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Street-Vended Foods Improvement: Contamination Mechanisms and Application of Food Safety Objective Strategy : Critical Review

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Abstract: Data collected from street-vended food enterprises and on vendors in west African countries revealed that, they provide a variety of ready-to-eat foods to a high proportion of the populations. Nevertheless, their handling and trading practices are not permit to obtain safe food. While, street-vended foods are easily contaminated by food borne pathogen and others chemicals compounds. The street-vended foods contamination mechanisms were identified and improvement pathways were suggested. Indeed, Food Safety Objective (FSO) concept developed by FAO and WHO, can be used as ideal strategy for safe street food production. However, to reach this goal, the Critical Control Points (CCP), Microbiological and Risks Assessment (MRA), and hygienic status during street food production and sale were gathered. By assembling and analyzing the data, the safety assurance for safe street food obtaining was evaluated at every step of production chain. The data were juxtaposed to FSO concept frame work and applied along the street-vended food production chain. We applied Performance Objective (PO) and Control Measure (CM) respectively at operational levels, measure at relevant points of risk and points, that permit reduction of all contamination risks along the chain, to enhancing safe food obtaining. The FSO concept could help government to elaborate guidance for street foods production, vending and consumption, producers and vendors, training about HACCP pre-requisites and information for global view on safe street-vended food (SSF) production. This will be an important task for the primary health care system aiming at "health for all".

Key words: Contaminants, safe street-vended food, improvement, food safety objective

Introduction

Food borne illness of microbial origin are a major international health problem associated to food safety and an important cause of death in developing countries (Rehydration Project, 2004; WHO, 2002a; 2002b). The problems of food safety in the developed countries differ considerably from those of developing countries. Whereas, in developing countries traditional methods of processing and packaging, improper holding temperature, poor personal hygiene of food handlers are still observed during food marketing and technology (Barro *et al*., 2002a; 2002b, Collins, 1997; Mensah *et al*., 2002). These observations refer to street-vended foods. The street food industry plays an important role in developing countries (Canet and N'Diaye, 1996; Ohiokpehai, 2003; Muinde and Kuria, 2005; Mwangi *et al*., 2002; van't Riet *et al*., 2003). They feed millions of people daily with a wide variety of ready-to-eat foods and beverages sold and sometimes prepared in the streets or public places, relatively cheap and easily accessible (Mensah *et al*., 2002; Barro *et al*., 2002b; FAO, 1989). The consumers who depend on such food are more interested in its convenience than in question of its safety, quality and hygiene (Barro *et al*., 2002b; Collins, 1997; Mensah *et al*., 2002).

According to street foods studies carried out in Africa, their tremendous unlimited and unregulated growth have placed a severe strain on city resources, such as water, sewage system and interferences with the city plan through congestion and littering adversely affecting daily life (Barro *et al*., 2002a; Canet and N'Diaye, 1996). FAO and several authors stipulated that street-vended food raise concerns with respect to their potential for serious food poisoning outbreak (Estrada-Garcia et *al*., 2002; 2004; Collins, 1997; King *et al*., 2000; Tjoa *et al*., 1977; Umoh *et al*., 1984), due to improper use of additives, the presence of pathogen bacteria, environmental contaminants and improper food handling practices based on unrespect of good manufacturing practices (GMPs) and good hygiene practices (GHPs) (Barro *et al*., 2002b; Canet and N'Diaye, 1996). Vendors are often poor level education,

unlicensed, untrained in food hygiene, technology and work under crude unsanitary conditions (Barro *et al.*, 2002a; 2006a; Muinde and Kuria, 2005). The hygienic aspects of street food processing and vending operations are a major source of concerns for food control. Street food safety management need a Hazard Analysis Critical Control Points (HACCP) and the pre-requisite system as good manufacturing practices (GMP) and good hygiene practices (GHPs) to instil professional face to street food operators (Bryan *et al.*, 1988; 1992). *Codex Alimentarius* Commission (CAC, 2004) formulated a concept of Food Safety Objective (FSO) argued by Gorris (2005) applicable to all food facilities for production of safe food. An attempt was made to apply this strategy to street food industry. The present paper aimed to determine the major problems that militating against the production of delivery of Safe Street Foods (SSF) and how to apply FSO for preventive measures, improving the sector and the products.

Organization of street-vended food enterprises, CCP and HACCP pre-requisites violations observed along production chain: Various safety assurance measures are implemented in throughout the food production chain to prevent street-vended foods from being contaminated with bacteria and viruses. The data that are generated from the implementation of these measures are dispersed and heterogeneous. While data were collected from street food enterprises in eleven towns belonging to ten west African countries (Burkina Faso, Mali, Cote d'Ivoire, Ghana, Guinea, Benin Niger, Nigeria, Senegal and Togo). It was identified eight main steps in street-vended foods general production chain. Street food enterprises tend to be satisfied with what they have achieved. Fig. 1 reports current hazards, CCP and Good Practices (GPs) violations observed at the main steps along the street food production chain. The others violations specific to one's steps are also described in Fig. 1. However, violations of these aspects means presence in food production chain, a risk that leads to direct or indirect contamination of food: the hazards of significance depend of the nature of the food, its intended use and production step. Steps 1, 3, 5, 6 and 7 are most important during uncooked foods processing and for cooked foods, steps 5, 6 and 7 are the main critical control points. Many small-scale food enterprises like street food industries, operates under a simple organizational structure, consisting of the manager-owner assisted by a few workers, who do not know modern techniques of management, including book-keeping and maintaining proper records. Street foods are processed generally by traditional methods of beverages and snack foods production. A poor manufacturing practices and personal hygiene of food handlers, a lacks of good-quality raw foodstuffs materials vegetables for processing are the main characteristics.

Holding cooked and uncooked foods at ambient temperature for 6 h or longer without any appropriate holding temperature (reheating in case of cooked food), constitute a major critical control point of street-vending (cook/hold) operations surveyed. In addition cooked foods were subjected to cross-contamination and contamination from various sources such as utensils, knives raw foodstuffs, flies that sporadically landing on the foods, by vendors bare hand serving and occasional food handling by consumers. Street food producers and vendors neglect food safety practices providing to population unsafe foods.

Street-vended foods contamination mechanism pathways: Hazards analysis consisted of existing microbiological assessment data processing, observing foods preparation, vending, serving and storage practices to identify sources and mode of contamination. The risk characterization and assessment, were carried out according to Lammerding, 1997; McKone, 1996, methodology. Their potential impact on the consumers were assessed and described as recommended by Vose, 1998; Marks *et al.*, 1998. We also took the principles of Risk Assessment as describe by *Codex Alimentarius* Commission (CAC, 2004), the FAO/WHO (FAO/WHO, 1997); Gorris (2005) and Woteki *et al.*, (2001). Diagram was built according street food process and risk points, as describe by Marks *et al.* (1998), Cassin *et al.* (1998) to determine the level of exposure of street foods to the hazards. In other, data on different vectors of street food contamination, were analyzed by using the interaction criterion to establish diagram of contamination complete routes from the raw foodstuffs, materials, transport, processing and storage to consumption. It allows to estimate the various levels of the hazard in various situations / circonstances. Fig. 2 shows the causes, routes and vectors of various contaminations of street-vended foods. Four main vectors can be distinguished. The first vectors include insects and animals, the second is constituted by environmental conditions (weather, dust, rains, winds, urbanization), the third vectors include peoples which acting in street food areas (government and his specific services low actions, hygiene controllers producers, growers, transporters, consumers and vendors). Finally, the last vectors is represented by natural contaminants as toxins contain in some raw foodstuffs and seafoods. The street-vended food improvement for sustainable quality are need the management at the level of these different vectors.

Several studies on street foods was done, but none report clearly an complete representative diagram of contamination mechanisms. In addition, early studies on improvement were targeted a specific street food. Ours finds are the first complete study identifying step by

step the mains problems discrediting street food as well, propose an improving through a global view of safe food production by using international concepts. Interestingly, in light of the attention, street food safety has received in media in recent years, consecutive to apparison of many diarrhoeal diseases as Cholera, Enterobacteriosis and Enterovirosis (Cardinale *et al.*, 2005; Estrada-Garcia *et al.*, 2004; Kuzuya *et al.*, 2003). Women in west Africa and other parts of the world are involved in a wide variety of food-processing activities. In the street food processing and vending activities there was a high illiteracy rate among women (Barro *et al.*, 2002a; Mensah *et al.*, 2002; Canet and N'Diaye, 1996; FAO, 1989). Action along educational line appeared as appropriate strategy to reach ALOP and it can be expected to improved the safety of street foods and thereby to heighten consumer protection (Abdussalam and Kaferstein, 1993).

The urbanization of west Africa towns and the associated dietary lifestyles, social and structural changes have caused and increased demand for street foods (Canet and N'Diaye, 1996). In the context of poverty, street food accounts for a part of the family income, daily diet and so contributes towards meeting nutritional requirements (Chakravarty and Canet, 1996; van't Riet *et al.*, 2003). This contemporary way of food consumption acts as impacting factor on food safety (Fig. 2). It is agreed that today, consumers are "time poor" and time spent preparing food is not considered quality time. Kurth (2000) reported that the two main issues that arose out were convenience and health. However, consumers could be a great of vendors behavior change the must exigi a right to basic good services which ensure quality of life: adequate food, clothing, shelter, health care, education and sanitation. Following the example of most developing countries urbanization, in west Africa generates many concerns as the difficult access to potable water, presence of different waste everywhere, lack of efficient drainage system. Indeed in most countries organic wastes and sewages are discarded on street. This healthiness provide harborage for insects and others animals and promote microorganisms growth. Under cover of flies, rain, wind wastes are dispersed and can be transported to uncover street-vended foods and cause the physical and microbiological contaminations. Considering the number of daily consumers of street foods, the number of diseased people will be important in case of consumption of contaminated foods. Thus, street food importance has consequence such as is association to epidemic and diseases outbreak in case of microbiological quality failure (Barro et *al.*, 2005; Cardinale et *al.*, 2005; Estrada-Garcia et *al.*, 2004; WHO, 2002b).

Many small-scale food enterprises operate as backyard industries located the owner's house, near the wastes, sewages, under trees without any sanitary system. They need better premises with a proper drainage and sewage system. The lack of appropriate site constitute a risk for food contamination. Small-scale food enterprises operators with an elementary school education are less receptive to new technologies compared to their counterparts who have a college education. This has made it difficult to transfer new technology to improve productivity and quality. Food stalls often lack the necessary storage, refrigeration and cooking facilities to prevent contamination with bacteria such as *Salmonella spp*, *E. coli*, which in warm, moist conditions, can duplicate into many disease-bearing organisms. A limited access to running water and waste disposal increases the potential for passing the problem on to many customers through street foods.

Safe Street Food production by application of FSO strategy: To perform a better understanding of the microbiological and hygienic problems associated to street-vended foods, hazard analyses and risks assessment data collected, were processed using FSO approach. By assembling and analyzing the data systematically, the safety assurance level can be evaluated at every step of the street-vended food production chain. This method allows the detection of strengths and weaknesses of the street food current safety system used. According to the risk assessors methodology (CAC, 2004; Lammerding, 1997; McKone, 1996; Reij and Van Schothorst, 2000) and in the Food Safety Objectives frameworks (Gorris, 2005; CAC, 2004; Woteki *et al.*, 2001). The data were processed to identify possible application points of HACCP pre-requisites as Performance objectives (PO) and control measure (CM) as describe by Gorris (2005), *Codex Alimentarius* (CAC, 2004), ILSI (ILSI, 2004) and ICMSF (2002). Fig. 3 shows how to apply FSO concept step by step during street-vended foods processing and exposure/vending. To reach this goal, street food production chain eight steps were considered including primary production step to serving step. At each steps advised good practices recommended by FAO/WHO (WHO, 2002a), CAC are indicated as well as HACCP pre-requisites. Fig. 3 also indicates sequential intervention of good practices, in accordance with quality assurance guidelines including HACCP and risk analysis to ensure safe street food (SSF). However, SSF obtaining through FSO concept need also Performance Objective and Control measure concepts. Their application points are located at steps 1, 4, 5, 6, 7 and 8. The street food safety actors responsible, for assistance to this sector by controlling the specific hazards, unhygienic aspects, risk possibly associated, need more guidance from food safety workers. To that end, within the Risk Analysis framework, vectors of contamination routes and setting the generic existing concepts as GAPs, GMPs, GHPs,

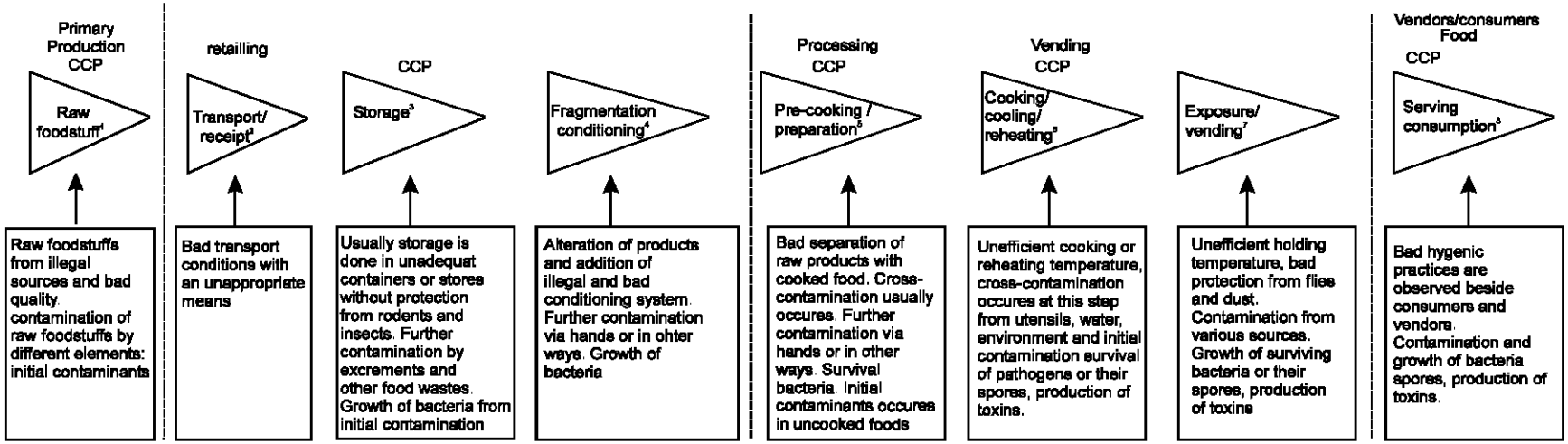

Fig. 1: Identification and description of Hazards, CP and Good Practices violations at different steps of street food production chain in reference to Advised Good Practices recommended by FAO /WHO along the street food chain of production. 1, 2, 3, 4, 5, 6, 7 and 8, indicate different steps in street food production chain. CCP = Critical Control Points

HACCP along the food chain of production, it was applied that when deemed appropriate, call Food Safety Objective (FSO) for street food safety and quality enhancing as detailed in Fig. 3.

Enforcement of the law banning street vendors, didn't work in the past and will not reach the core of problem, which is the lack of understanding of hazards and safe practices. A lack of knowledge of definition and causes of diarrhoea were important risk factors (Mensah *et al.*, 2002). The remedy is education: education of street food producers, vendors, food service personnel and education of public who either purchase street-vended foods. This constitutes a basic Performance Objective at the level of food operators, for enhancing street-vended food quality. Microbiological hazards and their solution, food processing and preparation technology, critical points, practical control measures and monitoring procedures (WHO/ICMSF, 1982) as well as the principles of food microbiology and food safety need to be incorporated into training programs for safe street food preparation. All these data should be sough and used to give direction for Food Safety Objective approach application to street food enterprises.

Several authors report, increasing in mesophilic aerobic bacteria when the duration of holding was prolonged, indicating microbiological deterioration of food (Bryan *et al.*, 1988; Bryan, 1978; El-Scherbeeny *et al.*, 1985). Cooked foods were subjected to cross contamination and contamination from varous sources (Fig. 2). The outbreak itself, is always associated with the initial contamination of a ready-to-eat food such as street-vended foods and re-contamination by inappropriate handling after cooking, consequently, in most cases , the street-vended food is subject to inadequate refrigeration during an excessive shelf-life. The responsibility of the food industry and the consumer is clearly engaged during this scenario of food borne diseases. The question is how to avoid the introduction of the contaminants in the food chain ? If a ready-to-eat, potentially hazardous food is improperly held or cooled, the potential for spore or toxin-forming bacteria growth increases. Some bacteria form spores that survive can germinate and grow if food is improperly held after cooking. Reheating is a "magic step" for eliminating hazards resulting from improper holding (Bryan *et al.*, 1988; 1992; Mensah *et al.*, 2002; Mosupye and van Holy, 2000; WHO, 1992). Reheating at 75°C for 15 seconds in important action to ensure the food's safety. Some risk factors or contamination routes might not have been directly linked to food preparation. The containers in which the food was served

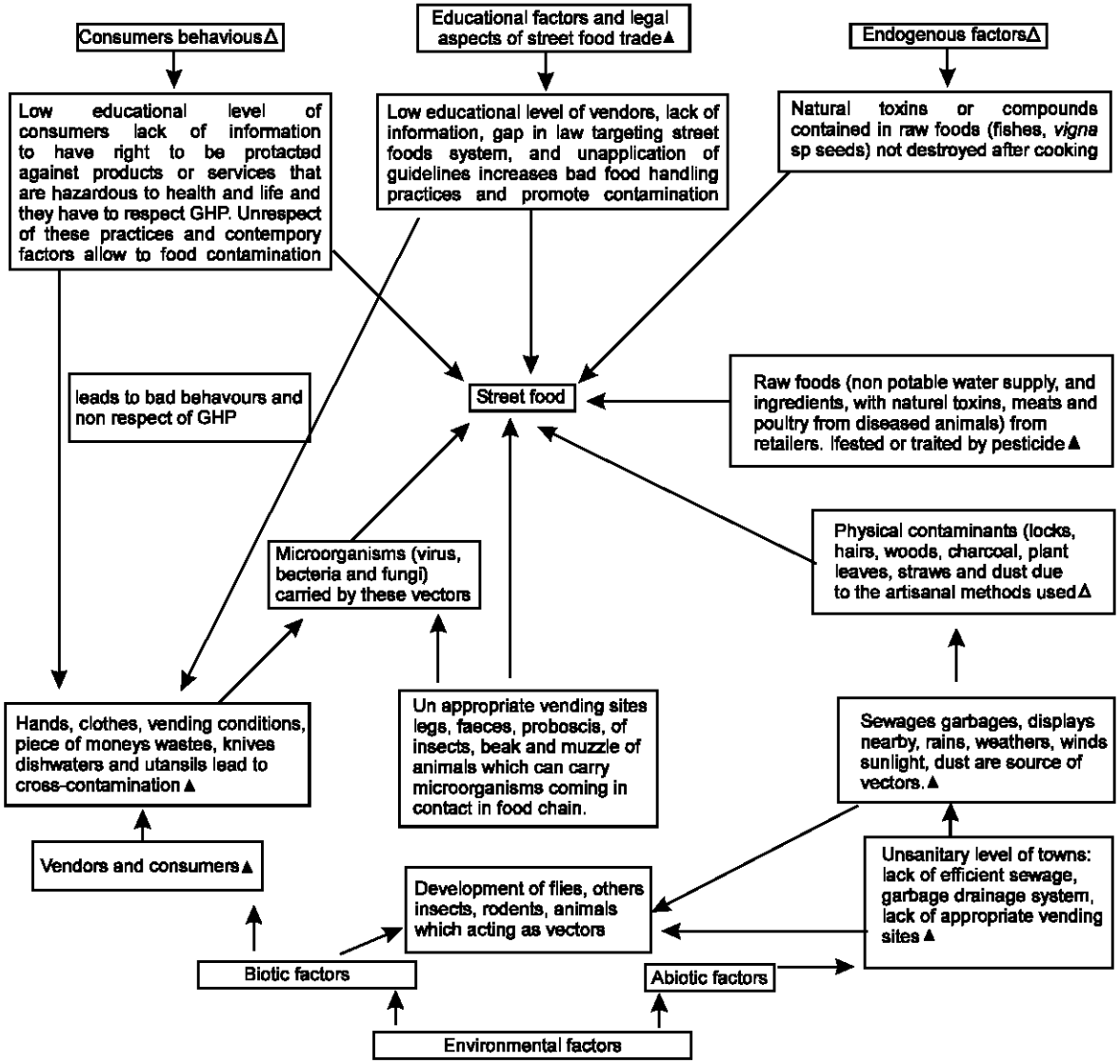

Fig. 2: Mechanism of street-vended foods microbiological and physical contamination vectors and their routes diagram. Arrows indicated contamination routes, dotted arrows indicate indirect routes. (▲) major factors and (Δ) minor factors of contamination

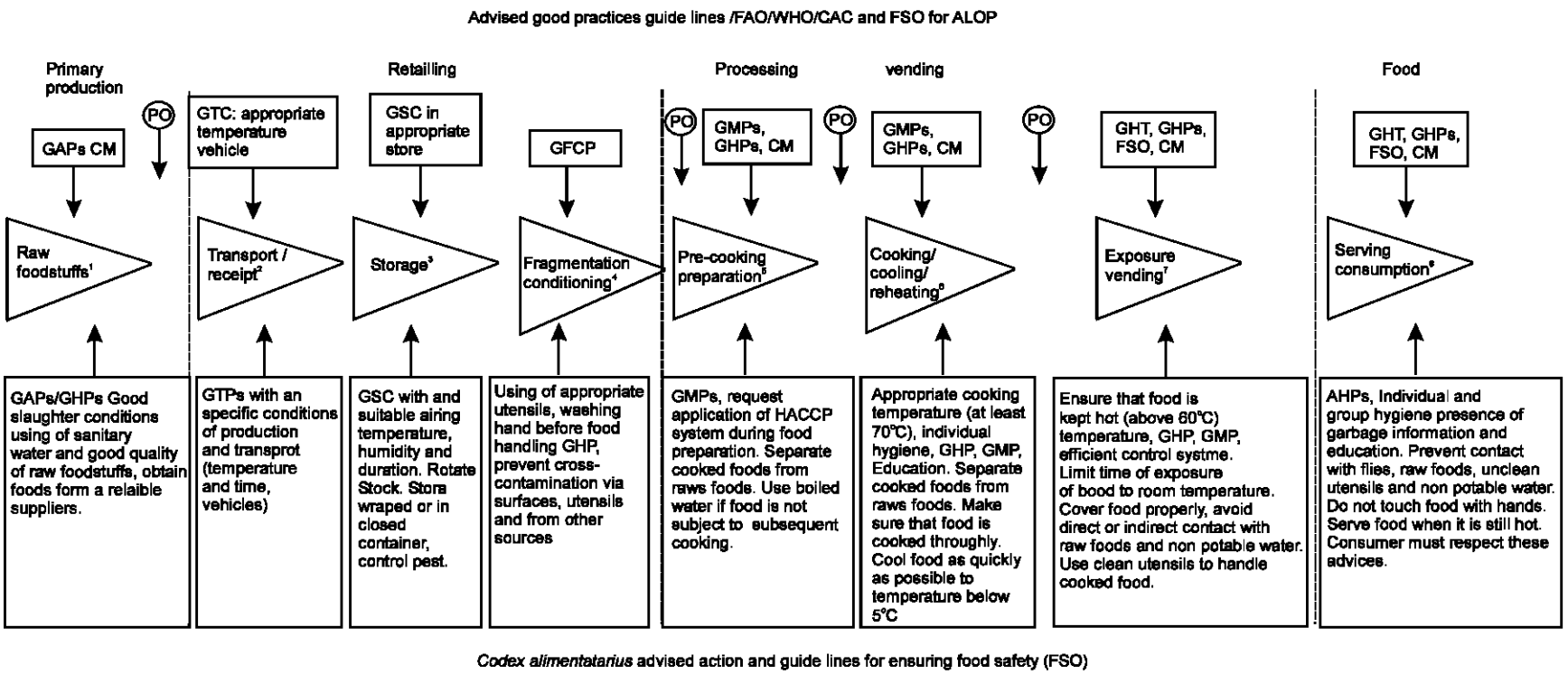

Fig. 3: Diagram of FSO concept and FAO (*Codex alimentarius* Commission) guidelines application for safe street foods guidelines. GAP= Good Agricultural Practices. GHP= Good Hygiene Practices, GMPs = Good Manufacture Practices, GSC = Good Storage Conditions; AHT = Appropriate Holding Temperature; GTC = Good Transport Conditions; GFCP = Good Fragmentation and Conditioning Procedure; GCC = Good Comportment of Consumers, ALOP = Appropriate Level of Protection, SSF = Safe Street Food, PO = Performance Objective, CM = Control Measure; 1, 2, 3, 4, 5 , 6, 7 and 8 indicate different steps in street food production chain.

are also important and the use of non-adequate wrapping paper and leaves increased the risk of contamination. The paper used for holding food was usually newsprint of questionable origin. Leaves were wiped with a piece of cloth and there was no disinfection (Mensah *et al*., 2002). In this connection it should be noted that the handling of food at ground level may lead to the contamination because dust could easily be blown on food thus handled. Pathogens can be passed mechanically by flies which are carried various microorganisms (Barro *et al*., 2006b; Khalil *et al*., 1994; Sulaiman *et al*., 2000; Sukontason *et al*., 2000). There is a real risk of contamination associated with exposure of street foods.

Viruses are somewhat resistant to heat and given their low infectious dose may not be reduced to safe levels using the reheating parameters in the Food Code. Several studies gave information on bacterial assessment, street food contamination risk factors or associated illness, while few data reports implication of viruses in street food contamination and associated diseases. Viruses can contaminated during processing and caused diarrhoeal diseases, toxi-infection as bacteria (Allwood *et al*., 2004; Barro *et al*., 2005; Borchardt *et al*., 2003; Nicand *et al*., 1998). Enteroviruses detection must be incorporated

in food microbiological risk assessment.

The natural toxins or antinutritional factors occurring in some raw foodstuffs can passed to food with is virulence during unappropriated culinary methods. In west African street food system, some food as "Benga" cooked seeds of *Vigna uniguiculata* "Souma" cooked seeds of *Voandzeia subterranea* (Nana *et al*., 2000), some cereal (*Penisetum sp*.) product as "Gapal", uncooked tubers of sweet potato (*Ipomea batatas*) and cassava (*Manihot esculentus*) contains antinutritional factors (ANFs) which cause bloating when improperly processing. Appropriate method of preparation reduce or elinate their negative impact of those contain in *Fabaceae* seeds (Nana *et al*., 2000).

Data described above were necessary to consider for FSO application to street food production chain. The relevant steps necessary to application of FSO in street food system derive from Appropriate Level of Protection (ALOP) approach (WTO, 1995). It acknowledged that street food production chain can be very different, but nevertheless should comply with a common target in this approach, the FSO applied to food facilities is as well as applicable specifically to street food production. FSO also become very important and deems necessary in street food system on the basis of public health goal, directed towards protecting a sub-population of illness. An epidemiological link between street foods and diarrhoea has been reported by several authors (Mensah *et al*., 1999; Estrada-Garcia *et al*., 2004; Heinze and Yackovich, 1988). One aspect interesting this FSO concept application in street foods system is his use as tool to reach ALOP without having an Microbiological Risk Assessment (Goris, 2005). Microbiological analysis are done rarely voluntarily by street foods vendors. Street foods risk and safety controllers may choice specific risk management measures as educating, hygiene code which seem to important in FSO targeting to specific food as street-vended foods.

An other aspect deriving from HACCP concept and providing or contributing to FSO or to ALOP and food safety control, and applicable for street food quality enhancing, is the management of individual specific step through the Performance Objective (PO) and CM concepts (CAC, 2004). In food safety management, the importance of PO, CM were clearly established (CAC, 2004; ILSI, 2004; Goris, 2005) and their application steps are indicated in Fig. 3. In street food system, PO can be use as milestone to obtains safe street food and the CM applied as precaution to reduce risk along the chain. Early data on street foods are indicated quality failure of raw foodstuffs involved in food preparation (Barro *et al*., 2002b; Bryan, 1988; Canet and N'Diaye, 1996). Indeed, PO concept shows that, having a target early on the food supply chain may be much more relevant in terms of guidance to hazard control than one at the end of chain (Goris, 2005). PO is very important and easy to use at different steps during street food processing. An irrefutable example was gave in case of prevention of cross-contaminations at a point where food is prepared for final consumption. Occurrence of cross contamination is a generic issue affecting the safety of all street-vended foods. Pathogen microorganisms present on raw foodstuffs, spices seasoning salads, could be in the process to ready to eat foods through manual handling, knives for cutting, cooking utensils or other surface (Barro *et al*., 2006a; Bryan, 1988; Gorris, 2005).

Three others approaches were deemed necessary to improve street food quality. The first approach is the "First-In-First-Out" (FIFO). Product rotation is important for both quality and safety reasons. FIFO means that the first batch of product prepared and placed in storage should be the first one sold. The FIFO concept limits the potential for pathogens growth, cross contamination, encourages product rotation, and documents compliance with time/temperature requirements. The second is Standard Operating Procedures (SOPs) - Following standardized, written procedures for performing various tasks that ensures quality, efficiency, and safety criteria, are met each time the task is performed. The quality and the taste of the same food produce by the same producer are very variable. They are a variety of street-vended foods and they are generally processed with one's hands and traditional culinary methods and various unstandard operations. The SOP in street food enterprise seems difficult to applied because of financial aspect, traditional equipments used, education level of vendors, lack of processing book and enterprise profile. It one of reason that it is difficult to establish a certificate of compliances with the quality to approved street food products. To set up of SOP, needs are education of vendors on preliminary notion and importance of standardization in their trade. Finally, the third approach is Risk Control Plans (RCPs) is a concisely written management plan developed by the retail or food service operators with input from the health inspector that describes a management system for controlling specific out-of-control risk factors. An RCP is intended to be a voluntary strategy develops to promote long-term compliance for specific out-of-control risk factors. The goal of this RCP could help to street food quality enhancing and reduce street food associated diseases. An RCP can ensure that new procedures are established to adequately treatment of street-vended food in the future. By implementing basic control systems, it is likely that the new controls will become "habits" that continue. This system are necessary in street food enterprises. By implementing an RCP, the retail or street food service operators will have the opportunity to determine the appropriate corrective action for the identified problem and design an implementation strategy to best suit their

facility and operation. Since the RCP is tailored to meet the needs of the establishment, the operator takes complete ownership of the plan and is ultimately responsible for its development and implementation. Inspector is to consult with the operator by suggesting ways that the risk factor might be controlled. By creating an RCP, the operator realizes that a problem exists in their food safety management system and commits to a specific correction plan rather than merely acknowledging a single violation.

Conclusion: Looking the complexity and is importance, is recurrent link to illness of street food system, Education and Information of different actors specifically in street food safety management, need to be incorporated clearly in FSO concept and might develop a new concept called Street Food Safety Objective (SFSO). The different steps defining the role of FSO agree with the current objectives of street foods safety managers (researchers, government, producers, vendors, consumers etc.). Risk assessment, risk management and risk communication as a mean of organizing available information, identifying data gaps, quantifying risk for specific pathogens and foods, and presenting strategies for improvement are need for education. Food safety education is a critical part of the overall strategy to reduce the incidence of food borne illness and complements regulatory and other activities. Meeting the huge challenge of food safety in the 21st century will require the application of new methods to identify, monitor and assess food borne hazards. Both traditional and new technologies for assuring food safety should be improved and fully exploited. This needs to be done through legislative measures where suitable, but with much greater reliance on voluntary compliance and education of consumers and professional food handlers. This will be an important task for the primary health care system aiming at "health for all".

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Review on Sequencing Batch Reactors

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Abstract: This review paper intends to provide an overall vision of SBR technology as an alternative method for treating wastewater. This technology has been gaining popularity through the years, mainly because of its single-tank design and ease of automation. The bibliographic review carried out here shows the efficiency and flexibility of this technology, as it is able to treat different kinds of effluents such as municipal, domestic, hyper saline, tannery, brewery, and dairy wastewater; landfill leachates; etc.; under different conditions. The review includes relevant experiments carried out at the laboratory, pilot-plant, and industrial scales.

Key words: Sequencing batch reactor, nutrient removal, laboratory SBR scale, pilot-scale SBR

Introduction

SBRs are used all over the world and have been around since the 1920s. With their growing popularity in Europe and China as well as the United States, they are being used successfully to treat both municipal and industrial wastewater, particularly in areas characterized by low or varying flow patterns. Municipalities, resorts, casinos, and a number of industries, including dairy, pulp and paper, tanneries and textiles, are using SBRs as practical wastewater treatment alternatives.

Improvements in equipment and technology, especially in aeration devices and computer control systems, have made SBRs a viable choice over the conventional activated-sludge system. These plants are very practical for a number of reasons:

In areas where there is a limited amount of space, treatment takes place in a single basin instead of multiple basins, allowing for a smaller footprint. Low total-suspended-solid values of less than 10 milligrams per liter (mg/l) can be achieved consistently through the use of effective decanters that eliminate the need for a separate clarifier.

The treatment cycle can be adjusted to undergo aerobic, anaerobic, and anoxic conditions in order to achieve biological nutrient removal, including nitrification, denitrification, and some phosphorus removal. Biochemical oxygen demand (BOD) levels of less than 5 mg/L can be achieved consistently. Total nitrogen limits of less than 5 mg/L can also be achieved by aerobic conversion of ammonia to nitrates (nitrification) and anoxic conversion of nitrates to nitrogen gas (denitrification) within the same tank. Low phosphorus limits of less than 2 mg/L can be attained by using a combination of biological treatment (anaerobic phosphorus absorbing organisms) and chemical agents (aluminum or iron salts) within the vessel and treatment cycle.

Older wastewater treatment facilities can be retrofitted to an SBR because the basins are already present.

Wastewater discharge permits are becoming more stringent and SBRs offer a cost-effective way to achieve lower effluent limits. Note that discharge limits that require a greater degree of treatment may necessitate the addition of a tertiary filtration unit following the SBR treatment phase. This consideration should be an important part of the design process.

The sequencing batch reactor (SBR) has received considerable attention since Irvine and Davis (1971) described its operation. The SBR system is a modern version of the fill and draw system, consisting of one or more tanks, each capable of waste stabilization and solids separation. The number of tanks may be varied, depending on the sophistication of the control system. Studies of SBR process were originally conducted at the University of Notre Dame, Indiana (Irvine and Busch, 1979). In biological wastewater treatment, each tank has several basic operational modes or periods. The periods are fill, react, settle, draw, and idle, in a time sequence. These operational modes can be modified, depending on the operational strategies desired.

Common SBR Characteristics

General: SBRs are a variation of the activated-sludge process. They differ from activated-sludge plants because they combine all of the treatment steps and processes into a single basin, or tank, whereas conventional facilities rely on multiple basins. According to a 1999 U.S. EPA report (Wastewater Technology Fact Sheet, 1999), an SBR is no more than an activated-sludge plant that operates in time rather than space.

Basic treatment process: In its most basic form, the SBR system is a set of tanks that operate on a fill-and-draw basis. Each tank in the SBR system is filled during

a discrete period of time and then operated as a batch reactor. After desired treatment, the mixed liquor is allowed to settle and the clarified supernatant is then drawn from the tank.

The cycle for each tank in a typical SBR is divided into five discrete time periods: Fill, React, Settle, Draw and Idle as shown in Fig.1. There are several types of Fill and React periods, which vary according to aeration and mixing procedures. Sludge wasting may take place near the end of React, or during Settle, Draw, or Idle. Central to SBR design is the use of a single tank for multiple aspects of wastewater treatment. A detailed discussion of each period of the SBR is provided in the following subsections, along with a description of typical process equipment and hardware associated with each (Irvine and Ketchum, 2004).

Fill: The influent to the tank may be either raw wastewater (screened and degritted) or primary effluent. It may be either pumped in or allowed to flow in by gravity. The feed volume is determined based on a number of factors including desired loading and detention time and expected settling characteristics of the organisms. The time of Fill depends upon the volume of each tank, the number of parallel tanks in operation, and the extent of diurnal variations in the wastewater flow rate.

Virtually any aeration system (e.g., diffused, floating mechanical, or jet) can be used. The ideal aeration system, however, must be able to provide both a range of mixing intensities, from zero to complete agitation, and the flexibility of mixing without aeration. Level sensing devices, or timers, or in-tank probes (e.g., for the measurement of either dissolved oxygen or ammonia nitrogen) can be used to switch the aerators and/or mixers on and off as desired.

React: Biological reactions, which were initiated during Fill, are completed during React. As in Fill, alternating conditions of low dissolved oxygen concentrations (e.g., Mixed React) and high dissolved oxygen concentrations (e.g. Aerated React) may be required. While Fig. 1 suggests that the liquid level remains at the maximum throughout react, sludge wasting can take place during this period as a simple means for controlling the sludge age. By wasting during React, sludge is removed from the reactor as a means of maintaining or decreasing the volume of sludge in the reactor and decreases the solids volume. Time dedicated to react can be as high as 50% or more of total cycle time.

The end of React may be dictated by a time specification (e.g. the time in React shall always be 1.5 h) or a level controller in an adjacent tank.

Settle: In the SBR, solids separation takes place under quiescent conditions (i.e., without inflow or outflow) in a tank, which may have a volume more than ten times that of the secondary clarifier used for conventional continuous-flow activated sludge plant. This major advantage in the clarification process results from the fact that the entire aeration tank serves as the clarifier during the period when no flow enters the tank. Because all of the biomass remains in the tank until some fraction must be wasted, there is no need for underflow hardware normally found in conventional clarifiers. By way of contrast, mixed liquor is continuously removed from a continuous-flow activated-sludge aeration tank and passed through the clarifiers only to have a major portion of the sludge returned to the aeration tank.

Draw (Decant): The withdrawal mechanism may take one of several forms, including a pipe fixed at some predetermined level with the flow regulated by an automatic valve or a pump, or an adjustable or floating weir at or just beneath the liquid surface. In any case, the withdrawal mechanism should be designed and operated in a manner that prevents floating matter from being discharged.

The time dedicated to Draw can range from 5 to more than 30% of the total cycle time. The time in Draw, however, should not be overly extended because of possible problems with rising sludge.

Idle: The period between Draw and Fill is termed Idle. Despite its name, this "idle" time can be used effectively to waste settled sludge. While sludge wasting can be as infrequent as once every 2 to 3 months, more frequent sludge wasting programs are recommended to maintain process efficiency and sludge settling.

Continuous-flow system: SBR facilities commonly consist of two or more basins that operate in parallel but single basin configurations under continuous-flow conditions. In this modified version of the SBR, flow enters each basin on a continuous basis. The influent flows into the influent chamber, which has inlets to the react basin at the bottom of the tank to control the entrance speed so as not to agitate the settled solids. Continuous-flow systems are not true batch reactions because influent is constantly entering the basin. The design configurations of SBR and continuous-flow systems are otherwise very similar. Plants operating under continuous flow should operate this way as a standard mode of operation. Ideally, a true batch-reaction SBR should operate under continuous flow only under emergency situations.

Plants that have been designed as continuous-inflow systems have been shown to have poor operational conditions during peak flows. Some of the major problems of continuous-inflow systems have been

Table 1: Operating conditions of the bench scale reactors (Keller *et al.*, 1997)

	Reactor Q	Reactor N
Pond 1 : Pond 2 feed mixture	1 : 1	3 : 1
HRT (hours)	18	24
SRT (days)	20	20
Reactor Sequence (hours)		
Non-aerated, non-mixed Fill	2.5	2.5
Aerated, mixed React 1	1.0	1.0
Non-aerated, non-mixed React	0.5	0.5
Aerated, mixed React 2	1.5	1.5
Settle	0.33	0.33
Decant	0.17	0.17

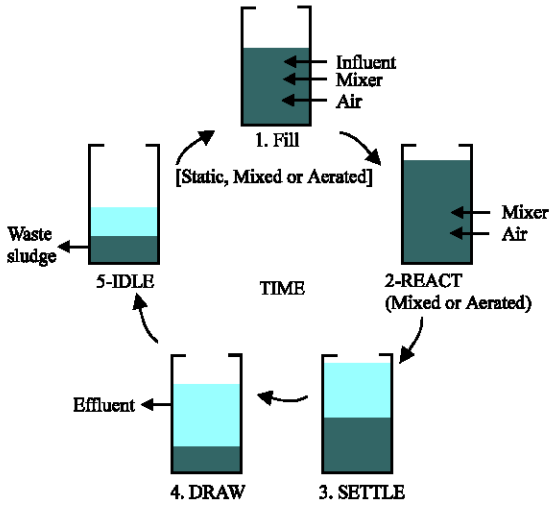

Fig. 1: SBR operation for each tank for one cycle for the five discrete time periods of Fill, React, Settle, Draw, and Idle (Irvine and Ketchum, 2004).

overflows, washouts, poor effluent, and permit violations (New England Interstate Water Pollution Control Commission, 2005).

Application SBR to treatment various wastewater (New SBR Technology): The Sequencing Batch Reactor (SBR) is an activated sludge process designed to operate under non-steady state conditions. An SBR operates in a true batch mode with aeration and sludge settlement both occurring in the same tank. The major differences between SBR and conventional continuous-flow, activated sludge system is that the SBR tank carries out the functions of equalization aeration and sedimentation in a time sequence rather than in the conventional space sequence of continuous-flow systems. In addition, the SBR system can be designed with the ability to treat a wide range of influent volumes whereas the continuous system is based upon a fixed influent flow rate. Thus, there is a degree of flexibility associated with working in a time rather than in a space sequence (Norcross, 1992).

SBRs produce sludges with good settling properties providing the influent wastewater is admitted into the aeration in a controlled manner. Controls range from a simplified float and timer based system with a PLC to a PC based SCADA system with color graphics using either flow proportional aeration or dissolved oxygen controlled aeration to reduce aeration to reduce energy consumption and enhance the selective pressures for BOD, nutrient removal, and control of filaments (Norcross, 1992). An appropriately designed SBR process is a unique combination of equipment and software. Working with automated control reduces the number of operator skill and attention requirement.

In this investigation we will overview recent experiments carried out by the laboratory SBR and pilot – scale plant SBR to treatment various wastewater.

Laboratory SBR scale: In recent times, the use of sequencing batch reactors (SBRs) in the biological treatment of wastewater has been widely extended from lab-scale studies to real WWTPs (wastewater treatment plants) (Mace and Mata-Alvarez, 2002; Steinmetz *et al.*, 2002). While lab-scale SBRs have been used for research on carbon and nutrient removal and the development of urban/industrial wastewater biodegradability assays, real plant applications are still mainly focused on carbon removal. Nevertheless, when operating real plant SBRs the efficiency of nitrogen removal sometimes turns out to be better than the legally required effluent standards (Teichgraber *et al.*, 2001).

Two bench scale SBR's were used by Keller *et al.* (1997) to investigate the effect of pretreatment abattoirs and process variations on the BNR (Biological Nutrient Removal) capacity. The operating conditions are shown in Table 1 the reactors were operated at room temperature (20±2°C) the maximum operating volume of the reactors was approximately 5 liters.

The summary of the effluent quality achieved in the two reactors is shown in Table 2. The overall removal efficiency of the incoming carbon was very good, particularly in terms of the effluent BOD which reached very low values during the whole reactor operation. The remaining COD has to be regard as nonbiodegradable. This fraction in fact quite small, representing around 2% of the COD initially present in the wastewater.

Ros and Vrtovsek (2004) also found that the removal of N was not dependent on initial P concentration, but P removal was related to P concentration in the original wastewater by using SBR laboratory pilot plant used in the study consisted of a 70 L rectangular reactor and operation of the pilot plant is monitored by five on-line measurements, i.e. pH, Redox potential (ORP), dissolved oxygen (DO) concentration, temperature (T) and water level. All experiments were carried out with synthetic wastewater to which different amounts of P were added. The optimal COD: N: P ratio was 100:11:2

Table 2: Effluent quality of the reactors (Keller *et al.*, 1997)

Parameters		Reactor Q	Reactor N
TCOD	(mg/L)	92-118	80-105
SCOD	(mg/L)	80-104	70-92
BOD5	(mg/L)	5-10	5-10
SS	(mg/L)	13-35	17-39
NH4-N	(mg/L)	1-5	0.2-3.0
NOX-N	(mg/L)	4-12	2-7
TN	(mg/L)	14-22	11-19
PO4-P	(mg/L)	3-10	0.5-5
TP	(mg/L)	5-14	2-7
pH		7.0-7.5	6.8-7.6

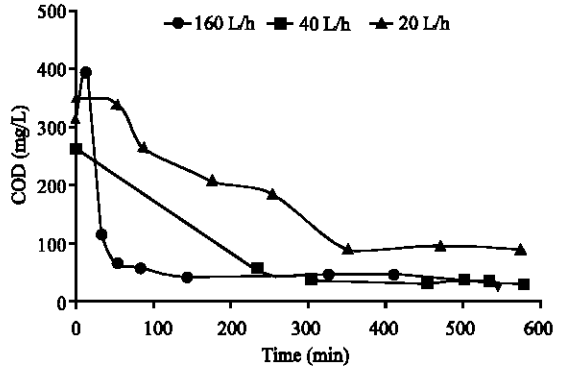

Fig. 2: Variation of COD with time under different air flux (Hu *et al.*, 2004).

Table 3: Ratios of COD: N: P and BOD5: N: P for different series of experiments (Ros and Vrtovsek, 2004).

Parameter	Series 1	Series 2	Series 3	Series 4
COD	100	100	100	100
N	10.1	10.3	10.5	11.1
P	1.0	1.9	2.0	2.2
BOD5	100	100	100	100
N	15.2	15.9	14.7	15.4
P	1.5	3.0	2.8	3.0

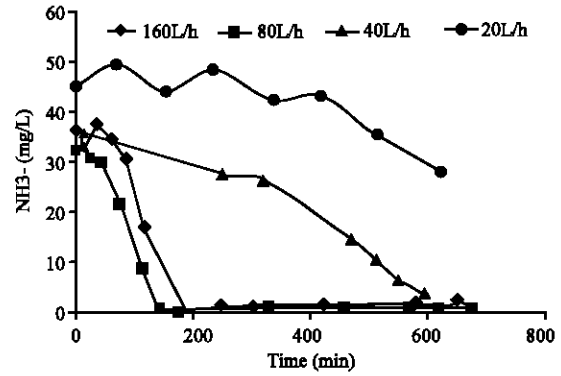

Fig. 3: Variation of NH4+-N with operation time under different air flux (Hu *et al.*, 2004).

and the BOD5: N: P ratio was 100:15:2.6 as shown in Table 3.

The performance of sequence batch reactor (SBR) was studied under four different air fluxes. Special attention was paid to the operating characteristics of SBR under limited aeration or low dissolved oxygen (DO) conditions. At the air flux of 40 l/h, COD and NH4-N had been removed just before the cycle was over, and during the cycle DO was about 0.5 mg/l most of the time Fig. 2, 3 and 4. Operational parameters, such as DO, ORP and pH, were monitored during the whole cycle. The effect of these parameters on the removal efficiency of COD and NH4-N was discussed (Hu *et al.*, 2004).

In a laboratory scale sequencing batch reactor (SBR) granules were cultured under aerobic conditions Fig. 5. To enhance the growth of granular the SBR was operated with very short sedimentation and draw phases resulting in the washout of slow biomass. Fast settling granules were retained in the reactor and thus had an advantage over flocs with a slower settling velocity. After 40 days of operation granules were the dominant from of microbial aggregates in the reactor, even though some pin- point flocs remained in the system. Granules taken from the reactor were stored for weeks without disintegrating. After about 130 days of operation the granule quality and COD- removal worsened. The reasons for that are yet to be investigated (Morgenroth *et al.*, 1997).

Kargi and Uyur (Kargi and Uygur, 2003) operated laboratory SBR to Nutrient removal from synthetic wastewater by sequencing batch operation was studied at different specific nutrient loading rates (SNLR). Nutrient removal in a sequencing batch reactor (SBR) was a five-step process consisting of anaerobic (An), anoxic (Ax), oxic (Ox), anoxic (An) and oxic (Ox) phases with hydraulic residence times (HRT) of 2/1/4.5/1.5/1.5 h, respectively. The settling step used at the end of the operation was 45 min for all experiments. The initial COD concentration was varied between 600 and 4800 mg/l at eight different levels with constant COD/N/P ratio of 100/3.33/0.7. Effects of SNLRs on COD, NH4-N and PO4-P removal were investigated. Percent nutrient removals decreased and effluent nutrient levels increased with increasing nutrient loading rates. The highest COD (99%), NH4-N (99%) and PO4-P (97%) removal efficiencies were obtained with the initial COD concentration of 600 mg/l at COD loading rate of nearly 40 mg COD /(g biomass)/ h. However, the sludge volume index (SVI) decreased with increasing COD loading rate resulting minimum SVI of 46 mg/l at COD loading rate of nearly 86 mg COD /(g biomass)/ h. Biomass concentration increased with increasing SNLR resulting in biomass concentration of 3.84 mg/l at COD loading rate of 86 mg COD /(g biomass) /h.

Sarioglu (Sarioglu, 2005) investigates the effect of pure cultures on the enhancement of biological phosphorus removal capability of a Sequencing Batch Reactor (SBR) inoculated initially with a mixed culture. For this purpose, three anaerobic/aerobic SBRs with mixed cultures were

Table 4: Chemical and biochemical properties of the influent and effluent (Zhu *et al.*, 2004).

Parameters	Influent	Effluent	Reduction (%)
TS (%)	1.053	0.237	77.5
TVS (%)	0.540	0.016	97.0
TSS (%)	0.766	0.001	99.9
TVSS (%)	0.442	0.004	99.1
COD (%)	8800	226	97.4
BOD (%)	3660	0	100
Turbidity (FTU)	2175	120	94.5

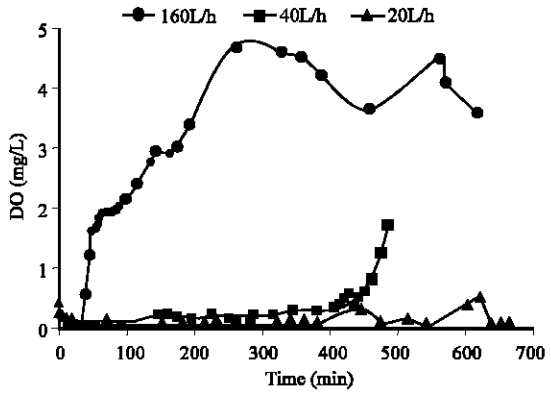

Fig. 4: Variation of DO with operation time under different air flux (Hu *et al.*, 2004).

started in parallel and operated for a while. At the end of this period, pure cultures of Acinetobacter lwoffii, A. lwoffii-Pseudomonas aeruginosa mixture and P. aeruginosa were added into the first, second and third reactors, respectively. All reactors were operated at a constant solid retention time (SRT) of 10 days and the food/microorganism (F/M) ratio was changed between 0.43-0.50 mg COD /mg VSS /day. The total cycle time was 14 h throughout the experimental study. The addition of A. lwoffii to the mixed culture in the first reactor significantly enhanced the biological phosphorus removal (EBPR) rate. Complete removal (E = 100%) of 20 mg /l PO4-P was achieved within 35 days of operation. Corresponding removal efficiencies obtained using A. lwoffii-P. aeruginosa mixture (second reactor) and P. aeruginosa alone (third reactor) were 25% and 20%, respectively. The COD removal efficiency was 90% in all reactors. Fig. 6 shows change of daily phosphate profile with the development of phosphorus removal during full cycle by using A. lwoffii culture and wastewater.

Zhu *et al.* (2004) developed and evaluated a lab-scale, $(AO)_2$ SBR for treating swine wastewater aiming at removing nutrients and organic materials. The SBR was operated on 3 cycles per day with 8 hours per cycle at constant 20°C. Unlike previous research, this SBR employs two alternating anaerobic/oxic phases to enhance nitrification and phosphorus removal. At the same time, sodium acetate is used as the external carbon source to promote denitrification in the latter part of each cycle. Other than nitrogen and phosphorus removal, discussions are also presented on changes resulted from the treatment in total solids (TS), total volatile solids (TVS), total suspended solids (TSS), total volatile suspended solids (TVSS), chemical oxygen demand (COD), and biochemical oxygen demand (BOD) as shown in Table 4.

An SBR operated with anaerobic and aerobic cycle stages could be considered a suitable technology for organic load removal from wool dyeing effluents. Soluble COD and BOD5 degradation efficiencies of 85 ± 6% and 95 ± 4%, respectively, were achieved. The residual suspended solids levels were in general acceptable (lower than 100 mg/l), and could be attributed to the operation with no biomass wastage, which led to high MLVSS concentrations (Goncalves *et al.*, 2005).

De Sousa and Foresti (De Sousa and Foresti, 1996) investigated treating domestic sewage in tropical regions by using a combined anaerobic-aerobic system composed of an USAB reactor followed by two sequencing batch reactors (SBR). In such a system, the USAB reactor removes considerable fraction of the influent organic matter, while the SBRs oxidize part of the remaining organic matter and ammonium nitrogen. A proper system operation would also permit the removal of nutrients (N and P). This system was efficient in removing COD (95%), TSS (96%) and TKN (85%). In order to investigate on the performance of this system for sewage treatment, a bench scale installation fed with synthetic substrate simulating domestic swage was operated continuously during 38 weeks. The results permit to confirm the hypothesis proposed, since the system has consistently produced high quality effluents (BOD5 and VSS lower than 10 mg/l). The result also indicates that such combined anaerobic-aerobic system compete favorably with conventional aerobic systems in three essential cost features: energy consumption, excess sludge production and nutrient removal.

A study was undertaken to examine the feasibility of biologically treating a combined waste stream of landfill leachate and municipal sewage. The ratio of sewage to leachate was 9 to 1 by volume. The combined waste had an average BOD5 430 mg/l, COD 1090 mg/l, and TKN 133 mg/l (80% of which was in the form of ammonia). A laboratory-scale sequencing batch activated sludge reactor was used to carry comparative performance evaluations of biological treatment, including nitrification and dentrification. The SBR reactor was operating in daily time cycles employing the following sequential operation phases: filling phase, anoxic phase, aeration reaction phase, settling phase, and drain phase. In particular, the anoxic and aeration periods were tailored

Table 5: Adjustment of phases duration according to the organic load in the activated sludge SBR (Rodrigues *et al.*, 1998)

Organic Load (kgCODt/kgTSS.d)	Influent Per cycle (l)	Fill (min)	Anaerobic-Anoxic phase (min)	Aerobic Phase (min)	Settling (min)	Draw (min)
0.13	212	7	218	218	30	7
0.25	421	15	210	210	30	15
0.35	602	25	200	200	30	25

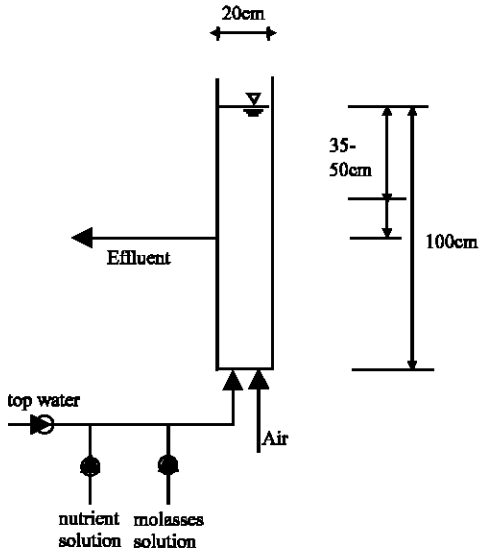

Fig. 5: Laboratory scale SBR (Morgenroth *et al.*, 1997).

in order to develop conditions conductive to desired nitrification and dentrification. During the reaction period, the process was operated under an extended aeration mode with the MLSS concentration being around 3500 mg/l the results indicated that successful biotreatment of combined leachate and sewage was possible, with the treated effluent being low in BOD5 and COD. The system was capable of BOD5 removal efficiencies exceeding 95%. Furthermore, nitrate removal during the anoxic phase was approximately 99% due to dentrification. However, the overall nitrogen removal during a full cycle was about 50%. The inclusion of an anoxic period right after the aeration phase enhanced the nitrogen removal efficiency, yet this phase required the addition of an external carbon source to the reactor due the low concentration of biodegradable carbon, and at the same time the process became less efficient in BOD removal (Diamadopoulos *et al.*, 1997).

Pilot-Scale SBR: Sequencing batch reactor (SBR) activated sludge processes are known to have several advantages over conventional continuous flow systems. Biological nitrogen and phosphorus removal is possible in a single tank SBR if operating conditions are selected to introduce anaerobic, anoxic and aerobic reactions during a cycle without any addition of separate reactors, recycling lines or clarifiers.

Previously, the laboratory scale SBRs and process conditions have shown a very high degree of biological nutrient removal (both N and P) even on very unfavorable domestic wastewater that was low in biodegradable COD (Ho *et al.*, 1993). Similarly, under conditions with extremely high nitrogen and phosphorus concentrations, such as in wastewater from abattoirs, very good preliminary results have been achieved (Subramaniam *et al.*, 1994).

Lin and Cheng (2001) investigated treatment of municipal sewage wastewater for possible agricultural reuse. The treatment method consisted of chemical coagulation and sequencing batch reactor (SBR) system. A new SBR reactor was designed based on this concept for treatment of municipal sewage wastewater, and experimental tests were performed to evaluate the performances of the modified SBR reactor for comparison with the traditional one Fig. 7. In addition, the final level of purification obtained with both chemical coagulation and SBR was evaluated in light of possible agricultural reuse.

To determine whether continuous flow SBR could provide efficient pollutant removal in synthetic wastewater. The experiment was carried out using pilot scale at Tehran University of Medical Sciences the reactor was separated into two zones (pre-react and main react) by a baffle wall Fig. 8. The pre-react zone acts as a biological selector enhancing the proliferation of the most desirable organisms while limiting the growth of filamentous bacteria, as an equalization tank and as a grease trap. In conventional SBRs there are five phases: fill, react, settle, draw and idle; but in this system there is only three phases: react, settle and draw. It must be noted again that influent never disrupts in any phase. The purpose of this research was to determine the best cycle capable to remove BOD, COD, N, P and TSS from synthetic wastewater. The results showed that the removal efficiency that has been achieved by the system were 97.7, 94.9, 85.4, 71.4 and 55.9% for BOD, COD, TKN, Total N and Total P, respectively could be achieved by the system. Maximum TSS concentration in final effluent was 6.3 mg /l (Mahvi *et al.*, 2005).

Mahvi *et al.* (2004) using the same pilot scale as mentioned before to determine whether continuous flow SBR could provide efficient nitrogen removal in synthetic and domestic wastewater. The experiment was carried out using pilot scale at Tehran University of Medical Sciences; into first stage at laboratory with synthetic wastewater and second stage in treatment plant with

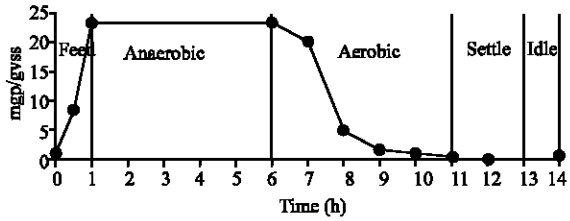

Fig. 6: Change of daily phosphate profile with the development of phosphorus removal (Sarioglu, 2005).

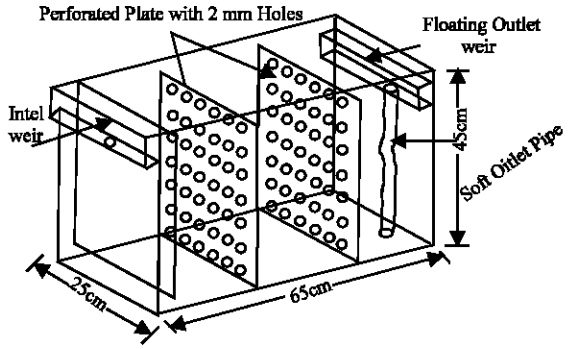

Fig. 7: Design of modified sequencing batch reactor (Lin and Cheng, 2001).

domestic wastewater. The results showed that in laboratory and treatment plant 80 and 70% of total nitrogen removal, respectively and 95 and 85% of total kjeldahl nitrogen removal, respectively could be achieved by the system.

Another pilot plant SBR investigated by Bernardes and Klapwijk (1996) aims to monitor a strategy for biological nutrient removal (nitrogen and phosphorus) in a Sequencing Batch Reactor (SBR) treating domestic wastewater. For this, the performance of an SBR with nitrification, dentrification, carbon oxidation and phosphorus removal is evaluated. During this study the influent used was pre-settled domestic wastewater from Bennekom Municipal Treatment Plant (The Netherlands). The average influent COD, TKN and phosphate were 443 mg COD/l, 71 mg N/l and 7 mg P/l, respectively. Acetic acid was added to this influent from a feed solution, to increase the COD by an extra 100 mg COD/l. in this study, a pilot plant SBR was operated during 5 months in order to have: i) a mixed culture able to perform carbon oxidation, nitrification, dentrification and biological phosphorus removal and ii) long term assessment of the biological nitrogen and phosphorus removal processes. Pilot plant SBR consists of two cylindrical polystyrene vessels, the first with total volume of 0.35 m^3 (reactor 1) and the second with total volume of 1.3 m^3 (reactor 2). The effluent had, in average, phosphate concentration lower than 1 mg P/l and nitrogen concentration lower than 12 mg N/l.

Rodrigues *et al.* (1998) project was conducted to analyze the performance of a SBR reactor when being fed with an aerobically fermented wastewater. Important was to determine the capacity of the system to remove nitrogen and phosphorus. Two SBR reactors, each one with a volume of 980 liters, were used: one used as fermented and the other as activated sludge SBR. Using 8-hour cycles, the reactors were operated and studied during 269 days. The fermented produced an effluent with an average value of 223±24 mg/l of volatile fatty acids. The activated sludge SBR was tested under 3 organic loading rates of 0.13, 0.25, and 0.35 kg COD total/kg TSS.d. Table 5. For three tested organic loading rates, PO4-P concentration under 1.1 mg/l and COD between 37 and 38 mg/l were consistently achieved. Exceptionally high NH4-N influent values not reaching in this case full nitrification. Dentrification was observed during the fill phase in every cycle. SVI values between 40 and 70 were determined during the experimental runs.

Wastewater originating from road and rail car cleaning installations is known to be potentially toxic/inhibitory. As a first step in the design procedure a pilot test was run for a period of 8 months. This pilot showed the SBR to be an appropriate technology for the treatment of the wastewater, with an option for powdered activated carbon (PAC) dosing, was selected The PAC option was not feasible. Based on the pilot results a full scale installation, comparing a batch reactor with a diameter of 10.4 m and a maximum water depth of 17.3 m, was designed and successfully started up. This paper presents the highlights of the total project (Zilverentant, 1997).

A pilot plant of SBR (Sequencing Batch Reactor) and MF (microfiltration) process was operated in order to treat and reuse the greywater produced from an office building. The performance of SBR for greywater was satisfactory as the effluent had 20 mg/l, 5 mg/l, and 0.5 mg/l of SCOD, BOD, and ammonia, respectively. The cyclic operation of SBR used in this study proved more effective in nitrification and dentrification than the conventional SBR operation. However, the most effective mode was step-feed SBR for dentrification. The decanting system of this SBR discharged the effluent fairly well without sludge washout. However, it was difficult to maintain constant concentration of suspended solid from the SBR process. Thus, additional filtration was needed to get adequate water quality for water reuse. MF could remove residual suspended solids and pathogens as well from the SBR effluent. The suspended solids of final effluent were around 1 mg/l and allowed using the treated water for some purpose (Shin *et al.*, 1998).

The design and operation of wastewater treatment systems for single houses, farms, hotels, leisure centers, small communities and small businesses are

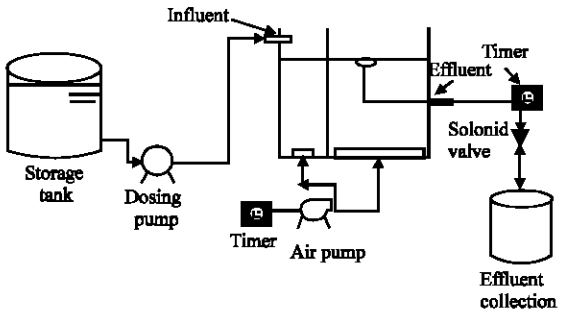

Fig. 8: Schematic of designed pilot (Mahvi *et al.*, 2005).

a challenge to wastewater engineers. A pilot-scale system comprising a vertically moving biofilm reactor (VMBR) followed by a stratified sand filter was constructed and its performance was evaluated. The vertically moving biofilm reactor was operated as a sequencing batch biofilm reactor (VMSBBR). The results show that the VMSBBR unit efficiently removed 94.8% of the filtered chemical oxygen demand (CODf) from a synthetic wastewater with the influent CODf of 1096 ± 425 mg/l, leaving 45 ± 16 mg/l CODf in the effluent, at an organic loading rate of 0.9 kg COD/m^3 day. After the system had been operated for 133 days, the removal efficiency of orthophosphate (PO4-P) reached 90%. A sand filter polished the effluent from the VMSBBR unit and reduced suspended solids (SS) to 4.4 mg/l and total bacterial by 3 log 10 units. The advantages of the treatment system studied for small wastewater flows include: (1) simple operation and maintenance-sludge was only disposed of once on Day 206 during the 7.5-month study period; clogging, which often happens in other attached-growth biofilm systems, did not take place; (2) efficient removal of COD and phosphorus; and (3) low-energy consumption-the electricity consumption was 4.6 kWh/population equivalent (p.e.) year, or 0.6 kWh/m^3 wastewater treated or 0.6 kWh/kg COD removed (Rodgers *et al.*, 2005).

Conclusion: Wastewater treatment has been a challenge throughout the years due to varying influent chemical and physical characteristics and stringent effluent regulations. Treatment systems using activated sludge have been able to handle many of these difficulties. Given the lack of on-line computer controls, continuous flow systems have been mostly used for these purposes versus sequencing batch processes. The availability of artificial intelligence has now made the option of a SBR process more attractive thus providing better controls and results in wastewater treatment. This is coupled by the flexibility of a SBR in the treatment of variable flows, minimum operator interaction required, option for anoxic or anaerobic conditions in the same tank, good oxygen contact with microorganisms and substrate, small floor space, and good removal efficiency.

Sequencing batch reactors operate by a cycle of periods consisting of fill, react, settle, decant, and idle. The duration, oxygen concentration, and mixing in these periods could be altered according to the needs of the particular treatment plant. Appropriate aeration and decanting is essential for the correct operations of these plants. The aerator should make the oxygen readily available to the microorganisms. The decanter should avoid the intake of floating matter from the tank. The many advantages offered by the SBR process justifies the recent increase in the implementation of this process in industrial and municipal wastewater treatment.

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Manipulation of Rumen Fermentation with Organic Acids Supplementation in Ruminants Raised in the Tropics

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Abstract: Locals feed resources are prime importance for ruminants raised in the tropic particularly low-quality roughages and agricultural crop-residues. Manipulating rumen fermentation through treatment of roughage, concentrate and strategic supplementation with organic acids could improve rumen efficiency by maintaining higher pH, optimum ammonia-nitrogen (NH_3-N), thus reducing methane (CH_4) and increasing microbial protein synthesis and essential volatile fatty acid (VFAs), for enhancing ruminant productivity in the tropics. The manipulation of rumen efficiency through the use of organic acids especially malate with local feeds would be an advantage. Indeed, organic acids potentially provide an alternative to currently used antimicrobial compounds by stimulating rather than inhibiting specific ruminal microbial populations. At the same time, local feed resources especially cassava chip could be used effectively at high level as an energy source for ruminants especially for beef and lactating cows. More recently, the combined use of concentrates containing high level of cassava chip with supplementation of sodium dl-malate and urea could improve rumen ecology and subsequent performance in dairy steers receiving urea-treated rice straw as a roughage. In addition, the high level of cassava chip in the diet resulted in increase population of bacteria and fungi, decreasing protozoal populations, and improving microbial protein synthesis and efficient microbial nitrogen supply in the rumen. Under these circumstances, malate was also effective in reducing the drop in ruminal pH normally seen 1 to 2 h after feeding a high-grain diet and improved cows performance efficiency. In summary, supplementation of organic acid like malate with local feed resources especially cassava chip or other carbohydrate sources with high rumen degradation would be a desirable alternative because there is no risk of developing antibiotic resistance or having unwanted residues appear in either meat or milk products as well as improving ruminal fermentation efficiency and productivity in ruminants in the tropics.

Key words: Organic acids, malate, feed resources, cassava chip, rumen fermentation, ruminants, tropics

Introduction

The rumen has been well recognized as an essential fermentation that is capable of preparing end-products particularly volatile fatty acids (VFAs) and microbial protein synthesis as major energy and protein for the ruminant host, hence, the more efficient the rumen is, the optimum the fermentation end-products are being synthesized. In recent years, there have been increasing interests, researches conducted as well as reviews in relation to rumen studies, rumen ecology and rumen manipulation (ørskov and Flint, 1989; Martin *et al*., 1999; Wanapat, 2000; Dann, 2005; Khampa *et al.,* 2006, 2006a). In the tropics, most of ruminants have been fed on low-quality roughages, agricultural crop-residues, industrial by - products which basically contained high levels of lingo-celluloses materials, low level of fermentable carbohydrate as well as low level of good-quality protein. In addition, long dry season and prevailing harsh environment especially high temperature, low fertile soil and less quantity of feeds available throughout the year-round feeding regimes would influence rumen fermentation of quantity and quality (Wanapat, 2005).

Rumen as a fermentation vat (Fig. 1): As it has been established, the rumen has an important role and function in preparing fermentation end-products for biosynthetic processes of ruminants. It is therefore essential that the rumen is healthy and be able to establish an optimum ecology in order to perform well in regards to rumen microorganisms (bacteria, protozoa and fungi), pH, substrates (e.g. roughage, energy, effective fiber etc), fermentation end-products (NH_3-N, VFAs), and microbial synthesis of VFAs, the major sources of energy, glycogenic and lipogenic compounds particularly propionate (C_3), acetate (C_2) and butyrate (C_4) while NH_3-N is essential source of nitrogen for microbial protein synthesis, respectively. As a

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consequence, it results in healthy rumen and preventing other unflavarable conditions e.g. acidosis, ketosis, mastitis, rumen-parakeratosis etc. Factors which contribute to the production and absorption of these compounds have been reported in a number of scientific reports as well as review papers. It was found that an established rumen be required and was affected by types of feeds, roughage to concentrate ratios, which consequently influenced on rumen microorganisms and fermentation pattern. As there were significant differences in type of feeds and quality between temperate and tropical feed resources which would remarkably influence on rumen microorganisms and fermentation-nutrient pool. Furthermore, different practical feeding systems prevailing in these regions would affect on rumen ecology. In ruminants fed on temperate feeds, increasing levels of concentrate feeding dramatically lowered rumen pH and resulted in acidosis. As a consequence, the VFAs could decrease rumen pH but lactic acid accumulated in the rumen had a more pronounced effect on lowering rumen pH. For the same reason, rumen pH was most affected by type of feeds and roughage to concentrate ratios in regards to saliva secretion, rumination, VFAs production and microbial population (Slyter, 1976). It was also shown that at lower rumen pH, increasing level of concentrate, on the contrary fiber digestion was inhibited and reduced in methanogenesis. The ultimate rumen pH value appeared to exert effect on type of rumen microorganisms. In addition, pure or mixed cultures could perform differently in fermenting available rumen substrates (Wallace, 1979).

Manipulation of ruminal fermentation with organic acids: A goal of rumen microbiologists and nutritionists is to manipulate the ruminal microbial ecosystem to improve the efficiency of converting feed to produce consumable products by humans. One approach that is used is the addition of feed additives (e.g. ionophores) to diets that alter the microbial ecosystem and decrease fermentation losses (e.g., methane (CH_4) or ammonia (NH_3)). Earlier studies investigating much of the research in the past 20 to 25 years has focused on the effects of antimicrobial compounds on rumen fermentation. Specifically, ionophore antibiotics are used in feedlot diets to reduce energy losses associated with methanogenesis in the rumen (Russell and Strobel, 1989). These compounds appear to inhibit hydrogen-producing microorganisms and gram-positive lactate-producing bacteria such as *Streptococcus bovis* (Russell, 1987; Russell and Strobel, 1989). Reductions in hydrogen production reduce ruminal methanogenesis and improve feed utilization by increasing the amount of metabolizable energy available to the animal as propionate (Bergen and Bates, 1984; Russell and Strobel, 1989). Recently, there has been increased public scrutiny about use of antibiotic feed additives in food animal production, especially in Europe (Castillo *et al.,* 2004). Use of organic acids, non-antibiotic feed additives may alleviate public skepticism. Organic acids may be beneficial feed additives for ruminants (Martin, 1998; Castillo *et al.,* 2004) because they have effects on ruminal fermentation analogous to ionophores (↓ CH_4, ↓ lactate, ↑ propionate; Castillo *et al.,* 2004). However, the mode of action for the organic acids is different than ionophores (Castillo *et al.,* 2004). Scientists have recently become interested in evaluating alternative means for manipulating gastrointestinal microflora in livestock. Their motivations come from increasing public scrutiny about the use of antibiotics in the animal feed industry. However, compared with the efforts to detail the effects of antimicrobial compounds on ruminal fermentation, little research has been conducted to evaluate alternatives to antimicrobial compounds. In the past 10 years, interest has increased in direct-fed microbial (DFM) products, and research has been conducted to examine the effects of DFM on ruminant performance. Some of these products have shown promise in favorably altering ruminal fermentation and improving animal performance, in these circumstances the effects have been variable and inconsistent. Organic acids stimulate rather than inhibit some specific ruminal bacterial populations (Castillo *et al.,* 2004). Organic acids that are currently being evaluated as feed additives are malic acid, fumaric acid, and aspartic acid. Malic acid and fumaric acid are four-carbon dicarboxylic acids that are found in biological tissues (e.g. plants) as intermediates of the citric acid cycle and are intermediates in the succinate-propionate pathway of ruminal bacteria, such as *Selenomonas ruminantium* (Castillo *et al.,* 2004). Aspartic acid is an alpha amino four-carbon dicarboxylic acid. In the above study, it was found that the organic acids can stimulate the growth of the prominent ruminal bacterium, *Selenomonas ruminantium*, can favorably alter the mixed ruminal microorganism fermentation, and can improve the performance of feedlot steers (Martin and Streeter, 1995; Callaway and Martin, 1996, 1996a; Martin *et al.,* 1999). Earlier studies investigating the antimicrobial compounds are routinely incorporated into ruminant diets to improve production efficiency (Callaway and Martin, 1996, 1996a). However, in recent years there has been an increasing concern regarding the use of antibiotics in ruminant feeding and the potential for selection of antibiotic-resistant pathogenic microorganisms. On the contrary, the organic acids (aspartate, fumarate, malate) potentially provide an alternative to currently used antimicrobial compounds (Newbold *et al.,* 1996). Thus, malate supplementation in ruminant diets has been shown to increase nitrogen retention in sheep and steers, and to improve average daily gain and feed efficiency in bull calves (Satacup,

Table 1: Summary of *in vitro* studies with mixed ruminal microorganisms that evaluated the response to supplemental malic acid

Culture system	Treatment	Response to supplementation of malate									References
		pH	Total VFA	Acetate	Propionate	Butyrate	Lactate	CH_4	NH_3-N	Digestibility	
Batch (steer)[a]	0, 4, 8, 12 mM[b]	↑	↑		↑						Martin and Streeter, 1995
Bach (steer)	0, 4, 8, 12 mM[b]	↑	NE[d]	NE	NE	↑	↓	NE	↑		Callaway and Martin, 1996
RUSTIEC (sheep)	0, 5.62 mmol[b]	NE	↑	NE	↑	NE	↓		↑	↑ DM,NDF	Carro *et al.*, 1999
Bach (sheep)	0, 8 mM[b]	NE	NE	NE	↑	NE	NE		↑	NE	Jalc & Ceresnakova, 2002
Bach (sheep)	0, 4, 7, 10 mM[c]	↑	↑	↑	↑	↑	↓				Carro & Ranilla, 2003
Bach (dairy cows)	0, 10, 20 mM	↑	↑	NE	↑	↑		↓	NE		Mohammed *et al.*, 2004
RUSITEC (sheep)	0, 6.55 mM[b]	NE	↑	NE	↑	↑		NE		↑ DM,NDF,ADF	Gomez *et al.*, 2005
Continuous (dairy cow)	0, 50, 100 g/h/d	NE	NE	NE	NE	NE	↓, NE		NE	↑ DM,NDF	Sniffen *et al.*, 2006

[a]Inoculum source; all animals fed a mixed (forage and concentrate) diet. [b]Disodium salt. [c]Disodium + calcium malate. [d]No effect of malic acid ($p > 0.10$). Source: Modified from Dann (2005).

1979; Sanson and Stallcup, 1984). In addition, a positive response in milk production by dairy cows fed diets supplemented with malate were obtained (Stallcup, 1979; Kung *et al.*, 1982). As a consequence, the malate altered the *in vitro* fermentation of soluble starch by mixed ruminal microorganisms or cracked corn resulting in changes in final pH, CH_4 and VFA that are analogous to ionophore effects (Martin and Streeter, 1995; Callaway and Martin, 1996). However, the mode of action of malate appears to be completely different, and in contrast with antimicrobial compounds, it appears to stimulate rather than inhibit some specific ruminal bacterial populations (Nisbet and Martin, 1993). In the same way, ionophores, such as monensin, are added routinely to beef cattle diets to increase the efficiency of production (Russell and Strobel, 1989). Ionophores decrease lactate and methane production by ruminal microorganisms, and these effects lead to increased ruminal pH and propionate concentrations. Recent research showed that a combination of organic acids (i.e., malate) and monensin was more effective at reducing lactate concentrations and increasing pH in mixed ruminal microorganism fermentation than the addition of organic acid or monensin alone (Callaway and Marrtin, 1996).

Manipulation of rumen fermentation by malate supplementation: In theory, malate is a four-carbon dicarboxylic acid that is commonly found in biological tissues because it is an intermediate of the citric acid cycle (Lehniger, 1975). Even though only aerobic bacteria are capable of respiration possess a functional citric acid cycle (oxidative), some strictly anaerobic bacteria use a reductive or reverse citric acid cycle known as the succinate-propionate pathway to synthesize succinate and (or) propionate (Gottschalk, 1986). Moreover, malate is also a key intermediate in the succinate-propionate pathway, and the predominant ruminal bacterium *Selenomonas ruminantium*, uses this pathway which, it could stimulate propionate production (Gottschalk, 1986).

In fact, propionate production has been increased by adding malate to *in vitro* cultures which the malate might act as an electron sink for hydrogen. However, the mechanism of action is not completely known. *Selenomonas ruminantium* is a common gram-negative ruminal bacterium that can account for up to 51% of the total viable bacterial counts in the rumen (Nisbet and Martin, 1991). Surprisingly, this bacterium can grow under a variety of dietary conditions and ferment many different soluble carbohydrates (Hungate, 1966). When *S. ruminantium* is grown in batch culture with glucose, homolactic fermentation occurs (Hobson, 1965). However, after the glucose is depleted from the medium, *S. ruminantium* then utilizes lactate as a carbon and energy source (Scheifinger *et al.*, 1975). Only some strains of *S. ruminantium* (subspecies *lactilytica*) are able to ferment lactate (Stewart and Bryant, 1988).

Several studies have shown that adding malate to *in vitro* fermentation of mixed ruminal microorganisms resulted in changes in pH, CH4, and volatile fatty acids (VFA) analogous to the addition of ionophores (Table 1). In batch cultures, pH was increased typically (Martin and Streeter, 1995; Callaway and Martin, 1996; Carro and

Table 2: Summary of *in vivo* studies that evaluated the response to supplementatal malic acid in dairy cattle

		Response to supplementation malic acid									
Animal	Treatment	DMI	Milk	Milk Comp.	Gain	Ruminal pH	Ruminal VFA[a]	Ruminal NH_3-N	Ruminal CH_4	Digestibility	References
Dairy cattle											
Holstein cows	0, 70, 105, 140g		↑	↑ fat							Alferez, 1978
Holstein cows	0, 28, 70g	↑ grain	↑								Stallcup, 1979
Holstein cows	0, 100g	↑	↑	↑ fat							Stallcup, 1979
Holstein cows	0, 70, 105, 140g			NE							Kung *et al.*, 1982
Holstein cows	0, 4g	NE	NE	NE		NE	↑ total, A,B,NE,P	NE			Vicini *et al.*, 2003
Holstein cows	0, 84g	↑ grain	↑	NE							Devan & Bach, 2004
Holstein cows	0, 50g		↑	NE		NE				NE DM, ↑ CP,ADF,NDF	Sniffen *et al.*, 2006
Holstein cows	10, 20g[c]	↑	↑	↑ fat		↑	↑ total P	↑	↑	↑ DM,CP, NDF,ADF	Khampa *et al.*, 2006

[a]A=acetate, P= propionate, B = butyrate. [b]No effect of malic acid (p>0.01). [c]Disodium salt Source: Modified from Dann (2005).

Ranilla, 2003; Mohammed *et al.*, 2004). This effect was not observed in semi-continuous (RUSITEC) (Carro *et al.*, 1999; Gomez *et al.*, 2005) or continuous culture systems (Sniffen *et al.*, 2006) due to the use of artificial saliva and its high buffering capacity. Martin (1998) suggested that supplementing beef cattle finishing diets or high-producing dairy cow diets with malate might be effective in reducing subclinical ruminal acidosis. Typically, total VFA and propionate production increased (Martin and Streeter, 1995; Carro *et al.*, 1999; Carro and Ranilla, 2003; Mohammed *et al.*, 2004) and lactate production decreased (Callaway and Martin, 1996; Carro *et al.*, 1999; Carro and Ranilla, 2003) with the addition of malate. Methanogenesis was reduced (Carro and Ranilla, 2003). Digestibility of dry matter (DM), acid detergent fiber (ADF), and neutral detergent fiber (NDF) increased in most studies (Carro *et al.*, 1999; Gomez *et al.*, 2005; Sniffen *et al.*, 2006). Recently, Gomez *et al.* (2005) and Sniffen *et al.* (2006) observed an increase in microbial N production with the addition the malate to semi-continuous and continuous culture systems, respectively. There was a numerical increase in efficiency of microbial synthesis (unit microbial N/unit DM digested) with the addition of malate. There were no differences among treatments for NH_3-N, non-ammonia N, or non-ammonia, nonmicrobial N. The inconsistent results observed with supplemental malic acid (Table 1) can partially be explained by the diet or substrate incubated and experimental conditions.

Based on earlier studies, it was found that growth of *S. ruminantium HD4* in a lactate-salts medium was stimulated by L-aspartate and the requirement for L-aspartate could be replaced with L-malate or fumarate Linehan *et al.*, 1978). More recent research showed that L-lactate uptake by *S. ruminantium HD4* was increased in the presence of 10 m*M* L-aspartate, fumarate, or L-malate and L-malate elicited the greatest response, especially uptake of lactate by S. ruminantium was increased fourfold by aspartate and fumarate and tenfold by malate (Fig. 2) (Nisbet and Martin, 1990). In addition, different concentrations (0.03 to 10 m*M*) of L-malate stimulated L-lactate uptake by *S. ruminantium* in a dose-response fashion. It also seems that both L-malate and Na^+ are involved in stimulating L-lactate utilization by *S. ruminantiurn HD4* (Nisbet and Martin, 1991). Lactate uptake and utilization by the predominant ruminal bacterium *Selenomonas ruminantium* was increased in presence of the dicarboxylic acid malate (Nisbet and Martin, 1990, 1991, 1994). For example, the addition of DL-malate to soluble starch and cracked corn fermentation with mixed ruminal microorganisms resulted in changes in final pH, methane, and VFA that are analogous to ionophore effects (Martin and Streeter, 1995). In some cases, it was demonstrated that dl-malate and monensin had an additive effect on these mixed ruminal microorganism fermentations (Callaway and Martin, 1996). Limited *in vivo* research has been conducted to evaluate the effects of malate on ruminant performance. In other studies, it was revealed that feeding 140 g of malate per day resulted in an increased milk persistency in lactating cows and increased total VFA during early lactation (Kung *et al.*, 1982). Other

Table 3: Summary of *in vivo* studies that evaluated the response to supplementatal malic acid in beef cattle and small ruminants

		Response to supplementation malic acid									
Animal	Treatment	DMI	Milk	Milk Comp.	Gain	Ruminal pH	Ruminal VFA[a]	Ruminal NH_3-N	Ruminal CH_4	Digestibility	References
Beef cattle											
Holstein steers	0, 100, 200 mg/kg BW	NE					↑ total, P ↑ NE A,B	NE		NE DM, ADF,CP	Kung *et al.*, 1982
Holstein steers	0, 200 mg/kg BW				↑		↑				Kung *et al.*, 1982
Crossbred steers	0, 27, 57, 80g				↑		↑				Martin *et al.*, 1999
Crossbred steers	0, 40, 80g	NE			↑						Martin *et al.*, 1999
Angle steers	0, 60, 120g	NE			NE						Martin *et al.*, 1999
Beef cattle	0, 100g	NE			NE						Martin *et al.*, 1999
Holstein steers	0, 80g					↑	NE		NE		Montano *et al.*, 1999
Holstein steers	0, 9, 18, 27gc	↑				↑	total, P	↑	NE	↑ DM,CP, NDF,ADF	Khampa *et al*, 2006
Small ruminants											
Dairy goats	0, 0.32%	NE	NE	NE							Salama *et al.*, 2002
Lambs	0, 0.2%	↓			↑	↑				↑ DM,CP, ADF, NDF	Flores *et al.*, 2003

[a] A=acetate, P= propionate, B = butyrate. [b] No effect of malic acid ($p>0.01$). [c] Disodium salt Source: Modified from Dann (2005).

variables, including ruminal pH, were not altered by malate treatment, however, ruminal fluid samples were collected by stomach tube and lactate concentrations were not reported. Feeding malate to Holstein bull calves improved average daily gain and feed efficiency but had little effect on blood serum constituents (Sanson and Stallcup, 1984). Even though *in vitro* studies have shown that dl-malate favorably alters ruminal fermentation which little information is available that details the effects of dl-malate on beef cattle performance (Martin and Streeter, 1995). In a later study it was also found that supplementation of malate concentrations between 0.3 and 10 m*M* increased lactate uptake in a dose-response manner. In addition, when mixed ruminal microorganisms were incubated in medium that contained cracked corn or soluble starch, malate treatment decreased lactate concentrations and increased final pH. This probably indicated that increasing dietary concentrations of malate might help to reduce problems associated with ruminal acidosis by stimulating lactate utilization by *S. ruminantium* (Martin *et al.,* 1999).

***In vivo* studies:** Although *in vitro* studies have shown positive effects of malic acid on ruminal fermentation, there are limited *in vivo* studies available to evaluate the effects of malic acid on dairy cow performance (Table 2, 3). Alferez (1978) fed early lactation Holstein cows an alfalfa hay, corn silage, and steam-rolled barley-based diet that was supplemented with malate (0, 70, 105, or 140 g supplemental malate per cow per day). Cows fed 105 g of malate had higher milk yield, fat-corrected milk yield, and fat yield and were more efficient in converting DM into milk than cows fed 0 or 70 g malate. Feeding malate above 105 g did not increase productivity or feed efficiency. Stallcup (1979) fed Holstein cows a sorghum-sudan forage and corn grain-based diet with 0, 28, or 70 g supplemental malate per cow per day. Cows fed 70 g of malate had higher milk yield than cows fed 0 g malate. In a second trial (Stallcup, 1979), cows fed an alfalfa grass hay and sorghum silage-based diet with 100 g supplemental malate had higher solids-corrected milk and milk fat content than cows fed the diet with no supplemental malate.

More recently, Vicini *et al.* (2003) observed no difference in milk yield between cows fed a corn-based control diet or the control diet supplemented with a commercial product containing soluble sugars and malate (estimated 4 g malate per cow per day). Martin *et al.* (1999) previously determined that the malate concentration in the commercial product was not high enough to stimulate lactate utilization by *S. ruminantium*, a predominant ruminal microorganism that utilizes lactic acid. In addition, Castillo *et al.* (2004) suggested that dietary factors, such as forage to concentrate ratio and forage type, are important in determining responses to

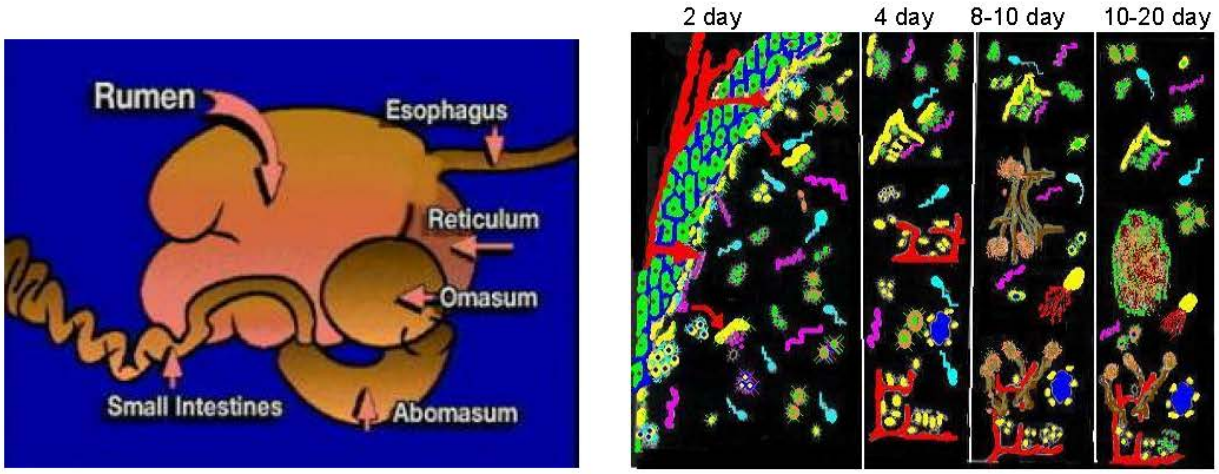

Fig. 1: Showing ruminant digestive tract component as well as rumen microbes (bacteria, protozoa and fungi).

malic acid supplementation because the content of malic acid in the basal diet will vary. The malic acid content of forage varies with forage type (legumes > grasses), forage variety, maturity (immature > mature), and processing (fresh > hay or pelleting; Callaway *et al.,* 1997). In a study with dairy goats (Salama *et al.,* 2002), supplementation with yeast and malate was not beneficial for lactational performance because of the high concentration of malic acid in the forages (high proportion of alfalfa) in the basal diet.

In early lactating cows fed a diet containing 84 g supplemental malate compared to cows fed a controled diet had increased milk yield during peak lactation, consuming more concentrate, but had similar ruminal pH (Devan and Bach, 2004). In a similar design, supplementation of malic acid at 50 g/cow/day would be effective in *in vivo* in altering ruminal fermentation and microbial efficiency; in addition, malic acid supplementation in lactating cow diets was effective in increasing microbial nitrogen production and microbial efficiency measured *in vitro* and milk yield (Sniffen *et al.,* 2006). A recent study by Khampa *et al.* (2006) supplementation of sodium dl-malate with concentrates containing a high level of cassava chip increased ruminal pH, and altered rumen fermentation by increasing propionate production and decreasing of acetate to propionate ratio. Moreover, the high level of cassava chip in the diet resulted in increased populations of bacteria and fungi, decreased protozoal populations, and improved rumen microbial N supply and efficiency microbial nitrogen. These results suggest that the combined use of concentrates containing high level of cassava chip with supplementation of sodium dl-malate at 18 g/hd/d could improve rumen ecology and subsequent performance in dairy steers. In a subsequent study it was found that the combined use of concentrate containing high level of cassava chip at 75 % DM with urea at 4 % in concentrate and sodium dl-malate at 20 g/hd/d with urea-treated rice straw as a roughage could improved rumen ecology and microbial protein synthesis efficiency in lactating in dairy cows (Khampa *et al.,* 2006a).

Implications: The rumen is an essential fermentation vat in which fermentation end-products are being prepared for the biosynthetic processes of the ruminant hosts. As could be seen in practical scale, ruminants raised in the tropics largely depend on seasonal feed resources that are relatively low in quality. Therefore, the manipulation of rumen efficiency through the use of organic acids especially malate with local feeds would be an advantage. Indeed, organic acids potentially provide an alternative to currently used antimicrobial compounds by stimulating rather than inhibiting specific ruminal microbial populations. Moreover, local feed resources especially cassava chip or other energy sources with high ruminal degradation could be used effectively at high level as an energy source with NPN (urea) for ruminants especially for fattening beef and lactating cows. Moreover, the high level of cassava chip in the diet resulted in increased population of bacteria and fungi, decreasing protozoal populations, and improving microbial protein synthesis and efficient microbial nitrogen supply in rumen. The use of malate was also effective in reducing the drop in ruminal pH. Therefore, supplementing high-producing dairy cows diets with malate might be effective in reducing subclinical acidosis. However, by selecting for and incorporating forage varieties that are high in malate into the ruminant diets, could be an alternative approach. Supplementation of organic acid, like malate, would be a desirable alternative because there is no risk of developing antibiotic resistance or having unwanted residues appear in either meat or milk products. Most

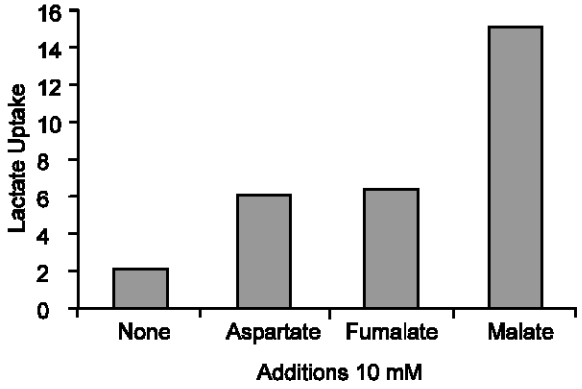

Fig. 2: Effects of aspartate, fumarate, and malate on lactate uptake (nmol/mg protein per min) by *S. ruminantium* (Nisbet and Martin, 1990).

importantly, any researches and development should be based on simplicity, availability of local feed resources, the cost-profit of production and the sustainability of ruminant production systems in the tropics particularly in fattening and lactating ruminants.

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Microflora of Pressurized Edam Cheese

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Abstract: Pressurization is a modern method of food processing and preservation. This paper discusses the effect of high pressure (200 and 400 MPa) on the microflora of ripening cheese. Cheese with a different ripening degree was subjected to a microbiological analysis which involved determination of the total bacteria count as well as the numbers of lactic streptococci, coli group bacteria, *Clostridium, Listeria* and *Salmonella*. After pressurization at 400 MPa, the number of lactic streptococci and total bacteria count decreased by 2-4 orders of magnitude. The high pressure did not result in the inactivation of technologically-undesirable bacteria.

Key words: High pressure technology, Edam cheese, microflora, ripening

Introduction

The high pressure technology is a modern method of food processing. It can be applied either as an alternative, non-thermal method of preservation of raw materials and food products, or as a means of modifying their organoleptic and technological properties. The term "HP technology" describes the application of pressures ranging from 100 to 1000 MPa at room temperature (Kolakowski *et al*., 1994; Trujillo *et al*., 2002), in industrial practice from 400 to 700 MPa (San Martin *et al*., 2002). This method can be modified, in order to inactive bacteria, through e.g. the application of high pressures at elevated temperatures or the addition of substances that lower a bacteria's resistance, e.g. lysozyme or nisin (Masschalck *et al*., 2000).

Food products subjected to high pressure treatment (e.g. jams, juices, sausages, ham, rice, cakes and desserts) emerged in the 1990s initially in Japan and then, successively, in the Member States of European Union and the United States (Kolakowski *et al*., 1994; Trujillo *et al*., 2002).

In the dairy industry, the high pressure technology may be applied for the preservation of milk and fermented drinks as well as for cheesemaking (Jankowska *et al*., 2005; Jankowska *et al*., 2003; Kolakowski *et al*., 1998; Trujillo *et al*., 2002). Pressurization of milk to be used for cheese making not only lowers the count of milk microflora (Buffa *et al*., 2001), but also affects the coagulation process. The selection of process conditions enables shortening the time of rennet coagulation and increasing the cheese yield (Huppertz *et al*., 2004; Lopez-Fandino *et al*., 1996). Cheeses produced from pressurized milk have been demonstrated to possess better organoleptic traits compared to those made of pasteurized milk (Buffa *et al*., 2001; Reps *et al*., 1998).

Pressurization accelerates the process of ripening, presumably due to the release of hydrolytic enzymes from bacterial cells inactivated upon pressure treatment (Kolakowski *et al*., 1998; O'Reilly *et al*., 2003; Saldo *et al*., 2000). It has also a considerable impact on the sensory and physicochemical properties of cheese (Messens *et al*., 1999; Messens *et al*., 2000; Saldo *et al*., 2000).

Materials and Methods

The object of the study was Edam cheese. Cheese samples, 6.5/5/6.5 cm in size, were cut out of cheese blocks after brining and paraffined. Prior to pressurization, the samples were packed under vacuum in additional barrier casings. Cheeses were pressurized for 30 min at 200 and 400 MPa, at room temperature, immediately after brining and after four, six and eight weeks of ripening. The non-pressurized cheese (control) and the pressurized cheese were subjected to microbiological analyses immediately after the pressurization and during ripening.

The cheeses were determined for:

- the presence of *Salmonella* and *Listeria* bacteria, with the use of TECRA UNIQUE *Salmonella* and TECRA UNIQUE *Listeria* tests;
- the total count of bacteria on plate count skim milk agar (Merck);
- the number of lactic fermentation streptococci on M17 agar according to Terzaghi (Merck);
- the number of coli group bacteria on the Chromocult coliform agar (Merck);
- the most probable number (MPN) of anaerobic proteolytic bacilli of the genus *Clostridium* on meat liver agar (Merck);
- the most probable number of anaerobic saccharolytic bacilli of the genus *Clostridium* on Bryant Burkey broth with resazurin and lactate (Merck) and with Durham tubes.

Microbiological analyses were carried out in triplicate.

Table 1: Total bacteria count in pressurized Edam cheese

Time of cheese ripening [weeks]	Control cheese	Period of cheese ripening before pressurization [weeks]							
		after brining		4		6		8	
		Pressure applied [MPa]							
		200	400	200	400	200	400	200	400
		[cfu/g]							
after brining	$2.3x10^9$	$2.6x10^9$	$1.3x10^6$	-	-	-	-	-	-
4	$4.2x10^8$	$4.0x10^7$	$1.4x10^7$	$1.4x10^{10}$	$4.2x10^6$	-	-	-	-
6	$9.2x10^9$	$8.8x10^7$	$1.2x10^6$	$5.1x10^8$	$7.5x10^5$	$2.5x10^9$	$9.0x10^5$	-	-
8	$1.8x10^9$	$5.0x10^8$	$1.6x10^6$	$4.0x10^8$	$1.8x10^6$	$4.6x10^8$	$1.4x10^7$	$9.8x10^8$	$1.5x10^5$

- not determined

Table 2: The number of lactic fermentation streptococci in pressurized Edam cheese

Time of cheese ripening [weeks]	Control cheese	Period of cheese ripening before pressurization [weeks]							
		after brining		4		6		8	
		Pressure applied [MPa]							
		200	400	200	400	200	400	200	400
		[cfu/g]							
after brining	$3.5x10^9$	$1.7x10^9$	$1.4x10^5$	-	-	-	-	-	-
4	$7.3x10^8$	$7.3x10^8$	$2.8x10^6$	$2.2x10^9$	$3.5x10^6$	-	-	-	-
6	$9.0 x10^8$	$7.6x10^8$	$4.9x10^6$	$8.4x10^8$	$1.8x10^6$	$1.1x10^9$	$4.1x10^6$	-	-
8	$8.5x10^8$	$7.7x10^8$	$1.0x10^7$	$8.6x10^8$	$6.2x10^6$	$7.7x10^8$	$3.2x10^6$	$8.9x10^8$	$1.7x10^6$

- not determined

Results and Discussion

Total bacteria count: In the control Edam cheese, the total bacterial count ranged from 10^8 to 10^{10} cfu/g (Table 1). The number and morphology of colonies obtained during determinations indicate that the main microflora of the cheese examined were lactic streptococci.

During analyses, it was noted that the pressurization of Edam cheese at 200 MPa immediately after brining did not affect the total bacteria count. Yet, during its ripening, the number of microorganism was observed to decrease distinctly, i.e. after four weeks of ripening the decreased by 90% and after the subsequent two weeks by 99%, as compared to the control cheese. The pressure treatment of cheese after four weeks of ripening caused an increase in the number of bacteria, whereas pressurization after six and eight weeks of ripening decreased the number of microorganisms in cheese by 73 and 45%, respectively. It should be noted that in eight-week cheeses pressurized at 200 MPa, the count of microflora was lower than that reported in control cheeses.

These results are confirmed by the findings of Kolakowski *et al.* (1998) who demonstrated the possibility of reducing the total bacteria count in Gouda cheese by 24% after threefold, pulsatory pressurization at 200 MPa for 5 min.

The pressurization at 400 MPa resulted in a tangible decrease in the total bacteria count ranging from 99.00 to 99.99% depending on the degree of ripening of the pressurized cheese. Nevertheless, the pressurization after brining and that after six weeks of ripening produced an increase in the total bacteria count during subsequent ripening. The greatest reduction of microflora was observed in cheese pressurized after eight weeks.

It was confirmed that the degree of ripening of cheese subjected to a pressure treatment affected its yield. Kolakowski *et al.* (1996) demonstrated that a threefold 5-min pressurization of two- and six-week Gouda cheese at 200 MPa reduced the number of microflora by 43 and 40.5%, respectively, whereas that carried out at 400 MPa – by 95.2 and 92.7%, respectively.

Lactic streptococci: The number of lactic streptococci in the control cheese reached 3.5×10^9 cfu/g after brining, whereas during an eight-week ripening period it fluctuated between 7.3 10^8 to 9.0 10^8 cfu/g. It should be emphasized that lactic streptococci are relatively resistant to the pressure of 200 MPa, since 20-min pressurization at 200 MPa caused a reduction in their number that depended on strain susceptibility, yet not greater than one order of magnitude (O'Reilly *et al.*, 2002).

The pressure treatment of cheese at 200 MPa immediately after brining reduced the number of streptococci by 52% (Table 2). In the other cheeses, the

Table 3: The presence of coli group bacteria in pressurized Edam cheese *

Time of cheese ripening [weeks]	Control cheese	Period of cheese ripening before pressurization [weeks]							
		after brining		4		6		8	
		Pressure applied [MPa]							
		200	400	200	400	200	400	200	400
		[% of samples]							
after brining	67	100	0	-	-	-	-	-	-
4	67	67	33	0	33	-	-	-	-
6	0	67	0	0	0	0	33	-	-
8	67	33	33	33	0	100	100	67	100

* in 0.01 g of cheese. - not determined

Table 4: The number of anaerobic saccharolytic bacilli of the genus *Clostridium* in pressurized Edam cheese

Time of cheese ripening [weeks]	Control cheese	Period of cheese ripening before pressurization [weeks]							
		after brining		4		6		8	
		Pressure applied [MPa]							
		200	400	200	400	200	400	200	400
		[MPN]							
after brining	14	32	2.1	-	-	-	-	-	-
4	25	1.9	n	5.6	5.4	-	-	-	-
6	3.6	2.1	7.5	2.1	2.1	5.6	2.1	-	-
8	18	51	45	15	$1.1x10^2$	$1.2x10^2$	$2.5x10^2$	$6.3x10^2$	$5.7x10^2$

n: not present in 0.1g. - not determined

Table 5: The number of anaerobic proteolytic bacilli of the genus *Clostridium* in pressurized Edam cheese

Time of cheese ripening [weeks]	Control cheese	Period of cheese ripening before pressurization [weeks]							
		after brining		4		6		8	
		Pressure applied [MPa]							
		200	400	200	400	200	400	200	400
		[MPN]							
after brining	8.2	4.3	n	-	-	-	-	-	-
4	2.0	$1.2x10^{-2}$	n	n	$8.7x10^{-2}$	-	-	-	-
6	3.8	5.0	1.1	2.4	1.2	5.0	0.75	-	-
8	0.26	0.1	2.2	2.7	1.7	n	$4.0x10^{-2}$	$4.0x10^{-2}$	$2.4x10^{-2}$

n: not present in 0.1g. - not determined.

pressure of 200 MPa was found to exert no distinct effect on the counts of lactic streptococci.

Although the pressure of 400 MPa has a considerable impact on the survivability of lactic streptococci strains, the degree of bacteria inactivation may be strain-dependent (Wick *et al.*, 2004).

After the pressure treatment at 400 MPa, the number of lactic streptococci decreased in the cheese by 99.5 to 99.99%, depending on its degree of ripening.

In the case of cheese pressurized after brining, the population number of streptococci was observed to increase during ripening. After eight weeks of ripening, it was noted that the extent of streptococci reduction became more distinct along with extended period of cheese ripening before pressurization, as compared to the control cheese.

Pathogenic and technologically-undesirable bacteria: The presence of pathogenic bacteria in milk and cheeses (De Buyser *et al.*, 2001) is likely to pose a risk to consumer health. Literature data indicate the possibility of a substantial reduction in the number of *L. monocytogenes* in cheese as a result of pressurization. Szczawiñski *et al.* (1997) demonstrated that the pressure treatment of cheese at 500 MPa for 15 min decreased the number of bacteria by six orders of magnitude. There are no data on the effect of pressure on *Salmonella* bacteria in cheese, however,

pressurization of milk at 350 MPa/10 min has been reported to cause their inactivation (Bozoglu *et al.*, 2004). In the study reported, bacteria of the genera *Salmonella* and *Listeria* were not detected in cheese after brining. Therefore, they were not determined in further stages of the experiment.

The growth of undesirable microflora may lead to numerous defects in cheese, the most common of which is the so-called "early blowing of cheeses". This defect is due to the growth of coli group bacteria (Wuytack *et al.*, 2002). In contrast, the so-called "late blowing of cheese" is probably caused by the growth of saccharolytic bacilli of the genus *Clostridium* (Su and Ingham, 2000).

The Gram-negative bacteria, including the coli group bacteria, are more susceptible to the effect of high pressure. Threefold 5-min pressurization of Gouda cheese at 200 MPa reduced the number of coli group bacteria by 90%, and that carried out at 400 MPa caused their complete inactivation (Kolakowski *et al.*, 1996). In the case of *Escherichia coli,* the susceptibility to high pressure may be a strain-specific trait, and individual strains are likely to demonstrate a considerable diversification in this respect (Linton *et al.*, 2001).

In the cheeses examined, it was impossible to determine the number of coli group bacteria. Their presence could only be detected in 0.01 g of cheese.

During analyses, in the control cheese the presence of coli group bacteria in 0.01 g of cheese was detected in 50% of the samples, on average, whereas in the cheeses pressurized at 200 and 400 MPa it was detected in 47 and 33% of the samples, respectively.

The spore-forming bacteria are likely to occur in pressurized products due to the considerable resistance of resting spores to high pressures. Hence, the application of combined methods is suggested, e.g. pressures over 600 MPa together with high temperatures (Jankowska, 2001). Simultaneous application of pressurization and nisin addition is also possible - the high pressure produces a change in the permeability of cellular membranes, which increases the efficiency of the activity of that bacteriocin (Buffa *et al.*, 2001).

The cheese examined contained a negligible (no more than 10 cfu/g) amount of proteolytic bacilli of the genus *Clostridium* (Table 5). Subjecting cheese to a pressure treatment at 200 MPa and 400 MPa has no impact on the number of these bacilli (fluctuations in the number of bacilli are within the standard deviation of the mean); only cheeses pressurized at 400 MPa and determined in the fourth and eighth week of ripening contained a smaller population of proteolytic bacilli compared to the control cheeses.

The control cheese, with a different degree of ripening, contained slightly more saccharolytic bacilli of the genus *Clostridium* (max. 25 cfu/g), (Table 4). Due to the low number of gas-producing bacteria it is difficult to characterize the effect of pressure on this group of bacteria. The high pressure positively affects the growth of anaerobic saccharolytic bacilli during the ripening of pressurized cheese. This results from the sporulation of the resting spores after pressurization.

The results obtained point to the need for further investigations into the effect of high pressures on cheese microflora.

Conclusions: Pressurization at 200 MPa for 30 min had no significant effect on either the total bacteria count or the number of lactic streptococci. After a pressure treatment at 400 MPa the number of those bacteria decreased by ca. 2-4 orders of magnitude. In the cheeses pressurized at 400 MPa, irrespective of the degree of their ripening, the total bacteria count and lactic streptococci after eight weeks of ripening was lower by 2-3 orders of magnitude than that in the control cheeses.

Pressures of 200 and 400 MPa did not result in the complete inactivation of technologically-undesirable microflora in cheeses, i.e. coli group bacteria and spore-forming bacteria.

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Estimation of Relationship Between Hot Carcass Weight and *Eye Muscle Area* Which Effects on Meat Production of Black Bengal Goats

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Abstract: A total of 16 castrated male goats were taken to measure hot carcass wt and *eye muscle area* to estimate meat production of goats. These goats were 12 months of age. In this experiment significant (0.1%) correlation was found between Hot carcass wt. and *eye muscle area*.

Key words: Hot carcass wt., *eye muscle area*, meat production

Introduction

Black Bengal goat is the only one livestock breed of Bangladesh. This goat is famous for its meat and skin quality. Chevon as well as goat meat production depends on many parameters but here I have considered only hot carcass wt. and *eye muscle area*. The "eye muscle" area is sometimes used as a predictor of the amount of carcass meat produced from an animal. Fat thickness directly above the greatest depth of the "eye muscle" and the "eye muscle" width and depth are the precise predictor of lean meat, subcutaneous fat and inter muscular fat (Wood *et al*., 1980).Objective of this experiment is to estimate meat production from carcass characteristics.

Materials and Methods

Data were collected from records maintained in the goat breeding project at the Department of Animal Breeding and Genetics, BAU, Mymensingh and the number of castrated male goats were 16. These goats were 12 months of age. Two main traits were considered to predict meat production.

The following parameters were considered for analysis:

a) Hot carcass weight, weight was taken within one hour of slaughter at warm condition.

b) *Eye muscle area*, For measuring *eye muscle area* the hot carcass was split between the 13th and 14th ribs. From the cross section the area was traced five times onto an acetate paper and from the weight-area relationship of the acetate paper the average area of each single 'eye' was estimated.

Eye muscle area (cm^2) = [Weight of acetate paper for total *eye muscle area* / Weight of acetate paper for one cm^2]

The collected data was compiled, tabulated and analyzed in accordance with the objectives of the study. Correlation co-efficient was calculated among the different measurements using the computer program Statgraf (1993). The correlation co-efficient used in Statgraf (1993) was:

$$r_{xy} = \frac{\Sigma(X-\bar{X})(Y-\bar{Y})}{\sqrt{\Sigma(X-\bar{X})^2(Y-\bar{Y})^2}}$$

Where, r_{xy} is the correlation co-efficient, $\bar{x}$ and $\bar{y}$ is the mean of the values of the variable of x and y. Linear, logarithmic models were used for expressing the relationship of two variables. Y= a+bx. Here, a is the intercept and b is the slope usually called the regression co-efficient of y on x. Y is dependent and x is independent variable.

Results and Discussion

The mean of hot carcass weight and *eye muscle area* were 5.82±0.27 kg and 6.50±0.25 cm^2 respectively. These results are in good agreement with Amin *et al*. (2000). Amin *et al*. (2000) found 4.9±0.21 kg hot carcass weight and 5.7±0.20 cm^2 *eye muscle area*.

There was significant correlation ($p<0.01$) between *eye muscle area* and hot carcass weight. Singh *et al*. (1983) and Raghavan (1988) reported that *eye muscle area* was highly associated with hot carcass weight. Hot carcass wt. can be used in estimating *eye muscle area* in goats.

Table 1: Average carcass characteristics in goats

Parameters	Average
Hot carcass weight (kg)	5.82±0.27
Eye muscle area (cm^2)	6.50±0.25

Table 2: Correlation co-efficient of eye-muscle area with hot carcass wt. in goats

Parameter	HCW
EMA	0.91***

EMA, *Eye muscle area*; HCW, Hot carcass weight;
***,Significant at 0.1%.

Table 3: Simple linear regression equation for estimation of eye-muscle area from hot carcass weight with their % reliability

Estimation	Regression equation
EMA from HCW	EMA=1.62+0.84 HCW

HCW, Hot carcass weight; EMA, *Eye muscle area*.

Table 4: Prediction chart for determining *eye muscle area* from hot carcass weight in Black Bengal goats

HCW (kg)	EMA (cm^2)
3.36	4.35
3.75	4.77
4.25	5.14
4.75	5.52
5.00	5.90
5.50	6.28
6.00	6.60
6.25	6.95
6.75	7.32
7.25	7.70
7.50	8.08

HCW = Hot carcass weight, EMA = *Eye muscle area*

Conclusion: In this experiment it is found that *eye muscle area* is highly associated with hot carcass weight. A prediction chart for determining *eye muscle area* from hot carcass weight in Black Bengal goats have been prepared and shown in Table 4. We may consider this positive correlation as selection criteria for selecting goats for meat production.

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Nutritional Quality of *Crassocephalum crepidioides* and *Senecio biafrae*

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Abstract: The nutritional potentials of two green leafy vegetables namely, *Crassocephalum crepidioides* (CC) and *Senecio biafrae* (SB) were evaluated through their proximate composition, mineral and amino acid profile. *Crassocephalum crepidiodies* contained 27.17±0.51% crude protein (CP) while that of *Senecio biafrae* was 28.93±0.68% but were not significantly different ($P \geq 0.05$). The crude fibre content for SB was 7.26±0.22% while that of CC was 8.13±0.06%. The ash content for SB was 16.30±0.21% and 17.31±0.02% for CC. The nitrogen free extract (NFE) for SB was 20.81±1.36% while CC recorded a value of 19.03±0.56%. The organic matter (OM) for SB was 83.70±0.24% and CC had 82.69±0.02%. The mean values of the CP, ash, NFE and OM were not significantly different ($P \geq 0.05$). Iron (Fe) in CC was 0.056±0.006% and 0.029±9.60 x 10^{-4}% These values were not significantly different ($P \geq 0.05$). The mean values for manganese (Mn), sodium (Na), potassium (K), magnesium (Mg) and calcium (Ca) in CC and SB were all significantly different ($P \leq 0.05$). Among all the minerals evaluated, K had the highest value of 0.07±2.0 x 10^{-4} % in CC while it is 0.04±6.0 x 10^{-3} % in SB. The amino acids values in CC ranged from 0.54±0.02 mg/g in threonine to 4.26±0.029 mg/g in tyrosine. In SB isoleucine had the lowest value of 0.51±7.37 x 10^{-4} mg/g while tyrosine recorded the highest, 3.69±0.07 mg/g. Valine, isoleucine and phenylalanine values in the two vegetables differ significantly ($P \leq 0.05$). The total amino acids (TAA) in CC was 19.01±0.08 mg/g and 17.41±0.081mg/g in SB while the total non-essential amino acids (TNEAA) was 11.23±0.06 mg/g and 9.89±0.02 mg/g in the two vegetables respectively which are also significantly different ($P \leq 0.05$). The percent TEAA and percent TEAA with histidine are also significantly different ($P \leq 0.05$). The study indicates that *Crassocephalum crepidioides* and *Senecio biafrae* are good sources of protein in the nutrition of both human and animal.

Key words: Proximate, mineral, amino acid, *Crassocephalum crepidioides, Senecio biafrae*

Introduction

Green leafy vegetables are one of the sources of nutrients for growth in man and animal. The roots and the leaves are also popular potherbs in many parts of the world. They constitute an indispensable part of human diets generally in most part of Africa. Studies have shown a progressive per capital daily consumption of vegetables from 65 g in 1977 to 360 g in 1989 (Fafunso and Basir, 1977; Oguntona *et al*., 1989). Even though there are no recent statistics on vegetable consumption, the abundance in Nigerian markets is a pointer to their use in human diets. *Crassocephalum crepidioides* (locally called "Ebolo") and *Senecio biafrae* (with local name "worowo" or Sierra Leone bologni) belong to this group of vegetables that grow in large quantity as undercover in tree crop plantation. Some of these leafy vegetables are also considered for their high medicinal value as the juice extracted from the leaves are wholly applied to fresh wounds or cuts as styptic in the rural community for man and animal use (Akah, 1996; Viana, *et al*., 2003; Gulliice *et al*., 2004; Okpara *et al*., 2006). The high edible mucilaginous fibre, leaves and stem are used to treat indigestion or as laxative and as purgative (Fowomola and Akindahunsi, 2005). With all these qualities, little is known as regards the quality of the amino acid content of most leafy vegetables except recently where information are provided on some for their nutritional importance. There had been reports on the characterization of cassava leaves as food and its amino acid composition (Lancaster and Brooks, 1983; Aletor and Adeogun, 1995; Fasuyi, 2005). Similarly, Ladeji *et al*. (1995) and Fasuyi (2006) both reported the nutritional significance of the amino acid profile of *Telferia occidentalis* while the latter worked further on the proximate characterization, the amino acid profile and the functional properties of *Talinum triangulare* and *Amaranthus cruentus*. There exist in the various ecological zones vegetables that are of natural endowment and of tremendous use as human and animal foods; especially in the present dispensation where vegetable protein sources in animal feeds are becoming increasingly expensive as a result of competition for use as human food. This study therefore was carried out to provide further information on the amino acid profile of some of the Nigerian vegetables of which *crassocephalum crepidioides* and *Senecio biafrae* belong.

Materials and Methods

The two edible vegetables *Crassocephalum crepidioides* and *Senecio biafrae* were obtained from "Oja- Oba" market in Ado-Ekiti, in Ekiti State of Nigeria

Table 1: Proximate composition of *Crassocephalum crepidioides* and *Senecio biafrae*

Parameters (%DM)	Mean Value *Crassocephalum crepidioides*	Mean Value *Senecio biafrae*	Standard Error	Coefficient of Variation	T Test | t | (P ≤ 0.05)
Dry Matter	85.08±0.19	87.5±0.35	0.20	0.39	0.04^{S}
Crude Protein	27.17±0.51	28.93±0.68	0.39	2.35	19.38^{NS}
Crude Fibre	8.13±0.06	7.26±0.22	0.13	2.98	0.26^{NS}
Ether Extract	12.45±0.02	14.21±0.09	0.05	0.63	0.01^{S}
Ash	17.31±0.02	16.30±0.21	0.12	1.26	0.10^{NS}
NFE	19.03±0.56	20.81±1.36	0.78	6.52	10.33^{NS}
Organic Matter	82.69±0.02	83.70±0.21	0.11	0.25	0.10^{NS}
Metabolizable Energy (Kcal./g)	2.64±0.03	2.91±0.05	0.0023	1.97	0.02^{S}

located in the rain forest zone on latitude 7°40^{1} North of the Equator and longitude 5°15^{1} East of the Greenwich Meridian. The samples were bought fresh, kept in a transparent polythene bag and transported to the University of Ado-Ekiti where they were sun dried. The foliage samples were hereafter collected and kept in a well labelled screw-capped sample bottles prior to analysis.

Analysis of the samples: The samples of *Cressocephalum crepidioides* and *Senecio biafrae* were divided into triplicate and the proximate analysis determined to obtain values for the dry matter, crude protein, crude fibre, ether extract, ash, nitrogen free extract and the organic matter as described by AOAC (1995). The mineral content analyzed were iron (Fe), manganese (Mn), sodium (Na), potassium (K), magnesium (Mg) and calcium (Ca). About 2 g of each of the dried samples in a crucible were ashed at 550°C in a Gallenkamp muffle furnace. The ash was later dissolved in 100 ml volumetric flask with de-ionized water. 10 ml of concentrated hydrochloric acid was added and filtered. The filterate was made up to 50 ml with 0.1M HCl. Fe, Mn, Mg and Ca values were determined using Atomic Absorption Spectrophotometer (Perkin-Elmer model 403, Norwalk CT, USA). Sodium and potassium were determined by using a flame photometer (model 405, Corning UK). Sodium chloride and potassium chloride were used to prepare the standards.

The amino acid profiles for the samples were determined by using a laboratory blender to blend the dried samples. They were hereafter hydrolyzed at 150°C for about 90 minutes and the solution used for the determination by the modification of Waters 'Pictotag' system as described by Bidlingmeyer *et al.* (1984) and Gardner *et al.* (1991). The amino acid score was calculated as a ratio of the mg. of amino acid per g of test diet to the mg. of amino acid per g protein in reference pattern as described by Crampton and Harris (1969) and WHO (1973). The metabolizable energy of the samples was calculated using the Pauzenga (1985) prediction equation.

Statistical analysis: All the triplicate data collected for each of the samples were subjected to statistical evaluation using the SAS (1987) computer soft ware package version six for t- test among the mean values, standard deviation, standard error and the coefficient of variation (CV).

Results and Discussion

The proximate composition of *Crassocephalum crepidioides* and *Senecio biafrae* is presented in Table 1. The dry matter, the ether extract and the metabolizable energy content of *Senecio biafrae* were significantly higher ($P \leq 0.05$) than that of *Crassosephalum crepidioides* with a coefficient of variation (CV) 0.39% and 0.63% respectively. Though the recorded values did not differ significantly ($P \geq 0.05$), the crude protein, fibre, ash, nitrogen free extracts (NFE) and the organic matter (OM) were all similar. The entire mineral element evaluated except Fe differ significantly ($P \leq 0.05$) among the mean values (Table 2). The macro minerals namely Ca, Mg, K, Na and Mn were all significantly higher in *Crassocephalum crepidioides* than *Senecio biafrae*. The mineral composition of the two vegetables revealed their Fe content to be higher than the other minerals analyzed.

The amino acid composition of the two vegetables is presented in Table 3. Proline, isoleucine and phenylalanine content of *Crassocephalum crepidioides* were significantly higher ($P \leq 0.05$) than the values recorded for *Senecio biafrae*. The total amino acids (TAA), total non-essential amino acids (TNEAA), total essential amino acids with histidine (TEAAH), percent total essential amino acids with histidine (%TEAAH) and percent total non-essential amino acids (%TNEAA) all differ significantly ($P \leq 0.05$) with *Crassocephalum crepidioides* having the higher values.

The dry matter content of the samples of the two vegetables evaluated in this study was higher than those reported by Oguntona (1988). This is because sun dried samples were used for the study. Fresh vegetables are generally characterized by high moisture hence the low dry matter content. However, the moisture content of dried vegetable leaves varies depending on the prevailing local environment and length of storage.

The crude protein content of the two vegetables compared favourably with the values obtained for *Amaranthus cruentus* (Fasuyi, 2006) and *Corchorus*

Table 2: Mineral content of *Crassocephalum crepidioides* and *Senecio biafrae* (%)

Mineral	Mean Value *Crassocephalum crepidioides*	Mean Value *Senecio biafrae*	Standard Error	Coefficient of Variation	T Test | t | (P ≤ 0.05)
Fe	0.056 ± 0.006	0.029±9.6 x 10^{-4}	5.5 x 10^{-4}	3.29	0.13^{NS}
Mn	7.87 x 10^{-4} ±1.52 x 10^{-5}	3.0 x10^{-4}±9.6 x 10^{-4}	5.77 x 10^{-5}	3.33	0.01^{S}
Na	0.09±0.002	0.005±1.01 x 10^{-5}	5.70 x 10^{-5}	2.0	0.01^{S}
K	0.07±2 x 10^{-4}	0.04±6.0 x 10^{-3}	3.51 x 10^{-4}	1.51	0.01^{S}
Mg	0.04±0.001	0.004±2.0 x 10^{-4}	1.15 x 10^{-4}	4.76	0.01^{S}
Ca	0.067±2.65 x 10^{-4}	0.004±1.0 x 10^{-4}	6.1 x 10^{-5}	2.45	0.01^{S}

Table 3: Amino acid composition of *Crassocephalum crepidioides* and *Senecio biafrae* (mg/g crude protein)

Amino acids	Mean Value *Crassocephalum crepidioides*	Ratio of amino acid in Crassocephalum *crepidioides* to egg /amino acid score (%)	Mean Value *Senecio biafrae*	Ratio of amino acid in *Senecio biafrae* to egg/amino acid score (%)	Standard Error	Coefficient of Variation	T Test | t | (P ≤ 0.05)
Lysine	0.60±0.007	8.30/91.26	0.61±0.001	8.47/91.53	6.44 x 10^{-4}	0.18	21.73^{NS}
Histidine	0.86±0.034	40.95/59.05	0.70±0.005	33.33/66.67	0.003	0.80	0.13^{NS}
Arginine	1.26±0.023	19.69/80.31	1.31±0.009	20.47/79.53	0.005	0.71	3.04^{NS}
Aspartic acid	0.71±8.5 X 10^{-4}		0.63±0.02		0.009	2.46	0.09^{NS}
Threonine	0.54±0.02	11.02/88.98	0.63±0.04	12.86/87.14	0.023	6.38	2.80^{NS}
Serine	0.61±0.005		0.61±0.004		0.002	0.73	63.50^{NS}
Glutamic acid	0.91±2.65 X 10^{-4}		0.97±0.02		0.01	2.50	0.85^{NS}
Proline	2.33±0.003		1.69±0.02		0.01	1.27	0.01^{NS}
Glysine	0.87±0.018		0.68±0.009		0.005	1.37	62.58^{NS}
Cystine	1.07±0.002	44.58/55.42	0.85±0.004	35.41/64.58	0.002	0.47	0.07^{NS}
Valine	0.55±0.0059	7.95/92.40	0.88±7*10^{-4}	12.05/87.95	4.0 x10^{-4}	0.08	0.01^{S}
Methionine	0.58±1.15 X 10^{-4}	14.15/85.85	0.58±0.004	14.15/85.85	0.002	0.73	28.82^{NS}
Isoleucine	0.91±0.01	11.38/88.63	0.51±7.37 x 10^{-4}	**6.38/93.63	4.25 x 10^{-4}	0.14	0.01^{S}
Leucine	0.62±0.004	**6.74/93.26	0.63±0.002	6.84/93.12	9.2 x10^{-4}	0.25	23.57^{NS}
Tyrosine	4.26±0.29	93.3/5.33	3.69±0.007	80.00/18.00	0.005	0.21	3.30^{NS}
Phenylalanine	1.91±0.001	30.31/69.68	1.65±0.006	26.19/73.81	0.003	0.36	0.01^{S}

S = Significant (P= 0.05): NS = Not Significant (P=0.05). **Limiting amino acid

Table 4: Total amino acid composition of *Crassocephalum crepidioides* and *Senecio biafrae* (%)

*TAA	19.01±0.081	17.41±0.08	0.045	0.45	0.01^{S}
*TNEAA	11.23±0.06	9.89±0.02	0.017	0.29	0.01^{S}
*TEAA	6.99±0.025	6.80±0.06	0.057	0.94	0.04^{NS}
*TEAA + Histidne	7.85±0.02	7.52±0.05	0.028	0.66	0.89^{NS}
%TNEAA	58.74±0.20	56.83±0.09	0.05	0.16	0.01^{S}
%TEAA	36.65±0.27	39.13±0.10	0.06	0.26	0.16^{NS}
% TEAA + Histidine	41.16±0.12	43.17±0.09		0.21	0.01^{S*}

TAA = Total Amino acid. *TNEAA =Total Non-Essential Amino acid. *TEAA = Total Amino acid. S = Significant (P= 0.05) ; NS = Not Significant (P=0.05)

(Oguntona *et al*., 1989), lower than the values reported for cassava leaf protein concentrate (Fasuyi, 2005) but higher than the protein content in its fresh leaves as reported by Oguntona *et al*. (1989) which is a function of the dry matter of the sample. *Crassocephalum crepidioides* and *Senecio biafrae* have crude fibre range of 7.26- 8.13% which is within the tolerable range for both infant and adult (Adeyeye, 1997). They can be good sources of succulent forages for rabbits and other pseudo ruminant animals. The dry matter and organic matter content of the two vegetables range between 85.08 and 87.51%, 82.69 and 83.70% which would be very good for the feeding of herbivores and other ruminants. The macro mineral content is higher in *Crassocephalum crepidioides* than in *Senecio biafrae*. The values obtained in this study are lower than those reported by Aletor and Adeogun (1995) and Oguntona (1998). With the higher dry matter content obtained in this study, the expectation is that the minerals evaluated would have higher concentration in the vegetables as reported by the earlier mentioned workers. However, it is known that the nutrient content of a crop is highly influenced by the soil nutrient or fertility and the type and quality of fertilizer that was applied at the time of cultivation. Nonetheless, the values obtained still follow the trend reported by these workers with Na having the highest value followed by K and Ca. The mineral content of vegetables often inform their use in human diets as purgative and as an agent that aid digestion process because some of them (in their divalent state) function as cofactors to enzymes in most of the nutrient metabolic processes for healthy living (Mackenzie *et al.,* 1985; Kim, 1995).

The amino acids profile indicates that *Crassocephalum*

crepidioides and *Senecio biafrae* are limiting in the essential amino acids. Tyrosine had the highest values among the amino acids in the two vegetable samples. The amino acids content is quite inferior to the values obtained for the legumes such as African yam beans, soybean and many others (Aletor and Adeogun, 1995; Adeyeye, 1997) and some vegetables as reported by Fasuyi (2006) for *Talinum triangulare, Amaranthus cruentus* and *Telfairia occidentalis*. The TAA was significantly higher in *Crassocephalum crepidioides* than *Senecio biafrae* and this trend was followed by the TEAA, TNEAA, percent TNEAA and %TEAA. The total essential amino acid with histidine (TEAAH) was also significantly higher in *Crassocephalum crepidioides* than *Senecio biafrae*. The environmental factors of the soil may also influence the essential amino acid composition. The limiting amino acid in *Crassocephalum crepidioides* is leucine with amino acid score of 6.74 or 93.26% while it is isoleucine in *Senecio biafrae* with a score of 6.38 or 93.12%. The use of these vegetables as food imply that they must be supplemented with other good sources of plant or animal protein for infant and adult feeding in man and farm animals. Since these vegetables grow unhindered under cocoa or kolanut plantation especially in the south west of Nigeria they can be further processed into leaf concentrate protein supplemented with soybean and cereal to enhance the protein and energy content. The calculated metabolizable energy content for *Crassocephalum crepidioides* is 10.58 MJ kg^{-1} while 11.65 MJ kg^{-1} was obtained for *Senecio biafrae* (Table 1). The values obtained are higher than those reported by Oguntona *et al.* (1989) but expectedly lower than those of cereals as a result of the lower dry matter and ether extract content. The total amino acids in *Crassocephalum crepidioides* (19.08±0.081 mg/g) and *Senecio biafrae* (17.41±0.08 mg/g) and the total essential amino acids values (with histidine) of 7.85± 0.02 mg/g and 7.52±0.05 mg/g respectively were lower than the values recorded for cow's milk (490 mg) and that of egg with histidine but no tryptophane (495 mg) (FAO WHO/UNU, 1985; Adeyeye and Afolabi, 2004). The percent TNEAA in the two vegetables were higher than the percent TEAA. This indicate that the vegetables are lower in the essential amino acids hence require supplementation with other rich sources of essential amino acids for effective use as animal or human diets. Traditionally, these vegetables are cooked as local delicacies either as mixture with *Colocynthis citrullis* or dried crayfish or fish that are rich in the essential amino acids. The deficiencies in amino acids composition are hence compensated for through the animal protein sources in this regards.

Conclusively, *Crassocephalum crepidioides* and *Senecio biafrae* are good sources of vegetable protein of which their quality could be enhanced through supplementation.

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Determination of Chemical Composition of *Gnetum africanum (*AFANG) Seeds

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Abstract: The study on the chemical composition of the seeds of one of the most popularly known and popularly consumed tropical plant, *Gnetum africana* (afang) has been carried out by analyzing samples of the plant seeds collected from some plantations located within Akwa Ibom State (South Eastern Nigeria) for chemical Composition. The proximate, elemental and toxicant composition of the seeds (*Gnetum africana*) were determined by analyzing samples of the identified seeds for their carbohydrate, protein, lipid, ash, fibre, moisture and caloric value (proximate composition), iron, zinc, lead, potassium, sodium, magnesium and calcium (mineral compositon) and tannin, hydrocyanic acid, oxalate and phytic acid (toxicant composition) using recommended method of analysis. The result of the analysis shows that the percentage moisture content, crude protein, crude fat, crude fibre, ash content and carbohydrate of this seeds are 31.60%, 17.50%, 3.15%, 0.80%, 1.20% and 87.62% respectively while its caloric value is 448.83kcal/100g. The percentage sodium, potassium, calcium, magnesium, iron, zinc and lead content of the seed were 15.57, 38.56, 7.01, 5.48, 1.50, 1.50, 1.07 and 0.03 respectively while its toxicant content were 540mg/100g, 100.74mg/100g, 209.00mg/100g and 238.26mg/100g for hydrocyanic acid, tannin, oxalate and phytic acid respectively. The proximate, mineral and toxicant composition of the seeds were also compare with values reported in literature for other edible vegetable and the results show that the seeds are poor source of essential elements compared with other vegetable seeds. The antinutritional content of the seeds were very high when compare to values reported for other vegetables. Based on the result of the present study, it has been found that afang seeds are rich in proximate composition (when compare with other edible vegetables). Their low mineral content and their high toxicant content therefore suggest that these seeds should be properly processed before consuming them and its consumption should be supplemented with other food whose elemental content are high.

Key words: *Gnetum africanum* seeds, chemical composition, green leafy vegetable

Introduction

Gnetum africanum is one of the most popular green leafy vegetable in Nigeria and is gaining equal popularity as a delicious food leaf in other African countries such as Cameroon, Gabon, Congo and Angola (Eyo and Abel, 1983). *G.africanum* leaves are widely consumed in the South Eastern Nigeria due to its palatability and taste. It is now eaten as a vegetable salad when mixed with palm oil. The popularly known afang soup that is often listed in many continental restaurant menu is prepared from these leaves which sometimes is cooked with water leaves (*Talicum traiangulare*) to give the soup a special savour.

Gnetum africanum (afang) grows as a wild evergreen climbing plant in the rainforest of Nigeria where it is searched for and highly priced in the regional markets. It is recently being cultivated in South Eastern Nigerian homes as exotic plants. It belongs to the family *Gnetacea* and the order *Gnetales* (Dutta, 1981). The seed of Afang is oval in shape and small in size, about 0.5cm in diameter. They are greenish in color when unripe and reddish when ripe.

Available literature reveals that both the leaf and the seed in particular have shown medicinal efficacy in the treatment of enlarged spleen, sore throats, deduction of pains of child-birth, antidotes to some forms of poison and snake bite. The seeds are specially used as fungicide for dressing fresh and septic wounds. It is also chewed raw in the management of excessive urination by infantile diabetic patients in Traditional Medical (Smith, 1983; Shiembo, 1984; Mialoundama, 1993).

The mineral element content, amino acid content and proximate composition of the leaves has been reported by (Eyo and Abel, 1983). Little or no information is available on the chemical composition of the seeds of *G. africanum*.

This study is designed to determine the chemical composition of the seed *G. africanum* for public and dietary awareness of its nutritional status.

Materials and Methods.

Matured seeds of *G.africanum* were collected in three batches between the months of August and September, 2005 from Agro-forestry Series-1, Mbiabong Ikono, Uyo L.G.A and randomly from a thick bush around the rain forest of Calabar in Southern Nigeria. Samples taken to the laboratory were washed with deionized water and spread on a clean paper for a two-hour air-drying.

Table 1: Proximate composition of *G.africanum*

Parameters	Percentage
Moisture	31.60±0.07
Crude Protein	17.50±0.03
Crude fat (lipid)	3.15±0.01
Crude fiber	0.80±0.02
Ash content	1.20±0.01
Carbohydrate	87.62±0.04
Caloric value	448.83kcal

Table 2: Elemental composition of *G.africanum*

Parameters	Percentage
Sodium (Na)	15.57±0.02
Potassium (K)	38.56±0.04
Calcium (Ca)	7.01±0.01
Magnesium (Mg)	5.48±0.02
Iron (Fe)	1.50±0.02
Zinc (Zn)	1.07±0.04
Lead (Pb)	0.03±0.03

Table 3: Toxicant composition of *G.africanum*

Parameters	Content (mg/100g)
Hydrogen cyanide	540.00
Tannin	100.74
Oxalate	209.00
Phytic acid	238.26

Table 4: Summary of the Results for Comparative Studies with Some Other Vegetable Seeds

Parameter	Afang Seeds (*Gnetum africanum)*	Fluted pumpkin seeds (Telferia occide-ntalis)*	B. horse 'eye; beans (M.ureans)**
Moisture content %	31.6	54.8	31.79
Crude protein %	17.5	7	24.33
Crude fat (lipid) %	3.15	50.9	4.3
Crude fibre %	0.8	4.6	4
Ash content %	1.2	6.9	6
Carbohydrate %	87.62	31.25	61.37
Caloric value (kcal)	448.83	60.53	381.5
Magnesium ppm	5.48	7.434	7.17
Calcium ppm	7.01	22.5	32.5
Iron ppm	1.5	4	3.318
Copper ppm	0.39	0.9	0.733
Zinc ppm	1.07	2.9	2.275
Potassium ppm	38.56	640	560
Sodium ppm	15.57	160	140
Lead ppm	0.03	-	-
Oxalate mg/100g	209	325.6	352
Hydrocyanide mg/100g	540	17.28	15.12
Tannin mg/100g	100.74	454.44	340.755
Phytic acid mg/100g	238.26	-	-

Source: *Etim (2000); **Ekop and Eddy (2005a,b)

Weighed samples were transferred into a hot air-drying oven (Gallen Kamp) set at a temperature 80-100°C for 48 hour-drying to constant weight. The outer shell of the seed was removed and the seeds ground into fine powder with a food blender (without metal contamination). The ground sample were stored in an air-tight labeled plastic container from which samples were removed for chemical analysis.

The hydrocyanide, tannin, oxalate and phytic acid content of the seeds were determined by titrimetric and colorimetric methods as described by Kirshna and Ranjhan (1980).

Sodium and potassium content of the seeds were determined by wet acid digested samples using flame emission spectrophotometry (A.O.A.C. 1984). Calcium, magnesium, iron, copper, zinc and lead were quantitatively determined from the digest using the Perkin Elmer Model 2280, Atomic Absorption Spectrophotometer with appropriate hollow cathode lamps. Accuracy was assessed by analyzing the samples in triplicates.

In the determination of the proximate composition of these seeds, the methods recommended by A.O.A.C (1984) were used.

Results

The results of the determination of the proximate, elemental and antinutients compositions of *G.africanum* seeds are presented in Table 1, 2 and 3 respectively.

Discussion

Comparing the chemical composition of the seeds of *G.africanum* with other edible vegetable seeds (presented in Table 4) shows that the seed is rich in carbohydrate with low crude fibre, ash and lipid content. The relatively low moisture contents of 31.60±0.07% promises a long shelf life for the plant seed before cultivation. Crude protein and crude fat are lower than what is obtained in most vegetable seeds. The low ash content of 1.12 correlated positively with the elemental composition of the seed ($P > 0.05$) implying that these seeds are not good source of mineral nutrition.

The crude protein content of 17.50% for the seeds is higher than the literature value of 5.6% recorded for its leaves. It is also higher than what is present in most vegetable seeds except legumes. For instance, fluted pumpkin leaves has an average of 7.00% and it is cherished by most people as being affordable source. Plant protein still remains a veritable source of food nutrient for the less-privileged population in developing countries, including Nigeria where the cost of animal protein is beyond their income per capita.

This research has uncovered *G.africanum* seeds to be a promising alternative source of food nutrient which hitherto had been ignored. The result of the elemental composition of afang revealed the seed to be a relatively poor source of all the mineral elements determined. It also has a low fat content compared to most tropical seeds. Higher values for mineral elements and crude fat are recorded in literature for other vegetable seeds as shown in Table 4. The need for supplementary diets rich in mineral content is necessary for a singular ration, to avoid metal deficiency syndrome like ricket and calcification of bones, as a result of calcium deficiency.

Distorted enzymatic activity and poor electrolyte balance of the blood fluid are related to inadequate Na, K, Mg and Zn; as they are the most required elements of living cells. Consumption of about 20 seeds of *G.africanum* a day may meet the daily requirements for poultry birds. Recommended daily requirement of 2.500- 3.00kcal per 100g weight have been documented (Maynard, 1997).

Table 3 shows the anti-nutritional factors of the studied seed. Values for all the four parameters are higher than tolerable limit permissible for children. Oxalate, 209.00; HCN, 540; Tannin 100.74 and phytic acid, 238.26mg/100g recorded for the seed is higher than 67.83, 5.40, 51.2 and 230mg/100g recorded respectively for afang leaves (Ifon and Bassir, 1980). On comparison, these toxic substances in the seed of *G.africanum* are also higher than those obtained for fluted pumpkin and M.*ureans* as shown in Table 4.

High level of HCN has been implicated for cerebral damage and lethargy in man and animals. Oxalate can complex with most essential trace metals therefore making them unavailable for enzymatic activities and other metabolic processes. Tannins are capable of lowering available protein by antagonistic competition and can therefore elicit protein deficiency syndrome, 'kwashiorkor'. Phytic acid has complicated effect in human system including indigestion of food and flatulence (Maynard, 1997). These relatively high anti-nutritional factors present in the afang seed studied in this work need not pose any threat of toxicity, because they can easily be detoxified by soaking, boiling or frying as rightly noted by Ekop *et al.* (2004); Eka and Osagie (1998); Ekop and Eddy (2005b); Ifon and Bassir (1980) among other researchers that most plant toxicants are drastically reduced to tolerable limits by proper processing. Total hydrogen cyanide in particular which is often present in food items both as free oxygen cyanide and bound cyanogenic glycoside from enzymatic hydrolysis of decarboxylated amino acids can be greatly reduced by boiling for more than 30minutes (Ekop and Eddy, 2005a).

Table 4 also shows the chemical composition of two other vegetable seeds namely; fluted pumpkin (*T.occidentalis)* and *Mucuna ureans* alongside the studied *G.africanum* seeds for easy perusal and fast casual comparison. It appears significant to note that *G.africanum* has the highest carbohydrate content of 84.62% which is significantly ($p<0.05$, $n = 4$) higher when compared with *M.ureans* (61.37%) and fluted pumpkin seed (31.25%). It should be remembered that most plant seeds are very poor source of carbohydrate contrary to the observation for afang seed. The sense of satiety experienced after eating 'afang soup' might be attributed to its high caloric value of 448.83kcal/100g. (Computed from its protein, fat and carbohydrate values by 4:6:4 factors respectively).

Conclusion: This research finding revealed *G.africanum* (afang) seeds as containing high percentage carbohydrate of 87.62 which is quite unique when compared with other vegetable seeds. The seed therefore promises a good nutritive supplementary source for rodents (rabbit) husbandry. Its mineral composition is relatively low and needs to be supplemented when utilized in isolation.

The results of this investigation also show that *G.africanum* seed contains substantial amounts of anti-nutrients. The preponderance of these toxic substances is presumed to be the main reason why the seeds of this popular vegetable is not consumed as is done to fluted pumpkin seeds except for medicinal purposes. Pretreatment and proper process of *G.africanum* seed is advocated before its incorporation into food formulation for animal and man. This work has provided research data which hitherto was very scanty about afang seed. Its high food caloric value of 448.83kcal/100g makes more research into its amino acids profile and characterization of its lipid content quite compelling.

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Comparing the Effects of Sodium and Potassium Diet with Calcium and Magnesium Diet on Sex Ratio of Rats' Offspring

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Abstract: Sex determination has scientific basis for prevention of genetic sex linked diseases in addition to social backgrounds. There are many methods for sex determination. This study was designed to investigate the effects of adding different ions to the drinking water of rats on determination of rats' offspring sexes. In total, 72 female Vistar rats were chosen and randomly divided into three groups. Group one (NaK) was supplied with sodium and potassium diet, group two (CaMg) was supplied with calcium and magnesium diet, and group (C) as the control group. After 15 days of special diet, the rats were mated with male rats and were separated after pregnancy. The newborn rats' sexes were determined after delivery. The data were entered and analyses by SPSS software using t. test. In CaMg group, 66 male and 97 female rats were born (sex ratio = 0.68), while these rates were 91 male and 73 female (sex ratio = 1.25) in the NaK group. In the control group, 72 male and 75 female rats were born (sex ratio = 0.96). The difference in the sex ratio between NaK and CaMg group was statistically significant (p-value = 0.007). While the differences between NaK group with control group (p-value = 0.251) and between CaMg group with control group (p-value = 0.133) were not statistically significant. Our results suggest that different amount of ions in the diet of rats can have significant effect on the sex ratio of delivered rats' offspring.

Key words: Sodium, potassium, calcium, magnesium, sex ratio, vistar rat

Introduction

Sex determination has been the subject of many human studies for a long time. Advances in genetic showed that some genetic diseases are sex linked and only occurs in certain sexes. Therefore, sex determination found scientific basis in addition to social backgrounds.

The sex is determined by the genetic elements of the sperm, but it is unclear that which factors decide whether a sperm carrying a Y or an X chromosome will fertilize the egg. It is believed that sperm carrying a Y chromosome had higher motility while sperm carrying an X chromosome are believed to be more resistant.

Overall, in human population, the ratio of male to female at birth is nearly equal to one (1.06). Countries with reliable demographic data report sex ratio at birth (SRB) of around 105 to 106 male births for every 100 female births.

Different race/ethnic groups have different SRB. For example, Blacks have lower SRB than Whites, while Whites have lower SRB than Asians in the U.S., especially Chinese and Filipinos (Jacobsen *et al.,* 1999). These differences are likely due mainly to the genetics background of different ethnic groups.

Human influence can change the SRB. For example, the SRB for the U.S. are biologically normal and are not influenced by human interventions. In contrast, the SRB for Taiwan, China and South Korea are higher than biologically normal, and likely represent various kinds of human interventions (Jacobsen *et al.,* 1999).

There are different methods for sex selection. Overall these methods can be divided into three different kinds;

1. Preconceptual methods such as flow cytometric separation of X and Y sperm are about 85% effective at producing a girl, and 65% effective in producing a boy (Jacobsen *et al.,* 1999; Jimenez *et al.*, 2003).
2. Periconceptual methods are based on the observation that conception close to ovulation is more likely to result in a boy. Other methods include positioning during intercourse, vaginal douching, and so on. The effectiveness of these methods has not been well documented.
3. Postconceptual medically assisted sex selection is possible by employing prenatal testing (chorionic villus sampling, amniocentesis, ultrasound) and termination of pregnancy. In-vitro fertilization (IVF) and preimplantation genetic diagnosis (PGD), provide an alternative which does not require abortion. After IVF, embryo biopsy is performed on Day 3 at the 8-cell stage to remove one or two cells for genetic examination. PGD can be used to detect chromosomal abnormalities (by fluorescence in situ hybridization [FISH]) and single gene disorders such as thalassemia or haemophilia (by DNA probe or polymerase chain reaction [PCR] amplification of specific DNA sequences).

There are many studies trying to investigate the efficacy of theses methods. Experiments on mouse showed that

both pre- and post-conceptional mechanisms of sex ratio distortion may explain, under different conditions, sex ratio deviations at birth (Jimenez *et al.*, 2003; Krackow and Burgoyne, 1997).

Some studies were based on theories that sperm carrying the X or Y chromosome favoured different vaginal pH. Therefore, it has been suggested that the pH of the vagina at the time of fertilization may have a differential effect on X- or Y- sperm and thereby affect the sex of the offspring. While, in one study, rabbit semen was collected, diluted 1:10 with a buffer of pH 5.4, 6.9, or 9.6, and after 20 minutes 0.5 ml of semen-buffer mixture was used for insemination in an ovulation-induced female. Newborn pups were examined both externally and internally for gender. The females inseminated with acidic semen had 6 litters, 50 offspring, with 48% males; those with neutral semen had 8 litters, 48 offspring, with 63% males; and those with alkaline semen had 7 litters, 49 offspring, with 49% males. There was no significant difference in these sex ratios from the expected 50% males (Muehleis and Long, 1976).

Krackow examined the effects of timing of mating and crowding of pregnant females on litter sex ratios in mice. Females either copulated during periods when no other female of the mating unit copulated simultaneously (single mating condition) or when more than one female copulated (multiple matings condition). Two crowding conditions were imposed on the animals: the females were placed into individual cages after mating (isolated condition), or they remained in the group until shortly before littering (crowded condition). The results showed a reduced sex ratio variance under single mating and crowded conditions (Krackow, 1997).

There are also methods which use different food combinations and especial diets to maximize the chance of having a baby with specific sex. The old believe is that eating salty, savoury foods leads to delivering a boy and calcium rich foods to a girl. Some believes that the ratio of the minerals sodium, potassium, calcium and magnesium are important in determination of baby's sex. It was shown that pregnant female house mice maintained on a consistent low-food diet give birth to a lower proportion of males than do control females fed ad libitum (Meikle and Thornton, 1995).

A study was undertaken to assess whether the use of clomiphene citrate in conjunction with albumin-separated sperm would alter the sex ratio towards females and, if so, whether this skewing was due solely to the induction of ovulation. The results showed that clomiphene citrate in conjunction with albumin-separated sperm decreased the sex ratio but this reduction was not exclusively due to induction of ovulation (Silverman *et al.*, 2002).

It is quite likely that, in the feature, parents will be able to use genetic engineering to select the sex of their child by directly manipulating the sex of an embryo. Some might think that this method would be a more ethical method of sex selection than present technologies such as PGD because, unlike PGD, it does not need to create and destroy embryos (Matthew Liao, 2005).

In this experimental prospective study, the effects of adding monovalan ions (sodium and potassium) and divalan ions (calcium and magnesium) to the drinking water of rats on determination of rats' offspring sexes was investigated.

Materials and Methods

In total, 72 female 10 weeks old Vistar rats (purchased from Razi Institute, Tehran, Iran), were chosen and randomly divided into three groups of 24 rats each. Four rats from each group were separated and the following protocol was applied on them. The protocol was repeated six times, with the intervals of two months, to include all the rats in each group. Group one (NaK) was supplied with drinking water mixed with 1% sodium and potassium, group two (CaMg) was supplied with drinking water mixed with 1% calcium and magnesium diet, and group C was chosen as a control group and pure drinking water was given to them. After 15 days, the rats were mated with male rats and were separated after they were pregnant.

Females inseminated in each session were housed together in 26.5 * 20.5 * 13.5-cm plastic cages until 17 days post pregnancy. At this point, pregnant females were housed individually and examined twice a day until parturition. Within the first 12 h after parturition, the number of litters and the gender of pups were recorded. Pups were sexed by means of the ano-genital distance, which is longer in males (Tarin *et al.*, 1999); this was confirmed in later examinations during preweaning development. The data were entered and analyses by SPSS software using t.test and ANOVA. The p-value less than 0.05 was considered as significant.

Results

In group NaK, under sodium and potassium diet, 23 rats out of 24 became pregnant which delivered 164 offspring. Their gender was 91 male and 73 female (55.5% male and 44.5% female). In group CaMg, under calcium and magnesium diet, all of the 24 rats became pregnant and delivered 163 offspring. From all pregnant rats, 66 male and 97 female rats were born (40.5% male and 59.5% female). Finally, in the control group (group C) all of the 24 rats became pregnant and delivered 147 offspring. From all pregnant rats, 72 male and 75 female rats were born (49% male and 51% female).

Overall, the sex ratio in the calcium and magnesium group was 0.68, while this ratio was 1.25 in the group under sodium and potassium diet. In the control group, the sex ratio was 0.96.

The difference between the sex of offsprings between

Table 1: The sex ratio in different groups of studied rats

Group	Total no of offspring	Male offspring		Female offspring		Sex ratio
		no	%	no	%	
NaK	164	91	55.5	73	44.5	1.25
CaMg	163	66	40.5	97	59.5	0.68
Control	147	72	49	75	51	0.96

group NaK and CaMg was statistically significant (p-value = 0.007). While the differences between group NaK with control group (p-value = 0.25) and between group CaMg with control group (p-value = 0.133) were not statistically significant (Table 1).

Discussion

There are many methods for sex determination, that among them specific diet could be the method of choice because of its simplicity, low expenses and the public approval. The American Society for Reproductive Medicine has ruled that it is proper and ethical to help couples to choose the sex of their babies (Gottlieb, 2001).

In societies with heavy preferences for male children, when couples are no longer able to have the number and gender mix of children they desire, they will use various kinds of human interventions to maximize the chances of realizing their fertility desires.

In most societies around 105 boys are born for every 100 girls. This biologically normal level of 105 is likely an evolutionary adaptation to the fact that males have lower survival probabilities than females. Therefore, 105 or so males are required at birth for every 100 females for there to be around equal numbers of males and females when the groups reach the marriageable ages in their twenties.

An abnormally high SRB have been reported in Taiwan, China, South Korea and a few other countries such as India since the 1980s (Park Chai Bin and Cho Nam-Hoon, 1995; Nair, 1996; Poston *et al*., 1997; Arnold, 1997; Clark, 2000). In China since the mid-1980s, the SRB has been on the increase, reaching 114.2 in 1992 (Gu and Roy, 1995) and 116.3 in 1994 (Tianlu, 1997). According to figures published in 2002 in state run Chinese media, 116.86 boys are born for every 100 girls in China (Plafker, 2002).

South Korea shows the highest SRB of 117.2 in 1990. These higher SRBs are likely due to prenatal sex identification followed by sex-selective abortion, and the concealment of female births (Zeng *et al*., 1993).

The disadvantages of sex selection techniques had big media coverage. Therefore, infertility specialists have expressed concern that media coverage of this technique might have raised unrealistic suspicions about its potential to prevent sex linked inherited conditions (Mayor, 2001).

Medically assisted sex selection for non-medical reasons is banned in many countries such as the United Kingdom and Canada. Paradoxically, it is legal to attempt preconceptual sex selection by "natural" means, even if these employ technology developed specifically for this purpose (Jansen, 1998).

Overall many important ethical issues must be addressed about sex selection. For example, sex selection may be beneficial to the child born if parents will treat a child of that sex more favourably. Sex selection might also cause psychological harm if the procedure does not produce a child of the desired sex. The other issues include; the importance of parent-child relationships, couple relationships, the right of individuals or couples to choice and personal desires (Hollingsworth, 2005). Concerns about these issues should be addressed by scientific investigation. In addition, the mutagenic risks of the sex selection procedure to children born must be evaluated (Benagiano and Bianchi, 1999).

Public opinion about the sex selection is another important issue which was the subject of many researches so far. In order to assess opinions and concerns about preconception sex selection for non-medical reasons, a social survey has been conducted in Germany on 1005 men and women 18 years and older. The results showed that only a minority of 11% approved of the use of preconception sex selection for non-medical reasons, while most of them were against it. In total, 87% of respondents believed that 'children are a gift and deserve to be loved regardless of any characteristics such as beauty, intelligence or sex', 79% argued that choosing the sex of children is 'playing God' and 76% were opposed because it is seen as 'unnatural' (Dahl *et al*., 2004). Similarly, an opinion poll of more than 2000 people in the United Kingdom showed that more than 80% of respondents were against the use of sex selection techniques for non-medical reasons (Kmietowicz, 2003).

Various methods now exist for attempting to choose to have a baby of a desired sex.

With the recent advent of flow cytometric separation of X and Y sperm and PGD, couples no longer have to employ abortion to select sex. PGD offers the only sure way of determining the sex of the offspring. This method is expensive and also requires the use of IVF. Currently, the most common reasons to request sex selection is family balancing and in case of certain genetic diseases (X-linked diseases) and the diseases that are not sex linked but are more common in boys such as autism. (Ralph, 1997).

Most of the non-invasive methods involve making the conception environment more attractive for specific type of sperm. Among different methods for sex selection, specific diet is the method of choice because of its simplicity, low expenses and the public approval. Our results suggest that different amount of ions in the diet of rats can have significant effect on the sex ratio of delivered rats' babies. It seems that relative excess of sodium and potassium ions in the diet would favour the birth of males, while relative excess of calcium and magnesium ions in the diet would favour the birth of females. In a similar experiment on sows to check that if mineral imbalance in the diet of the female before fertilization affects the sex ratio of the progeny, out of a total of 677 births, the sex ratio was 55.7 with the sodium and potassium diet and 48.3 with the calcium and magnesium diet (Bolet *et al*., 1982). These results show that sodium and potassium diet is in favour of male birth, which is similar to our results. More studies on more cases and different species are needed to confirm these results. In one study on human, the intervention of ions in ovarian metabolism was obtained by controlling the diet of the woman; the decisive factor was the ratio of sodium and potassium to calcium and magnesium in the daily diet. High values of this ratio lead to boys, and low values to girls. Using this method since 1970 in 47 births, only 7 of them failed to produce the expected sex (Stolkowski and Choukroun, 1981). These results were confirmed by another study on 281 couples, who adhered to a diet specific in its amounts of calcium, magnesium, sodium and potassium. This dietary method of preconceptional selection of fetal sex resulted in the anticipated outcome in about 80% of the cases (Stolkowski and Lorrain, 1980).

In conclusion, our study showed the importance of special diet with the right proportion of different ions on determination of sex ratio in rats. Because of the safety of this method before and during pregnancy, it can apply to pregnant women to investigate the effects of different ions on the sex ratio of human beings.

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Socio-Economics Characteristics and Food Security Status of Farming Households in Kwara State, North-Central Nigeria

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Abstract: In recent times, the global focus has been on food security and poverty alleviation. This is in response to increasing food insecurity and poverty in the world. The incidences of food insecurity and poverty are particularly devastating in the developing countries and a lot of resources are being channelled towards programmes aimed at eradicating food insecurity and poverty by various international organizations and government of the developing nations. In terms of food insecurity, 852 million people worldwide are still chronically undernourished. In Africa, an estimated 200 million or 27.4% of the people on the continent are undernourished. This figure is expected to increase to 30% by 2010. In Nigeria, over 40% of the estimated population of 133 million people are food insecure. To achieve the Millennium Development Goals of halving the proportion of hungry people by 2015, it was projected that 22 million people must achieve food security every year. In consonance of the above, this study examined the socio-economic characteristics and determinants of the food security status of rural farming households in Kwara State of Nigeria. The study utilized a three-stage random sampling technique to obtain a sample of 94 farm households and a cross sectional data in year 2005. Descriptive analysis was carried out to describe the socio-economic characteristics of the households. Econometric tools were used to determine factors affecting the food security status of household. Using the recommended calorie required approach; the study revealed that 36% and 64% of the households were food secure and food insecure respectively. The Shortfall/Surplus index showed that the food secure households exceeded the recommended calorie intake by 42%, while the food insecure households fell short of the recommended calorie intake by 38%. A logit regression model made up of eight regressors was specified. Household income, household size, educational status of household's head and quantity of food obtained from own production were found to determine the food security status of farming households in the study area. It is concluded that the design of food security strategies should be multi-dimensional such that would focus on and address the identified determinants in order to achieve the target set by the Millennium Development Goals.

Key words: Farming household, food security, food insecurity, logistic regression, north central Nigeria

Introduction

In the last decade, attention has been focused on means of eliminating food insecurity and hunger worldwide. The 1992 International Conference on Nutrition and the 1996 World Food Summit both stressed the urgent need to reduce food insecurity and hunger. The 1996 World Food Summit specifically brought back to center-stage in the development debate, the issue of hunger and food insecurity as both the cause and effect of poverty and slow growth. In the wake of this new push, the Millennium Development Goals were launched bringing the international communities to work together to achieve the set goals by 2015 (Migotto *et al*., 2005). The first Millennium Development Goal is to eradicate extreme poverty and hunger. The targets here are to halve between 1990 and 2015, the proportion of people who suffer from extreme hunger and people whose income is less than $1 a day (FAO, 2005). According to FAO (2005), the achievement of these targets is very important to reducing hunger and poverty. This is because it is believed that hunger perpetuates poverty by reducing productivity and poverty in turns prevents people from producing or acquiring the food they need. Less than 10 years to the target year, available statistics still cast doubt on whether this goal could be achieved by 2015.

A progress monitoring report released by FAO (2005), indicated that even though most Millennium Development Goals could be achieved, eliminating hunger and poverty is a pre-requisite for achieving all the other targets of the Millennium Development Goals. Although, the percentage of hungry people in the world has fallen between 1981 and 2001, an estimated 852 million people worldwide are still chronically undernourished; among them are 170 million children under 5 years of age (IFPRI, 2005). The MDGR (2006),

Table 1: Prevalence of under nourishment in the world, 1990-92, 1995-97 and 2001-03

Sub-regions	1990-1992	1995-1997	2001-2003	2015 target
Sub-Saharan Africa	33	34	31	18
Southern Asia	25	23	21	14
CIS, Asia	16	N.A	20	9
Eastern Asia	16	12	12	9
South East Asia	18	14	12	10
Oceania	15	14	12	8
Latin America and Caribbean	13	11	10	7
Western Asia	6	9	9	4
Northern Africa	4	4	4	3
CIS, Europe	4	N.A	3	2.8

Source: MDGR (2006), CIS is Commonwealth of Independent States; N.A is data not available

reported that in 1990, more than 1.2 billion people (28%) of the developing world's population lived in extreme poverty. By 2002, the proportion decreased to 19%. During that period, rate of extreme poverty fell rapidly in Asia, where the number of people living on less than $1 a day dropped by nearly a quarter of a billion people. Progress was not so rapid in Latin America and the Caribbean, which has a larger share of people living in poverty than south-eastern Asia and Oceania. Poverty rates in Western Asia and Northern Africa remained almost unchanged between 1990 and 2002 and increased in the transition economies of southern-eastern Europe and the Commonwealth of Independent States (CIS). These two regions had previously nearly eradicated the worst form of poverty, and survey data suggest that their poverty rates are again dropping.

In Sub-Saharan Africa, although the poverty rate declined marginally, the number of people living in extreme poverty increased by 140 million. Many Sub-Saharan African countries are now showing potentials for long-term growth that could bring up the standard of living (MDGR, 2006). Chronic hunger, measured by the percentage of people lacking the food needed to meet their daily needs has declined in the developing world. However, overall progress is not fast enough to reduce the number of people going hungry, which increased between 1995-1997 and 2001-2003. An estimated 824 million people were affected by chronic hunger in 2003. The worst affected regions were Sub-Saharan Africa and Southern Asia. These regions though have made progress in recent years, their advances have not kept pace with those of the early 1990s and the number of people going hungry is increasing. Of particular concern is Eastern Asia; in the early 1990s, the number of hungry people declined, but again is on the rise (MDGR, 2006). Table 1 shows the proportion of people living in extreme hunger compared with the 2015 target of the Millennium Development Goals across different sub-regions of the world.

In many African countries, food security at both the national and the household level is dismal. Though there are more undernourished individuals in India alone than Africa, it is in Africa that one finds the highest prevalence of under nourishment. Whereas 14% of the Global population is undernourished, 27.4% of the population of Africa as a whole are undernourished (FAO, 2003). In more than a dozen countries, the rate of under nourishment is above 40% while it exceeds 50% in those countries experiencing or emerging from armed conflicts (Todd, 2004). In West Africa sub-region, about 16% of the people are undernourished. The proportion is lower than the regional figure of 27.4%, however, two countries of the West African sub-region-Liberia and Sierra Leone[1] are amongst those with the highest rate of under nourishment in the continent. Table 2 shows the prevalence of under nourishment among West African countries.

In Nigeria, the percentage of food insecure households was reported to be 18% in 1986 and over 40% in 2005 (Sanusi *et al.,* 2006). Although, figures released by Food and Agricultural Organization in 2005 on the state of food insecurity in the world, indicated that 9% of Nigerian population was chronically undernourished between 2000 and 2002 (FAO, 2005). This was less than the regional average of 33% for Sub-Saharan Africa. However, the 9% or about 11 million undernourished Nigerians translate to about 5.4% of total number of undernourished people in Sub-Saharan African as a whole. On the national level, per-capita growth of production of major food items in Nigeria has not been sufficient to satisfy the demand of an increasing population. The result is a big gap between national supply and demand for food. Several reports have been published that show a consistent increased in the production of staple food in the country especially between 1999 and 2005, but there is still an observable gap between food demand and food supply (Sanusi *et al.,* 2006).

The socio-economics characteristics and resources of individual households have been identified as basic factors influencing the food security status of households (Sanusi *et al.,* 2006). Rural households continue to face poor economic conditions which impact on their living standard and food security situation. The returns to land in terms of output have been on the decrease especially where increased population and non-agricultural uses compete for land use. This further creates gaps in resource availability among the poor households. The impact of this is that the food situation gets worse; farms are being abandoned to the elderly or for off-farm jobs. The income from off-farm activities has not been proven to be adequate to meet households' needs (Akinsanmi and Doppler, 2005). This situation requires that the socio-economic conditions are known for a guided change to take place. The particular factors which affect households differently must also be examined and understood. The aim of this paper is to

Table 2: Prevalence of under nourishment among West African countries

Countries	No. of under nourished People (in million)		Proportion of under nourished people in total population	
	1990-1992	2000-2002	1990-1992	2000-2002
Sub-Saharan Africa	170.4	203.5	36	33
West Africa	37.2	36.4	21	16
Benin	1.0	0.9	20	15
Burkina Faso	1.9	2.3	21	19
Cape Verde	N.A	N.A	N.A	N.A
Cote d'Ivoire	2.3	2.2	18	14
Gambia	0.2	0.4	22	27
Ghana	5.8	2.5	37	13
Guinea	2.5	2.1	39	26
Guinea Bissau	N.A	N.A	N.A	N.A
Liberia	0.7	1.4	34	46
Mali	2.7	3.6	29	29
Mauritania	0.3	0.3	15	10
Niger	3.2	3.8	41	34
Nigeria	11.8	11.0	13	9
Senegal	1.8	2.3	23	24
Sierra Leone	1.9	2.3	46	50
Togo	1.2	1.2	33	26

Source: FAO, (2005); N.A means data not available

examine the socio-economics characteristic and food security status of rural farming households in Kwara state, north central zone of Nigeria. The objectives of the paper are to; (1) Measure the food security status of farming households using the 7-days calorie intake recall method. (2) Identify the determinants of food security among farming households. (3) Suggest measures that could help to reduce the level of food insecurity in the area.

Food insecurity in Nigeria: Among the developmental problems facing Nigeria, food insecurity rank topmost. The level of food insecurity has continued to rise steadily since the 1980s. It rose from about 18% in 1986 to about 41% in 2004 (Sanusi *et al.,* 2006). The daily per capita calorie supply as a proportion of recommended requirement was 90% between 1988-90 and 85% between 1992-96 (FOS, 1999). According to FAO (2000), Nigeria was able to reduce the prevalence of under nourishment by more than 30% between 1979-81 and 1996-98. The prevalence dropped from 44% to 8% between these periods[2]. However, the average per capita daily calorie intake remained 2050 kcal during the 1979-81 periods while the diet comprised of 64% cereals and root and tubers (Agboola *et al.,* 2004). National food expenditure data showed that almost two thirds of total expenditure of households in 1980 was on food. This food share rose by about 10% points by 1985, but dropped during the period 1985-1992. In subsequent four year period, 1992-1996, a further drop of 5% points took place. The figures were 63.4%, 74.1%, 72.8% and 63.6% for 1980, 1985, 1992 and 1996 respectively. Also, trends in poverty reveal that the incidence of poverty increased sharply both between 1980 and 1985 and between 1992 and 1996. The figures were 27,2%, 46.3%, 42.7% and 65.6% for 1980, 1985, 1992 and 1996 respectively. The figure for 1996 was translated to 67.1 million (Agboola *et al.,* 2004). The overall national average household income in 1996 prices indicate a very significant downward trend, moving from N13, 454 in 1980 to N6, 252 in 1996, over 50% reduction. The average household in the rural areas earned N5,590 (FAO, 2000).

Agriculture is one of the most important sectors of the Nigerian economy. This is because it contributes more than 30% of the total annual GDP, employs about 70% of the labour force, accounts for over 70% of the non-oil exports and, perhaps most important, provided over 80% of the food needs of the country, (Adegboye, 2004). Given the role of agriculture in the Nigerian economy, food insecurity and poverty could be attributed to the poor performance of the agricultural sector, which in turns creates food availability and accessibility problems at the household and national levels. In other words, the poor performance of the sector directly creates supply shortages and indirectly creates demand shortages by denying the households access to sufficient income. As the food situation in Nigeria worsened after the 1960s, a number of agricultural development institutions were set up and special programmes and projects were launched, these include: National Accelerated Food Production Programme, NAFPP (1973); Agricultural Development Project, ADP (1975); Operation Feed the Nation, OFN (1976); River Basin Development Authorities, RBDA (1977); National Seed Service, NSS (1977); Agricultural Credit Guarantee Scheme, ACGS (1977); Rural Banking Scheme, RBS (1977); Green Revolution, GR (1979); Directorate of Food Road and Rural Infrastructure, DFRRI (1986); National Agricultural Land Development Authority, NALDA (1992); National

Fadama Development Project, NFDP (1992); Nigerian Agricultural Cooperatives and Rural Development Bank, NACRDB (2000); National Agricultural Development Fund, NADF (2002); National Special Programme on Food Security, NSPFS (2002); Commodity Marketing and Development Companies, CMDC (2003). However, according to Idachaba (2004), empirical records of many of these programmes and projects are not impressive enough to bring about the expected transformation of the sector.

Recently however, Nigeria made some progress in the areas of per capita daily calorie intake and the proportion of under nourished people. The average national per capita daily calorie intake increased from 2050 kcal in 1979-81 to 2430 kcal in 1989-91 and to 2700 kcal in 2000-02. Though cereals and root and tubers accounted for 65.3% of the diet in 2000-02 compared to 64% in 1979-81 period (FAO, 2004). The figure represents an 11% increase in per capita daily calorie intake between 1991 and 2002. Also the proportion of under-nourished people decreases from 13% in 1990-92 to 9% in 2000-02 (FAO, 2005). The poverty level according to Kpakol (2005), also fell from 70.8% in 2003 to 54% in 2005.

Conceptual framework of household food Security: Food security has been defined as a situation when all people, at all times, have physical and economic access to sufficient, save and nutritious food needed to maintain a healthy and active life (FAO, 1996). This definition implies that food security is a broad concept that is more than food production and food accessibility. In reality it revolves round four pillars namely, food availability, food accessibility, nutritional factors and stability of food supply (Gross *et al.,* 1999). Fig. 1 shows the elements of food and nutrition security. The implication of this definition is that, achieving food security requires that the aggregate availability of physical supplies of food is sufficient, that households have access to those food supplies through their own production, through the markets (given sufficient purchasing power) or through other sources, and that the utilization of those food supplies is appropriate to meet the specific dietary needs of individuals households or individuals in the households. Fig. 2 shows the framework of food and nutrition security. Food accessibility is ensured when all households and all individuals within those households have sufficient resources to obtain appropriate foods for a nutritious diet. It is dependent on the level of household's resources-capital, labour and knowledge and prices.

Materials and Methods

The study area: This study was conducted in Kwara state in the north-central zone of Nigeria. Nigeria, presently made up of 36 states is divided into six geo-political zones for political, agricultural, industrial and educational planning. These zones are; north central[3], northwest, northeast, southwest, southeast and south. The north-central zone is under the moist savannah agro-ecological zone. The state lies between latitude 7° 15′ and 6° 18′ N of the equator. The state shares boundaries with Osun, Oyo, Ondo, Kogi, Niger and Ekiti states. It shares an international boundary with the Republic of Benin. The state presently comprises of sixteen Local Government Areas. A humid tropical climate prevails over the state and it has two distinct seasons; the wet and dry seasons. The wet season last between April and October during which there is rain and the dry season with no rain is between November and March. The rainfall ranges between 50.8mm during the driest months to 2413.3mm in the wettest months. The minimum average temperature throughout the state ranges between 21.1°C and 25.0°C while, maximum averages temperature ranges from 30°C to 35°C. The state is primarily agrarian with great expanse of arable land and rich fertile soils.

The typical cropping systems in the state are maize-based system, yam-based system, cassava-based system and rice cultivation in areas located along river Niger, the major river in the state. The major crops cultivated in the state include yam, maize, cassava, groundnut, cowpeas, sorghum, melon, okra, pepper and some leafy vegetables. Majority of the food produced are eaten, while some households sell small amount of the food in the market to earn additional income for household upkeep. Some households grow cash crops such as cashew, palm oil and rice (KWADP, 1996).The total estimated population of the state is about 2.2 million people in 2004 out of which farmers account for about 70%. The state has a total land area of about 32,500km^2, which is about 3.5% of the total land area of the country, which is put at 923,770km^2 (FAO, 1995). Approximately 25% of the land area of Kwara State is use for farming. The average population density of the state as at 2004 was about 68 people per square kilometer. Agricultural production is largely peasant and small-scale relying heavily on the use of manual labour equipped with crude implements, while fertilizers, mechanical implement, improved seeds and agrochemicals are also used to some extent. Landholding in the state is very small and most of the households have less than two hectares of land for farming. The output from this land is low and most households have to buy food when what they produce from their own land is finished. Some of the rural households also engage in off-farm wage or self-employment to supplement their household's income.

Data source and sampling procedure: The data for this study were obtained from a sample survey of farming households conducted in 2005 in Kwara State of Nigeria. A three-stage random sampling technique was

used to select a sample of 94 farming households from twelve villages across four local government areas of Kwara State. The survey instrument was designed to gather general information about household's characteristics, food consumption and expenditure and non-food consumption and expenditure.

Analytical techniques and variables measurement: To identify the determinants of the food security status of farming households, we carry out two stages of analyses; one, we constructed a food security Index (Z) and determine the food security status of each household based on the food security line using the recommended daily calorie required approach and second, we used the Logit regression model to estimate the food security status of households as a function of a set of independent determinants. A household whose daily per capita calorie intake is up to 2260 kcal was regarded as food secures and those below 2260 kcal were regarded as food insecure households.

$$Zi = Yi / R \quad (1)$$

Zi = food security status of ith households which take values 1 for food secure households or 0 for food insecure households.
Yi = daily per capita calorie intake of ith household
R = recommended per capita daily calorie intake (2260Kcal)
Zi = 1 for Yi greater than or equal to R
Zi = 0 for Yi less than R

Based on the household food security status (Z), the Logit model was estimated to identify the determinants of food security among farm households. The implicit form of the model was expressed as:

$$Zi = \beta Xi + Ui \quad (2)$$

Zi = the food security status of ith household
Xi = vector of explanatory variables
Ui = the error term
β = vector of the parameter estimates

The dependent variable and the explanatory variables that were included in the model are:

Food security: Two objective methods of food security measurement have been widely used in most food security studies (Maxwell, 1996). One is to estimate gross household production and purchases over time, estimate the growth or depletion of food stocks held over that period of time and presume that the food that has come into the households possession and "disappeared" has been consumed. The other method is to undertake food consumption recall for individual members of a household or for the household as a whole and analyze each type of food mentioned for calorie content. In this study, a 7-day recall method was used. The food security line was the recommended daily per capita calorie intake of 2260 kcal. The household's calorie intake was obtained through the household's consumption and expenditure data. From the data we estimated the quantity of every food items consumed by the households in the 7 days period. The quantities were converted to gram and the calorie content was estimated by using the nutrient composition table of commonly eaten food in Nigeria (Appendix 1). Per capita calorie intake was calculated by dividing estimated total household calorie intake by the family size after adjusting for adult equivalent using the consumption factors for age-sex categories (Appendix 2). To get the household's daily per capita calorie intake we divided the household's per capita calorie intake by seven. A household whose daily per capita calorie intake is up to 2260 kcal was regarded as food secure and those below 2260 kcal were regarded as food insecure households. The food security status is bivariate, taking the value 1 for food secure households and 0 for food insecure households.

Household income (X_1): This refers to the sum total of the earnings of the household in a year from farm and off-farm sources. The income is expected to boost household's food production and also access to more quantity and quality food. The expected effect of this variable (β_1) on food security is positive

Farm size (X_2): Farm size is the total farmland cultivated by the household measured in hectares. The larger the farm size, the higher the production level. It is thus expected that households with larger farm size are more likely to be food secure than those with smaller farm size. The expected effect on food security (β_2) is positive.

Membership of cooperatives (X_3): Cooperatives are vehicle for development in the rural areas. Access to cooperative loans depends on membership of the society and it is expected that access to credit should increase household's income, food production and food consumption. Membership of a society = 1 and non-membership = 0, the expected effect on food security (β_3) is positive.

Quantity of food from own production (X_4): This is the total quantity of food output by the household from their own farm measured in kilogram grain equivalent. It consists of both food and cash crop outputs. Cash crops are included because, money realised from their sale could be used to buy staple food for household's consumption. The expected effect on food security (β_4) is positive.

Access to consumption credit (X_5): This is the ability of the household to obtain credit for household's consumption. This could be from cooperatives, government, friends and relatives and moneylenders. Consumption credit could increase household income in the short run and could allow it to possess and consume more food. Households that have access to consumption credit in the last one year are coded =1 and those without access = 0, the expected effect on food security (β_5) is positive.

Age of household head (X_6): The age of household's head in year is expected to have impact on his labour supply for food production. It is also expected to have impact on ability to seek and obtain off-farm jobs and income, which could increase household income. Young people are stronger and are expected to cultivate larger-size farm than old people. However, the expected effect of age on food security (β_6) could be positive or negative.

Educational status of household head (X_7): Education is a social capital, which could impact positively on household ability to take good and well-informed production and nutritional decisions. Some scholars have argued that spouse education could be more important in food security than household's head educational status. Household head that are educated = 1 and those not educated = 0, the expected effect on food security (β_7) is positive.

Household size (X_8): The number of adult individual members in the household measures household size. Since food requirements increase with the number of persons in a household, the expected effect (β_8) is negative.

Additionally, the shortfall/surplus index and the headcount ratio of food security were calculated for the sampled households based on the food security line. The shortfall index (P) measures the extent to which poor households are food insecure while the headcount ratio (H) measures the percentage of the population of household that are food insecure/secure.

$$\text{Shortfall Index (P)} = \frac{1}{M}\sum_{i}^{m} G_i \qquad (3)$$

Where:

M = number of food insecure households

Gi = per capita calorie intake deficiency for ith household

$$G_i = (Yi - R) / R \qquad (4)$$

$$\text{Headcount Index (H)} = M / N \qquad (5)$$

N = the number of households in the sample

Results and Discussion

Socio-economic description of farm households: The human resources available to the farm household determine the minimum level of subsistence and cash requirement (Akinsanmi and Doppler, 2005). The educational level in the area is low which limits opportunity for better off-farm jobs. 52.1% have one form of education or the other ranging from primary education- 10.6%, secondary education -4.3%, Arabic education -37.2%. About 47.9% of the household head have no education at all. The summary of the socio-economic characteristics of the household is presented in Table 3.

The household size could have great implications for labour supply for farm work and also food security. A large household is expected to cultivate large farm size, but the contrary is also possible especially when there are many children dependants and elderly people in the family. The average household size was 7.34 and more than half of the households have between 1 and 5 people. In a country where access to land by women is still very low, the few household's head that were female could be facing problems in securing agricultural land. The average age of household's head was 50 years and 11% of the heads were over 65 years of age. This result probably shows that majority (89%) of the farming population were young and in the active age group. Nonetheless, this was not reflected in the average farm size cultivated as 97.5% of the farmers cultivated between 0.1 and 2.5 hectares of land. Only 2.5% cultivated more than 2.5 hectares.

Access to consumption credit and membership of cooperatives are factors that could increase household's income *ceteris paribus,* however, the result shows that only 14.8% of the farm households had access to consumption credit and 6.4% were member of cooperatives. This situation has negative impacts on household's income and food demand in the short and long run. The total household income was converted into average monthly income and the mean was N5,180 which is equivalent to about $43. This translates to $1.4 per day and $0.2 per adult person per day in the area[4].

Food security indices of households: Based on the recommended daily calorie intake (R) of 2260 Kcal, it was observed that 37.2% of the households were food secure and 62.8% were food insecure. Table 4 presents the summary statistics and food security indices among the sampled households.

Table 4 shows that the study area could be regarded as food insecure given the fact that only 36% of the population were able to meet the recommended calorie intake of 2260 Kcal per capita per day, while 64% could not. The shortfall/surplus index (P) which measures the extent of deviation from the food security line, show that the food secure households exceeded the calorie

Table 3: Descriptive Statistics of Farm Households in the Study Area

Variables	Frequency (N = 94)	Percentage
Gender of household head		
Male	85	90.4
Female	9	9.6
Age of household head		
18 - 65	84	89.0
> 65	10	11.0
Household size		
1 - 5	52	55.1
6-10	31	32.9
>10	11	12.0
Farm size		
0.1- 2.5	92	97.5
>2.5	2	2.5
Education of household head		
Tertiary education	0	0
Secondary education	4	4.3
Primary education	10	10.6
Arabic education	35	37.2
No education	45	47.9
Household income		
N20,000 - N80,000	46	48.8
N80,001 - N200,000	42	44.5
> N200,000	6	6.7
Membership of cooperatives		
Members	6	6.4
Non-member	88	93.6
Access to consumption credit		
Have access	14	14.8
No access	80	85.2

Source: field survey, 2005

Table 4: Summary Statistics of Food Security Indices for the Study Area

Variables	Mean		
Food Security Indices	Food Secure	Food Insecure	All
Recommended per capita daily calorie intake (R) is 2260 Kilocalorie			
Percentage of households	37.2	62.8	100
Number of households	35	59	94
Age of household head	46.6	53.2	50
Household size (Adult equivalent)	6.26	8.98	7.34
Household monthly income	N5,964	N4,215	N5,180
Farm size	1.16	0.87	1.02
Food Security Index (Z)			
Mean	1.41	0.62	0.92
Std	0.35	0.22	0.47
Per capita daily calorie availability	3269	1318	2021
Shortfall/surplus Index (P)	0.42	0.32	-
Head count ratio (H)	0.36	0.64	-

-Source: Computed from Field Survey, 2005

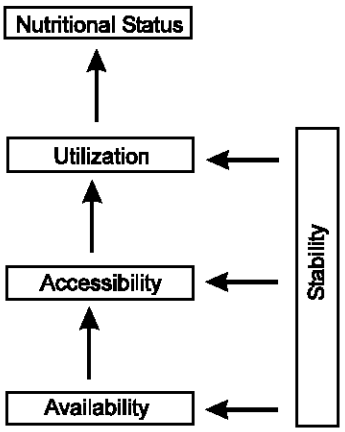
requirement by 42%, while the food insecure households feel short of the calorie requirement by 38%.

Determinants of the food security status of households in the study area: Analysis of the survey data revealed that four out of the eight variables included in the model were significant in explaining the variation in the food security status of households in the study area. These variables are total households income, quantity of food from own production, educational status of household head and household size. The result of the estimation of the Logistic model is presented in table 5.

Table 5: Regression Estimates for Determinants of Food Security Status of Households.

Variables	Coefficients	t-statistics
Constant	-5.060	0.999
Households monthly income	0.488	6.104**
Farm size	-0.498	0.558
Membership of cooperatives	3.959	0.759
Quantity of food from own production	0.001	12.801**
Access to consumption credit	-0.230	0.139
Age of household head	-0.44	1.810*
Educational status of household head	1.334	4.050**
Household size	-0.310	7.069

**Source: Computed from Field Survey Data, 2005; Dependent variable: food security status Asterisks **indicate significant at 5% level;
*indicates significant at 10% level



Source: Gross *et al.*, 1999

Fig. 1: Elements of Food and Nutrition Security

Household income: This variable was positive and significant at 5% level. This indicates that the higher the household income, the higher is the probability that the household would be food secure. This could be expected because increased income, other things being equal, means increased access to food.

Quantity of food from own production: This variable has a low but positive coefficient that was significant at 5% level. This indicates that the higher the amount of food obtained from own production, the higher the likelihood of food security.

Education status of household head: This variable was found to be positive and significant at 5% level. This implies that households with an educated head are more likely to be food secure than one with an uneducated head.

Household size: This variable has a negative coefficient

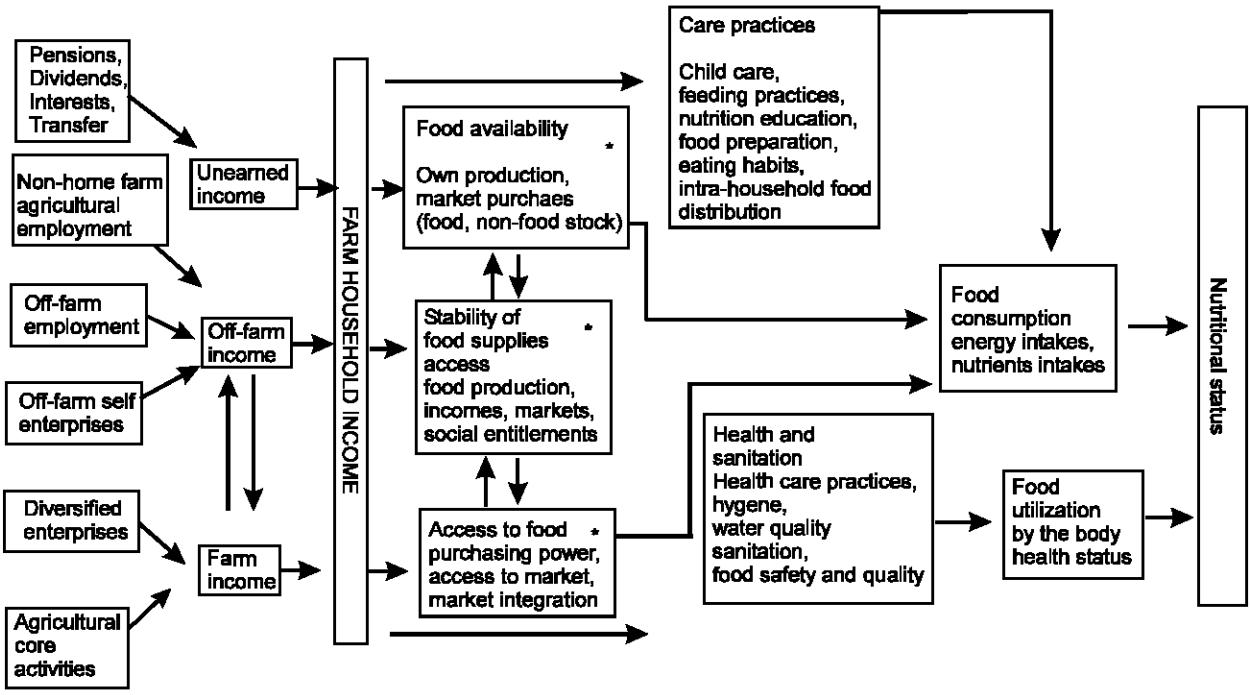


Fig. 2: Conceptual Framework of Household Food Security

that is significant at 5% level, implying that as the household size gets larger, the probability of food security decreases. In another language, large size households are more likely to be food insecure than small size households.

Other variables: The age of the household head, has a negative coefficient that was significant at 10% level. This probably indicates that the older the household head, the lower the probability that the household would be food secure. The coefficient of farm size was negative but not significant. The negative coefficient was contrary to expectation, and this could be due to reason such as inefficiency in the use of land resources. Membership of cooperatives has a positive coefficient, which though not significant, but agrees with a priori expectation. The coefficient of access to consumption credit was negative and not significant. This is not in agreement with expectation and could probably be as a result of non utilization of consumption credit for the purpose it was meant for.

Conclusion and recommendations: This paper has shown that the socio-economic variables of the farm households are important determinants of their food security or insecurity status. The paper showed that household's income, quantity of food from own production, education of household's head and the household's size are important determinants of food security among rural households. It further showed that majority of the households were headed by men (85%) while women headed only 9%. About 89% of the household heads were less than 65 years of age. This was expected to probably allow the young farmers to cultivate large hectare of land, however, the average farm size showed that 97.5% cultivated between 0.1 and 2.5 hectare of land. The average household's size was approximately 7 people and about 44.9% of the household have more than 5 people in their household. Among the 52.1% of the household heads that have education, none had tertiary education and 47.9% had no formal education at all. This low level of education among household has impacts on their nutrition and food security, since the study also showed that household's head education is one of the significant determinants of food security.

Household income, which was identified as one of the significant determinants of food security, was very low in the study area. The average monthly income was N5,180 and this translates to an average adult income of $0.2 per day, even lower than the $1 poverty line. Cooperatives membership was low as only 6% of the households' head were members. Given the role of cooperatives in rural development generally and agricultural development specifically, the introduction and promotion of cooperatives organization should be

Appendix 1: Calorie content of some commonly eaten food items in Nigeria

Food items	Kcal/kg	Food items	Kcal/kg
Staple foods		Mango	590
Cassava Tuber	1500	Pawpaw	300
Cassava flour	3870	Pineapple	320
Cassava chips	3000	Apple	570
Garri	3840	Coconut	580
Yam Tuber	1100	Guava	730
Yam flour	3810	Sugar cane	360
Yam chips	3000	Meat and animal products	
Sweet potato Tuber	1100	Cow meat	2370
Sweet potato chips	900	Goat meat	2370
Irish potato	1200	Sheep meat	2370
Cocoyam Tuber	3830	Pork	2370
Maize green	3100	Bush meat	2370
Maize grain	4120	Chicken	2380
Maize flour	4120	Turkey	2380
Sorghum grain	3500	Fish	2230
Sorghum Flour	3500	Snail	2245
Millet grain	3500	Shrimps	2230
Millet flour	3500	Crayfish	2200
Rice	1230	Crabs	2200
Wheat grain	3400	Eggs (pieces)	1400
Wheat flour	3300	Dairy products	
Cowpea (beans)	5920	Milk	4900
Ground nut	5950	Cheese	4000
Soybeans	4050	Yoghurt	4100
Soybean flour	2600	Ice cream	4100
Melon (shelled)	5670	Beverages	
Plantain	770	Cocoa	1200
Banana	960	Tea (leaves)	1200
Vegetables		Tea (liquid)	1200
Okra	4550	Coffee (powder)	1340
Tomato	880	Coffee (liquid)	1340
Pepper	3930	Drinks	
Onion	440	Soft drinks	620
Carrot	400	Orange juice	400
Egg plant	440	Apple juice	550
Cucumber	270	Pineapple juice	560
Cochorus/ewedu	500	Local beer	740
Spinach	220	Bottled beer	460
Bitter leaf	220	Wine	330
Water leaf	180	Condiments and spices	
Cabbage	230	Maggi	220
Pumpkin	440	Salt	180

Appendix 2: Adult equivalent scale for adjusting the household size

Age category	Male	Female
0 – 1	0.33	0.33
1 – 2	0.46	0.46
2 – 3	0.54	0.54
3 – 5	0.62	0.62
5 – 7	0.74	0.70
7 – 10	0.84	0.72
10 – 12	0.88	0.78
12 – 14	0.96	0.84
14 – 16	1.06	0.86
16 – 18	1.14	0.86
18 – 30	1.04	0.80
30 – 60	1.00	0.82
> 60	0.84	0.74

Source: Stefan and Pramila, (1998)

given adequate priority by government and intervention agencies. Household's access to consumption credit should also be given adequate attention, as 85.2% of the households have no access from any source, to consumption credit.

The food security indices estimated in this study, in our opinion, is a fair representation of the extent and dimension of food security/insecurity in this part of the country. It could serve as reference benchmark with which food security measures elsewhere in the country could be compared-especially against the background of recently launched agricultural programmes such as the National Special Programme on Food Security and the National Fadama Development Projects Phase II. To achieve the Millennium Development Goal of eradicating hunger in Nigeria, it is recommended that food security strategies should be designed in a way that would focus on and address the identified determinants.

Specifically, government and farmers group should provide agricultural inputs to farming households at

affordable prices to be able to increase farm size and food production since own food production was one of the significant determinants of food security in the area. In addition efforts that could boost households' income generation should be promoted. For example, the provision of village infrastructures like motor able road, water, electricity, telephone etc could increase the possibility of off-farm activities that could generate more income for the households.

Enlightment programmes on health education and birth control measures should be directed at the farming households. This is to reduce the risk of consuming unbalanced diets and large family size observed in the area, which could have effects on households' food security. Reducing the negative impact of middlemen who buy food cheaply from farmers but in turn sell at prohibitive price even to the farmers from whom they buy the food should enhance the access to market by rural households. Market access could also be improved by provision of good rural transportation system that would assist farmers to convey their farm produce to the market at cheaper cost.

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[1]Both countries have suffered long-lasting armed conflicts that have undermined the well-being of the people leading to extreme hunger and poverty (Flores, 2004).

[2]An important factor for this was the rapid increase in the supply of cassava products during the period, which benefited mostly the poor and under nourished people.

[3]In the north-central zone, there are six states namely, Kwara, Kogi, Niger, Nassarawa, Plateau and Benue.

[4]As at 2005 and 2006 the exchange rate was N120 to One US dollar

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Hypercholesterolemic Effect of Drug-Type *Cannabis sativa* L. Seed (Marijuana Seed) in Guinea Pig

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Abstract: *Cannabis sativa* L. has two drug and nondrug varieties. Nondrug varieties of *Cannabis* are hemp but drug varieties commonly referred to as marijuana. Marijuana is considered nutritional and narcotic plant. Marijuana has not been studied extensively for its nutritional potential in recent years but whole hempseed typically contains over 30% oil (3%saturated, 28% unsaturated fatty acids) and about 25% protein. The objectives of the present study were to evaluate the effects of whole marijuana seed on lipid profile of guinea pig. This study was experienced on fourteen guinea pigs as case and control groups. In control group, guinea pigs were fed with normal diet while case group had free access to normal diet and marijuana seed for 60 days. At the end of experiment the feeding of animal stopped and after 12hr fasting, the animal anesthetized by ketamine/xylazine combination and 5 ml of blood of heart was taken. The blood parameters were measured by automated biochemical analyzer. Marijuana seed significantly increased total cholesterol and LDL-c levels (p=0.00) while HDL-c and triglyceride levels didn't significantly change. In spite of omega-3, omega-6 and omega-9 fatty acids that highly incorporated in hempseed, short term feeding of marijuana seed has not improved blood lipid profile. It may be due to hidden orexigenic phytocannabinoids that found in drug-type seed. In the light of this research, it is recommended that individuals who are affected to cardiovascular diseases and atherosclerosis, they should not use unclean marijuana seed that cultivated in Isfahan province of Iran in their food preparation on regular basis.

Key words: Marijuana, lipid profile, hypercholesterolemia, tetrahydrocannabinol, guinea pig

Introduction

Cardiovascular disease continues to be the leading cause of morbidity and mortality in the industrial world (Grundy *et al*., 2004). Reducing plasma low-density lipoprotein cholesterol (LDL-c) concentration is a primary strategy for reducing coronary heart diseases (CHD) risk, and the new guidelines for very high risk individuals indicate a LDL-c goal of <1.8 mmol/L (70 mg/dL) (O'Keefe *et al*., 2004; Tavazzi, 1999). It is unlikely, however, that maintaining such an extremely low LDL-c concentration can be achieved without therapeutic intervention. Statin drugs provide effective cholesterol-lowering therapy and are widely prescribed (Stein, 2003), but they are expensive and can cause side effects and even death (Evans and Rees, 2002; Farmer, 2003). Lowering plasma LDL cholesterol through nondrug strategies, such as ingesting specific food components, would therefore be most desirable. Several dietary constituents of plant origin are effective cholesterol-lowering agents, including plant sterols (Katan *et al*., 2003; Ostlund, 2004) and b-sitosterol (Callaway *et al*., 2002; Malini and Vanithakumari, 1990). Consequently, plant foods and manufactured products rich in these phytochemicals are being promoted to consumers as cardioprotective and beneficial to overall health but sometimes plant products are hazardous waste. *Cannabis sativa* L. is a dioecious and annual plant (Adams and Martin, 1996a).The most important *Cannabis sativa* L. products in the food and drug trade are whole hempseed, hulled hempseed, hempseed oil, hempseed milk, marijuana seed, marijuana leaf (grass), and hashish (Adams and Martin, 1996a). In China, Iran, Pakistan and Turkey roasted salty *Cannabis* seeds (Per: Shahdaneh that means king of seeds) with wheat still sold by street vender and herbal stores and it is very popular especially among children as a nut (Callaway, 2004; Karimi and Hayatghaibi, 2006). Hemp seed has been a valuable industrial crop for both food and fiber in Canada and European countries during the last decade (Callaway, 2004). In Iran *Cannabis* plant (both drug and nondrug types) is annihilated by government to avoid narcotic abusing. Whole hempseed contain approximately 25% protein, 31% fat, and 34% carbohydrate, in addition to an interesting array of vitamins and minerals (Callaway, 2004; Darshan and Rudolph, 2000; Leizer *et al*., 2000). The oil contained in hempseed is 75-80% polyunsaturated fatty acids (PUFAs) and only 9-11% of the lesser desired saturated fatty acids (Callaway, 2004; Darshan and Rudolph, 2000). *Cannabis* seed is a rich source of phytochemicals that include terpenoid compounds such as phytocannabinoids, plant sterols, and high content of

polyunsaturated fatty acids, yet this abundant crop has been largely overlooked for its benefits to human abusing. The intoxicating properties of *Cannabis sativa* L. reside in sticky resin produced most abundantly in the flowering tops of female plants before the seeds mature. The main psychoactive compound in this resin is Δ^9-tetrahydrocannabinol (Δ^9- THC) (Elliot and Mechoulam, 2002). The concentration of THC in the seeds depends on the type of plant (fibre or drug hemp) as well as on the degree of contamination at the harvest (Ross *et al*., 2000). As a consequence only very low THC concentrations are found in the inside of the seeds (less than 2 mg/kg with drug hemp(marijuana) and less than 0.5 mg/kg with fibre hemp) (Ross *et al*., 2000; Small and Marcus, 2003). Therefore, the tidiness of the seeds plays the most decisive part in the concentration of THC in the seeds. According to the claim of marijuana abusers, drug-type variety of *Cannabis* that grown illegally in Isfahan province of Iran has a high intoxicating (euphoric) potency, albeit the increased rate of psychosis among the young people proves this claim. We were, therefore, motivated to critically evaluate the possible effect of hempseed on lipid profile of guinea pig. In this study we used unclean, resin contaminated marijuana seed. As a result THC is found in unclean marijuana seed. THC is an appetite promoting agent and may be cause to overeating and metabolic syndrome in normal human and animals.

Materials and Methods

Experimental animal: All procedures that involved animals were approved by the Animal Care and Use Committee of Western Azerbaijan Veterinary College. The animals were housed individually in polycarbonate cages with sawdust bedding. Guinea pigs were kept in a 25°C room with a 12h light: dark cycle and had free access to feed and clean water and stabilized for two week before the start of experiment. The fourteen healthy guinea pigs were divided into two equal groups. In control group, guinea pigs were fed with normal diet (fresh vegetables) while case group had free access to normal diet and marijuana seed for 60 days. At the end day of study the overnight fasting animals were anesthetized with ketamine/xylazine combination and blood samples for sera preparation were collected by cardiac puncture into sterile plain tubes. Serum samples were separated from the clot by centrifugation at 3000rpm for 15 min using bench top centrifuge (MSE Minor, England). Serum samples were separated into sterile plain tubes and stored in the refrigerator for analyses. All analyzed were completed within 24h of sample collection. Determinations of lipid parameters were performed using an automated biochemical analyzer (Multianalyzer Technicon RA-XT, Bayer do Brazil).

Statistical analysis: The results were expressed as mean ± SEM. Differences between means analyzed using independent sample-t-test. P values of 0.05 or less were taken as being statistically significant. Data were analyzed using version 13 of SPSS software.

Results

Table 1 shows the lipid parameters of control and case groups respectively at the end day (60th day) of study. The data expressed as mean± SEM. Plasma high-density lipoprotein cholesterol (HDL-c) was not affected by the consumption of marijuana seed during the 2-month study (Table 1), although there was a trend for HDL-c to increase with marijuana seed intake (P = 0.07). In contrast, the plasma non-HDL-c fraction, containing mainly low- density lipoprotein cholesterol (LDL-c), increased significantly (P = 0.00). LDL-c is the predominant fraction in humans. The majority of plasma cholesterol in guinea pig fed purified diets is typically carried in the LDL-c fraction. Therefore, guinea pig is the best model for studying the effect of diet on LDL-c level (Fernandez and McNamara, 1991). The total Plasma cholesterol in guinea pigs fed marijuana seed increased significantly (P = 0.00). Triglyceride also was affected falling non-significantly (p = 0.86).

Discussion

In the 1960s, Prof. Rafael Mechoulam isolated and identified the cannabinoids and the chief cannabinoid chemical in the marijuana plant: delta-9-tertrahydrocannabinol (Δ^9-THC) (Adams and Martin, 1996a). Approximately 20 years later, in the late 1980s, Allyn Howlett, identified a receptor (CB1) for THC that is a G-protein coupled receptor (GPCR) (Devane and Mechoulam, 1992).CB1 is a ubiquitous receptor found in the central nervous system and periphery, and in both neural as well as non-neural tissues (Breivogel and Childers, 1998; Di Marzo *et al*., 2001). The CB2 receptor has a more limited distribution, principally in cells associated with the immune system, such as leukocytes, spleen, thymus, and tonsils (Goutopoulos and Makriyannis, 2002). Thirty years after the identification of THC, William Devane, identified a brain chemical, anandamide which binds to the cannabinoid receptor and causes changes which are qualitatively similar to those provoked by THC (Devane and Mechoulam, 1992). In the present study, a significant increase in levels of LDL-c and total cholesterol and also a non-significant change in levels of HDL-c and triglyceride were observed upon treatment with marijuana seed during 60 days. Major component of total cholesterol is LDL-c which is directly related to coronary artery disease (CAD) (Brousseau and Schaefer, 2000). It is recognized as major atherogenic lipoprotein and primary target of lipid lowering therapy (Kawahiri *et al*., 2000). The serum LDL-c increased significantly after treatment with marijuana seed. HDL-c has preventive role in CAD and epidemiological studies, as well as, studies in animal models of atherosclerosis,

Table 1: Serum lipid profile of normal adult guinea pigs in two groups

Group	Total cholesterol*(mg/dl)	Triglyceride(mg/dl)	LDL-C*(mg/dl)	HDL-C(mg/dl)
Control	25.28±.746	56.71±2.98	10.28±0.42	12.42±1.17
Case	52.57±2.66	55.85±3.78	30.85±2.58	15.28±0.918

Note: Values are expressed as mean ± SEM. The significant level was 0.05 or less.*P<0.05.

support the cardioprotective role of HDL-c (Gordon *et al*., 1997; Kawahiri *et al*., 2000). The HDL level was non-significantly raised after treatment with marijuana seed. In spite of high content of omega-6, omega-3, omega-9 fatty acids and the relatively high phytosterol content of hempseed(Callaway, 2004), that makes it beneficial to cardiovascular health, short term of marijuana seed feeding didn't improve lipid profile (Table 1). Although there are many causes for the increased prevalence of cardiovascular diseases, it appears that nutritional factors, notably increased saturated fat consumption, play important role in promoting premature atherosclerosis (Keys, 1997) however, one of the apparent paradoxes has been the observation that the low saturated fat consumption recommended by several organization including the American Heart Association, the American Diabetes Association, and the American Dietetics Association, often results in decreasing HDL-c levels (Mensink *et al*., 2003). These data may be related to fatty acid profile of marijuana seed or some unidentified factors present in natural plant products but present evidences support subjective concerning of Δ^9-THC, as an appetizer. Although Δ^9-THC-fed mice continued to have elevated serum lipid levels (Paul *et al*., 2006). A growing body of evidence has also established that appetite is modulated by the cannabinoid system of the brain (Kirkham and Williams, 2001). The administration of the marijuana constituent Δ^9 - THC stimulates appetite (Adams and Martin, 1996b; Koch, 2001) whereas the cannabinoid receptor antagonist SR 141716 reduces food intake (Arnone *et al*., 1997; Colombo *et al*., 1998; Simiand *et al*., 1998). Furthermore, recent studies have indicated that appetite is increased by the endogenous cannabinoid ligands 2-arachidonoylglycerol and anandamide (Verty *et al*., 2004; Williams and Kirkham, 2002). This provides additional evidence that the cannabinoid system is a positive modulator of food intake. Today, rimonabant (CB1 receptor antagonist) shows promise as a new approach to address cardiovascular risk factor management, specifically in the areas of obesity and metabolic syndrome (Van Gaal, 2004). According to other studies that have been done in our laboratory, using of very clean hempseed of nondrug *Cannabis sativa* L. cultivar that grown in Khorasan province of Iran improved lipid profile of rat and has cardioprotective and hepatoprotective effects (Karimi and Hayatghaibi, 2006). However this study shows that drug cultivar of *Cannabis sativa* L. that grown illegally in Isfahan province of Iran lead to hypercholesterolemia. We expect development of plurimetabolic syndrome after long term feeding of unclean, THC-contaminated marijuana seed or its byproducts including of marijuana seed oil, marijuana seed milk and hashish syrup in normal human and animals. Additional studies are underway in our laboratory to isolate the effects of lipid-soluble *Cannabis* seed phytochemicals such as THC, THC oil and hashish on lipoprotein metabolism and biomarkers of atherogenesis.

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Changes in Oxidized Groundnut Oil and its Effect on Na^+/k^+ - Atpase in Rat Tissues

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Abstract: Groundnut oil was thermally oxidized at the temperature range of 180-200°C in open air for a period of 10 days at 4 hours per day. The extent of deterioration of thermoxidized groundnut oil were investigated spectroscopically, using infrared, ultra violet and atomic absorption spectroscopy and compared with fresh oil. The themoxidised oil samples differed in composition to the fresh oil, which served as control. Weanling rats were fed diet containing thermally oxidized groundnut oil while control rat were fed diet containing unheated fresh groundnut oil at 15% dietary level. The activity of Na^+K^+ ATPase were studied in the brain, liver, kidney, lungs and heart of experimental animals. The brain and the kidney had a relatively higher enzyme activity when compared to other tissues. Na^+K^+ ATPase activities of the liver, lungs and heart were not significantly affected (P> 0.05) while the ingestion of thermoxidised oil led to significant reduction (p< 005) of Na^+K^+ ATPase activity in the brain and kidney. It is considered that (a) thermoxidized oil causes reduced synthesis and structural changes in membrane phospholipids and (b) a probable impairment of kidney active transport and cerebral transmission of nerve impulse might have occurred due to reduced Na^+K^+ ATPase synthesis.

Key words: Thermal oxidation, groundnut oil, spectroscopy, Na^+K^+ ATPase, membrane function

Introduction

Most foods are subjected to processing before being consumed to improve their palatability and in some cases enhance their digestion (Sanders, 1993). Various food processing techniques have been found to leave deleterious effects on the processed foods and fats and oils are no exception (Gurr and James, 1975; Kubows, 1992, Ononogbu, 2002). Although fat and oil serve as the principals and inexpensive source of essential fatty acids and vitamins, during processing, they are subjected to oxidative degradation (Alexander, 1978; Frankel, 1980; Kubows, 1992; Ologan, 2002).

In the economically developing nations of the world, the intermittent use of reprocessed thermoxidised oil is common and uninhibited. Moreover, the semi-refined oils, which are predisposed to auto-oxidative deterioration, even without thermal processing, are the most cheaply and readily available. The compounds formed as a result of thermal oxidation are of special interest, since deep fried fat is continuously or repeatedly used at elevated temperatures in the presence of air and moisture. The peroxides and hydro peroxides do not survive the heating process while the non-volatile products that remain in the oil are absorbed into the food and subsequently ingested (Thomson and Aust, 1983). Degradative products that accumulate have been shown to be potentially toxic (Izaki *et al*., 1984; Okiy, 1988; Isong *et al*., 1996; Odutuga *et al*., 1997; Odutuga *et al*., 1999; Jimoh and Odutuga, 2002).

Most of the studies that have been carried out have involved the use of highly abused oxidized oils whose mode of oxidation cannot be compared to normal culinary practices (Andrew *et al*., 1960; Fujimoto *et al*., 1984; Mac Gregor *et al*., 1988). In this study therefore, groundnut oil was thermally oxidized in a way to simulate normal culinary practice, characterized and its effect on the activity of Na^+K^+ATPases in selected rat tissues was investigated.

Materials and Methods

Groundnut oil was obtained from Ipata market, Ilorin Nigeria. All chemical and solvents are of analytical grace (BDH and Aldrich).

Treatment of groundnut oil: Groundnut oil was divided into three portions and treated as follows:

(a) No thermal treatment and served as control.

(b) One litre groundnut oil was poured into a stainless steel pot and used intermittently to fry yam chips at a temperature range of 180-200°C in open air 4 hourly for 10days. The oil sample was left overnight to cool and was replenished with fresh oil 10 hourly This portion of groundnut oil was poured into a stainless steel pot and used to fry yam chips at a temperature range of 180-200°C in open air for a period of 4hrs daily for 10days. The sample was left overnight and not replenished throughout the period of use.

These treatments (b) and (c) simulated the process of repeated use of frying oil.

Spectroscopical analysis: A change in quality and the extent of deterioration of the oil samples were observed spectroscopically. The room temperature infrared, electronic and atomic absorption data were determined as reported previously (Obaleye and Orjiekwe, 1992; Obaleye and Orjiekwe, 1995). Infrared spectra were obtained neat while electronic and atomic absorption spectra were run in petroleum ether. The measured frequencies in infrared were accurate to $0.1cm^{-1}$. In the spectroscopy, all spectra data obtained between 400 and 200nm were corrected for background by solvent subtraction.

Animals and diet: Thirty six female albino rat (*Rattus norvegicus*) with mean weight of 40.5±2.22 obtained from the Animal Breeding Unit, Department of Biochemistry, University of Ilorin, Ilorin, Nigeria. They were divided into 3 groups of 12 animals each and were maintained respectively on:

(a) Control diet containing fresh groundnut oil - Group A (fresh)
(b) Diet containing oil replenished 10 hourly after use - Group B (replenished).
(c) Diet containing oil used for frying but not replenished all through the period of use - Group C (not replenished)

The diets were isoprotenous and isocaloric the composition of the diet is shown in Table 1. The appropriate diets and water were given *ad libitum* for 12 weeks. The animals were kept in plastic metabolic cages at room temperature.

Table 1: Composition of Experimental Diets (g/Kg)

Component	Group A	Group B	Group C
Soymeal	500	500	500
Lipid (oil)	150	150	150
Sucrose	100	100	100
Methionine	10	10	10
*Vitamin / mineral mix	30	30	30
Com Starch	200	200	200
Lysine	10	10	10

A - Diet of animals fed with fresh groundnut oil. B - Diet of animals fed with replenished groundnut oil. C - Diet of animals fed with not-replenished groundnut oil. * Mineral mix contained (g/kg diet): $CaCO_3$ (15.258); $CoCl_2.6H_2O$(0.001); $ZnCl_2$ (0.001); $CuSO_4.5H_2O$ (0.019); $FeSO_4.7H_2O$ (1.078); $MgSO_4$ (2.929); $MnSO_4.2H_2O$ (0.178); KI (0.032); KH_2PO_4 (15.559) and NaCl (5.573). The vitamin mix contained (g/kg diet): Thiamine (0.02); Riboflavin (0.03); Pyridoxine (0.01); p-Aminobenzoic acid (0.20); Myo-inositol (2.00); Biotin (0.001); Menadione (0.01); Ergocalciferol (0.4); Choline-HCl (2.0) and Cellulose (3.31).

At the end of the experimental period, the animals were sacrificed while still under anaesthesia by cervical dislocation. They were quickly dissected and the tissues of interest brain, liver, kidney, lungs and heart were removed into ice cold 0.25M sucrose solution. Each tissue was then homogenized separately in ice-cold 0.25M sucrose buffer solution. The homogenates were kept frozen overnight before enzyme assay to allow unbroken cells to lyse (Ngaha, 1982).

Enzyme and protein measurement: Inorganic phosphate was determined using the methods described by Fiske and Subbarow (Fiske and Subbarow, 1925).

Protein concentration was measured by the Biuret method (Plummer, 1978). All measurements were done using Spectronic 20 spectrophotometer. All results were subjected to an analysis of factorial experiments and the mean were separated using Duncan's multiple range test.

Results

Ultraviolet spectroscopy: The electronic spectra data of the three oil samples are shown in Table 2. In complete analogy to the fresh oil sample, both replenished and not-replenished oil samples show a substantial red shift in the electronic absorption band at 224.3nm whereas both the two other samples exhibited peak at ~238nm. There are variations in the absorbance values of these three oil samples. The n →Λ* transition is observed at ~260nm for the fresh sample while the two treated oil samples exhibit this transition around 272nm.

There are additional peaks for both the treated oil samples at around λ max ~ 280nm. This band however cannot be resolved for fresh oil.

Infrared spectroscopy: The major infrared bands and their assignments are shown in Table 3.

In analogy to the fresh oil sample which shows very strong and sharp band at $1724cm^{-1}$ due to (C=O) both the replenished and not –replenished groundnut oil samples undergo a red shift to 1715 cm^{-1} and 1720 cm^{-1} (which represents carbonyl functions, ester linkages) probably from fatty acyl glycerol bonding characteristics) respectively, for v (C=O) due to their thermal oxidation. On the other hand, the very weak band near $1369cm^{-1}$ in fresh with subsequent red shift ~ $1350cm^{-1}$ in both the other processed oils, apparently belong to the vibration of the 0-H of the carboxylic group. Bands in the finger print region, which undergo changes upon oxidation, are at 1430,1369,1220,1140 and $695cm^{-1}$ (fresh).

Atomic absorption spectroscopy

Metal analysis: Table 4 shows the metal constituents of the oil samples. The variance in composition of the three oil sample is also confirmed from the difference in traces of metal constituents of the oil samples. The percentage of heavy metals is higher in both the replenished and not replenished oils when compared with the fresh one. On the other hand there is a decrease in the percentage of alkali metal (Na) for both the affected oil samples.

Table 2: Electronic spectral data (nm) of the oil samples in the region 400-200nm

Fresh oil		Replenished oil		Not-replenished oil	
λ max	Absorbance	λ max	Absorbance	λ max	Absorbance
224.3	1.897	238.9	1.674	238.7	1.639
260.2	0.650	272.1	0.581	272.0	0.545
		281.0	0.484	280.1	0.451

Table 3: Prominent infrared absorption bands (cm^{-1}) observed in fresh, replenished and not-replenished groundnut oil

Fresh oil	Replenish-ed oil	Not-replenished	Tentative assignment	Remark
3422vw	3440vw	3400w	0-H stretch ; carboxylic group	A shift
2980w,sh	2942vs, sp	2960w,sh	C-H stretch (COOH, OH, Mono-and diacyl-glycerides and hydro-peroxide group)	A shift
2900 vs, sp	2879 vs, sp	2894 vs, sp	C-H asym. stretch (CH_2, for unsaturated aldehydes)	Bathochromic shift
2823 s,sp	2804s,sp	2820s,sp	CH_3, sym. stretch (for carboxylic group, ester linkages) probably from fatty acyl glycerol bonding characteristics, anhydrides, aldehydes, ketones, acid peroxides, aldehydes, ketones, acid peroxides in descending orders.	Bathochromic shift
1724 vs,sh	1715vs,sp	1720vs,sp	C=O stretch, carboxylic group, (ester linkages probably from fatty acyl glycerol bonding characteristics anhydrides, aldehydes, ketones, acid peroxides in descending orders	Barthochromic shift
1430m	1442m	1432m	C-H bend, CH_3 group (alkanes, aldehydes, alcohol, aldehyde	Hypsochromic shift
1369w	1350vw	1350w	O-H bend	Barthochromic shift
1220w	1222w	1221w	C-O stretch; C-OH carboxylic group	Hypsochromic shift
1140s	1143s	1130s	C-O stretch (carbonyl compounds alcohols)	A shift
695w	705w	697m	C-OH carboxylic group	Hypsochromic shift

Abbreviations: w-weak; vw-very weak; b-broad; m-medium; s-strong; sp-sharp; sh-shoulder.

Effect of diet on Na^+K^+ ATPase activity: Na^+K^+ ATPase activity of the various rat tissues is shown in Table 4. The ingestion of replenished groundnut oil caused a significant $P<0.05$ reduction in the activity of the enzyme from the brain and lungs, the reduction being 32.0% and 32.5% respectively. The liver, kidney and heart Na^+K^+ ATPase activities were however not significantly affected. On the other hand, the ingestion of not-replenished groundnut oil containing diet led to a significant ($P<0.05$) reduction in Na^+K^+ ATPase activity in the brain, kidney and heart. It was noted that although a 32.0% reduction was recorded in the brain enzyme activity in animals fed replenished oil, the reduction was 71.56% in animals fed not- replenished groundnut oil.

Discussion

The present investigations demonstrated that the thermally oxidized groundnut oil samples were different in composition to the fresh (control, unheated) oil and also had many different spectral features. In the UV analysis, the peak ~260-272nm corresponds to secondary or end products formed by subsequent degradation of alkyl or acyl chains (Odutuga *et al.*, 1997). This absorption appears weak in the fresh oil because of partial autoxidation of hydrocarbon chains exposed to atmospheric oxygen. It has been previously noted that secondary products characteristics of lower hydrocarbons such as carbonyl compounds were detected by an abrupt change in intensity of the 270nm peak (Lamba *et al.*, 1991).

In the various oil complexes during IR analysis, the greater shifts in the v (C=0) and v+δ (O-H) bands coupled with slight changes in associated v (O-H) band are strong evidences of thermal effect on both the replenished and not replenished groundnut oil. There is very high increase in intensity in oil sample C compared with others which showed highest oxidation and highest number of conjugated bands formed. (Rouxhet *et al.*, 1950; Kemp, 1979 and Williams and Fleming, 1980).

Bands on the finger print region, which undergo changes upon oxidation, are at 1430,1369, 1220, 1140, and 695cm^{-1} (fresh). Changes observed here for the treated samples confirmed the difference or oxidation induced change in the physical state of the treated samples. It also shows the reduction in the *van der* walls force / interactions of the oxidized products.

Table 4: Metal analysis of the oil sample

Metals Analyzed	Na (ppm)	Mn (ppm)	Fe (ppm)	Zn (ppm)	Mg (%)	Ca (%)	K (%)
Fresh oil	29.61	0.00	0.00	2.10	0.001	0.017	0.007
Replenished oil	17.27	0.00	0.00	2.38	0.001	0.007	0.007
Not –replenished oil	7.83	0.02	3.98	2.13	0.002	0.023	0.026

(Rouxhet *et al.*, 1950; Williams and Fleming, 1980; Kemp, 1979).

The fact that the different functional groups were identified in the fresh oil confirms the fact that most of the oils retailed in the market even without thermal treatment has already started deteriorating. This may because of exposure to environmental factors such as sunlight and air, it could also be due to the fact that the oils are not usually refined after extraction (Leo, 1983; Nnadozie *et al.*, 1990).

The concentration of the various functional groups obtained by calculating the relative areas occupied by such peaks show the accumulation of anhydrides, aldehydes, ketones, acid peroxides and alcohols in the oil that were subjected to thermal treatments.

Increase in heavy metal content and other representative metals in the thermoxidized oil sample as recorded in this study is likely to increase the toxicity effect of the affected samples. The pro-oxidant materials in oil are the trace amounts of these metals (Lamba *et al.*, 1991).

The activity of Na^+K^+ ATPase in the brain and kidney were found to be relatively higher when compared to the other tissues. This is due to the fact that these organs are highly membranous and are also involved in active transport processes than the others. Lehninger *et al.* (1993) reported that the activities of the ATPases are usually highest in tissues where it constitutes the main mechanism for producing physiologic work.

Na^+K^+ ATPase is involved in active transport across the plasma membrane within virtually all cell types; the sodium concentration is relatively low while that of potassium is high. Most animal cells maintain intracellular K^+ at relatively high and constant concentration between 120mM and 160mM, whereas the intracellular Na^+ concentration is usually less than 10mm (Wills, 1985). The cell requires a high intracellular level of K^+ for correct conformation and function of proteins / enzymes, a defect in the activity of Na^+K^+ ATPase will affect various metabolic processes. The reduction in the activity of Na^+K^+ ATPase in these organs might therefore affect the transmission of nerve impulse, a function to which it is directly involved in the brain (Wills, 1985). In the kidney, it is involved in the re-absorption of substances such as sugars, amino acids and electrolytes back to the blood. An impairment in the function of this energy dependent pump in the kidney could lead to loss of sugars, amino acids and electrolytes in the wine.

The decrease in the activity of Na^+K^+ ATPase in the brain and kidney observed in the present study might be a consequence of (a) incorporation of deoxidized fatty acids into membraine phospholipids and (b) increased lipid peroxidation in the membrane.

Table 5: Na^+K^+ ATPase activity of tissues of rats maintained on diets containing fresh and oxidized groundnut oil

Tissues	Group A	Group B	Group C
Brain	11.90 ± 1.85^a	8.09 ± 0.23^b	$3.46+1.09^c$
Liver	0.81 ± 0.23^a	1.38 ± 0.47^a	$0.73+ 0.15^a$
Kidney	10.75 ± 2.4^a	10.40 ± 2.01^a	$3.62+1.03^b$
Lungs	2.37 ± 0.66^a	1.60 ± 0.29^b	$3.03+1.05^{ab}$
Hear	2.11 ± 0.63^a	1.98 ± 0.55^b	$1.32+0.45^b$

Enzyme activities are expressed as specific activity in moles Pi / tir / mg protein. The results are the values for 5 determinations (± SEM). a,b,c values along the same row with different superscripts are statistically significant

The substantial red shift in electronic absorption (peak at 238nm) exhibited by the heated oil samples would indicate the presence of a conjugated double bond band (also known as k band) in the fatty acid molecule (Lamba *et al.*, 1991) Incorporation of this altered fatty acid molecule into membrane phospholipids may likely lead to loss of essentially of the phospholipids and affect lipid membrane structure and function relationship in biological systems (Odutuga, 1977; Odutuga *et al.*, 1997).

Odutuga and Ajayi (1998) reported reduced alkaline phosphatase (a membrane bound enzyme) synthesis as well as loss of this enzyme in essential fatty acid and zinc deficiencies due to changes in the organization of membrane phospholipid matrix. The reduction in Na^+K^+ ATPase activity observed in the present investigation, therefore is considered to be a result of the ingestion of peroxidized groundnut oil affecting the phospholipids matrix and changing the structure and function of brain or kidney cells and membranes (Odutuga, 1977) and probably impairing the proper coupling of oxidative phosphorylation.

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Supplementation of Cassava Hay as a Protein Replacement for Soybean Meal in Concentrate Supplement for Dairy Cows

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Abstract: Three crossbred Holstein-Friesian dairy cows in mid-late lactation were randomly allocated to three ratio of cassava hay (CH) and soybean meal (SBM) (CH:SBM) in concentrate supplement treatments(0:100, 60:40, 100:0) according to a 3 x 3 Latin square design. Concentrate mixture containing 16 %CP was given to animals at two equal parts (2 % of body weight per day) while urea-treated rice straw (5 %urea) (UTRS) was given on *ad libitum*. The experiment revealed that increasing CH:SBM ratio in concentrate had no effect on dry matter intake and digestibility while reduced concentrations of ruminal ammonia nitrogen (NH_3-N) and blood urea nitrogen (BUN) were reduced. Milk yield across treatments were similar (8.0-8.5 kg/hd/d) while fat contents of milk tended to linearly increase as ratio of CH to SBM in concentrate increased. Moreover, increasing levels of CH to SBM ratio in concentrate linearly increased income over feed thus resulted in more milk income return. Conclusions can be made that CH should be recommended used as a protein source replacement a soybean meal in concentrate for a sustainable dairy production in the tropics.

Key words: Cassava hay, soybean meal, rumen fermentation, milk yield and composition, dairy cows

Introduction

The substitution of traditional feeds in the diets of dairy cows is common as economic condition changes. Soybean meal (SBM) has long been used as a prominent source of CP for dairy cows, however, with its increasing price, the use results in higher cost of production. Cassava (*Manihot esculenta*, Crantz) is an important cash crop, widely grown in sandy loam soil, low fertility and under hot long dry season. Its' leaves collected at harvesting time, contained high level of protein, and could be used as a protein supplement in ruminants (Wanapat *et al*., 2000a, 2000c). In comparison with SBM, CH has a higher concentration of RUP (Wanapat *et al*., 2000b) cand is beneficial because it can supply more total AA for absorption in the lower-gut. The AA profiles of CH were relatively comparable with SBM (Wanapat, 2003). Lysine, glutamine, asparagine and arginine were higher in SBM but in CH methionine were higher. Therefore, the objectives of the experiments were to study the effect of cassava hay and soybean meal ratio in concentrate on rumen fermentation, digestibility, milk yield and composition and economical return.

Materials and Methods

Animals and design: Three, crossbred Holstein-Friesian dairy cows in mid-late lactation were randomly allocated to three ratio of CH and SBM (CH:SBM) in concentrate treatments(0:100, 60:40, 100:0) according to a 3 x 3 Latin square design. Experimental periods were 21 d in length per each period.

Table 1: Ingredient mixtures (%) and chemical compositions of concentrate, urea-treated rice straw (UTRS) and cassava hay (CH) for dairy cow

Item	CH:SBM				
	0:100	60:40	100:0		
Cassava chip	67.5	62.9	64.4		
Soybean meal (SBM)	20	9.6	0		
Dried brewery's grain	7.3	6.1	6.1		
Cassava hay (CH)	0	14.8	20		
Urea	1.5	2	3.1		
Molasses	2	2	3		
Sulphur	0.5	0.5	0.5		
Salt	0.5	0.5	0.5		
Mineral mix	0.5	0.5	0.5		
Vegetable oil	0.2	1.2	2		
Chemical compositions, (%)				UTRS	CH
	------% dry matter ------				
DM	90.5	90.2	91	51.6	92.5
OM	94.8	95.1	94.9	86.5	92.8
CP	16.2	16	15.9	8.3	22.5
NDF	18.1	22.7	26.9	79.7	57.2
ADF	12.5	14.8	17.5	53.3	34.3
Ash	5.1	5	5.2	13.5	7.2

Experimental feeds and management: Ingredient compositions of concentrate feed, CH (CH making could be found in Wanapat *et al*., 1997, 2000a) and roughage (urea-treated rice straw 5% urea; UTRS) (Wanapat, 1985) are shown in Table 1. Animals were individually penned and water was available at all times. Concentrate mixture containing 16 %CP with different ratio of CH and SBM and given to animals at two equal parts (2 % of body weight per day) during morning and

Table 2: Effect of cassava hay (CH) and soybean meal (SBM) ratio in concentrate on dry matter (DM) intake, ruminal fermentation, blood urea nitrogen (BUN), in mid-late lactating dairy cows

	CH:SBM				Contrast[1]	
Item	0:100	60:40	100:0	SEM	L	Q
DM Intake						
Concentrate						
kg/hd/d	8.2	7.5	6.9	0.3	NS	NS
%BW	1.5	1.4	1.4	0.2	NS	NS
Urea-treated rice straw						
kg/hd/d	6.7	6.2	6.9	0	NS	NS
%BW	1.3	1.2	1.4	0	NS	*
Rumen ecology,						
pH	6.8	6.7	6.7	0.1	NS	NS
NH3-N (mg/dl)	15.4[a]	14.5[ab]	14.4[b]	0.1	*	NS
BUN (mg/dl)	14.8[a]	13.8[b]	13.6[b]	0.1	*	NS
Total VFA (mmol/L)	72.8	73.9	73.8	0.4	NS	NS
VFA (mol/100 mol)						
Acetate (C2)	70.8	72.7	73	0.4	NS	NS
Propionate (C3)	19.9	18.1	17.6	0.5	NS	NS
Butyrate (C4)	9.3	9.2	9.5	0.3	NS	NS
C2:C3 ratio	3.5	4	4.2	0.3	NS	NS

[a, b]Values on the same row with different superscripts differed ($p<0.05$).
[1]L = Linear, Q = quadratic, NS = non-significant, *P<0.05, ** P<0.01

afternoon milking times. UTRS (5%) was given on *ad libitum*.

Sampling procedure, data collection and analysis: UTRS and concentrate were sampled for chemical composition analyses. Rumen fluid was taken by stomach tube with vacuum pump at 0 and 4 h-post feeding and analyzed for pH immediately, and for later analysis of NH_3-N (Bremner and Keeney, 1965), ruminal volatile fatty acids (VFAs) (Samuel *et al.,* 1997). Bood samples were collected from jugular vein of each cow at 0 and 4 h-post feeding and serum was removed and analyzed for BUN composition according to the method of Croker (1967) using automated clinical chemistry analyzers. Milk yield was recorded daily and composited samples from mornings and afternoon were analyzed for milk compositions using Milko Scan.

Statistical analysis: All data were subjected to analysis of variance using Proc. GLM and treatment means were statistically compared by Duncan's New Multiple Range Test (SAS 1998). Trend analysis for increasing CH and SBM ratio was compared using orthogonal polynomial analysis.

Results and Discussion

Table 1 illustrates details of chemical compositions of experimental feeds. UTRS contained 51.6% DM and 8.3% CP on dry matter basis. CH had 22.5% CP but was slightly lower than the value earlier reported by Wanapat *et al.* (1997). The reasons have been stated by Wanapat *et al.* (2000a) that the lower value may have been attributed by having higher portion of stem to leaf containing in the CH itself. The CP contents of concentrate were similar among dietary treatments, (16.2%) (0:100, CH:SBM), 16.0% (60:40, CH:SBM) and 15.9 % (100:0, CH:SBM), respectively. Data on intakes, ruminal fermentation parameters are presented in Table 2. For both concentrate and UTSR intakes, there were no significant differences among treatments while CH intakes were 1.11 and 1.38 kg DM/hd/d for CH to SBM ratio at 60:40 and 100:0, respectively. This roughage level was sufficient for dairy cattle as reported by many researches (Kumer and Singh, 1984; Barry, 1989; Wanapat *et al.,* 2000a). Increasing CH to SBM ratio in concentrate particularly at 100:0 ratio remarkably enhanced UTRS intake. This result has also been found in previous study by Wanapat *et al.* (2000c).

Ruminal NH_3-N and BUN were similar in concentrate with CH to SBM ratio at 60:40 and 100:0 and were higher than in CH to SBM ratio at 0:100 while ruminal pH were similar among treatments. Wanapat *et al.* (1997) reported that CH contained tannin-protein complex that could provide ruminal by-pass protein and thus lowered ruminal NH_3-N and BUN. In addition, supplementation of cassoy-urea pellet as a protein source in concentrates for cattle resulted in improvement of digestibility, ruminal fermentation and rumen ecology (Wanapat *et al.,* 2006). Milk yields were similar among treatments while fat contents of milk tended (P = 0.061) to linearly increase as ratio of CH to SBM in concentrate increased (Table 3). Wanapat *et al.* (2000c) stated that CH could have provided additional VFA necessary for milk fat synthesis. This is supported by the recent study, where acetate tended (P = 0.073) to linearly increase as ratio of CH to SBM in concentrate increased which would act as substrates for milk fat synthesis.

As clearly demonstrated, as levels of CH to SBM ratio in

Table 3: Effect of cassava hay and soybean meal ratio in concentrate on digestibility of nutrients, milk yield and compositions in mid-late lactating dairy cows

	CH:SBM				Contrast[1]	
Item	0:100	60:40	100:0	SEM	L	Q
Apparent total-tract digestibility (%)						
DM	78.3	77.5	77.6	0.7	NS	NS
OM	80.4	79.8	80.2	0.7	NS	NS
CP	77.1	73.6	73.5	0.9	NS	NS
NDF	64.4	64.6	67.2	1.3	NS	NS
ADF	62.4	61.6	62.0	1.6	NS	NS
Milk yield (kg/hd/d)	8.5	8.0	8.1	0.3	NS	NS
3.5% FCM (kg) [2]	10.1	9.3	9.3	0.4	NS	NS
Milk composition (%)						
Fat	3.7	3.9	4.2	0.5	NS	NS
Protein	3.3	3.5	3.1	0.1	NS	NS
Lactose	4.9	4.8	4.7	0.2	NS	NS
Solids-not-fat	9.3	9	8.6	0.2	NS	NS
TS[3]	14.5	14.4	14.3	0.7	NS	NS
Income over feed (US$[4]/hd/d)	0.3[a]	0.5[ab]	0.6[b]	0.9	*	NS
US$/hd/d	2.1[a]	3.5[ab]	4.2[b]	0.9	*	NS

[a, b]Values on the same row with different superscripts differed (p<0.05).

[1]L = linear, Q = quadratic, NS = non-significant, *P<0.05, ** P<0.01

[2]3.5% FCM = 0.4*(kg of milk)+15*(kg of fat),[3] TS = total solids, [4] 1 US$ = 39 Baht

concentrate linearly increased income over feed (P<0.05). This provides important data that CH can be an alternative source of protein. As reported by Wanapat and Khampa (2006) reported that cassava hay could not only provide as a protein source but also serve as an anthelmintic in ruminants. In addition, condensed tannin (CT) containing forages have the potential to help control anthelmintic resistant gastro-intestinal parasites (GIP). The CT may have direct or indirect biological effects on the control of GIP. Butter *et al.* (2000) reported that direct effects might be mediated through CT nematode interaction, thereby affecting physiological functioning of GIP. Condensed tannins also may react directly by interfering with parasite egg hatching and development to infective stage larvae (Athanasiadou *et al.*, 2000, 2001). The findings of Seng Sokerya and Rodriguez (2001) and Sokerya and Preston (2003) showed that eggs per gram (EPG) counted in goats fed the cassava and cassava + grass treatments steadily declined during the experiment from about 4000 – 5000 eggs/g of fresh feces in the first 30 days to about 1500 eggs/g after 70 days. The CT can improve protein nutrition by binding to plant protein in the rumen so preventing microbial degradation and increasing amino acid flow to the duodenum.

Increasing ratio of CH to SBM in concentrate for dairy cows resulted in similar nutrient digestion coefficients among treatments (Table 3). Level of CH intake in recent study was in good level and was in agreement of other studies (Kumer and Singh 1984; Barry, 1989; Wanapat *et al.*, 2000c).

Based on this experiment, the results suggest that CH as a protein replacement for soybean meal in concentrate for dairy cows which resulted in similar milk yield while milk fat was improved. Increasing levels of CH to SBM in concentrate significantly increased income over feed. CH can be used as a protein source replacement a soybean meal in concentrate for a sustainable dairy production as well as could result in more economical return and more sustainable dairy production in the tropics.

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A Comparison of Serum Omega - 3 Fatty Acid Concentrations Between Patients with Coronary Heart Disease and Healthy Subjects

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Abstract: Blood levels of eicosapentaenoic (EPA) and docosahexaenoic acids (DHA) are independently associated with elevated risk of death from coronary heart disease (CHD). The aim of this study was to compare the serum omega 3 and omega 6 fatty acid levels between patients with CHD and control subjects. The mean serum levels of triglyceride (TG) total cholesterol (TC) and low density lipoprotein cholesterol (LDLC) were higher in patients as compared with health subjects. The mean serum levels of alpha-linolenic acid (ALA), EPA, and DHA were significantly lower in the patients than in the control subjects. The mean serum concentrations of the ALA, EPA and DHA in patients were 3.04±0.75, 3.33±2.04, and 6.50±1.79 µg/dL, meanwhile the mean serum concentrations of the ALA, EPA and DHA in healthy subjects were 3.43± 0.50, 4.19±1.79, and 9.78±2.47 µg/dL. In contrast, the mean serum level of arachidonic acid (AA) was higher in the patients as compared with control subjects. The mean serum concentration of AA in patients and healthy subjects was 7.58±1.09 and 6.80±1.31 µg/dL, respectively. The ratio of serum omega-3/omega-6 fatty acids (FAs) was significantly lower in the patients than in the control subjects. In conclusion, the patients with CHD had low concentrations of Omega-3 FAs as compared with health subjects. In contrast, the level of AA is high in patients as compared with healthy subjects. These data suggest that it is important to maintain omega-3 fatty acids level in serum of subjects, especially who are at risk of CHD for the purpose of preventing CHD.

Key words: Omega 3, omega 6, eicosapentaenoicacid, docosahexaenoic acid, arachidonic acid, coronary heart disease

Introduction

The relationship between intake of omega-3 fatty acids and risk of developing Cardiovascular diseases (CVD) began to emerge in the late 1970s (Dyerberg *et al*., 1978; Bang *et al*., 1980; Balk *et al*., 2006). Many studies have reported a negative relation between intake of omega-3 fatty acids (FAs) and CVD incidence and/or mortality (Osler *et al*., 2003; Ervin *et al*., 2004; Psota *et al*., 2006). Omega-3 polyunsaturated fatty acids (PUFAs) are one of dietary substrates that have reported cardioprotective benefits. Omega-3 PUFAs generally exert their cardioprotective effects through changes in lipids and lipoproteins. In addition, omega-3 FAs especially EPA and DHA contribute benefits through their antiarrhythmic, anti-inflammatory, antithrombotic effects. Moreover, EPA and DHA also improve vascular endothelial function and help lower blood pressure, platelet sensitivity (Wijendran and Hayes, 2004). There are three major types of omega 3 fatty acids including alpha-linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA). The major sources in human diet are fish, especially dark fleshed fish, and, if consumed, fish oil supplements. ALA is a plant form of omega-3 fatty acid (Kris-Etherton *et al*., 2002; Balk *et al*., 2006). In Saudi Arabia there are insufficient data on which to draw conclusions about the status of omega-3 FAs in patients with CHD. Therefore, the objective of this study was to compare the serum omega 3 and omega 6 fatty acids of the patients with coronary heart disease (CHD) and of the control subjects.

Materials and Methods

The study was carried out in 30 females who had coronary heart disease (CHD), defined as those who had ever been told by a doctor that they had a heart attack, heart failure, or used medicine for a weak heart during the 6 months prior to the baseline study. Patients were randomly chosen from the 2 hospitals in Almdenah Almonorah city. A total of 57 age-matched healthy females also were provided blood samples for comparison of serum lipids and FA analysis. Concentrations of serum total cholesterol, TGs, LDLC, and HDLC were measured by enzymatic methods on an auto-analyzer. The serum concentrations of fatty acids were measured by gas liquid chromatography. The data are presented as means ± SD. The statistical analysis included means; standard deviations were analyzed by SSPS version 10 and differences were tested for significance ($P < 0.05$).

Results

Characteristics of the subjects are presented in Table 1. Mean body weight and BMI were slightly higher in

patients as compared with healthy subjects. A comparison of serum lipids of the patients and the controls is presented Table 2. The mean serum levels TG, TC, and LDLC were higher in patients as compared with health subjects. In contrast, the mean serum concentration of high density lipoprotein cholesterol (HDLC) was slightly higher in health subjects as compared with patients. The fatty acid concentrations in the serum of the study populations are shown in Table 3. The mean serum concentrations of the ALA, EPA and DHA were significant higher in healthy subjects as compared with patients. In contrast, the mean serum concentrations of the AA was significant higher in patients as compared with health subjects. The mean serum concentrations of the ALA, EPA and DHA in patients were 3.04 ±.75, 3.33 ± 2.04, and 6.50 ±1.79 µg/dL, meanwhile the mean serum concentrations of the ALA, EPA and DHA in healthy subjects were 3.43± 0.50, 4.19 ±1.79, and 9.78 ±2.47 µg/dL respectively. In contrast, the mean serum concentrations of AL and AA in healthy subjects were 36.26 ± 9.83 and 6.80 ±1.31, while the mean serum concentrations of AL and AA in patients were 35.99 ±8.90 and 7.58 ±1.09. The ratio of serum omega-3 / omega-6 fatty acids was significantly lower in the patients than in the control subjects. The ratio of serum omega-3/omega-6 fatty acids in the control subjects was 0.4, while the ratio of serum omega-3 / omega-6 fatty acids in patients was 0.3.

Table 1: Characteristics of the study population (mean ± S.D.)

Parameter	Patients (n=30)	Controls (n=57)
Number	30	57
Age (year)	53.23±6.71	51.7±6.5
Hight (cm)	156.3±5.6	158.4± 6.1
Wight (kg)	65.24±14.3	58.2±11.5
BMI (kg/m^2)	26.8±6.6	25.2±3.66

BMI ; body mass index

Table 2: Serum lipid concentrations of the study subjects (mean ± S.D)

Parameter	patients (n=30)	controls (n=57)
TG (mg/dl)	137.89±70.09	119.7±63.10
Cholesterol (mg/dl)	191.78±45.38	173.8±36.85
LDLC (mg/dl)	116.62±40.01	106.74±27.91
HDLC (mg/dl)	43.91±16.34	46.6±13.04

Table 3: Serum omega-3 and omega-6 FAs of study populations (mean ± S.D.)

Parameter	patients (n=30)	controls (n=57)
Omeg-3		
ALA (µg/dL)	3.04±0.75	3.43±0.50*
EPA (µg/dL)	3.33±2.04	4.19±1.79*
DHA (µg/dL)	6.50 ±1.79	9.78 ±2.47*
Omega-6		
LA (µg/dL)	35.99±8.90	36.26±9.83
AA (µg/dL)	7.58±1.09	6.80±1.31*
omega-3/omega-6	0.3±0.06	0.4±0.08*

ALA, alpha-linolenic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid. LA, linoleic acid; and AA, arachidonic acid . * significant difference patients and controls ($p < 0.05$)

Discussion

The primary finding of this study was that healthy subjects had higher concentrations of omega-3 including EPA, DHA, and ALA than did patients. In contrast, patients had the concentrations of AA higher as compared with healthy subjects. We could attribute the low serum levels of omega-3 among patients as compared with healthy subjects to low intake of omega-3 among patients as compared with healthy subjects (data not shown). Our findings support those of Hojo *et al.* (1998) who found that eicosapentaenoic acid was significantly lower in the patients than in the control subjects, whereas, arachidonic acid was significantly higher in the patients than in the control subjects. In addition, Albert *et al.* (2002) found that whole blood levels of EPA and DHA were lower in cases of sudden cardiac death (3.8%) than in controls (4.2%), The evidence for a beneficial coloration between omega-3 FAs and lower risk for death from CHD is strong (Wang *et al.*, 2006), and it was reported that EPA might independently have a protective effect on the progress of coronary atherosclerosis. Moreover Low blood concentration of EPA and DHA is an independent predictor of elevated risk for acute coronary syndrome (Harris *et al.*, 2006). The ratio of serum omega-3 / omega-6 fatty acids was significantly lower in the patients than control subjects. Similarly Hojo *et al.*, 1998 found that the ratio of serum omega-3 / omega-6 fatty acids was significantly lower in the patients with stenosis than in the control subjects. The most likely mechanism by which omega-3 FAs may reduce risk for CVD is through a reduction in myocardial susceptibility to lethal arrhythmias (Leaf *et al.*, 2003). In addition, EPA and DHA may promote plaque stability (Thies *et al.*, 2003), and may be anti-atherosclerotic through a variety of other mechanisms (Von Schacky, 2003). In addition, omega-3 FAs especially EPA and DHA contribute benefits through their antiarrhythmic, anti-inflammatory, antithrombotic effects. Moreover, EPA and DHA also improve vascular endothelial function and help lower blood pressure, platelet sensitivity (Wijendran and Hayes, 2004). Potential Mechanisms by which omega-3 Fatty Acids may reduce risk for cardiovascular disease include reduce adhesion molecule expression, reduce platelet-derived growth factor, promote nitric oxide-induced endothelial relaxation, and reduce susceptibility of the heart to ventricular arrhythmia (Connor, 2000). Supplemental omega-3 FAs have long been known to reduce serum TG levels and TG concentrations are inversely associated with endogenous EPA and DHA levels (Harris *et al.*, 2006). Vasandi *et al.* (2002) observed that dietary omega-3 PUFA markedly reduced TGs and cholesterol ester levels in the liver and the level of apoB-containing lipoproteins in the plasma of LDL-

receptor-deficient mice. These data suggest an important mechanism by which dietary (n-3) PUFAs lower plasma TGs. Omega-3 FAs lower plasma TG concentrations, particularly in people with hypertriglyceridemia by suppressing the synthesis of TG and very-low-density lipoprotein (VLDL) cholesterol in the liver (Harris *et al*., 1997).

In this study, the mean serum level of TG was higher in patients than control subjects and the mean serum concentrations of AA was higher in the patients as compared with healthy subjects. AA levels relate to CHD because of the influence on blood coagulation rather than the progress of atherosclerosis because AA is the precursor of thromboxane A_2 (Hojo *et al*., 1998).

In conclusion, the results of this study support the conclusion that patients with CHD had low concentrations of omega-3 fatty acids as compared with health subjects. In contrast, the level of AA is high as compared with healthy subjects. These data suggest that it is important to maintain omega-3 fatty acids level in serum of subjects who at risk of CHD for the purpose of preventing CHD. Therefore, it is important to increase intake of omega-3 to maintain omega-3 fatty acids level in serum for the purpose of preventing CHD.

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Physicochemical Studies on Oils from Five Selected Nigerian Plant Seeds

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Abstract: Oils were extracted from four underutilized seeds of the Nigerian plants *Chrysophylum albidum, Dacryoides edulis, Landolphia owariensis* and *Napoleona imperialis* using n-hexane and their physicochemical properties compared with oils from seeds of *Elaeis guinensis*. Percent oil yield were 12.00, 15.80, 6.40 and 8.00 for *C. albidum D. edulis, L.* owariensis and *N. imperialis* respectively while the value for *E guinensis* seed is 28.00. The four seed oil were odourless and at room temperature liquids as against *E. guinensis* seed oil that were semi-solid under the same condition. Specific gravity of the seed oils ranged from 0.82-0.94 while peroxide value for all the oil extracts except that from *D. edulis* seed were less than three. Saponification values were as low as 42.40 in *L.* owariensis and as high as 246.60 in *E. guinensis* seed oils. Iodine values were between 15.10 and 45.00 in the extracts. These results suggest that *C. albidum* and *D edulis* seeds may be viable sources of oil going by their oil yield. However the studied characteristics of all oils extracts in most cases compared favourably with *E. guinensis* seed oil which is presently used for many domestic and industrial purposes in Nigeria.

Key words: Nigerian seeds oils, physicochemical properties, palm kernel oil, conventional seed oils

Introduction

Trees and shrubs with medicinal and nutritional potentials abound in Nigeria (Burkill, 1985). Several of these plants have fruits which have been identified to be nutritionally important (Ihekoronye and Ngoddy, 1985).

In recent times, the desire to conserve resources spent on importation of oil for domestic and industrial use gave renewed impetus in the search for novel sources to complement the traditional ones. Attention has therefore, been focused on under-utiliised local seeds for possible development and use. There are several of these under-exploited plant seeds in Nigeria.

Chrysophyllum albidum Linn (African Star apple) belongs to the family sapotaceae. It is found in many ecozones of Africa, Nigeria inclusive (Bada, 1997). Its leaves are used in ethnomedicine (Adewusi, 1997). The fruit pulp is rich in iron and vitamin C and is good source of raw material for some industries (Asenjo, 1946; Cenrad, 1999; Adisa, 2000). While the pulp is eaten, the seeds are usually thrown away.

Dacryodes edulis G.don (African pear) is a Burseracea and has many medicinal and nutritional uses (Burkill, 1985). The fruit pulp is eaten and the seeds usually thrown away (Ajayi and Oderinde, 2002). Obasi and Okolie (1993) studied the potential of the seeds for food supplement.

Landolphia owariensis P.Beauv (Vine rubber) belongs to the family apocynacea. It has many medicinal and nutritional uses (Gill, 1992; Ebi and Ofoelue, 1997; Owoyele *et al*., 2001). The fruit pulp which is contained in a pod is eaten and the seeds usually thrown away.

Napoleana imperidis is of the family Lecythidacea. Its leaves have many medicinal uses. (Obute, 2005) the pulp is eaten and the seeds, thrown away.

Eleais guineansis (Palm tree) is commercially very relevant. The nutritional uses. The palm kernel all (PKO) is particularly useful.

Therefore, except for *E.guinensis* seed, the potential of the other four plant seeds are presently under-exploited. The present study was therefore undertaken to explore their potential as possible sources of oil for domestic and industrial uses, relative to the established potentials of palm kernel oil (PKO) from *E.guineensis* seed.

Materials and Methods

Healthy seeds of *C. albidum*, *D. edulis*, *E. guineanisis L. Owariensis* and *N. imperialis* were collected from Isiala Ngwa, Abia State Nigeria between January and June, 2006. They were taken to the department of Biochemistry Abia State University, Uturu. The seeds were authenticated by a taxonomist. They were dehauled (where applicable) sun-dried, wrapped in polyethylene bags and kept in desiccators until needed.

Extraction of oil: 250g each of the seed samples were milled into a paste using thermal Willey Mill (Model ED-5), The paste was transferred into a thimble and oil extracted using normal hexane in vacou with soxhlet apparatus. The extracting solvent was evaporated leaving the concentrated oil sample for analysis.

Extracted oil was quantified gravimetrically.

Chemicals used: All chemicals used were of the analytical grade and products of British Drug House, Poole England.

Statistical analysis: All extractions and analysis were

performed in triplicates. Results were expressed in mean±SD. Statistical significance was established using Analysis of Variance (ANOVA). Means were separated according to Duncan's multiple range analysis ($P < 0.05$).

Experimental: Specific gravity was determined using specific gravity bottle according to the method described by Pearson, 1980.

Iodine value (Wiji's method), saponification number, peroxide values were as recommended by the AOAC 1984.

For iodine value of each sample, 0.20g of oil was dissolved in 15ml carbon tetrachloride in 100ml glass stoppered flask. 25ml of Wiji's solution was added, the flask stoppered and allowed to stand for 2 hours in the dark at 25°C. 20ml of 10% potassium iodide (KI) solution was added and the mixture titrated with 0.2N sodium thiosulphate ($Na_2S_2O_3$) using starch indicator. A blank determination was carried out and the iodine value calculated using the formular:

Iodine value = $12.69N \quad V_2\text{-}V_1 / W$

Where N = Normality of thiosulphate
V_1 = Volume (ml) of thiosulphate solution used in test
V_2 = Volume (in ml) of thiosulphate solution used in blank
W = Weight of sample (0.20g)

Saponification value of the oil samples were determined as described below:

1g of each oil was dissolved in 12.5ml of 0.5% ethanolic KOH and the mixture refluxed for 30 minutes. 1ml of phenolphthalein indicator was added and the hot soap solution titrated with 0.5NHCL. A blank determination was also carried out under \the same condition and saponification value determined using the equation;

Saponification value = $56.1N (V_1\text{-}V_2) / W$

Where N = Normality of Hydrocloric acid used
V_1 = Volume of Hydrochloric acid used in test
V_2 = Volume of Hydrochloric acid used in blank
W = Weight of oil used (1g)

For peroxide value (PV),1g of each oil sample was weighted into a 200ml conical flask, then 25ml of 2:1 v/v glacial acetic acid: chloroform solvent was added. 1ml of saturated potassium iodide was then added and mixture left in the dark for 1 minute. Next, 30ml of water was added and the mixture titrated with 0.02N thiosulphate solution using 5ml starch as indicator. A blank determination was similarly carried out. PV was calculated from the equation.

Peroxide value (PV) = $[100(V_1\text{-}V_2)$ meg/Kg] / W
W = weight of sample
V_1 = Volume (ml) of thiosulphate used in test
V_2 = Volume (ml) of thiosulphate used in blank
N = Normality of thiosulphate ($Na_2 S_2O_3$).

Acid Value was determined for each oil sample by dissolving 0.20g of each oil in 2.5ml of 1:1 v/v ethanol: diethylether solvent and titrating with 0.1N sodium hydroxide while swirling using phenolphthalein as indicator. Calculation is as follows:

Acid Value = [56.1 x N x V] / W
Where N = Normality of NaOH used
V = Volume (ml) of NaOH used
W = Weight of sample used

Percentage free fatty acid (% FFA) (as oleic) was etermined by multiplying the acid value with the factor 0.503. Thus % FFA = 0.503 x acid value.

Results and Discussion

The studied physical properties of seed oil from the five plant are shown in Table 1. The n-hexane-extractable oil from the four underexploited seeds were lower than the 28.00% obtained for palm kernel in this study. The percentage oil yield were 15.30±0.10% for *D.edulis;* 12.00±0.90 for *C. albidum;* 8.00±0.05 for *N. imperialis* and 6.40±0.80 for *L.Owarieusis* The oil yield obtained for *D edulis* seed in this study is higher than the 10.44±0.80% reported by Ajayi and Oderinde 2002).

The oil yields for the five studied seeds (except *E guinensis* seed) are lower than 18% reported for soybean seed and 43% for groundnut seed (Ene-Obong and Carnovale 1992; Apata and Ologhobo, 1994). They are however, higher than 1.42±0.03% reported for seeds of *Piliostigma thonningii* - another under exploited plant found in Nigeria (Jimoh and Oladiji, 2005). The oil yields for *C. albidum* and *D. edulis* seeds may classify them as average yielding while *L. owarienssis* and *N. imperialis* seeds are low yielding: *E guinensis* seed is high yielding.

At room temperature (29°C) all the seed oil (except palm kernel oil which is semi solid) are liquids. They palm kernel oil is milk white while the other seed oil are pale to dark yellow in colour. The specific gravity of the oils ranged between 0.82 for *L. owariensis* seed oil to 0.92 for *N. imperalis* seed oil. These values compare with 0.82 and 0.84 reported for the pulp and seed oil of *D. edulis* respectively by Ajayi and Oderinde (2002). Non of the seed oils had offensive odour.

The chemical properties of the studied seed oils are shown in table 2. It indicate that the iodine values ranged from 15.10±0.08 in *L. owariensis* to 45.86±80±2.00 in *N. imperialis*. These values classify the oils as non drying. Similar non-drying oil values have been reported for

Table 1: Physical Properties of five selected Nigerian Seed Oils

	Percent oil yield	Specific gravity	Slate at 29°C	Colour	Odour
Chrysophyllum albidum	12.00±0.28^{c}	0.92±0.17^{a}	Liquid	Pale yellow	agreeable
Dacryodes edulis	15.30±0.10^{d}	0.87±0.03^{a}	Liquid	Dark Yellow	agreable
Elaeis guineensis	28.00±0.17^{e}	0.88±0.01^{a}	Semi solid	Milk white	agreable
Landolphia owariensis	6.40±1.00^{a}	0.82±0.04^{a}	Liquid	Yellow	asgreable
Napoleana imperialis	8.00±0.22^{b}	0.90±0.17^{a}	Liquid	Dark yellow	agtreable

Figures are mean ±SD. Figures bearing different alphabets differ significantly (P< 0.05): N=3

Table 2: Chemical Properties of five selected Nigerian seed oils

Plant	Acid value mEqKg^{-1}	Percent Free Fatty acid	Peroxide value	Iodine value	Saponification value
C. *albidum*	3.56±0.20^{a}	1.76±0.10^{a}	1.80±0.28^{a}	31.06±0.80^{c}	
D. edulis	5.56±0.07^{b}	2.78±0.02^{b}	21.23±1.50^{c}	40.20±2.50^{d}	191.10±3.80^{c}
E. guinensis	14.04±0.22^{c}	7.06±0.01^{c}	2.12±0.41^{a}	18.30±1.10^{b}	246.60±4.20^{d}
L. owariensis	15.33±0.27^{c}	7.70±0.11^{c}	2.80±0.50^{b}	15.10±0.80^{a}	42.40±3.00^{a}
N. imperialis	15.15±0.16^{b}	2.60±0.07^{b}	1.55±0.35^{a}	45.80±2.00^{e}	77.06±40^{b}

Figures are mean ±SD. Figures bearing different alphabets differ significantly (P< 0.05) according to Duncan's multiple range analysis N = 3.

D. edulis pulp and seed and *Cucurbita maxima* seed (Ajayi and Oderinde, 2002; Amoo *et al.* 2004). This non-drying" attribute qualifies them for use in the paint industry (Dosumu and Ochu, 1995). The iodine value is also an index for assessing the ability of an oil to go rancid (Eka, 1980, Amoo *et al.*, 2004). The iodine values obtained in this study indicate that the oils contain appreciable level of unsaturated bonds. Storage procedure used should ensure protection of oil from oxidative deterioration.

Acid value is used as an indicator for edibility of oil and suitability for used in the paint industry.

The acid values of the seed oils ranged from 3.56±0.20 for *C albidum* to 15.33±0.20 for *L. owariensis*. The obtained acid value for palm kernel oil is 14.04±0.022. Pearson (1976), reported acid values of 4 for sesame, soybean, sunflower and rape seed and 7 for olive oil.

Free fatty acid values of less than 3 were obtained for *C. albidum, D. edulis* and *N. imperalis* seed oils are within allowable limits for edible oils while the values for *E. guinensis* and *L. owariensis* are slightly above (Eckey, 1954). The oils could therefore be used as edible oils.

Peroxide values of less than 3 (except for *D. edulis*) seed oils were obtained. The high value (21.23±1.5) recorded for *D. edulis* seed oil can be reduced by alkaline refining. The peroxide value is used as an indicator of deterioration of oils. Fresh oils have values less than 10 mEq Kg^{-1}. Values between 20 and 40 result to rancid taste.

Saponification value is used in checking adulteration. Saponification values were *L. owariensis* 42.40, *N. imperalis* 77.40 C. albidum 126.30, *D. edulis* 191.10, and *E. guinensis* 236.70. The low saponification value obtained for. *L. owariensis* and *N. imperalis* suggests that they may not be industrially, useful. The relatively high value recorded for the other three seed oils is indicative that they have potential for use in the industry. (Amoo *et al.*, 2004).

Conclusion: Based on the extractable oil the seeds can be classified as high (*E. guinensis*, average (*C. albidum, D. edulis*) as low (*L. owariensis*), *N. imperalis*) yielding. Many of physicochemical properties of the seed oils studied compared favourably with palm kernel oil and other conventional seed oils such as groundnut oil, and soybean. Their colour and odour are agreeable. The seed oils therefore have potential for development for use as domestic and industrial oils.

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Blood Profile of West African Dwarf Goats Fed *Panicum maximum* Supplemented with *Afzelia africana* and *Newbouldia laevis*

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Abstract: The haematological and biochemical status of twenty West African dwarf goats fed *Panicum maximum* supplemented with foliage from *Afzelia africana* and *Newbouldia laevis* was investigated. Values for PCV, Hb concentration and RBC count differed significantly ($P < 0.05$) between the diets. Average PCV value was highest in 25Nwb:75Pm diet and least in 25Afz:75Pm diet. Hb concentration was significantly higher in diet 25Nwb:75Pm than in diet 25Afz:75Pm but not different from all the other dietary treatments. RBC counts observed differed between the dietary treatments. Apart from the values obtained for sodium, potassium, bicarbonate, total protein and aspartate transaminase, differences between the measured biochemical parameters were not significant ($P > 0.05$) between the diets. Sodium was highest in 25Afz:75Pm diet and varied significantly ($P < 0.05$) compared to 50Nwb:50Pm diet. Potassium in the serum of the studied animals was significantly higher ($P < 0.05$) in 25Afz:75Pm diet than in 25Nwb:75Pm and 100Pm (control) diets but did not differ from diets 50Afz:50Pm and 50Nwb:50Pm. In terms of total protein level, only the 100Pm (control) diet differed significantly from the supplemented diets. Activities of the enzymes alanine transaminase, aspartate transaminase and alkaline phosphatase in the sampled sera did not vary between the diets except for aspartate transaminase where, only the 50Nwb:50Pm diet differed from the other diets. These results to a large extent suggest the positive potential of the studied plant leaves in the feeding of goats without adverse effects.

Key words: Goats, haematological indices, serum biochemistry, *Afzelia africana*, *Newbouldia laevis*

Introduction

The West African dwarf (WAD) goat is the dominant breed of small ruminants and make up 38 percent of the 38 million goats found in the West African humid zone (Gall, 1996). It is well adapted to this environment and trypanotolerant (Steele, 1996). Generally, the feeding pattern of these dwarf goats is characteristic of the native husbandry practice whereby they scavenge for food to meet their daily nutrient requirements (Daramola *et al*., 2005). However, due to scarcity of enough green fodder for these natural browsers, particularly in the dry season, attempts have focused on the utilization of the abundant but unconventional foliages by goats in this eco-zone which tend to be green all-year-round. With a large proportion of plants being used for the nourishment of various domestic animals (Rehm and Epsig, 1991), naturally occurring browse species thus appear a vital component in the diet of sheep and goats, with goats particularly dependent on them to meet their nutrient requirements.

Afzelia africana (Sm.) is a legume common to the savannah of tropical humid Africa (Nielson, 1965) and quick growing (Prinsen, 1986). It's foliage can be lopped and fed to ruminants (Aye and Adeyeye, 2002) and have a high nutritive value for goats (Anugwa *et al*., 2000). *Newbouldia laevis* (P. Beauv.) is a fast growing non-leguminous shrub or small tree common in the forest area of West Africa (Akobundu, 1984) and planted around the homestead (Okigbo, 1980) with a widespread distribution. Haematological and biochemical determinations in animals have been well documented by Oduye and Adadevoh (1976) and Taiwo and Anosa (1995). According to Karesh and Cook (1995) examining blood for their constituents is used to monitor and evaluate disease prognosis of animals. Much of the available information on the haematology and biochemistry for goats in the humid tropics has mostly been on disease prognosis. Thus, information on blood parameters of goats offered foliages from these unconventional plants as feed have mostly been scanty. The objective of this study therefore is to provide information on some haematological and biochemical parameters of the West African dwarf goat fed a basal diet of *Panicum maximum* (Pm) supplemented with *Afzelia africana* (Afz) and *Newbouldia laevis* (Nwb).

Materials and Methods

Animal management and feeding: This experiment was carried out in the Teaching and Research Farm of Ambrose Alli University, Ekpoma, Nigeria. It involved 20 WAD female goats purchased from the open market and weighing between 5.5 and 9.5Kg. The goats were vaccinated against Peste de Petit Ruminant (PPR), dipped and dewormed. They were housed individually and randomly allotted to one of five dietary treatment groups and in replicates of four animals per treatment.

The dietary treatments comprised animals solely on *Panicum maximum* (100Pm), 25%Afz:75%Pm, 50%Afz:50%Pm, 25%Nwb:75%Pm, 50%Nwb:50%Pm. Quantity of the diet offered the animals was calculated on the basis of 50g DM Kg^{-1} d^{-1} and had free access to water.

Blood collection: On the last day of an 84-day experimental feeding trial period, two sets of blood samples were taken from all the animals via jugular venipuncture using a 10ml 20guage syringe. One set of the blood samples (5ml) was collected into plastic tubes containing the anti-coagulant ethylene diamine tetraacetic acid (EDTA) for the determination of haematological parameters. The other set of blood samples (10ml) was collected into anti-coagulant free plastic tubes, allowed to coagulate at room temperature and centrifuged for 10mins at 3000 rmp. The supernatant sera were then stored in a freezer for subsequent biochemical analysis.

Analysis: The proximate chemical composition of the basal and supplemental foliages offered the animals were determined according to the procedures of AOAC (1990).

Haematological values of the blood samples were estimated for packed cell volume (PCV), haemoglobin (Hb) concentration following the procedures outlined by Schalm *et al.* (1975). Red blood cell (RBC) and total white blood cell (WBC) as well as the differential WBC counts were determined using the Neubauer haemocytometer after appropriate dilution. Values for the constants: mean corpuscular haemoglobin (MCHC), mean corpuscular haemoglobin (MCH) and mean corpuscular volume (MCV) were calculated from RBC, Hb and PCV values as described by Jain (1986).

Biochemical constituents of the serum samples estimated include calcium (Lorenz, 1982), sodium and potassium (Berman, 1975), inorganic phosphorus, chloride and bicarbonate (Toro and Ackermann, 1975), urea (Tietz, 1970), creatinine (Bonsnes and Taussky, 1945) and cholesterol (Allain *et al.*, 1974). Total protein and albumin were by the method of Peters *et al.* (1982) and globulin according to Coles (1986).

The activity of the enzymes alanine transaminase (ALT) and aspartate transaminase (AST) was measured using the method of Reitman and Frankel (1957) and alkaline phosphatase (ALP) was by the method of Roy (1970).

Resulting haematological and biochemical data obtained from the samples were then compared statistically on the basis of the different dietary treatments using analysis of variance procedure for completely randomized design and differences between means separated using the Duncan's multiple range test (SAS, 1994).

Results and Discussion

The chemical composition of the leaves from *Afzelia africana* and *Newbouldia laevis* offered the animals as well as that of *Panicum maximum* are presented in Table 1. The crude protein content for *A. africana* and *N. laevis* were quite high compared to that of *P. maximum* and comparable to the average of 12.50% reported for some tropical native browse plants (Le Houérou, 1980). Relative to the suggested requirement range of 0.19-0.77% for calcium (McDowell, 1997), 0.01-1.0% for potassium (McDowell, 1992), 0.01-0.25% for sodium (Fettman *et al.*, 1984) and 1.20-2.70% for phosphorus (Akinsoyinu, 1986) the inorganic mineral contents of the plant leaves appeared adequate for ruminants.

Table 2 shows the haematological values of the goats solely on *Panicum* (100Pm) and those on 25 and 50 percent supplementation with *Afzelia* (25Afz:75Pm and 50Afz:50Pm) and *Newbouldia* (25Nwb:75Pm and 50Nwb:50Pm).

The observed haematological values showed that except for PCV, Hb concentration and RBC count, where the mean values between the five diets significantly ($P < 0.05$) differed; all the other haematological parameters measured did not. Mean PCV was highest in 25Nwb:75Pm diet and least in 25Afz:75Pm diet. However, these values were within the range of 21-35% reported for WAD goats by Daramola *et al.* (2005). In contrast, Taiwo and Ogunsanmi (2003) reported higher values of 36.9% and 35.5% for clinically healthy WAD goats and sheep respectively. The implication of this observed PCV values, going by the reports of Dargie and Allonby (1975), is that only the goats on 25Nwb:75Pm diet could probably have the high tendency for a return of PCV to normal level following an infection through compensatory accelerated production. This is in view of the fact that only the goats on this diet had values above the 32% PCV documented to be normal for circulatory system in sheep (Frandson, 1974). A comparison of the PCV values in this study to that from other investigators for Zarabi and Baladi goats (El-Barody and Luikart, 2000), Red Sokoto goats (Tambuwal *et al.*, 2002), WAD sheep (Taiwo and Ogunsanmi, 2003) and WAD goats (Daramola *et al.*, 2005) however, support the observation of Azab and Abdel-Maksoud (1999) that PCV values for ruminants varies from breed to breed.

The haemoglobin concentration in the blood of the studied goats showed a similar pattern of variation as with PCV. Mean Hb concentration was significantly higher ($P < 0.05$) in diet 25Nwb:75Pm than in diet 25Afz:75Pm but did not differ from the other dietary treatments. Nevertheless, the Hb range in this study was similar to the mean value for goats fed *Prosopis juliflora* by Misri *et al.* (2000) and fell within the range of 7-15g/dl reported by Daramola *et al.* (2005). It however was lower than the value of 11.40g/dl reported for Red Sokoto goats (Tambuwal *et al.*, 2002) and in cattle fed different

Table 1: Chemical composition (g/100gDM) of the leaves offered the experimental goats

Nutrient	Leaves		
	Afzelia africana	*Newbouldia laevis*	*Panicum maximum*
Dry matter	30.50	42.24	34.72
Crude protein	29.85	15.57	8.44
Ash	6.66	2.49	12.86
Crude fibre	24.24	12.38	21.34
Ether extract	7.95	13.59	6.46
Nitrogen free extract	31.31	55.98	50.90
Gross energy (Kcal/g)	3.25	4.09	3.68
Calcium	0.31	0.51	0.30
Phosphorus	0.32	0.26	0.34
Potassium	1.14	1.02	5.34
Sodium	0.14	0.17	1.86

levels of extracted rice bran (Singh *et al.*, 2002). With the relatively higher Hb concentration observed in this study, the dietary treatments generally seemed to be capable of supporting high oxygen carrying capacity blood in goats.

Although the RBC counts observed in this experiment differed significantly ($P < 0.05$) between the dietary treatments they however, were much lower than the 9.2-13.5g/l range reported for Red Sokoto goats (Tambuwal *et al.*, 2002) and 9.9 and 18.7g/l (Taiwo and Ogunsanmi, 2003) for goats and sheep respectively. Red blood cell indices aid in the characterization of anemia (Merck's Veterinary Manual, 1979). Thus, the low RBC counts recorded for the goats in the different diets present a likely high susceptibility to anemia-related disease conditions by these goats. This is corroborated by the fact that the goats in this study recorded MCV values that were relatively high compared to the normal range of 18-34fl for goats, which could have resulted from the release of immature red blood cells into the blood system (Merck Veterinary Manual, 1979).

WBC counts in all the diets was higher than reported values by Tambuwal *et al.* (2002) for Red Sokoto goats, Taiwo and Ogunsanmi (2003) for WAD sheep and Daramola *et al.* (2005) for WAD goats.

According to Otesile *et al.* (1991) serum biochemistry is a generalized medium of assessing the health status of animals. Variations in the biochemical indices of the WAD goats placed on the different dietary treatments are shown in Table 3. Aside from the values for sodium, potassium, bicarbonate, total protein and arspartate transaminase, differences between the measured biochemical parameters were not significantly ($P > 0.05$) different between the diets.

The concentration of sodium was highest in the 25Afz:75Pm diet and varied significantly ($P < 0.05$) compared to 50Nwb:50Pm diet. The observation was comparable to that reported for Red Sokoto goats (Tambuwal *et al.*, 2002). However, this study reported serum sodium means that were lower than levels observed with mange infested and non-infested WAD goats by Adejinmi *et al.* (2000) and the value of 149.9mmol/l for temperate sheep (English *et al.*, 1969). Oduye and Fasanmi (1971) had earlier attributed low sodium levels to be the case in tropical environment. The range of 129.3-138.1mmol/l reported for WAD goats by Daramola *et al.* (2005) were lower than observed in this study. It is thought that the marked variation in sodium levels in the serum of the goats on diets 25Afz:75Pm and 50Nwb:50Pm may have resulted from variable intake of sodium in these diets based on the sodium content of the plant leaves as earlier shown in Table 1. However, the generally higher sodium level in the *Afzelia* than the *Newbouldia* supplemented diets may be attributed to cellular dehydration characterized by haemo-dilution as observed by Zilva and Pannall (1984).

Mean potassium levels in the serum of the studied animals was significantly higher ($P < 0.05$) in 25Afz:75Pm diet than in 25Nwb:75Pm and 100Pm (control) diets but did not differ from that of diets 50Afz:50Pm and 50Nwb:50Pm. Whether the presence of *Panicum maximum* at high levels, that is 75% and above in this case resulted in the readsorption of potassium (Tanwar *et al.*, 2000) is not too clear in this study. The potassium level reported by Tanwar *et al.* (2000) for non-ketotic healthy goats was comparable to diets with high *P. maximum* content in this study.

Bicarbonates which are chiefly chemical buffers are responsible for maintaining acid/base balance in the animal body. According to Jain (1986) this is usually regulated by the kidney and the digestive tract. In this study, the control group and 50Nwb:50Pm diet had significantly ($P < 0.05$) higher values than 50Afz:50Pm diet but did not differ from that of diets 25Afz::75Pm and 25Nwb:75Pm respectively.

Serum proteins are important in osmotic regulation, immunity and transport of several substances in the animal body (Jain, 1986). However, in this experiment, apart from the control diet (100Pm) the supplement diets did not differ significantly in terms of their total protein levels in the serum of the goats. Besides, the statistically non-significant ($P > 0.05$) difference between the control and 25Afz::75Pm diet may be related to the findings of Tewe and Maner (1980) that serum protein is not related to the amount of calories contained in diets but to the availability of protein. The diets in this study did not significantly affect the globulin levels in the serum of the goats thus indicating the safety of these leaves as supplements for goats. The higher values for total protein, albumin and globulin in this study compared to reports by Esugbohungbe and Oduyemi (2002) suggest that the studied plants could contain low levels of tannin known to diminish nutrient permeability in gut walls as well as increase excretion of endogenous protein which is subsequently passed out in the faeces and so may not alter protein metabolism (Mitjavila *et al.*, 1977).

Table 2: Effect of supplementing *Panicum maximum* with *Afzelia africana* and *Newbouldia laevis* on the hematological parameters of WAD goats

Parameters	Dietary treatments					
	100Pm	25Afz:75Pm	50Afz:50Pm	25Nwb:75Pm	50Nwb:50Pm	Mean±SE
PCV (%)	30.25^{ab}	25.75^{b}	30.50^{ab}	34.00^{a}	29.50^{ab}	30±2.97
Hb (g/l)	9.80^{ab}	8.45^{b}	10.13^{ab}	11.00^{a}	9.75^{ab}	9.83±0.92
RBC (x10^{6}il)	3.13^{ab}	2.70^{b}	3.25^{ab}	3.65^{a}	3.18^{ab}	3.18±0.34
MCHC (g/dl)	32.80	32.83	33.48	32.70	33.05	32.97±0.31
MCH (i/g)	30.80	31.35	31.40	30.28	31.38	31.04±0.49
MCV (fl)	94.25	95.50	93.75	93.00	93.00	93.90±1.04
WBC (x10^{3}il)	27.98	29.70	20.95	26.30	29.98	26.98±3.68
Lymphocytes (%)	67.50	65.50	58.00	66.50	56.25	62.75±5.22
Neutrophils (%)	32.25	32.75	39.50	31.25	41.00	35.35±4.54
Eosinophils (%)	0.25	0.25	0.00	0.00	0.25	0.15±0.14
Monocytes (%)	0.50	1.25	2.50	2.25	2.25	1.75±0.85
Basophils (%)	0.00	0.25	0.00	0.00	0.00	0.05±0.11

100Pm= 100% Panicum maximum; 25Afz:75Pm= 25% *Afzelia Africana* + 75% *Panicum maximum*; 50Afz:50Pm= 50% *Afzelia Africana* + 50% *Panicum maximum*; 25Nwb:75Pm= 25% *Newbouldia laevis* + 75% *Panicum maximum*; 50Nwb:50Pm= 50% *Newbouldia laevis* + 75% *Panicum maximum*. SE = standard error of the means.

Table 3: Effect of supplementing *Panicum maximum* with *Afzelia africana* and *Newbouldia laevis* on serum biochemical values of WAD goats

Parameters	Dietary treatments					
	100Pm	25Afz:75Pm	50Afz:50Pm	25Nwb:75Pm	50Nwb:50Pm	Mean±SE
Sodium (mmol/l)	138.00^{ab}	142.00^{a}	138.50^{ab}	136.50^{ab}	135.25^{ab}	138.05 ±2.55
Potassium (mmol/l)	4.80^{b}	5.58^{a}	5.05^{ab}	4.85^{b}	5.07^{ab}	5.07±0.31
Chloride (mmol/l)	94.83	92.03	96.00	97.70	92.58	94.63±2.36
Bicarbonate (mmol/l)	16.50^{a}	15.00^{ab}	13.00^{b}	15.25^{ab}	16.75^{a}	15.30±1.49
Calcium (mmol/l)	2.20	2.33	2.43	2.28	2.30	2.31±0.08
Phosphorus (mmol/l)	1.70	2.15	2.15	1.68	2.03	1.94±0.24
Urea (mg/dl)	37.30	37.20	34.35	32.25	32.85	34.79±2.37
Creatinine (mg/dl)	0.25	0.30	0.25	0.25	0.25	0.26±0.02
Cholesterol (mg/dl)	103.55	102.58	117.05	106.48	126.93	111.32 ±10.45
Total protein (g/dl)	7.53^{b}	8.48^{ab}	9.20^{a}	9.55^{a}	9.25^{a}	8.80±0.81
Albumin (g/dl)	3.13	3.05	3.28	3.20	3.30	3.19±0.10
Globulin (g/dl)	5.88	5.38	6.00	6.48	5.95	5.94±0.39
ALT (IU/l)	10.63	10.50	12.03	10.73	8.00	10.38±1.47
AST (IU/l)	46.40^{ab}	49.33^{ab}	59.00^{a}	43.45^{ab}	41.05^{b}	47.85±6.97
ALP (IU/l)	60.93	56.25	79.18	65.55	30.73	58.53 ±17.74

100Pm= 100% *Panicum maximum*; 25Afz:75Pm= 25% *Afzelia Africana* + 75% *Panicum maximum*; 50Afz:50Pm= 50% *Afzelia Africana* + 50% *Panicum maximum*; 25Nwb:75Pm= 25% *Newbouldia laevis* + 75% *Panicum maximum*; 50Nwb:50Pm= 50% *Newbouldia laevis* + 75% *Panicum maximum*.SE = standard error of the means.

Urea and creatinine levels did not differ between the diets in this study. However, compared to values reported for apparently healthy Marwari goats (Tanwar *et al*., 2000), this study reported high serum urea values. This may probably have been due to persistent hypoglycemia since according to Radostits *et al*. (1994) catabolic activity is increased for gluconeogenesis thus resulting in higher serum urea levels.

The monitored activities of the enzymes alanine transaminase (ALT), aspartate transaminase (AST) and alkaline phosphatase (ALP) in the sera of the goats studied did not vary widely between the diets except for AST where only the mean from the 50Nwb:50Pm treatment differed significantly from the other diets. Enzymes are protein catalysts present mostly in living cells and are constantly and rapidly degraded although, renewed by new synthesis (Coles, 1986). According to Zilva and Pannall (1984), normal enzyme level in serum is a reflection of a balance between synthesis and their release, as a result of the different physiological processes in the body. Transaminase enzymes are those mostly responsible for the synthesis of non-essential amino acids through the process known as transamination according to Carola *et al.* (1990). In this study, the relatively close and but low mean levels observed for the transaminases could be an indication that the test diets did not differ in their effects on enzyme secretion mechanism. According to Keele and Neil (1971), serum levels of AST are significantly high under disease and morbid conditions involving injuries to large numbers of metabolically active cells. However, the result of this study suggests a contrary situation in this regard thus indicating the potential of the studied plant leaves in the feeding of

goats. Guyton (1991) observed that ALP level in the blood is usually a good indicator of bone formation since osteoblasts secrete large quantities of this enzyme. Thus, since the diets in this study did not differ from especially the 100Pm diet, it may be deduced that the leaves under study did not adversely disrupt the activity of these osteoblasts. Although the level of ALP can be influenced by disease, pregnancy as well as blood pH (Kelly, 1974), since apparently healthy and non-pregnant goats were used for this study, this parameter cannot be said to have been affected by these factors.

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Studies on Microbial and Sensory Quality of Mango Pulp Storage with Chemical Preservatives

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Abstract: The effect of chemical preservatives of sodium benzoate, potassium metabisulphite and potassium sorbet used individually and in combination, was studied on the microbial and sensory quality of the mango pulp (packed in 1kg glass and plastic containers) stored at ambient temperature (30-36°C) for 90 days with an interval of fifteen days. Mean score of taste panel for color, flavor and overall acceptability significantly ($p<0.01$) decreased, while microbial growth significantly ($p<0.01$) increased during storage. Results showed that samples with 0.2% potassium metabisulphite packed in plastic containers had negligible microbial growth, maintained maximum nutrients stability and best quality characteristics during storage.

Key words: Mango pulp, chemical preservatives, sensory quality

Introduction

Mango (*Mangifera indica* L.) is one of the oldest and most important tropical fruit. It is cultivated in almost every tropical and sub tropical country. It has originated in a tropical to sub tropical monsoon area in the Himalayan foot hills especially Burma and eastern India. Later on it spread to Africa, Brazil, Caribbean and Central America (Iagtiani *et al*., 1988).

Pakistan produces mangoes in large quantities. The main varieties grown in Pakistan are Dusehri, Katha, Chonsa, Anwar Ratual, Malda, Fajri, Saroli, Sindheri, Langra, Desi, Almas, Totapari, and Ting etc (Iagtiani *et al*., 1988). Total area under mango cultivation in Pak. was 102.7 thousand hectares and total production was 1.04 million tonnes during the year 2002-2003, while the total area and production of mango in N.W.F.P was 306 hectares and 3224 tonnes respectively. (Agri. Stat. of Pak., 2002-2003).

Mango is one of the cherished fruit not only for taste but also for nutritional values. It serves as a good source of energy and vitamin A & C, Iron & phosphorus etc. (Watt and Merril, 1963). Mango fruit is also beneficial in the treatment of nephritis as well as other kidney troubles (Islam, 1986). Due to such qualities, perhaps mango is considered as the king of fruits.

Mango is mostly consumed as fresh fruit, but due to its perishable nature it cannot be stored for long time. In order to make the mango fruit available during the off season it is processed to make juices, jams, squashes, nectars, chutney, pickles, toffees, canned mango slices etc. (Hussain *et al*., 2003).

During peak harvest season large quantities (30-50%) of this valuable fruit is wasted due to limited storage life (Wills and Scott, 1972). It is important to prepare such products, which can be preserved for longer time. Keeping in view this fact, this study was undertaken to know the effect of different chemical preservatives on the microbial and sensory quality of mango pulp stored in bulk at ambient temperature (30-36°C). In this way the producers will get their proper return at peak harvest season and the consumer will enjoy a variety of products in off season.

Materials and Methods

Preparation of mango pulp: Fresh mature mangoes were purchased from Peshawar fruit market and were brought to Department of Food Sci. and Tec. NWFP Agricultural University Peshawar, where the research was carried out. After washing, peeling and coring, the flesh was cut into small pieces with stainless steel knives and pulp was made by using an electric blender.

Pasteurization: Pulp was then pasteurized in a water bath at a temperature of 82±2°C for 30 minutes to reduce the microbial load.

Addition of acid: Acidity was increased by addition of 1% commercial grade citric acid.

Treatments: The product was divided in to different treatments and was stored in 1 kg glass and plastic containers. Following are the treatments combinations.

T_1 = Control + glass container
T_2 = Control + plastic container
T_3 = 0.2% sodium benzoate + glass container
T_4 = 0.2% sodium benzoate + plastic container

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Table 1: Total Bacterial Count (cfu/g) of mango pulp

	Storage interval (Days)								
Trts	Fresh	15	30	45	60	75	90	% Inc	Means
T_1	7	23	72	135	216	309	384	5385.71	163.71[a]
T_2	7	18	66	127	203	295	356	4985.71	153.14[a]
T_3	5	12	27	44	53	64	83	1560	41.14[b]
T_4	5	10	25	40	50	61	78	1460	38.42[b]
T_5	5	10	22	37	45	58	74	1380	35.85[b]
T_6	5	9	23	34	43	56	70	1300	34.28[b]
T_7	5	10	28	47	53	66	83	1560	41.71[b]
T_8	5	10	27	42	52	61	78	1460	39.28[b]
T_9	5	11	25	40	50	62	77	1400	38.57[b]
T_{10}	5	9	24	37	48	60	75	1400	36.85[b]
Means	5.4[d]	12.2[d]	33.9[cd]	58.3[bc]	81.3[ab]	109.2[ab]	135.9[a]		

Mean followed by different letters are statistically different (P<0.01) using LSD test.

Table 2: Mean score of judges for color of mango pulp

	Storage interval (Days)								
Trts.	Fresh	15	30	45	60	75	90	%Dec	Means
T_1	9	7.6	6.2	4.3	4.3	3.0	1.8	83.33	5.13[b]
T_2	9	7.6	6.4	5.1	4.8	3.1	1.8	80.00	5.40[b]
T_3	9	8.1	7.3	5.5	4.5	3.6	3.3	63.33	5.90[b]
T_4	9	8.3	7.4	5.7	4.7	3.8	3.5	61.11	6.05[b]
T_5	9	8.5	8.1	7.5	7.3	6.8	6.2	31.11	7.63[a]
T_6	9	8.6	8.3	8.1	7.8	7.5	6.9	23.33	8.03[a]
T_7	9	7.9	6.5	5.7	4.8	3.7	2.8	68.88	5.77[b]
T_8	9	7.9	6.6	5.6	4.8	3.8	3.1	65.55	5.83[b]
T_9	9	8.4	7.8	7.3	7.0	6.5	5.5	38.88	7.36[a]
T_{10}	9	8.4	7.9	7.8	6.8	6.6	5.8	35.55	7.47[a]
Means	9[a]	8.1[ab]	7.2[b]	6.3[c]	5.7[cd]	4.8[de]	4.0[e]		

Table 3: Mean score of judges for flavor of mango pulp

	Storage interval (Days)								
Trts.	Fresh	15	30	45	60	75	90	%Dec	Means
T_1	8.8	6.6	4.5	3.3	1.8	1.5	1.3	85.23	3.97[d]
T_2	8.8	6.6	5.5	3.8	2.4	1.6	1.5	82.95	4.31[cd]
T_3	8.6	6.1	7.3	5.6	3.5	3.4	3.0	65.11	5.35[bc]
T_4	8.9	6.3	7.5	5.8	4.3	3.9	3.1	65.16	5.68[b]
T_5	8.9	8.6	7.9	7.5	7.3	6.6	5.8	34.83	7.51[a]
T_6	8.8	8.8	8.5	7.8	7.5	6.8	6.2	29.54	7.77[a]
T_7	8.6	6.9	6.3	5.5	4.7	3.5	2.5	70.93	5.42[bc]
T_8	8.7	6.9	6.1	5.7	4.8	3.7	3.2	62.79	5.58[b]
T_9	8.8	8.3	7.7	6.6	6.3	5.7	5.3	39.33	6.95[a]
T_{10}	8.8	8.3	7.7	6.9	6.5	6.3	5.5	37.50	7.14[a]
Mean	8.8[a]	7.3[b]	6.9[b]	5.8[c]	4.9[d]	4.3[d]	3.7[e]		

Mean followed by different letters are statistically different (P<0.01) using LSD test.

T_5 = 0.2% potassium metabisulphite + glass container
T_6 = 0.2% potassium metabisulphite + plastic container
T_7 = 0.2% potassium sorbet + glass container
T_8 = 0.2% potassium sorbet + plastic container
T_9 = Mix (0.066% each) + glass container
T_{10} = Mix (0.066% each) + plastic container

Storage: Preserved mango pulp was stored for a period of 3 months at ambient temperature (30-36°C). The product was studied for microbial and sensory evaluation at 15 days interval for a total storage period of 90 days.

Total bacterial count (TBC): The samples were analyzed for microbiological evaluation by the total plate count method as described by Diliello (1982).

Sensory evaluation: Ready to serve mango drinks were prepared with selected treatments and were evaluated by a panel of 10 judges for sensory characteristics like color, flavor and over all acceptability as described by Larmond (1977).

Table 4: Overall acceptability of mango pulp

	Storage interval (Days)								
Trts.	Fresh	15	30	45	60	75	90	%Dec	Means
T_1	8.8	6.8	4.9	3.5	1.8	1.6	1.5	82.95	4.12^{d}
T_2	8.8	6.6	5.5	3.8	2.4	1.6	1.6	81.81	4.32^{cd}
T_3	8.6	6.1	7.3	5.6	3.5	3.4	3.3	61.62	5.40^{bc}
T_4	8.9	6.3	7.5	5.8	4.3	3.9	3.5	60.67	5.74^{b}
T_5	8.9	8.6	7.9	7.5	7.3	6.6	6.2	30.33	7.57^{a}
T_6	8.8	8.8	8.5	7.8	7.5	6.8	6.5	26.13	7.81^{a}
T_7	8.6	6.9	6.3	5.5	4.7	3.5	2.8	67.44	5.47^{b}
T_8	8.7	6.9	6.1	5.7	4.8	3.7	3.5	59.30	5.62^{b}
T_9	8.8	8.3	7.7	6.6	6.3	5.7	5.5	37.50	6.98^{a}
T_{10}	8.8	8.3	7.7	6.9	6.5	6.3	5.9	32.95	7.20^{a}
Means	8.8^{a}	7.4^{b}	6.9^{b}	5.9^{c}	4.9^{d}	4.3^{d}	4.0^{d}		

Mean followed by different letters are statistically different (P<0.01) using LSD test. Each figure is the mean of observation of 10 judges

Statistical analysis: The results were analyzed statistically by using Randomized Complete Block Design as recommended by Steel and Torrie (1980) and means were separated by applying LSD test.

Results and Discussion

Total bacterial count: The mean total bacterial count (TBC) of mango pulp was significantly (p<0.01) increased from 5.4 cfu/g to 135.9 cfu/g during storage. For treatments maximum mean value was recorded in sample T_1 (163.71 cfu/g), while minimum mean value was observed in sample T_6 (34.28 cfu/g). During storage maximum increase in TBC was observed in sample T_1 (5385.71%), while minimum increase of micro-organisms was recorded in sample T_6 (1300%) (Table 1). Our results showed that samples with added 0.2% potassium metabisulphite had overall best results in controlling the microbial growth due to its more effectiveness in controlling microorganisms (Brenndor *et al.*, 1985). In another study Hussain *et al.* (2003) reported that application of potassium metabisulphite reduces the growth of microorganisms in mango pulp.

Organoleptic evaluation: The samples were sensory evaluated for color, flavor and overall acceptability at the storage interval of 15 days for a total period of 90 days.

Color: The mean score of judges for color were significantly (p<0.01) decreased from 9 to 4.04 during storage. For treatments maximum mean score was observed in sample T_6 (8.03), while minimum mean score was recorded in sample T_1 (5.13). Maximum decrease was observed in sample T_1 (83.33%), while minimum decrease was recorded in sample T_5 (23.33%) (Table 2). Saini *et al.* (2000) observed that application of potassium metabisulphite control browning in fruit pulp. The reduction in color scores might be due to maillard reaction accelerated during storage. In another study Heikal and El-Sidawi (1972) observed that reducing sugars and amino acids help in browning of fruit pulp during storage.

Flavor: The mean score of judges for flavor were significantly (p<0.01) decreased from 8.76 to 3.74 during storage. For treatments maximum mean score was observed in sample T_6 (7.77), while minimum mean score was recorded for sample T_1 (3.97). Maximum decrease in score was observed in sample T_1 (87.5%) while minimum decrease was recorded in sample T_6 (33.33%) (Table 3). Hussain *et al.* (2003) observed similar results in mango pulp.

Overall acceptability: The mean score of judges for overall acceptability were significantly (p<0.01) decreased from 8.76 to 4.03 during storage. For treatments maximum mean score was observed in sample T_6 (7.81), while minimum mean score was recorded in sample T_1 (4.13). Minimum decrease was observed in sample T_6 (26.13%), while maximum decrease was recorded in sample T_1 (82.95%) (Table 4). In a similar study Saini *et al.* (2000) observed that pulp preserved with potassium metabisulphite either individually or in combination with other preservatives retains maximum overall acceptability, maintains maximum nutrients stability and negligible microbes.
Statistical analysis showed that storage intervals and treatments had a significant (p<0.01) effect on TBC and overall mean score of judges for color, flavor and overall acceptability of all mango squash samples during storage.

Conclusion: It could be concluded that addition of 0.2% potassium metabisulphite helps in controlling microbial growth and maintaining sensory characteristics of mango pulp, packed in bulk plastic containers.

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Chemical and Physio-Chemical Characterization of the Flours and Oils from Whole and Rejected Cashew Nuts Cultivated in Southwest Nigeria

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Abstract: Processed whole as well as rejected cashew nuts were obtained from a processing plant located at Owo, Ondo State, Nigeria. Each of them were divided into two, a part defatted while the other part were undefatted. They were thereafter characterized with respect to their proximate constituents, energy, mineral contents, anti-nutritional factors and functional properties. The physio-chemical properties of the oil from the cashew nuts were also determined. On the average, crude protein (CP) of the whole nut ranged from 200.0 ± 0.3 g kg^{-1} DM in undefatted to 304.0 ± 0.1 g kg^{-1} DM in the defatted sample while it ranged from 176.0 ± 0.6 g kg^{-1} DM to 323.0 ± 0.5 g kg^{-1} DM with 30% and 45.5% increments, respectively. The crude fat of the undefatted samples ranged: 436.0 – 452.0 g kg^{-1} DM while the gross energy content averaged 106 MJ kg^{-1} (range: 39 – 206 MJ kg^{-1}). Apart from Cu and Co, which was not detected, cashew nut meal (CNM) contained appreciable nutritionally needed mineral elements, which were higher in most cases in defatted than in the defatted samples. The phytic acid content averaged 24.6 mg g^{-1} and tannin as total phenols averaged 12.1g kg^{-1}. The water absorption capacity (WAC) was enhanced by 35% and 50%, respectively in the whole and rejected samples by defatting while oil absorption capacity (OAC) were enhanced by 18% and 42%, respectively by defatting. There were wide variations among the foaming capacity (FC) and foaming stability (FS) as evident in the high values of coefficient of variation (CV) of 127.7% and 115.5% respectively. The protein solubility curves generally had multiple maxima and minima peaks. The oil from the CNM had iodine value of 115.0 wijs in rejects and 136.9 wijs in whole nut and the peroxide value varied widely with a CV of 41.85% when compared with other properties of the oil analyzed. The analytical data indicate that the good and rejected cashew nut could be important alternative protein and energy contributors to compound non ruminant animal feed in this region.

Key words: Cashew nut oil, flours, ruminant animal feed

Introduction

The nutritional inadequacies that arise from high cost of animal proteins (milk, eggs, meat) in developing countries have given rise to various forms of malnutrition such as the kwashiorkor, maramus, infant blindness, mortality and morbidity (Tee, 1992; Aletor *et al*., 2002). This has necessitated the use of non-conventional protein food source, especially from plant origins and compelled the need to harness the potentials of the so-called 'wastes' as part replacement for the more expensive protein resource in monogastric feeding (Agbede and Aletor, 2004 and 2005).

The cashew plants are either trees or shrubs, monoecious or diecious and usually radially symmetrical. Cashew is chiefly tropical in distribution while some are strictly in temperate areas of the North hemisphere. There are two types viz: the principal representative in the North America called the Rhus and the mango (*Mangifera indica*) is cultivated in the South of America. The latter represents the most common type in West Africa especially Nigeria (Benson, 1972).

Cashew fruits (*Anacardiaceae*) are among the widely cultivated fruits in southwestern Nigeria. The fruit juice is squeezed and made into fruit juice and the seeds, which contain the nuts, are processed into cashew nuts, pistachio, resin and oils. Although, the nutritional values of cashew nuts have long been recognized (Fetuga *et al*., 1974), cashew nut meal has more recently assumed greater importance due to the fact that its use has been extended from human consumption to the feeding of poultry, especially in layers (Onifade, *et al*., 1998). The upsurge in the consumption of the cashew in this region has resulted in its large-scale production for local consumption and export during which, large quantities of the kennels are broken, bruised or burnt. Such grades of nuts, which do not meet local or export requirements are usually discarded as wastes in several industrial sites in southwest of Nigeria. Conceivably, rejects could be exploited as alternative protein food resources in monogastric feeding after a careful evaluation of the nutritional potentials.

While some of the nutritive values of cashew nuts of other regions have been well detailed, information on the nutritional and physico-chemical characterization of the nuts cultivated in southwest Nigeria remains scanty. This study was therefore designed to assess both

whole and rejected cashew nuts produced in Nigeria with respect to the chemical composition and physico-chemical properties of the flour and oil with a view to increasing their utilization.

Materials and Methods

Good and 'wastes' or reject cashew nuts were collected from Ideal Family Farm in Owo, Nigeria. Ideal Family Farm is one of the major exporters of cashew nuts in Nigeria. At the farm the raw cashew nuts were usually washed manually with sand and water. Thereafter, they were soaked, steamed and dried in the sun for 4 to 5 days. The nuts were then roasted to facilitate the release of the kernels when shelling was done. The shelling was done manually with a locally fabricated Sheller. The whole nuts after shelling were normally dried in the sun. The whole nuts were roasted with sand in a locally constructed frying pan. Thereafter, the nuts were allowed to cool and the nuts separated manually from the sand. In the process of roasting some nuts were burnt and considered as wastes or rejects. The good ones were packed for export. The whole and rejects nuts were finely ground using a laboratory hammer mill (DIETZ, 7311 Dettingen-Teck, Germany). Half of either the whole or rejects were defatted and considered as defatted samples. In all, four samples viz: undefatted whole, defatted whole, undefatted reject and defatted reject were used for analysis. They were kept in airtight container and deep frozen (-18^{o}C) prior to analysis.

Extraction of oil from cashew meal (deffated cashew meal): The ground cashew nut meals were extracted for 6 hours with diethyl ether in a soxhlet extractor. Thereafter, the solvent was evaporated under reduced pressure (AOAC, 1990).

Chemical analysis: The defatted and undefatted samples were analyzed for proximate composition. Crude protein was calculated by multiplying the nitrogen content of the samples with the factor of 6.25. The Na and K contents were determined by Flame photometry (Jenway Ltd, Dunmow, Essex, UK), and P by Vanado-molybdate method (AOAC, 1990). Ca, Mg, Fe, Zn, Cu and Mn were determined after wet digestion with a mixture of nitric, sulphuric and hydrochloric acids, using atomic absorption spectro-photometer (Buck Scientific, East Norwalk, CT06855, USA). The gross energy was determined against thermo-chemical-grade benzoic acid using a Gallenkamp Ballistic bomb calorimeter (Cam Metric Ltd, Cambridge, England).

Analysis of anti-nutrients

Tannin and phytin: For the determination of tannin, finely milled defatted and undefatted samples (200mg in 10ml of 70% aqueous acetone) were extracted for 2 h at 30^{o}C in water-bath using Gallenkamp orbital shaker (Surrey, U.K) at 120 revolutions per minute. Pigments and fat were first removed from the samples by extracting with di-ethyl ether containing 1 % acetic acid. Thereafter, the total polyphenols (as tannic equivalent) was determined as described (Makkar and Goodchild, 1996). The amount of total polyphenols (as tannic equivalent) was calculated from the standard curve.

For the quantification of phytin, eight (8) grams of each finely ground defatted and undefatted samples was soaked in 200ml of 2% hydrochloric acid and allowed to stand for three hours. The extract was thereafter filtered through two layers of hardened filter paper. Thereafter, Phytin-Phosphorus was determined and phytin content was calculated by multiplying the value of phytin-Phosphorus by 3.55 (Young and Greaves, 1940).

Physico-chemical properties of cashew nut oil: The physico-chemical properties of the crude oils extracted viz: the specific gravity, acid value, saponification value, peroxide value, iodine value, free fatty acid (as oleic acid) were determined (AOAC, 1990). Refraction index at 29^{o}C was determined using Abbe refractometer.

Determination of the functional properties of the cashew nut meal: The protein solubility (PS) of the cashew nut meal (whole and rejects) was determined as described (Oshodi and Aletor, 1993; Adeyeye *et al.*, 1994) while the water absorption capacity (WAC) and fat emulsion stability were also determined (Beuchat, 1977). The fat absorption capacity (FAC) was determined as described (Solsulki, 1962) and the lowest gelation concentration (LGC), foaming capacity (FC) and foaming stability (FS) were determined using a standard technique described elsewhere (Coffman and Garcia, 1977).

Data analysis: Mean values for all parameters measured between the cashew nut (whole and rejects) were assigned a coefficient of variation (CV) as described (Snedecor and Cochran, 1973).

Results

Table 1 reveals that defatting enhanced the crude protein (CP) in both the whole and rejected samples by 34% and 45.5%, respectively. Ether extract varied from 436.0 to 452.0 g kg^{-1} DM in undefatted samples while 23.0 to 76.0 g kg^{-1} DM in the defatted samples. The ash contents of the defatted samples (51.0 – 54.0 g kg^{-1}DM) were higher than those of undefatted samples (13.0 g kg^{-1}DM). The gross energy (GE) of the undefatted whole sample (206.2 MJ kg^{-1}) was reduced by 68.8% in the defatted whole sample while that of the undefatted rejected sample was reduced by 66.7 % in the defatted rejected sample.

The mineral elements (P, Ca, Mg, K, Na, Zn and Fe) were more abundant in whole and rejected defatted

Table 1: Proximate Composition (g kg^{-1}DM) and gross energy (MJ kg^{-1}) of Cashew nut meal

	Dry Matter	Crude Protein	Ether Extract	ASH	Gross Energy
Whole undefatted	961.0±0.0	200.0±0.3	452.0±0.0	13.0±0.1	206.2
Whole defatted	938.0±42	304.0±1.1	23.0±0.0	51.0±0.4	64.4
Reject undefatted	953.0±0.1	176.0±0.6	436.0±0.0	13.0±0.3	116.0
Rejected defatted	946.0±5.6	323.0±0.5	76.0±0.0	54.0±0.8	38.6
Coefficient of variation CV%	1.03	29.32	92.75	69.63	69.61

+Values are for triplicate determinations

Table 2: Mineral Composition (mg kg^{-1}) of Cashew Meal

Minerals	Whole undefatted	Whole defatted	Reject undefatted	Reject defatted	(CV%)
Macro minerals					
Phosphorus (P)	685.4	878.6	3610.3	5468.8	86.43
Calcium (Ca)	1887.8	2208.7	1326.8	2427.3	24.37
Magnesium (Mg)	1704.1	1805.8	1240.2	1956.7	18.42
Potassium (K)	1323.1	2504.9	1131.3	2331.7	38.17
Sodium (Na)	1976.2	2577.1	1354.7	1678.8	27.41
Micro minerals					
Iron (Fe)	132.7	170.0	67.0	139.4	34.01
Zinc (Zn)	17.0	534.0	36.3	25.1	165.97
Manganese (Mn)	10.2	9.7	8.4	11.2	12.12
Copper (Cu)	NDT	NDT	NDT	NDT	NDT
Cobalt (Co)	NDT	NDT	NDT	NDT	NDT

+Values are for triplicate determinations; NDT = Not detected

Table 3: Anti nutritional Constituents of Cashew nut meal

Samples	Phytin (mg g^{-1})	Phytin-Phos-phorus (mg g^{-1})	Tannin (g kg^{-1})
Whole undefatted	14.9.0±0.6	4.2±0.2	Nd
Whole defatted	33.7.0±4.1	9.5±1.5	11.4
Reject undefatted	16.7±0.3	4.7±0.1	Nd
Reject defatted	33.0±0.0	9.3±0.0	14.3
Mean	24.6	6.9	12.9
Standard deviation (sd)	10.2	2.9	2.1
Coefficient of variation (%)	41.5	42.0	16.3

+ Values are for triplicate determinations; nd = not determined

samples than the undefatted samples (Table 2). Mn was the least abundant in all while Cu and Co were not detected in the samples. The coefficient of variation (CV) with regard to minerals between the whole and rejected samples varied from 12.1 to 166.0 %.

Table 3 shows the phytin, phytin-P and Tannin constituents of the cashew nut meal. Phytic acid varied from 14.9±0.6 mg g^{-1} in whole undefatted sample to 33.7 ± 4.1 mg g^{-1} in whole defatted with CV of 41.5%. Phytin-P varies widely from 4.2±0.2 mg g^{-1} in whole undefatted to 9.5±1.5 mg g^{-1} in whole defatted while tannin in the defatted samples ranged between 11.4 mg kg^{-1} and 14.3 mg kg^{-1} (Table 3).

Table 4 presents the functional properties (%) of cashew nut meal. The defatted samples (whole and rejects) showed higher percentage of water absorption and oil absorption capacity while foaming capacity and foaming stability were 0% in the reject defatted and undefatted samples. All the samples showed varying solubility with changes in pH (Fig. 1).

Table 5 shows the physico-chemical properties of cashew nut oil. The oil from the whole sample had lower specific gravity (0.90); acid value (12.66) and free fatty acid (0.73), respectively while saponification and peroxide values were higher in the rejected sample. Both had similar refractive index.

Discussion

The present study showed that with respect to the crude protein, ether extract and ash, cashew nut meal could be used as source of good quality food for human and or monogastric animals in this region. For example the nutritive components of the defatted cashew nut samples compared favourably, with and in some instances, surpassed those reported for most legumes grown in West Africa (Ologhobo, 1980 and Aletor and Aladetimi, 1989). The removal of fat enhanced the crude protein in the products by 34-45.5 %, thus suggesting that defatting process could be used to enhance its use as a protein food resource. However, the high fat content of this nut suggests that it could be used as veritable source of energy in human and/ or monogastric animal diets.

This study further confirmed that, like most tropical legumes, cashew nut meal irrespective of whether it is whole or rejected samples contains both major and minor mineral elements. However, the values observed fall within the range reported for several cowpea varieties (Oke *et al.*, 1995) and more recently for differently processed *Canavalia ensiformis* and *Mucuna pruriens* (Agbede and Aletor, 2005). The defatted cashew nut meal (CNM) had the higher values of the minerals evaluated than the undefatted samples.

Table 4: Functional properties (%) of cashew nut meal

	Whole undefatted	Whole defatted	Reject undefatted	Reject defatted	CV
Water absorption capacity	142.5±3.5	219.5±0.7	139.5±0.7	279.0±1.4	34.4
Oil absorption capacity	167.4±0.0	203.7±1.3	129.3±1.3	222.3±1.3	22.8
Foaming capacity	3.0±0.0	6.0±0.1	0	0	127.7
Foaming stability	2.0±0.1	2.0±0.1	0	0	115.5
Emulsion capacity	49.0±0.1	48.6±0.2	49.1±0.1	46.50±0.1	2.6
Least gelatin	8.0±0.0	10.0±0.0	12.0±0.0	10.0±0.0	16.3

+ Values are for triplicate determinations.

Table 5: Physico-chemical Properties of Cashew nut Oil

Parameters	Whole	Reject	CV
Specific gravity (at 20°C)	0.90	0.91	0.66
Acid value (mg/KOH/g)	12.66	22.72	40.19
Saponification value (mg/KOH/g)	17.60	10.01	38.88
Peroxide value (meg/kg)	2.94	1.60	41.85
Refractive index (at 29°C)	1.47	1.47	0.07
Iodine value (Wij's)	136.89	115.01	12.28
Free fatty acids (as oleic acid)	0.73	1.18	33.33

Conceivably, while the whole defatted and undefatted CNM could be used in human food formulations especially in low hypertension risk areas where quality food shortage is endemic, the rejected samples could be used to replace the more expensive conventional plant protein food resources such soy bean and groundnut cake, in monogastric animal diets to enhance animal protein production and consumption in this region.

In spite of the potential contribution of cashew nut meal to the amelioration of protein dearth in most under developed countries, their endowment with ability to synthesize anti-nutritional factors remains a major drawback to their direct use as food by man and livestock. For instance, the phytin and phytin-P of CNM varied from 14.9mg g^{-1} to 33.7mg g^{-1} and 4.2 mg g^{-1} to 9.5 mg g^{-1}, respectively while tannin-averaged 12.9g kg^{-1}. This further confirms the wide occurrence of these two anti-nutritional factors in most leguminous seeds.

The values of the functional properties of the cashew nut meal from this study suggest its potential for the development of different food products. The water absorption capacity (WAC) ranged: 139.5-279.0% for the rejects and the whole ranged: 142.5-279.0% and this compared favourably with those reported for some edible legume seeds (Oshodi and Ekperigin, 1989). The high WAC is considered important in viscous foods such as soups and gravies. The observed values for fat absorption capacity (FAC) were high especially in the defatted whole and rejected (219.5-279.0%) samples and this were higher than the one reported for pigeon pea flour (89.7%) (Oshodi and Ekperigin, 1989), thus indicating the nutritional potentials of CNM as flavour retainers. The foaming capacity and foaming stability (FS) for the rejects were 0% and the values for the whole were equally low (2.0%) at 30 minutes when compared with the values reported elsewhere (Oshodi and Adeladun, 1993) for dehulled varieties of lima bean flour

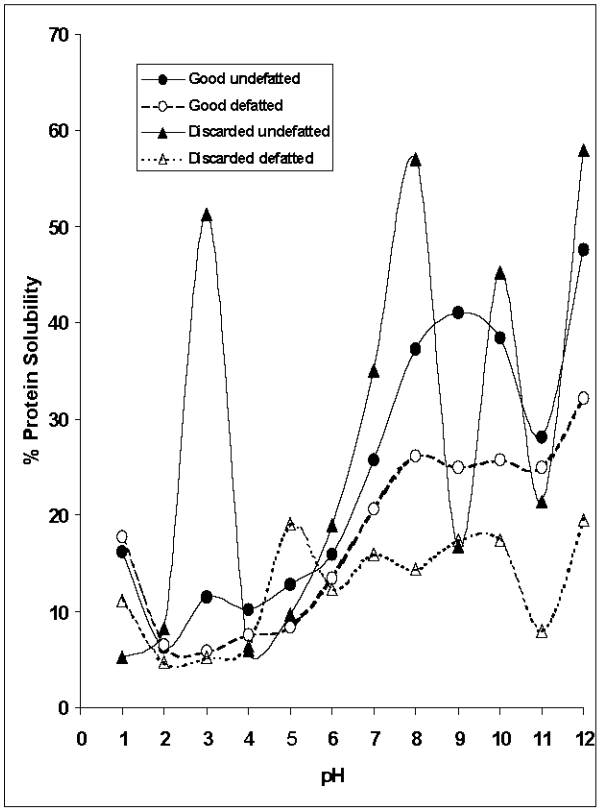

Fig. 1: Protein solubilities as a function of pH

whose FS ranged from 8.80 to 15.20%. This indicates that the cashew nut meal may not be a suitable whipping agent. The whole and rejected samples showed different solubility at different pH ranges in both the acidic and basic regions (Fig. 1), implying that they could be useful in industrial food applications in both acidic and basic regions.

The physico-chemical properties of the oil from the CNM showed that the whole nuts had a higher iodine value (IV), thus suggesting that it has higher proportion of unsaturated fatty acid than in the rejects. The study further showed that the oil is not a drying one as previously indicated (Pearson, 1947). However, the peroxide values obtained for the oils in whole and rejects were still below the recommended standards (Codex Alimentarius Commission, 1970) for all edible oils.

Conclusion: The study further confirmed the nutritive potentials of CNM. Defatting the nut enhanced the protein content but increased the level of phytic acid in the nut. Also the functional properties of the nut indicated their potential usefulness in various food preparations. The Iodine value of the oil suggests that the oil is not a drying one thus implying its edibility. While defatted and undefatted whole cashew nut meal are recommended for human consumption in this region, the rejected nuts could be used to replace the more expensive conventional pulses used in animal food formulations with a viewing to reducing the cost of finished foods which, often derives from the high cost of pulses in this region.

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Detection of Cow Milk in Water Buffalo Cheese by SYBR Green Real-Time PCR: Sensitivity Test on Governing Liquid Samples

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Abstract: A real-time polymerase chain reaction (PCR) assay was developed to detect a bovine-specific mitochondrial DNA sequence in "buffalo" Mozzarella cheese by using primers targeting the cytochrome oxidase subunit 1 (COI) gene. Hot-start PCR, primer design, annealing and signal acquisition temperatures were exploited to obtain reliable analytical conditions which yielded a 134-bp amplicon from cow's DNA only. Water buffalo's DNA didn't originate any amplification product. DNA isolated from blood was used to test primers' specificity and to construct a calibration curve in order to quantify bovine DNA concentration in governing liquid. The method was capable to detect low amounts of cow's DNA in governing liquid samples. Due to low detection limit and fast, simple execution, the analytical protocol described in this work is suitable to become a common tool to detect the fraudulent addition of cow milk in water buffalo Mozzarella cheese.

Key words: Real-time PCR, sensitivity test, species identification, mitochondrial DNA, Mozzarella cheese

Introduction

Italian Mozzarella is a PDO "pasta filata" cheese made using water buffalo milk only and natural whey cultures as fermentation starters. It is produced in Southern Italy since ancient times with little or no changes in the main technological aspects, thus representing a major traditional and genuine Italian dairy product. Cow milk's lower cost and larger availability deriving from greater productions, in conjunction with the frequent breeding of both cows and buffalos in the same farm, encouraged the undeclared addition of cow milk in violation of the Italian law and the European PDO certification (Anonymous, 1996).

The need to protect both producers and consumers from this fraud prompted the development of several chromatographic (Pellegrino *et al*., 1991), electrophoretic (Addeo *et al*., 1989; Cartoni *et al*., 1998), immunological (Addeo *et al*., 1995) and mass-spectrometric (Cozzolino *et al*., 2002) analytical techniques, most of which rely on protein analysis to discriminate the two species. To date, methods based on the isoelectrofocusing of gamma-caseins after plasminolysis (Addeo *et al*., 1989) and based on HPLC (Pellegrino *et al*., 1991), which are the official methods in EU (Anonymous, 2001) and in Italy respectively (Gazzetta Ufficiale della Repubblica Italiana, 1996), have a minimum detection limit of 1 % cow milk.

In the last years, some molecular methods for bovine species identification, mostly based upon PCR technology (Bardin *et al*., 1994; Lopez-Calleja *et al*., 2004) were presented. The DNA assays consist essentially in extracting DNA from governing liquid (Lipkin *et al*., 1993) and amplifying a mitochondrial *cyt b* locus (Kocher *et al*., 1989), while species identification relies upon PCR reaction multiplexing by means of a primer pair for each one (Branciari *et al*., 2000; Rea *et al*., 2001; Bottero *et al*., 2002).

We previously reported bovine species discrimination in water buffalo Mozzarella by PCR (Feligini *et al*., 2005).

We now describe a real-time PCR assay capable to detect bovine DNA in water buffalo Mozzarella cheese. The most important aim of this work was to demonstrate the high potential of this technique in frauds' recognition. Real-time PCR uses a fluorescence detection system that can collect fluorescence measurements during the amplification cycles, thus monitoring the specific product's accumulation, and, unlike conventional PCR, relate them to template's initial quantity. This SYBR green I-based assay allows the simple and fast detection of a specific 134-bp amplicon located in the bovine mitochondrial cytochrome oxidase subunit 1 (COI) gene in samples containing down to 0.5 % of cow milk.

Materials and Methods

Samples: Experimental water buffalo Mozzarella cheeses were manufactured in a traditional fashion, except for adding bovine milk in 30, 20, 10, 5, 1 and 0.5% proportions. The governing liquid (i.e. the pickle in which Mozzarella is packaged) of experimental samples was preserved at -20°C.

Water buffalo and bovine reference DNA were extracted from blood using phenol and chloroform (1:1 vol/vol) (Sambrook *et al*., 1989). DNA was precipitated by adding 3 M sodium acetate at pH = 5.2 and absolute ethanol (1:2.5 vol/vol), then washed with 100 µL of an ethanol/double distilled water solution (70:30 vol/vol) and finally suspended in distilled water. DNA from blood

was spectrophotometrically quantified in a GeneQuantPro apparatus (Amersham Pharmacia Biotech, Uppsala, Sweden) at 260/280 nm. After DNA extraction, samples were preserved frozen at -20°C.

DNA extraction: Governing liquid (40 mL) was centrifuged (4°C) for 30 min at 2000 x g and the pellets were resuspended in 1 mL lysis buffer (1 mM EDTA, 10 mM Tris/HCl pH 7.5, 50 mM NaCl, 5 g/L SDS). 100 µL of a 10 mg/mL Proteinase K (Sigma-Aldrich, St. Louis, MO) were added to the solution, which was then incubated on a linear shaker at 42°C overnight. 1 mL of the digested suspensions was solvent-extracted once using 1 mL phenol and three times using a phenol/chloroform (1:1 vol/vol) volume equal to the supernatant's one, each time followed by centrifugation for 15 min at 18,000 x g and transfer of the supernatant to a new tube. DNA was pelleted by adding sodium acetate (3 M, pH 5.2) and absolute ethanol (-20°C), incubating for 120 min at -20 °C and centrifuging (4°C) for 30 min at 18,000 x g. The pellet was finally resuspended in 200 µL sterile deionized water and frozen until analysis.

Real-time PCR amplification: The real-time PCR amplifications were performed in a DNA Engine Opticon® 2 System for continuous fluorescence detection (MJ Research, Boston, USA). The reaction was conducted in a final volume of 30 µL containing: 200 µM dNTPs, 10 mM Tris/HCl pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 100 nM primers, 0.9 U DNA polymerase (AmpliTaq Gold, Perkin Elmer) and 6 µl of DNA solution an unknown concentration. The primer pair consisting of primer BT3 [5'-GAACTCTGCTCGGAGACGAC-3'] and primer BT4 [5'-AGCACCAATTATTAGGGGAAC-3'] was used for the amplification of a bovine-specific 134-bp region of the COI gene (Feligini *et al.*, 2005). Thermocycling was performed as 45 cycles of 30 s at 95 °C (denaturation), 30 s at 56°C (annealing) and 30 s at 72°C (extension). The final extension step was carried out as 5 min at 72°C. SYBR Green I (Stratagene, La Jolla, CA, USA) was used to monitor the accumulation of double-stranded 134 bp amplicon; it was added to the PCR reagents as a 1:2,000 aqueous solution in order to obtain a final concentration of 1:50,000. The dye was diluted prior to each run because of its unstability to freezing and thawing according to Wittwer *et al.* (2001). PCR negative controls were run along with samples, in order to detect false positives due to possible contaminations.
Results of the real-time PCR assay were analyzed with Opticon® 2 software supplied with the Opticon cycler, obtaining fluorescence intensity *vs* cycle number curves.

Calibration curve: After spectrophotometrical quantification, reference bovine DNA extracted from blood was diluted to obtain a series of concentration standards set at 50 ng/µL, 5 ng/µL and 0.5 ng/µl respectively. Standards were amplified four times along with samples, in triplicate for each run. Fluorescence data from all standards' amplifications were processed in order to construct a concentration vs Ct semilog calibration curve. This calibration curve was then used to quantify samples from governing liquid.

Results and Discussion

A real-time polymerase chain reaction amplifying a fragment of the cytochrome oxidase subunit I (COI) gene was developed for the detection of bovine milk in buffalo Mozzarella cheese. Primers' specificity and functionality were tested by amplifying reference DNA from blood of both cow and water buffalo. The amplification plot, showing the changing in measured fluorescence along PCR cycles, is reported in Fig. 1. The former sample showed a clear increase in emitted fluorescence starting at cycle 24.16, while the latter one didn't reach the threshold intensity. PCR products were subsequently analyzed by agarose gel electrophoresis and sequencing in order to obtain further confirmation regarding the expected size (134 bp) of the specific amplicon and the absence of nonspecific amplification products.
Fig. 2a shows the fluorescence profiles obtained from one of the four standards' triplicate amplifications. The observed average threshold cycle (Ct) values were 22.84, 26.89 and 30.33 and were inversely related with standards' concentration.
Mean Ct and confidence interval (P = 0.05) were calculated for each concentration from all standards runs' data (Table 1). Standards' fluorescence profiles and Ct values were evidently grouped according to their concentration (Fig. 2a) and the calibration curve featured a good linear correlation coefficient (Fig. 2b), notwithstanding the confidence intervals slightly widened along with concentration's lowering.
Logarithms of the DNA concentration were plot against the calculated means (Fig. 2b), obtaining a straight line of equation y = - 0.27087 x + 8.1669 (where y is the log of DNA concentration and x is the Ct), with a linear correlation coefficient (r^2) of 0.99993. This equation was used to quantify DNA from governing liquid.
To evaluate method's effectiveness and reliability on DNA isolated from governing liquid, duplicate real-time PCR amplifications were performed. Fig. 3 shows the fluorescence profiles obtained in a single run. Although the bovine-to-buffalo ratio was known, the DNA concentration in samples was not assessed prior to PCR reaction whilst it was calculated by means of the Opticon Monitor® software applying the calibration curve. The estimated concentrations of bovine DNA from governing liquid samples were low (down to the calibration curve's limit of 0.5 ng/µl) and very close to each other regardless to the bovine-to-buffalo ratio, thus

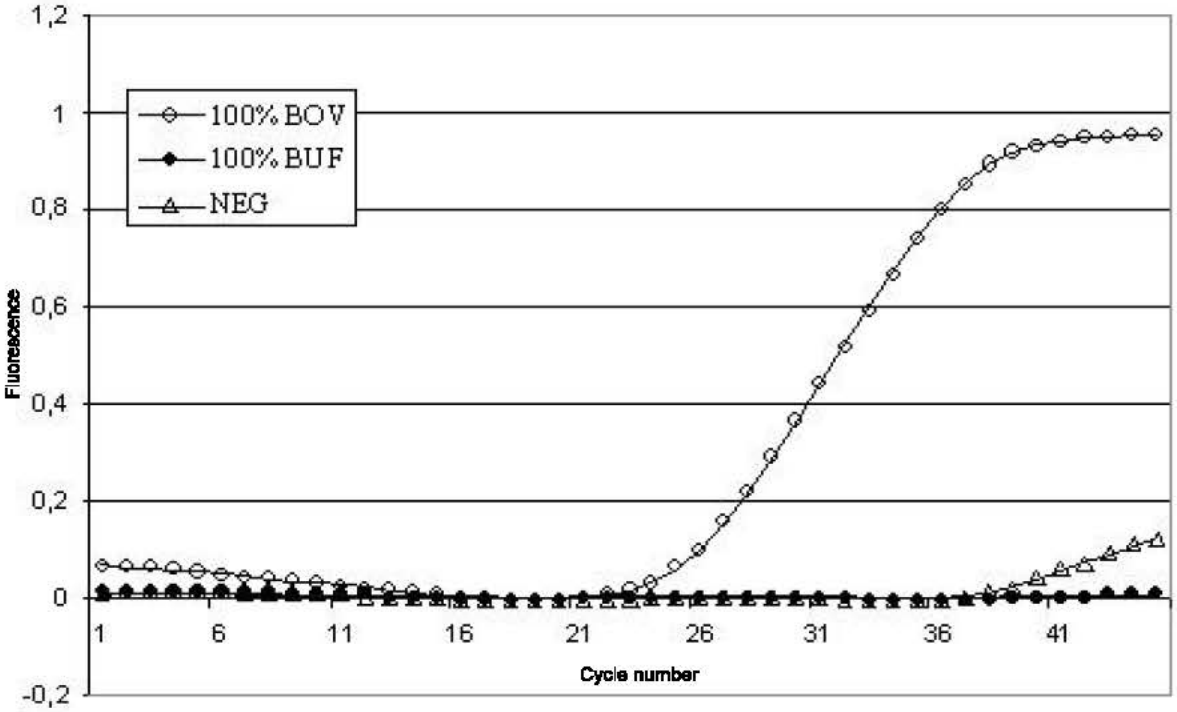

Fig. 1: Real-time PCR of bovine and water buffalo reference DNA isolated from blood. The reaction was carried out using BT3/BT4 bovine-specific primer pair. SYBR Green I fluorescent dye was used to monitor the amplification of a 134-bp fragment in CO1 gene from mitochondrial bovine DNA.

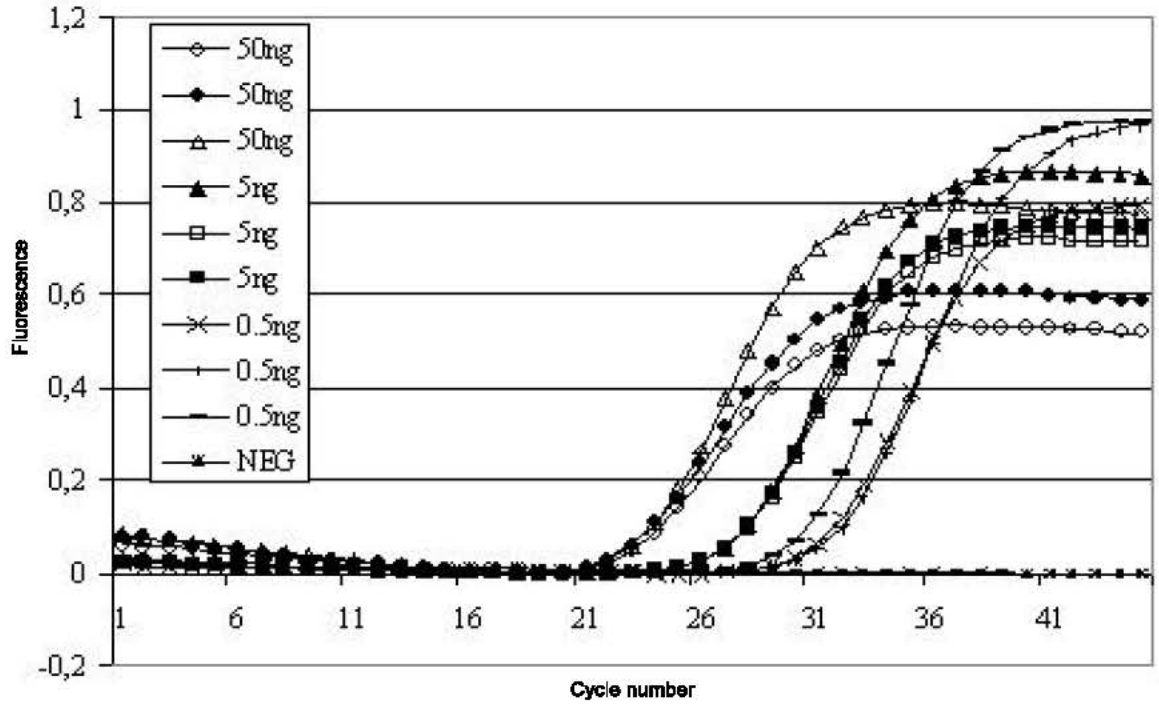

Fig. 2 a: Fluorescent profiles obtained from one triplicate real-time PCR of bovine DNA standards (50, 5 and 0.5 ng/µL).

Table 1: Relation between bovine DNA standards' concentration and threshold cycle (Ct)

Concentration (ng/µl)	Repeats	Ct (P = 0.05)
50	12	23.90 ± 0.48
5.0	12	27.54 ± 0.62
0.50	11[a]	31.28 ± 1.08

[a]Plus one false negative

Table 2: Quantification of DNA samples from governing liquid by SYBR Green-based real- time PCR

Sample	Ct (P = 0.05)	Bovine DNA[1] (ng/µl)	Total DNA[2] (ng/µl)
0.5% bovine	31.26 ± 0.19	0.5	100.15
1% bovine	28.29 ± 0.70	3.19	319
5% bovine	28.8 ± 1.1	2.36	47.1
10% bovine	29.2 ± 1.1	1.8	18
20% bovine	29.8 ± 1.5	1.25	6.3
30% bovine	25.26 ± 0.25	21.2	70.5

[1]Calculated from mean Ct by applying the calibration curve

[2]Calculated dividing bovine DNA content by bovine proportion reported in "Sample" column

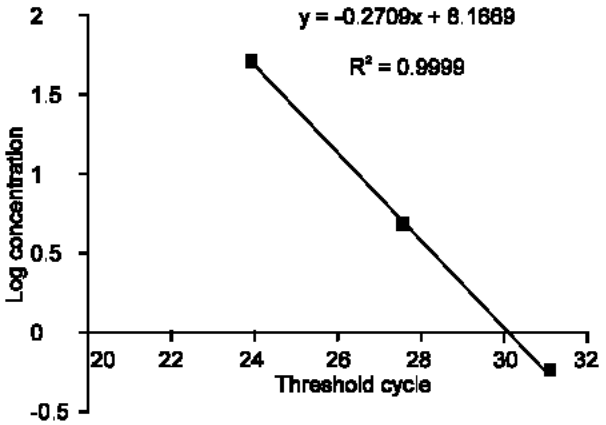

Fig. 2b: Standard curve obtained by plotting the logarithm of the DNA concentration vs threshold cycle (Ct) mean values. The curve was constructed using data from all the four triplicate standards' amplifications.

resulting in a wide range of calculated extraction yields (Table 2). The small DNA concentration loaded confirmed the real-time PCR's high sensitivity and low detection limit.

The analytical strategy we describe in this work differs from the ones developed by other authors focusing on bovine DNA detection in milk (Maudet and Taberlet, 2001; Lopez-Calleja *et al.*, 2004) or other matrices (Tartaglia *et al.*, 1998; Herman, 2001; Lahiff *et al.*, 2002) for primers' target sequences and for the real-time PCR experimental approach. We chose to analyze DNA samples from governing liquid at unknown concentration, performing "inline" absolute quantification by means of the calibration curve instead of

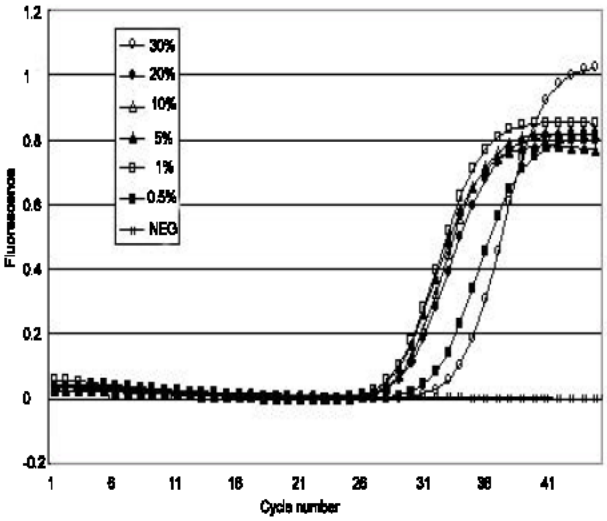

Fig. 3: Fluorescence profiles obtained by real-time PCR of DNA samples isolated from governing liquid of experimental cheeses manufactured using different percentages of cow milk.

spectrophotometrical one prior to analysis, in order to speed up the method and to verify its reliability in routine applications. This implies that samples were not normalized for total DNA concentration before the amplification. Because of this, a direct relation between the percentage of added bovine milk and the absolute DNA concentration was not expected (Fig. 3). Samples' fluorescence profiles and Ct values resulted to be markedly similar, implying that loaded amounts of bovine DNA were very close to each other in spite of the corresponding bovine-to-buffalo ratios. Since DNA quality and yield are among the most important variables in real-time PCR applications, obtaining DNA susceptible to be amplified from governing liquid represents this work's first result. The presence of DNA in governing liquid is due to the cheese matrix's exfoliation that slowly occurs during the whole preservation period. DNA was found in all experimental samples. Real time amplification of DNA from governing liquid proved the method's actual applicability for species detection purposes. Hot-start PCR and fluorescence signal acquisition were optimal at 56°C, allowing SYBR Green I-based real-time PCR to be sensitive and specific (Morrison *et al.*, 1998), although amplification was obtained in conditions of greater stringency as well.

The possibility to detect small quantities is important, in addition to recognizing frauds, also in protecting consumers allergic to cow milk proteins. Due to its low detection limit, the assay here described is suitable to routine analysis and can be applied to detect fraudulent cow milk addition in cheeses. In the future, this real-time PCR application could be extended to the identification of frauds in other kinds of cheese.

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Comparative Evaluation of the Nutritive and Functional Attributes of Some Traditional Nigerian Snacks and Oil Seed Cakes

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Abstract: The proximate composition, the content of nutritionally valuable minerals and functional properties of some traditional Nigerian snacks: peanut ball *(Kulikuli)*, maize-groundnut ball *(Donkwa)* and melon ball (Robo) were analyzed along with some oilseed cakes: groundnut cake (GNC), palm kernel cake (PKC) and soya bean cake (SBC). On the average the samples contained 31.7 g/100 g DM crude protein (range, 25.2-34.3 g/100 g DM); 20.6 g/100g DM crude fat (range, 9.2-29.6 g/100g DM); 8.0 g/100g DM crude fibre(range, 2.5-22.4g/100g DM) and ash 10.5 g/100g (range, 2.0-20 g/100g DM) The protein content of the snacks were generally similar to those of the oilseed cakes, while the fat content of the snacks were much higher. The crude fibre content was least in *kulikuli* while PKC had the highest value. The ash content of the snacks were generally much higher than those of the oilseed cakes. The gross energy ranged between 310.8 kcal/100 g in PKC to 559.2 kcal/100 g in *kulikuli*. Ca, Mg, P, K and Na were the most abundant minerals in both the snacks and oilseed cakes, while the Zn, Cu and Mn were the least abundant. The snacks were particularly much higher in their Na content. Among the functional attributes, the water absorption capacity (WAC) ranged from 70 to 220% in the traditional snacks, and from 200 to 260 % in the oilseed cakes. Foaming absorption capacity (FAC), varied from 128 to 147% in the snacks while it varied from 184 to 221% in the oilseed cakes. The least gelation concentration of *Kulikuli, Robo* and palm kernel cake were identical. Fat emulsion capacity and emulsion stability were also similar in all the products. All the samples had varying solubilities with change in pH. The proteins generally had multiple maxima and minima in their solubilities.

Key words: Nigerian snacks, oilseed cakes, palm kernel cake

Introduction

It is well documented that most leguminous plant seeds are rich in nutrients such as digestible protein with a good array of amino acids and minerals (Fagbemi *et al*., 2004; Agbede and Aletor, 2003). It is also regarded as the cheapest source of proteins especially in the diets of resource-poor classes of the population in West Africa (Altschul and Wilcks, 1985; Oshodi and Aletor, 1993; Fagbemi *et al*., 2006). It has been suggested that more than anything else, the lack of adequate information on the composition and processing effects on the food value of the many and varied protein sources indigenous to the tropics is the major problem, rather than a real shortage of protein feed resources (Aletor and Aladetimi, 1989; Adeyeye, 1995; Agbede and Aletor, 2003)

Apart from the indigenous tropical edible legumes which serve as dietary protein sources for a large segment of the population in Nigeria, there are also a wide variety of traditional snacks and appetizers which contribute to the overall dietary protein intake. These include *Kulikuli* (Pea nut ball), *Donkwa* (Maize-peanut ball) and *Robo* (Melon ball). Although these snacks and appetizers are popular food items, with a long history of consumption especially among the low income populace, there exists a paucity of information on their nutritive and functional attributes. While the nutritive potentials of the oilseed cakes such as groundnut cakes (GNC), palm kernel cake (PKC) and soya bean cake (SBC) commonly used as components of livestock feed are well documented (Aletor and Aladetimi, 1989; Amefule and Obioha, 1998), their functional properties like those of traditional Nigerian snacks remain lesser-known. It was therefore the objective of this study to characterize these traditional Nigerian snacks and oil-seed cakes with regard to their proximate constituents, nutritionally valuable mineral content and physico-chemical (functional) properties.

Materials and Methods

Sample collection: The oil seed cakes – Groundnut cake (GNC), palm kernel cake (PKC) and soya bean cake (SBC) were all purchased in fresh condition from Olukayode Feedmill – a commercial feedmill located in Akure metropolis.

Preparation of the Nigerian traditional snacks: These snacks were prepared in simulation of the methods used in most homes as follows:

***Kulikuli* (Peanut ball):** About I kg of groundnut is first roasted and milled, 20 ml of savoil oil is added and make into a paste, this is followed by addition of about 2 grams of pepper (*Capsicum spp*.) this is optional. The paste is moulded into different shapes and sun-dried. It is ready for consumption.

Table 1: Proximate Composition (g/100g DM) and Energy Values (kcal/100g) of Some Traditional Nigerian Snacks and oilseed cakes

Traditional Snacks	DM	CP	EE	CF	Ash	NFE	GE
Peanut ball *(Kulikuli)*	91.8±0.2	32.4± 0.2	31.1±0.1	2.5±0.2	6.0±0.3	19.8	559.2
Maize-groundnut ball *(Donkwa)*	92.0±0.1	28.5±0.2	23.9±0.2	6.2±0.1	20.0±0.1	13.4	443.3
Melon ball*(Robo)*	94.0±0.3	32.4±0.1	29.6±0.2	5.3±0.2	20.0±0.2	6.7	493.0
Oil Seed Cakes							
Groundnut cake (GNC)	97.0±0.1	34.2±0.3	16.5±0.1	6.9±0.5	5.0±0.3	34.4	489.4
Palm kernel cake(PKC)	85.0±0.2	25.2±0.2	9.2±0.4	22.4±0.1	10.0±0.1	18.2	310.8
Soy bean cake (SBC)	95.0±0.2	34.3±0.1	13.3±0.6	4.9±0.2	2.0±0.2	40.5	483.7
Mean	92.5	31.7	20.6	8.0	10.5	22.2	463.2
SD	4.1	3.6	8.9	7.2	7.8	12.8	85.5
CV (%)	4.4	11.4	43.2	90.0	74.2	57.7	18.0

Means are for triplicate determinations. DM, dry matter; CP, crude protein; EE, ether extract; CF, crude fibre; NFE, nitrogen free extract; GE, gross energy.

***Donkwa* (Maize-groundnut ball):** About 1kg of maize and 200g of groundnut are roasted and milled together into powdery form. About 3g of ground pepper (*Capsicum spp.*), 20g of granulated sugar and 2g common salt are added to taste. About 20 ml savoil is added to form a paste and then rolled into ball shape or any other desired shape and ready for consumption.

***Robo* (Melon balls):** To about 1kg of melon is added 20g of onion, 5g of ground pepper (*Capsicum spp.*) and milled. About 25 ml of oil savoil is added and the paste formed is rolled into ball shape and deep-fried in savoil and ready for consumption.

Sample preparation: About 500 g each of the oil seed cakes and traditional snacks were finely milled using a hammer mill to pass through 0.5 mm sieve size. The milled samples were packed in airtight containers and refrigerated prior to analysis.

Chemical and physico-chemical analyses: Proximate analysis of the samples was carried out in triplicate, using method described by AOAC (1995). Nitrogen was determined by micro-kjeldahl method, described by AOAC (1995) and percentage nitrogen was converted to crude protein by multiplying by 6.25. The minerals were analyzed after dry-ashing at 550^{0}C in a Muffle furnace and dissolved in de-ionized water to standard volume. Sodium and potassium were determined by flame photometry while phosphorus was determined by the vanado-molybdate method (AOAC, 1995). The other minerals - Mg, Ca, Zn, Mn, Fe and Cu - were determined using an atomic absorption spectrophotometer (AAS; Vogel, 1962). The gross energy content of the different samples was computed from the proximate constituents as described by Ng and Wee (1989).

Determination of the functional properties: The variation of protein solubilities with pH was determined as described by Oshodi and Aletor (1993) while the water absorption capacity (WAC) and fat emulsion stability (FES) were determined by the procedure of Beuchat (1977). The fat absorption capacity (FAC) was determined as described by Sosulki (1962). Similarly, the lowest gelation concentration (LGC), foaming capacity (FC) and foaming stability of the samples were determined using the technique of Coffman and Garcia (1977).

Data analysis: All data used were means of triplicate (n = 3) determinations. The coefficients of variation (CV) between the different products were also determined (Steel and Torrie, 1980).

Results and Discussion

Table 1 presents the result of proximate composition, carbohydrate fraction and gross energy values of the traditional snacks (peanut ball, maize-groundnut ball and melon ball) and oilseed cakes (groundnut cake, palm kernel cake and soy bean cake). The mean crude protein (CP) content of the samples were 32.4 ± 0.2 g/100 g DM for *kulikuli*; 28.5 ± 0.2 g/100 g DM Donkwa; 32.4 ± 0.1 g/100g DM for *Robo,* while groundnut cake was 34.2 ± 0.2 g/100 g DM; palm kernel cake 25.2 ± 0.2 g/100 g DM and soy bean cake, 34.3 ± 0.1 g/100 g DM. The similarity in these values is attested to by low coefficient of variation (CV) of 11.4%. These results compare favourably with, and even surpassed those of most edible legumes such as lima bean and cowpeas reported by Aletor and Aladetimi (1989); Oshodi and Adeladun (1993). The fat content of the traditional snacks was markedly high, and ranged from 23.9±0.2 g/100 g DM in *Donkwa* to 31.1±0.1 g/100 g DM in *Kulikuli.* Among the oil seed cakes, the fat content ranged from 9.2±0.1 g/100 g DM in PKC to 16.5±0.1 g/100 g DM in GNC. The high fat content of the snacks were results of the processing techniques which generally involved the addition of cooking oils and, or deep frying. The fat values were generally below those of *Adenopus breviflorus, benth* seed (51.1±0.2 g/100 g DM) reported by Oshodi (1992). The crude (CF) ranged from 2.5±0.2 g/100 g DM in *Kulikuli* to 22.4±0.1 g/100 g DM in PKC. The high fibre content in PKC has generally been implicated for low protein digestibility of PKC especially in monogastric animals, including humans. The NFE (carbohydrate) content of the traditional snacks

Table 2: Major and trace mineral components (mg/kg) of some Traditional Nigerian Snacks and oilseed cakes

Traditional Snacks	Major minerals						Trace minerals		
	Ca	Mg	P	K	Na	Fe	Zn	Cu	Mn
Peanut ball (*Kulikuli*)	124.8±0.3	82.5±0.5	171.2±0.2	247.3±0.2	195.7±0.4	12.6±0.3	4.2±0.1	1.7±0.2	0.6±0.1
Maize-groundnut ball (*Donkwa*)	122.7±0.2	60.0±0.0	114.2± 0.2	302.3±0.5	364.5±0.2	22.3±0.2	4.8±0.1	-	0.8±0.2
Melon ball (*Robo*)	130.7±0.1	100.5±0.2	216.3±0.2	322.2±0.3	369.5±0.1	14.8±0.1	5.1±0.1	0.2±0.2	0.9±0.3
Oil Seed Cakes									
Groundnut cake (GNC)	132.7±0.2	185.0±0.1	198.2±0.4	311.5±0.5	117.5±0.4	9.7±0.3	8.0±0.2	0.3±0.1	1.0±0.1
Palm kernel cake(PKC)	163.0±0.2	76.4±0.2	198.2±0.3	72.0±0.1	134.0±0.1	14.7±0.2	4.6±0.1	-	0.8±0.4
Soy bean cake (SBC)	327.3±0.5	73.4±0.6	165.4±0.4	288.0±0.3	170.0±0.6	11.2±0.4	6.5±0.7	-	0.7±0.3
Mean	166.9	96.3	177.3	257.2	225.2	14.2	5.5	0.7	0.8
SD	79.9	45.4	36.2	94.4	113.2	4.4	1.4	0.8	0.1
CV (%)	47.9	47.1	20.4	36.7	50.3	31.0	25.5	14.3	12.5

Means are for triplicate determinations; CV, coefficient of variation

Table 3: Water and Fat absorption capacities, Bulk density and Least gelation capacity of some Traditional Nigerian Snacks and oilseed cakes

Traditional Snacks	Water absorption (%)	Fat absorption (%)	Bulk Density	Least gelation capacity
Peanut ball (*Kulikuli*)	120.0±0.1	147.2±0.2	0.7±0.2	2.0±0.2
Maize-groundnut ball (*Donkwa*)	220.0±0.1	128.0±1.6	0.6±0.2	3.0±0.3
Melon ball (*Robo*)	70.0±1.2	128.8±0.7	0.9±0.1	2.0±0.2
Oil Seed Cakes				
Groundnut cake (GNC)	225±0.1	184.0±0.2	0.5±0.3	4.0±0.2
Palm kernel cake (PKC)	200.2±0.5	202.0±0.9	0.5±0.1	2.0±0.1
Soy bean cake (SBC)	260.0±0.2	220.8±0.2	0.4±0.1	8.0±0.1
Mean	182.5	168.5	0.6	3.7
SD	72.2	39.4	0.2	2.1
CV (%)	39.6	23.4	33.3	56.8

Means are for triplicate determinations; CV, coefficient of variation.

was generally lower than the oil seed cakes. This is attributed to the higher fat content of the snacks relative to the oil seed cakes. The GE values were least in PKC (310.8 kcal/100 g) and highest in *Kulikuli* (559.2 kcal/100 g). Apart from PKC, both the snacks and the oil seed cakes had similar energy values. The similarities in energy value between the snacks and the cakes is attributed to the fact that while the snacks generally had higher high fat content, the oil seed cakes generally had much higher carbohydrate values. These two food constituents are associated with high calorific values of foods.

Table 2 presents the mineral contents of these products. The values compared well with those reported for leguminous seeds (Oke *et al*., 1995; Agbede and Aletor, 2003). Of all the nutritionally valuable minerals analyzed, K and Na were most abundant while Cu was the least abundant, or not detected in some of the products. Apart from the effect of processing on the traditional snacks which brings about increase in Na content, K was also relatively high, especially in the snacks and in agreement with earlier observation of Olaofe and Sanni (1988) that K is an abundant mineral in Nigerian agricultural products.

Table 3 shows the data on the functional properties of the traditional snacks and the oil seed cakes. The water absorption capacity (WAC) ranged from 70±1.2 to 220±0.1% in the traditional snacks and from 200±0.3 to 260±0.2% in the oil seed cakes. This compares favourably with the (180±0.5%) reported by (Tagode and Nip, 1994) for Taro (*Colocasia esculenta*) flour. The high WAC in this study suggests that all the traditional snacks (except melon ball with 70±1.2%) and oil seed cakes may be suitable in the formulation of some foods such as sausages, doughs and soups (Oshodi *et al*., 1997). The FAC ranged from 128±0.7 to 147.2±0.2% in the selected snacks while it ranged from 184±0.2 to 147.2±0.2% in the oilseed cakes. These values were higher than (138 %) reported for soy bean (Ogunsipe, 2000), and suggests that these products may be useful in food formulation especially as flavour retainers. The least gelation concentration of *Kulikuli, Robo* and PKC showed identical value of 2.0% while soybean cake had a much higher value of 8.0±0.2%. This value was higher

Table 4: Foaming Capacity, Foaming Stability, Emulsion Capacity and Emulsion Stability of Some Traditional Nigerian Snacks and oilseed cakes

Traditional Snacks	Foaming Capacity (%)	Foaming Stability (%)	Emulsion Capacity (%)	Emulsion Stability (%)
Peanut ball *(Kulikuli)*	6.0±0.3	3.0±0.6	49.5±0.1	45.7±0.4
Maize-groundnut ball*(Donkwa)*	4.0±0.3	0.0±0.0	49.5±0.7	45.7±2.0
Melon ball *(Robo)*	5.0±0.2	3.0±0.9	44.7±1.2	44.7±0.9
Oil Seed Cakes				
Groundnut cake (GNC)	4.0±0.2	0.0±0.0	45.7±1.5	45.7±0.5
Palm kernel cake (PKC)	4.0±0.1	0.0±0.0	44.0±0.4	44.0±0.6
Soy bean cake (SBC)	20.0±0.1	10.0±0.5	46.5±0.6	45.7±1.5
Mean	7.3	86.3	3.9	46.7
SD	6.3	2.7	144.4	2.4
CV (%)	5.1	45.3	0.1	1.5

Means are for triplicate determinations; CV, Coefficient of variation.

than the 2% reported for soybean (Ogunsipe, 2000) but lower than the 12% reported for Pigeon pea by (Oshodi and Ekperingin, 1989). The low gelation may be an asset in the use of these oil seeds cakes and traditional snacks as additives to other gel forming materials in food product (Altschul and Wilcks, 1985; Fagbemi *et al*., 2006). The foaming capacity of all these products were generally lower than those reported for soy bean flour (70%) and sunflower (230%) by (Ogunsipe, 2000). The foaming stability (Table 4) of these products were also low, which suggests their unsuitability as a whipping agent in food systems. The fat emulsion capacity and fat emulsion stability were relatively high in all the products with a means of 46.7±2.4% and 45.3±0.7%, respectively. This is an important attributes in their potential use for the stabilization of fat emulsion such as in the production of mayonnaise, milks, commuted meats and salad dressings (Adeyeye, 2004).

These products have variable solubilities with varying pH ranges, in both the acidic and alkaline regions which could be useful in industrial applications. *Donkwa* had maximum solubility at pH 1, and maximum solubility at pH 11 while SBC had minimum solubility at pH 5 and maximum solubility at pH 1.

Conclusion: Judging from the proximate composition, mineral contents, gross energy and functional properties, it is of interest that these Nigerian snacks compare well with the oil seed cakes frequently used in Animal feed industries. They also compare favourably with animal protein sources and also implies that they may be useful as supplements to low nitrogen foods such as cereals, tubers and maize gruel.

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