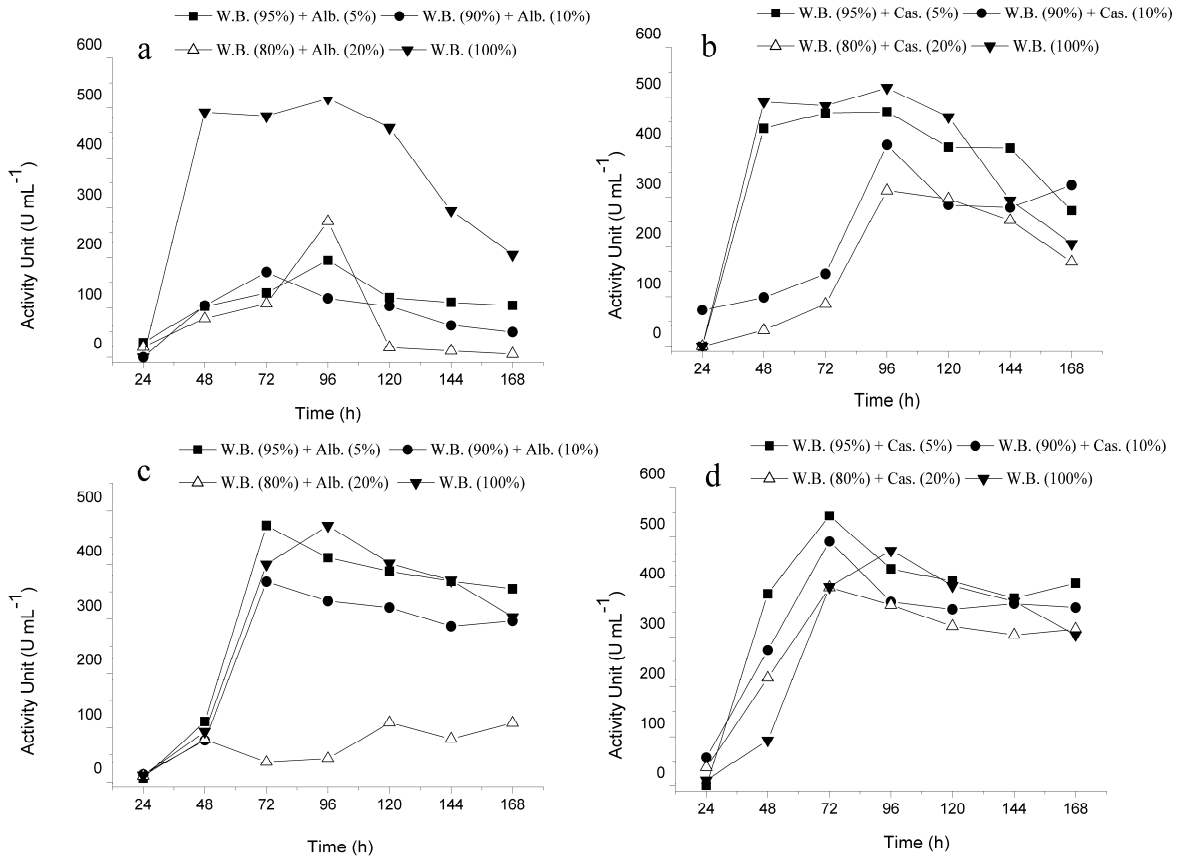

Fig. 1 Effect of medium composition on peptidase production in SSF using wheat bran (W.B.), egg albumin (Alb.) and casein (Cas.), inoculum size of 5 × 10^6 spores per 5 g substrate at 30 °C. SSF by *P. corylophilum*: in medium containing W.B. and Alb. (a), medium containing W.B. and Cas. (b); SSF by *P. waksmanii*: using W.B. and Alb. (c), and W.B. and Cas. (d).

more homogeneous in the medium and may provide greater contact surface and accessibility to the fungus.

3.2 Effect of Spore Concentration in Peptidase Production

Fungal growth and the consequent production of peptidase are related to the amount of spores inoculated into the fermentation media. Therefore different concentrations of spores (5 g substrate) (1 × 10^6, 2.5 × 10^6 and 5 × 10^6) were used to investigate the best conditions for the production of peptidase in wheat bran (100%) (*P. corylophilum*) and wheat bran (95%) with casein (5%) (*P. waksmanii*), both at 30 °C.

The results showed that the highest efficiency of enzyme production occurred at the concentration of 5 × 10^6 spores (5 g substrate) for both species, *P. corylophilum* (Fig. 2a) and *P. waksmanii* (Fig. 2b) indicating that at this concentration, the fungi had greater exploitation of the nutrient in the medium, resulting in greater induction of peptidase. Result with similar behavior of increased production of peptidase with an exponential increase in the concentration of spores was also observed in *Penicillium* sp. [12].

3.3 Effect of Temperature on Peptidase Production

Temperature is a limiting factor for fungal growth and production of enzymes and may influence mycelial dispersion and metabolic activity. Thus the effect of temperatures (35, 40 and 45 °C) on the production of peptidases was investigated. The largest production for both fungi was achieved at 30 °C in the medium containing wheat bran (100%) for *P. corylophilum* (Fig. 2c) and wheat bran (95%) and casein (5%) for *P. waksmanii* (Fig. 2d), both with an

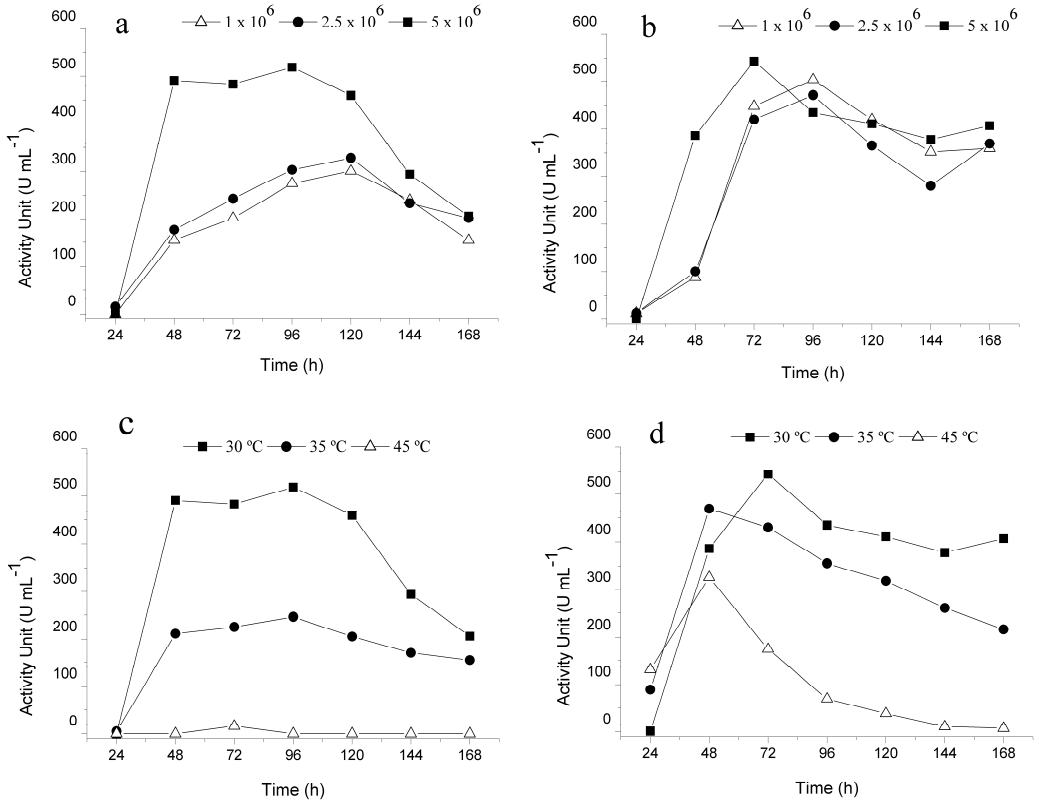

Fig. 2 Effect of spore concentration on peptidase production in SSF by *P. corylophilum* (a) and *P. waksmanii* (b), at 30 °C. Effect of temperature on peptidase production by *P. corylophilum* (c) and by *P. waksmanii* (d), inoculum size of 5 x 10^6 spores per 5 g substrate. SSF with 100% wheat bran (*P. corylophilum*) and 95% wheat bran + 5% casein (*P. waksmanii*).

inoculum of 5 × 10^6 spores per 5 g substrate. It was observed that with the gradual increasing of temperature, there was a reduction in the production of peptidases, which confirmed the temperature was a modulator factor in peptidase production. A similar result with less production of peptidase in relation to the increase in cultivation temperature was also demonstrated by *Penicillium chrysogenum* [23].

3.4 Effect of pH on Activity and Stability of Peptidase

In order to understand the peptidase produced from wheat bran, the enzymatic properties of the partial purified peptidase were studied.

It was observed that the maximal activity of the peptidase secreted by *P. corylophilum* was in the pH range from 7 to 8. For peptidase produced by *P. waksmanii*, maximal activity at pH 7.5 was noted, and the catalytic performance was strongly influenced by the pH of the reaction condition, since it showed great sensitivity to changes in the concentration of the proton (H^+) (Fig. 3a). The production of alkaline peptidase by *Penicillium* species has also been reported by other researchers [12, 24].

Regarding the effect of pH exposition, we observed that both the peptidases demonstrated a greater range of stability. It was also noticed that the peptidase produced by *P. corylophilum* showed to be slightly more stable in acidic condition than the peptidase produced by *P. waksmanii*, which better stability was observed at pH range from 8 to 9 (Fig. 3b). Other studies of peptidases secreted by *Penicillium* species also showed high stability in the pH range from 6 to 9 [9].

The production of active and stable peptidases in alkaline pH suggests important industrial application in detergents, foods, pharmaceuticals and leather [25].

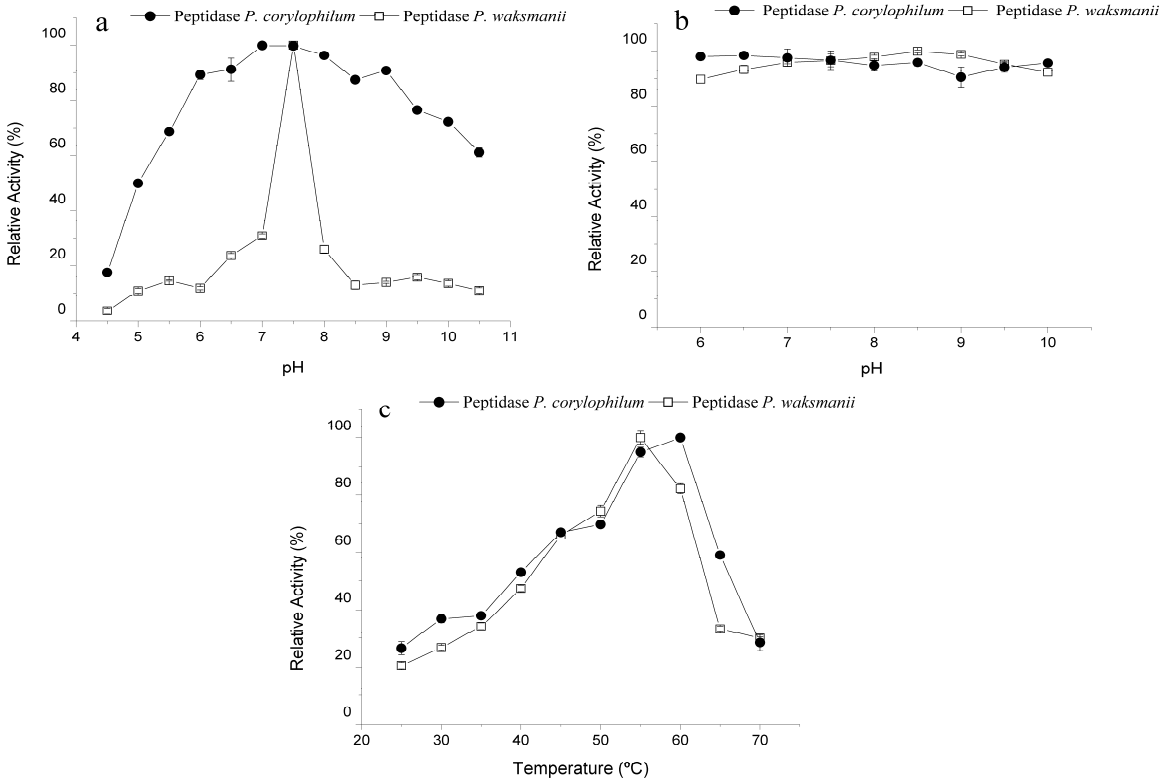

Fig. 3 Effect of pH and temperature on activity and stability of peptidases produced in SSF by *P. corylophilum* and *P. waksmanii*. Optimum pH for proteolytic activity (a); pH stability (b); optimum temperature for proteolytic activity (c).

3.5 Effect of Temperature on Activity and Stability of Peptidase

The effect of temperature on the activity of peptidases produced by *P. corylophilum* and *P. waksmanii* showed the optimal activity at 60 and 55 °C, respectively (Fig 3c). The enzymes were mainly stable at temperature until 45 °C for 60 min of incubation. In temperature up to 45 °C, it was also pointed out the activity of the peptidase produced by *P. waksmanii*, retaining 50% of residual activity after 30 min of incubation at 50 °C (Fig. 4b), unlike the thermal stability exhibited by peptidase produced by *P. corylophilum*, which showed 37% residual activity under the same time and temperature conditions (Fig. 4a).

The optimum temperatures of peptidases produced by *P. corylophilum* and *P. waksmanii* were higher than reported by Germano et al. [9] in *Penicillium* sp., which the optimal activity was observed at 45 °C. It also demonstrated to be less stable compared with the peptidases described in the present work, since Germano et al. [9] showed residual activity below 20% after 30 min of incubation at 50 °C. Agrawal et al. [12] also reported the production of peptidases by *Penicillium* sp. with optimal activity at lower temperatures than those described in this paper.

3.6 Effect of Inhibitors on Peptidase Activity

The study on the effect of inhibitors on proteolytic activity is important for better understanding the catalytic behavior of peptidases. In this work, the produced peptidases were almost completely inhibited by PMSF (10 mM), retaining only 10% and 2% of enzymatic activity for peptidases produced by *P. corylophilum* and *P. waksmanii*, respectively (Table 1). These results suggest that these enzymes belong to the subclass of serine peptidases, and they are dependent on a serine residue in their active site. Similar results with production of serine peptidases by species of *Penicillium* were also reported by Day et al. [10] and Germano et al. [9].

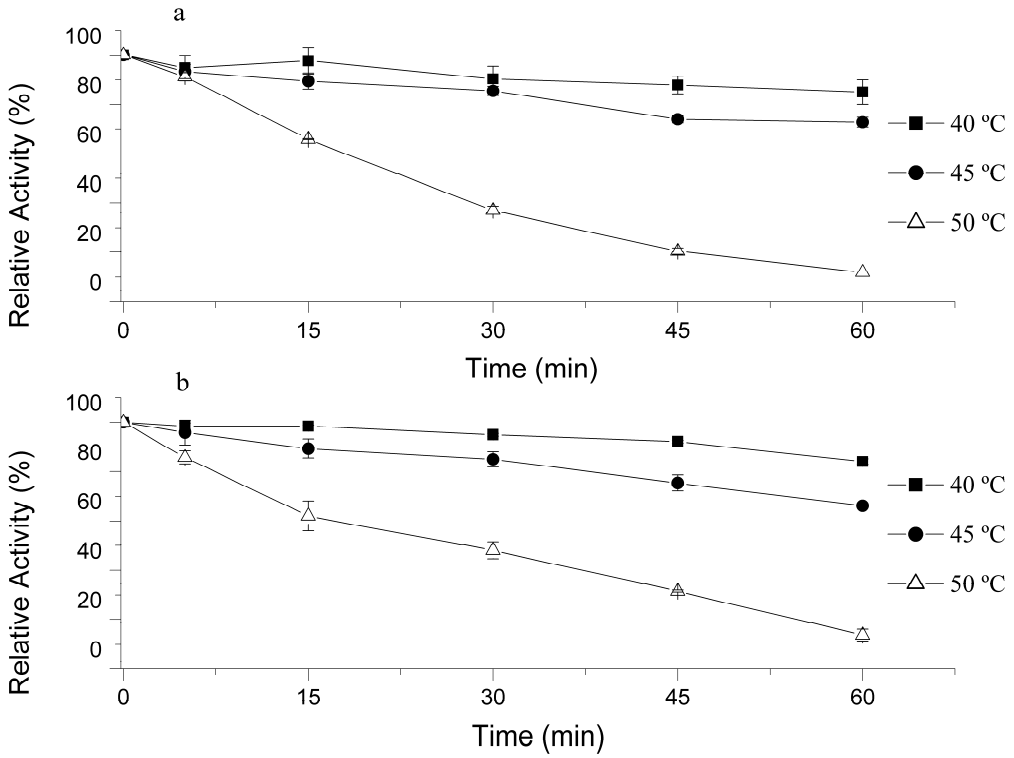

Fig. 4 Effect of temperature on the stability of peptidases produced in SSF by *P. corylophilum* (a) and *P. waksmanii* (b).

Table 1 Effect of inhibitors of proteolytic activity on peptidases produced in SSF by *P. corylophilum* and *P. waksmanii*. The enzymatic assay was realized in pH 6.0 and 40 °C, using azocasein 1% as substrate.

Inhibitors (10 mM)	(%) Residual activity peptidase *P. corylophilum*	(%) Residual activity peptidase *P. waksmanii*
None	100 ± 7.5	100 ± 1.0
IAA	98 ± 3.5	96 ± 4.2
EDTA	97 ± 3.0	86 ± 2.5
Pepstatin	92 ± 3.0	87 ± 2.0
PMSF	10 ± 5.0	2 ± 1.2

Values are the average of three independent experiments ± standard deviations. IAA (iodoacetic acid); EDTA (ethylenediamine tetraacetic acid); PMSF (phenylmethanesulfonyl fluoride).

4. Conclusions

The results presented in this paper regarding the production and characterization of peptidases secreted by *P. corylophilum* and *P. waksmanii* established the comparative parameters of these enzymes and highlighted that peptidases production was unique to each fungus. Notably, we found that SSF yielded production of different peptidase, being the wheat bran an excellent substrate for peptidase production. In this work there was production peptidases, which optimum activity was on the range of alkaline pH and temperatures of 55 and 60 °C. These results reinforce the importance of this study, demonstrating the biochemical potential of the studied species, providing more functional biochemical information on the produced enzymes, and allowing the consideration of possible potential applications of these peptidases.

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