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Applied Biochemistry

1st Semester 2019
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Agricultural Biochemistry II

- Leaf Protein Concentrate (LPC)
- คือ โปรตีนที่พบในใบของพืช ทำให้อยู่ในรูปเข้มข้น
- ใช้เป็นแหล่งอาหารของมนุษย์และสัตว์
- มีศักยภาพสูง ราคาถูก หาง่าย
- มนุษย์สามารถกินพืชผัก เพื่อให้ได้โปรตีนได้โดยตรง แต่ระบบย่อยอาหารไม่สามารถจัดการกับพืชปริมาณมาก ๆ ให้ได้โปรตีนเพียงพอได้

Agricultural Biochemistry II

- ประวัติการศึกษา LPC
- ศึกษามาตั้งแต่ยุค 1960s แต่ไม่ค่อยสำเร็จมาก
- เริ่มศึกษาจริงจังในยุค 1970s
- ปัจจุบันมีการเลี้ยงสัตว์แบบขุน (feedlot) ก่อนสู่ผู้บริโภค ทำให้ต้องการแหล่งโปรตีนราคาถูกสำหรับใช้เป็นอาหารสัตว์ โดยไม่ต้องใช้พืชผักที่เป็นอาหารของมนุษย์

Agricultural Biochemistry II

- ประเด็นด้านสุขภาพ
- เป็นแหล่งที่ดีของกรดแอมิโน มี methionine เป็น limiting factor
- มีสารประกอบ polyphenols สูง
- ถั่ว Lucerne และมันสำปะหลัง จะใช้เป็นแหล่งอาหารของมนุษย์ได้ มีข้อจำกัดคือ มี fiber สูง และมีสารต้านโภชนะ เช่น phytate, cyanide และ tannins

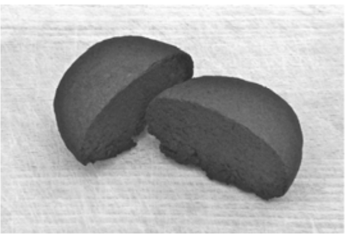
Agricultural Biochemistry II

- ประเด็นด้านสุขภาพ (ต่อ)
- "Leaf for Life" เป็นองค์กรที่ไม่หวังผลกำไร รณรงค์ต่อสู้กับภาวะทุพโภชนาการ โดยส่งเสริมให้บริโภค LPC
- ศึกษาการผลิต LPC ในระดับ small scale และการกำจัด fiber โดยใช้กระบวนการ low tech.

Agricultural Biochemistry II

- กระบวนการผลิต
- Pulping leaves and pressing juice out (บดใบพืชแล้วคั้นเอาน้ำออกมา)
- เอาน้ำคั้นใบพืชมาให้ความร้อน เพื่อให้โปรตีนจับตัวกัน (coagulation)
- กรองโปรตีนออกแล้วทำให้แห้ง

Leafu จาก stinging nettle



- How to make Leafu (Leaf Curd)
  Leafu is the protein curd extracted from edible leaves such as nettle leaves.Leafu can be made at home with low-tech kit, although the yield is low for the effort required
  Makes around 100g curd from 800g leaves

Gather 800g (dry weight) of clean young nettle (*Urtica dioica*) leaves.

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Review

Fruit and vegetable waste management and the challenge of fresh-cut salad

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A B S T R A C T

Background: The fruit and vegetable sector generates large amounts of waste. In industrialized countries, **fruit and vegetable waste** (FVW) is mainly generated before reaching consumers, due to programmed overproduction and unfulfillment of retailer quality standards. FVW poses environmental problems due to its high biodegradability, represents a loss of valuable biomass and an economic cost for companies. Different reduction, reuse and recycle strategies to tackle FVW have been proposed.
Scope and approach: This review paper summarizes these strategies, underlying their main advantages and pitfalls. In particular, **fresh-cut salad** waste was considered as a particularly challenging FVW, due to its low concentration of nutrients (e.g. polyphenols, pigments, fiber).
Key findings and conclusions: Different management strategies can be successfully applied to FVW. Among them, the extraction of specific functional compounds was found to be one of the most studied in the last years. This suggests that FVW can be considered a source of valuable ingredients and products. To maximally exploit these FVW potentialities, a rational strategy is required. The latter should be developed using a step-procedure including waste characterization, output definition, process design and feasibility study. The application of this procedure to the case of fresh-cut salad waste was presented. Based on the review of currently applied and potential salad **waste management** strategies, an operational scheme for the development of alternative strategies was proposed. This scheme considers the exploitation of traditional and **novel technologies**, even applied in combination, for salad waste valorization.

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1. Fruit and vegetable waste (FVW)

Around 89 million tons of food are wasted annually in the European Union (Stenmarck, Jensen, Quested, & Moates, 2016) and this value is expected to further increase by 40% in the next 4 years. Moreover, the World and Agriculture Organization calculated that one-third of the edible parts of food intended for human consumption get lost or wasted (FAO, 2011). The term "food loss" identifies the decrease in edible food mass throughout the part of the supply chain that specifically leads from raw material to food for human consumption. Food losses, thus, take place at production, post-harvest and processing stages in the food supply chain. Food losses occurring at the end of the food supply chain (retail and final consumption) are rather called "food waste", which relates to retailers' and consumers' behavior (Manzocco, Alongi, Sillani, & Nicoli, 2016; Parfitt, Barthel, & Macnaughton, 2010). Moreover, the term "food by-products" has been increasingly used. This term notifies that biomass and waste can be properly treated and converted into valuable marketable products (Galanakis, 2012).

In the fruit and vegetable sector definitions are more controversial. A widely-used term is "fruit and vegetable waste" or FVW. The latter has been defined as the inedible parts of vegetables that are discarded during collection, handling, transportation and processing (Chang, Tsai, & Wu, 2006). According to the definitions reported above, it should be defined fruit and vegetable loss rather than waste. Panda, Mishra, Kayitesi, and Ray (2016) affirmed that FVW can be generated in different steps of the food supply chain, from farm to fork, including thus both pre- and post-consumer stages. Similarly, Galanakis (2012) used this term to indicate a specific group of plant food wastes, generated along the entire food supply chain (agricultural production, postharvest handling, storage and consumer phase).

In this paper, the term FVW will be used to generally indicate

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fruit and vegetables from processing plants and production sites which are required or intended to be discarded.

2. Main causes of FVW

According to FAO estimation (FAO, 2011) pre-consumer phases are particularly critical in terms of FVW generation. To this regard, Segrè and Falasconi (2011), reported that, in Italy, up to 87% of fruit, vegetable and cereals are discarded before reaching consumer. Causes may be different. In developing countries, wastes are mainly generated in agricultural production, post-harvest and distribution stages, due to seasonality that leads to unsaleable gluts and to the absence of proper conservation strategies for perishable crops. Wastes in agricultural production dominate also in industrialized countries. In this case, however, they are mostly due to post-harvest evaluation of crops on the basis of quality standards requested by retailers and to programmed overproduction (FAO, 2011; Segrè & Falasconi, 2011).

3. FVW management

FVW poses disposal and environmental problems, due to its high biodegradability. In addition, it represents a loss of valuable biomass and nutrients as well as an economic loss. For these reasons, in the last years, great attention has been focused on the development of policies and methods for its management (Laufenberg, Kunz, & Nystroem, 2003, 2006/12/EC). In general, waste management "is the collection, transport, recovery and disposal of waste, including the supervision of such operations" (2006/12/EC) and the waste management system consists of "the whole set of activities related to handling, disposing or recycling waste materials". Waste management strategies can be classified with respect to the final disposition of waste and ordered according to their priority: minimization and prevention (reduction) of waste generation, recycling and reuse, energy recovery and landfilling. This option list in order of priority is commonly known as waste hierarchy (Demirbas, 2011).

In the past, FVW was mixed into municipal waste streams and sent to landfills or incinerators (without energy recovery) for final disposal (Nawirska & Kwaśniewska, 2005). However, this is not a good option for FVW, due to its high water content which is, in turn, responsible for microbiological instability, formation of off-odors and leachate (Abu-Qudais, 1996; Lin et al., 2011; Zhang et al., 2007). On the contrary, FVW has a great potential for reuse, recycling, and energy recovery. To this regard, Table 1 reviews the main strategies recently proposed for reducing and valorizing FVW in industrialized countries.

3.1. Reduction of FVW

Reduction has the top priority in the waste hierarchy and mostly depend on production practices (Demirbas, 2011). Some of them cannot be easily modified. For example, agricultural production has necessarily to be higher than sales forecast, in order to face eventual harvest losses due to natural phenomena (Segrè & Falasconi, 2011). On the contrary, some practices can be definitely modified. It has been estimated that huge amounts of fruit and vegetables are wasted because products do not fulfill quality standards set by retailers or consumers (Mena, Adenso-Diaz, & Yurt, 2011). This small-sized or misshaped fruit and vegetables are usually defined "substandard". Different strategies have been proposed and implemented to tackle waste of substandard fruit and vegetables. The latter have been traditionally downgraded to the production of alternative fruit and vegetable derivatives (e.g. juices, vinegar) (Grewal, Tewari, & Kalra, 1988). Moreover, an interesting initiative in this direction is being carried out by the campaign "Inglorious Fruit and Vegetables" and the line "No Name® Naturally Imperfect™", launched in 2015 by the French retailer Intermarchè and the Canadian one Loblaw, respectively. They address the FVW issue by selling substandard fruit and vegetables, while reducing costs for consumers (Table 1). In addition, the so defined "food rescue programs" collect perishable food, including fruit and vegetable surplus, and donate it to hungry people.

3.2. Reuse of FVW

Reuse indicates the use of waste materials for other purposes without or with minor modification of their properties (Manzocco et al., 2016). Reuse strategies for FVW are nowadays limited to soil amendment and animal feed (Table 1). Direct reuse of FVW for soil amendment has been reviewed by Clemente, Pardo, Madejón, Madejón, and Bernal (2015). This practice is based on the ability of organic waste to increase properties of polluted soil by immobilizing trace metals and metalloids, preventing their transfer to groundwater and living organism, and promoting the establishment of plants. However, this reuse strategy is often difficult to put into practice due to the high biological instability of FVW, responsible for pathogen growth risk and off-odors generation (Ajila, Brar, Verma, & Prasada Rao, 2012). Fiber content of FVW can be exploited to formulate animal feeds with increased nutritional value (San Martin, Ramos, & Zufía, 2016). However, also this reuse strategy is limited by some drawbacks. The high water content, often exceeding 80%, makes these wastes prone to microbiological contamination. A partial drying is thus usually required. In addition, low protein content and high presence of indigestible compounds are not always suitable for animal feed (Clemente et al., 2015). Moreover, composition of vegetable products varies according to season, forcing manufacturers to often change feed formulations (San Martin et al., 2016).

3.3. Recycle of FVW

Strategies based on the recovery of waste materials after a major modification of their characteristics are defined as recycle (Williams & Anderson, 2006). Because of its intrinsic characteristics (high content of water and fiber, low protein content), a substantial modification of FVW is usually required to maximally exploit its potentialities. Recycle of FVW offers thus more possibilities than its reuse (Table 1). Recycle strategies for FVW can be divided into strategies in which the whole waste mass is recycled (composting, processing to flour, conversion into water) and strategies in which specific compounds are extracted.

Aerobic composting is an ancient eco-friendly method to convert organic waste into organic fertilizer. However, it is well established that anaerobic digestion (§ 3.4) is a more attractive strategy to produce fertilizers from FVW, due to the energy recovery as biogas (Sharma, Testa, Lastella, Cornacchia, & Comparato, 2000). Processing into flour of FVW has been exploited with different purposes. The fibrous structure and the high contact surface of FVW flour has been used to adsorb pollutants such as dyes and heavy metals from water and ground. To this regard, adsorption is due to both physical entrapment into the porous structure of the vegetable and to specific interaction with the functional groups of cellulose, hemicellulose and lignin (Azouaou & Mokaddem, 2008; Hashem, Abdelmonem, & Farrag, 2007). FVW flour has also been used as an ingredient for the formulation of products rich in functional compounds such as polyphenols and fiber (Ferreira et al., 2015). The main advantage of this recycle strategy is that valuable products such as adsorbents and functional flours are obtained from low-cost raw materials. Moreover, after

Table 1
Main strategies of FVW management according to different authors.

Strategy	Output	Waste origin	References
Reduction			
Alternative processing of substandard items	Fruit and vegetable derivatives	Substandard apple and grapes	Grewal et al., 1988
Market of substandard items	Low cost fruit and vegetables	Substandard fruit and vegetables	http://itm.marcelww.com/inglorious/ media.loblaw.ca/English/media-centre/press-releases/press-release-details/2016/More-products-more-locations-no-name-Naturally-Imperfect-produce-line-expanded-to-meet-customer-demand/default.aspx
Food rescue programs	Fruit and vegetables distributed to hungry people	Fruit and vegetable surplus	Schneider, 2013
Reuse			
Direct use	Products for soil amendment	Olive, mushrooms	Clemente et al., 2015
Minor changes (partial dehydration, trimming)	Fiber-enriched animal feed	Mixed fruit and vegetables	San Martin et al., 2016
Recycle			
Composting	Fertilizers	Mixed fruit and vegetables	Chang et al., 2006; Choy et al., 2015
Processing into flour	Green and low cost adsorbents for pollutants in wastewaters	Orange, citrus, banana, olive, apricot	Annadurai, Juang, & Lee, 2002; Azouaou & Mokaddem, 2008; Daifullah & Girgis, 1998; Pavlovic et al., 2015
	Flour rich in antioxidants, phenols, minerals and fiber	Tropical fruit, orange	de Oliveira et al., 2009; Ferreira et al., 2015; Larrauri, 1999
Conversion into water	Water for industrial facilities	Mixed fruit and vegetables	http://www.eco-wiz.com/ecoDigester.php http://www.enviropuresystems.com/index.php http://www.wastetowater.com.au/
Extraction of specific compounds	Bioactive extracts		
	Flavonoids and bio-sugars	Onions	Choi, Cho, Moon, & Bae, 2015
	Antioxidants and antimicrobials	Fresh-cut fruit	Ayala-Zavala, Rosas-Domínguez, Vega-Vega, & González-Aguilar, 2010
	Oils	Citrus fruit	Bustamante et al., 2016
	Essential oils		
	Oils for food, biodiesel, pharmaceutical and cosmetic sectors	Watermelon, melon, red currant, pomegranate, grape, apple	Górnaś, Soliven, & Segliņa, 2015; Górnaś & Rudzińska, 2016
	Fiber extracts		
	Reinforced biopolymers	Banana	Zini & Scandola, 2011
	Bioplastics	Pineapple, banana	Elain et al., 2016; Jabeen, Majid, Nayik, & Yildiz, 2015
	Cellulose nanofibers	Carrot	Piccinno, Hischier, Seeger, & Som, 2015
	Dietary fiber	Apple, cherry, chokeberry, black currant, pear, carrot	Nawirska & Kwaśniewska, 2005
	Natural dyes	Raspberries, black carrots, currants, onions	Bechtold, Mussak, Mahmud-Ali, Ganglberger, & Geissler, 2006
	Structuring agents	Apple, carrot	McCann et al., 2011; Roversi et al., 2016; Roversi, Radaelli, & Piazza, 2015
Energy Recovery			
Anaerobic digestion	Biogas Fertilizers	Mixed fruit and vegetables	Han & Shin, 2004; Kim, 2004; Lin et al., 2013; Shen et al., 2013; Zhang et al., 2007
Bio-electrochemical systems	Electrical energy	Carbohydrate-rich vegetables	ElMekawy et al., 2015

processing to flour, no residual waste has to be disposed of. However, the main issue is the high cost required for FVW drying, due to the high water content. As a consequence, the production of FVW flour is affordable only if high value-added ingredients and products are developed (Ratti, 2001). Water can also be considered a valuable output of a recycle strategy. To this regard, patented or patent-pending systems able to convert organic material into water are already applied in companies, supermarkets and restaurants. They are based on the hyper-acceleration of aerobic decomposition through the activity of naturally-occurring microorganisms with enhanced degradation capabilities under tightly controlled environmental conditions (Table 1).

The extraction of specific functional compounds from FVW has been largely studied (Table 1). Bioactive compounds as well as oils, fibers and natural dyes are the main targets of this recycle strategy. Structuring agents, mainly referring to colloidal polymers with interesting gelling or viscosant properties, can also be selectively extracted from FVW (McCann, Fabre, & Day, 2011). These compounds are high value-added ingredients derived from a low-cost, easily-available material. The efficiency and sustainability of their extraction has been significantly increased by the application of novel technologies, which guarantee high extraction rate and yield and by concomitantly reducing the need for organic solvents (Herrero, Plaza, Cifuentes, & Ibáñez, 2010). Some recent studies relevant to bioactive extraction (e.g. carotenoids, essential oils, polyphenols, anthocyanins) from FVW using novel technologies include the use of ultrasounds, supercritical carbon dioxide, microwaves and pulsed electric fields (Amiri-Rigi, Abbasi, & Scanlon, 2016; Baysal, Ersus, & Starmans, 2000; Jacotet-Navarro et al., 2016; Rabelo, MacHado, Martínez, & Hubinger, 2016; Zhou, Zhao, & Huang, 2015). For these reasons, extraction of specific compounds from FVW could be an affordable, sustainable and even profitable recycle strategy for industries (Galanakis, 2012; Laufenberg, Kunz, & Nystroem, 2003). However, it should be considered that novel

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Nutritional Significance of Cowpea Leaves for Human Consumption

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ARTICLE INFO | **ABSTRACT**

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Cowpea is a legume whose grains are widely consumed as rich sources of protein and other nutrients in some parts of the continent. Recent surveys have also shown that cowpea leaves are relished as vegetable in Southern and Eastern Africa as well as Middle belt areas of Nigeria. Protein, calorie and mineral deficiencies such as marasmus, kwashiorkor and anaemia are reported to characterize malnutrition in many parts of Africa. For instance, amongst the health benefits of cowpea leaves, low glycemic index carbohydrates and vegetable-derived nutrients are known to prevent or combat cancer, hypertension, stroke, diabetes, insomnia, osteomalacia, osteoporosis, anencephaly, rickets and could enhance cardiac health and metabolic wellbeing. However, in eastern Nigeria, consumption of cowpea as leafy vegetable is largely non-existent probably due to dearth of information on its nutrients composition and possible health benefits amongst other factors. As part of efforts to create awareness to stimulate and warrant its adoption, utilization and consumption as leafy vegetable in eastern Nigeria, this study aims to document the nutrient composition of cowpea leaves grown in the area; and to highlight the potential human health benefits attendant from consumption of these nutrients. The study was carried out in the University greenhouse, with cowpea variety IAR-48 grown in heat sterilized topsoil contained in 20 cm pots arranged in completely randomized design replicated four times. The whole experiment was repeated twice. At 8 weeks after planting, tender leaves of the crop were harvested from the mid section of the crop, enveloped and later used for nutrient analyses. Chemical and nutrient profiling of the cowpea leaves tissues were done based on analytical and standard spectrometric methods and was carried out in triplicate determinations. Findings from this study indicated that cowpea leaves contained protein (34.91%), low glycemic index carbohydrates (31.11 %), prebiotics (19.46 %), fat (5.42 %), iron (65.21 mg), calcium (1.62 g), phosphorus (0.56g), magnesium (1.66 g), potassium (13.445 g) and sodium (2.22 g). Based on literature, these amounts of nutrients were sufficient to offset most of the recommended daily intakes (RDIs) of these nutrients in human diets. Though the anti-nutrient factors were not conducted in this study, previous investigators have shown that cowpea leaves contain low amounts of anti-nutrient factors, hence, findings from this study therefore support the adoption, utilization and consumption of cowpea leaves as vegetable in eastern parts of Nigeria.

INTRODUCTION

Cowpea (*Vigna unguiculata* L. Walp.) a member of the family fabaceae, is an annual legume thought to have originated in Africa (Davis *et al*., 2007). The crop which may be trailing, erect or bushy in growth habit is widely cultivated in Africa, Asia and the Americas (Awurum and Enyiukwu, 2013a). Cowpea is drought and salinity tolerant. It is an excellent intercrop in tuber and cereal-based farming systems where it is reported to improve yield of the component crops by 30 %. In addition to lowering aluminum ion toxicity in tropical soils, it controls erosion and plays an important role in soil fertility restoration fixing by symbiosis with nodular *Bradyrhizobia spp.* up to 200 kgN/ha per annum (source: www.leafforlife.org>VIGNAUNG; Enyiukwu and Awurum, 2013a; 2013b). It also functions in sinking greenhouse gases (GHGs) and ameliorating climate change effects (FAO, 2016).

Cowpea is used as medicine, fodder, feed and food (Awurum, 2000; Okwu and Njoku, 2009). In ethno-botany, the root is prepared as an antidote against snake bite; its clustered porridge for treating chest pain, epilepsy and dysmenorrhea, infusion of the seed for amenorrhea, while that of the whole plant for fever and schistosomiasis (HBT, 2016). In addition, the role of pulses and pulse-derived vegetables as tonics, appetizers, stimulants, aphrodisiacs and anthelmintics are well documented in literature (FAO, 2016). Preparations from cowpea also play important roles in reducing the risk of lymphoblastic leukemia and aberrant foci cyst development (Campos-Vega *et al*., 2010). As food, the protein-rich grains provide cheap sources of protein for millions of consumers of the crop in meat-scarce communities of the tropics (Aveling, 2007). Besides contributing valuable grains for human consumption, the mild-flavoured leaves are veritable sources of nutrient-rich edible vegetable which could play significant roles in fighting malnutrition (Alemu *et al*., 2016; FAO, 2016). In a trial on displaced people it was found that six (6) tonnes of fresh leaf (1 tonne dried leaf) of cowpea could supply about 800 children 4-6 years old with 10 g leaf concentrate and provide them 7.75 mg of iron per week year round (source: www.leafforlife.org>VIGNAUNG, 2016).

Fig. 1: Leaves of cowpea (Var. IAR-48) growing in the greenhouse

Nielsen *et al*. (1997) reported that compared to the grains, the leaves of the crop contain amino acids such as isoleucine, leucine, lysine, methionine, cysteine, phenylalaminine, valine, tryptophan and histidine in significantly higher amounts which are able to meet the recommended daily averages of these nutrients than the seeds. Many amino acids in its leaves have been tied to good health benefits. The amino acid trytophan counters social anxiety, insomnia and improves sleep and in concert with histidine assists neurotransmitters involved in appetite, mood and energy production. Isoleucine assists the body in raising endurance, fixes muscle tissues and promotes blood clotting and in conjunction with valine, isoleucine and leucine stabilizes blood sugar and enhance muscle performance. Also, lysine in cowpea is reported to help in healing cold and genital sores whereas phenylalanine functions to prevent depression and raises levels of biochemicals such as noreptrophine and dopamine which alleviate migraine and insomnia. In addition threonine in the leaves improves antibody production, enhancing the production of serine and glycine which are essential requirements for synthesis of collagen and elastin in the body (HBT, 2016).

Pulses and legumes' carbohydrates are rated as low glycemic index nutrients which could contribute to reducing inflammation, diabetes, obesity and/or preventing coronary heart diseases in both healthy individuals and persons challenged with metabolic syndrome (Rizkalla *et al*., 2014; BHM, 2016). The nutrient-rich status of cowpea leaves could therefore strengthen the immunity, vision, heart and circulatory health, fight depression and diabetes, prevents cold sores, and enrich the blood of its consumers and contribute to offsetting protein-calorie deficiency in sub-Saharan Africa (Hallensleben *et al*., 2009; Okonya and Maas, 2012; HBT, 2016).

Leaves of the crop (Fig. 1) which is reported to rank among the top 4 vegetables in 24 countries of Africa and 7 in Asia is suited for production in high rainfall agro-ecologies (SADAFF, 2013). In such rain fed systems up to 6 tonnes fresh leaf yield per ha have been reported (source: www.leafforlife.org>VIGNAUNG). One hundred grams (100 g) of this edible leaf is reported to contain protein 4.2 g, Ca 110 mg, iron 4.7 mg, beta carotene 2.4 mg and ascorbic acid 35 mg and delivers 34 calories of metabolizable energy, phosphorus 383.20 mg, zinc 12.91 mg and carbohydrate 39.11.mg (Nielsen *et al*., 1997; Olayiwole *et al*., 2012; Chikwendu *et al.,* 2014). Generally, Mamiro *et al*. (2011) reported that its leaves are higher in nutrients than the seeds. Compared to the grains, cowpea leaf is reported to produce 9 times more calories, 1.5 times more protein, 90 times more calcium and a thousand times more ascorbic acid and beta carotene than the grains of the crop (source: www.leafforlife.org>VIGNAUNG; Nielsen *et al*., 1997).

In terms of organoleptic properties, cowpea leaves are mild and good tasting comparing favourably with other tropical potherbs and vegetables such as amaranth, taro, spinach, pumpkins, lettuce and sweet potato (Nielsen *et al*., 1997; Hallensleben *et al*., 2009). In an evaluation at the University of Nigeria Nsukka, Igbatim *et al.* (2014) reported that cowpea leaves enhanced the acceptability and organoleptic attributes of three cowpea-based soups. The husks and leaves of the crop according to these authors provided nutrient-dense food that could be exploited, diversified and promoted in sub-Saharan Africa.

However, consumption of this valuable vegetable though increasing in many other parts of Africa including Tanzania, Uganda and Southern Africa (Hallensleben *et al*., 2009; Okonya and Maas, 2012) so far is at very low ebb especially in eastern Nigeria. This poor utilization and consumption of the crop as vegetable in eastern Nigeria, is reasoned to be due largely in part to poor awareness about the nutrients and chemicals it contains and the possible health benefits of the crop amongst others.

Hence, in a bid to create and step up awareness that could contribute to offsetting protein-calorie deficiency as well as mineral imbalances in the nation through use of lesser known vegetables; this paper sought to determine and document the nutrient composition of cowpea leaves grown in eastern Nigeria and to relate same to their potential health benefits.

MATERIALS AND METHODS

Preparation of plant samples for analyses.

This experiment was conducted at the greenhouse of the Michael Okpara University of Agriculture, Umudike (MOUAU), Nigeria. Cowpea seeds (Var. IAR-48) were sown 4 per stand in 20 cm diameter plastic pots containing heat sterilized top soil (4 kg). Two weeks after planting (WAP) the seedlings were thinned to 3 per stand, and watered twice daily. At maturity (8 WAP) leaves of the test crop were harvested from the mid section of the crop enveloped and taken to the Crop Science Laboratory of the University (Chikwendu *et al*., 2014). The whole experiment was repeated twice. Collected samples were air-dried at the Laboratory bench for 1 day and then re-enveloped and oven-dried at 60^{o}C for 3 days. One hundred grams (100 g) of the specimen were weighed out with a digital balance and ground into powder using a hand milling machine (Corona Lavesh 250) (Amadi and Oso, 1996). The powder was stored in an air-tight bottle and kept in a dark cupboard until required for biochemical analyses.

Biochemical composition of healthy leaves of cowpea.

Biochemical analyses of the specimens were conducted at the Analytical Laboratories of the Federal Institute for Industrial Research Oshodi, (FIIRO) Lagos. Standard methods based on the protocols of AOAC (2000) were employed in the determination of the proximate composition of the specimen while the elemental nutrients were determined based on the absorbance of the specimen from atomic absorption spectrometer (AAS) (Model: AA 7000, Schimadzu, Japan). The biochemical composition of the leaves specimen was conducted in triplicate determinations in a completely randomized design (CRD) to ascertain the nutrient values of the leaves of the crop.

Moisture content evaluation of samples

The sample was first scanned from 100^{o}C to 200^{o}C with the moisture analyzer (Model: MS-70, A & D Company limited, UK) to obtain the optimum temperature suitable for the specimen to be dried and it was found to be 140^{o}C. The percentage moisture contents of the sample were then determined and shown automatically on the light emitting diode (LED) of the analyzer.

Ash content

One gram (1 g) of the samples was weighed separately into a dried and preweighed crucible. The samples were then charred on a hot plate to decarbonize them. After complete decarbonization, they were put in the muffle furnace (Model 186A, Fisher Scientific Co.) for 3 h at 560^{o}C to obtain the ash contents using the formula by Kayode *et al.* (2008):

$$\text{Percentage ash content (\%)} = \frac{W2 - W1}{W} \times \frac{100}{1}$$

Where, W_2 is the weight of crucible + ash
W_1 is the weight of dried crucible
W is the weight of sample taken

Protein contents

Using the kjehdahl apparatus, 0.5 g of each of the samples were separately weighed into a digestion tube, to which 1 tablet of kjehdahl catalyst and 10 ml of concentrated sulphuric acid were added and placed in the digestion block to digest at 430^{o}C for 2 h and then placed separately in the distiller before 80 ml of 40% sodium hydroxide was dispensed into each digestate and distilled. The resulting nitrogen (N) from each digestate was trapped inside 50 ml boric acid indicator (a mixture of 40 g boric acid, 0.03 g methyl red stain, 0.06 g bromocresol green in 2 liters of ethanol water) which changes to green indicating the presence of protein in the sample.

The trapped nitrogen in the boric acid was then titrated against 0.1N HCl which gave a red coloration at the end point. The protein content of each sample was determined using the formular by Kayode *et al.* (2008):

$$\text{Protein content (\%)} = \frac{\text{Titre value x Normality of acid x 1.4007 x 6.25}}{\text{Weight of sample taken}} \times \frac{100}{1}$$

Fat contents

Two grams (2 g) of each of the samples were weighed separately onto filter papers, inserted with Soxhlet and put in the Soxhlet apparatus and then 150 ml of 60 % petroleum ether were added in already dried and weighed round bottom flasks placed on a hot plate and set to 80°C and then allowed to extract for about 3 h. The resulting extracts were then dried in an oven and weighed. The fat content was calculated based on the fomular adopted from Kayode *et al*. (2008):

$$\text{Percentage fat content of sample (\%)} = \frac{W2 - W1}{W} \times \frac{100}{1}$$

Where, W_2 is the weight of flask + extract
W_1 is the weight of dried flask
W is the weight of sample taken

Crude fibre (Prebiotics) contents

The method as described by the Tecator Application Manual Number 1978.03.15 ANO/78 (*www.dairyknowledge.in/sites/default/files/7.11.pdf*) for crude fibre determination which involved hot extraction using Fibretec 1020 Tecator System Technology was employed in this study. One gram (1 g) of the de-fatted samples was weighed separately into clean crucibles to which 150 ml each of 0.256 N sulphuric acid was added with 3 drops (0.15 ml) of octanol (to prevent foaming) and heated for 30 min. Thereafter, they were washed in de-ionized water and 150 ml of potassium hydroxide added and the samples were then dried at 130°C for 2 h. The resulting residues were ashed at 500°C for 3 h. the crude. The cude fiber was determined using the formula from the above manual as:

$$\text{Percentage crude fibre content of sample (\%)} = \frac{W2 - W1}{W} \times \frac{100}{1}$$

Where, W_2 is the weight of crucible + extract
W_1 is the weight of dried crucible
W is the weight of sample taken

Determination of total carbohydrate contents of sample

This was determined based on the protocol adopted by Kayode *et al.* (2009) and Afolabi *et al.* (2012) where its value was calculated by difference using the formula:

Percentage carbohydrate content of sample (%) = 100-{∑DC}.
Where,
∑DC is the summation of the determined proximate contents of other variables (crude protein, crude fibre, fat and moisture) in 100 grams of the sample.

Determination of some micro and macronutrients of the samples

The ashes previously obtained above from the specimens were dissolved in 1N nitric acid (about 3 drops of concentrated nitric acid and made up to 100 ml with distilled water). The resulting sample solutions were taken to the AAS (Atomic Absorption Spectrometer) (Model: AA 7000, Schimadzu, Japan) for the analysis of the elements present in the samples. Standards were prepared and run on the AAS, and a calibration curve generated. From the calibration curve the comparative amounts of the elements were determined (Kayode *et al.,* 2008).

STATISTICAL ANALYSIS

Data collected from this study were analyzed using Analysis of Variance (ANOVA) with the general linear model procedure in Genstat Release (PC Windows Vista, Version 12.10) at 5% level of significance. Means were separated and compared using Fisher's least significant difference (FLSD) at probability level of 0.05.

RESULTS

The results presented in Table 1 indicated that cowpea leaves used in this study contain protein 34.91 %, fat 5. 42 %, carbohydrate 31.11 %, ash 11.15 %, and crude fibre 19.46 %. It showed that protein was the highest proximate nutrient present in the test samples. The leaves specimens also contain high amounts of carbohydrate which differed significantly (P≤ 0.05) but compares well with the protein content of the test samples (Table 1). However both were statistically superior to the values of 5.42 %, 19.46 % and 11.15 % recorded for fat, crude fibre and ash respectively. Also, crude fibre recorded at 19.46 % was statistically (P≤ 0.05) superior to 11.15 % obtained for ash content in the study. In the overall fat recorded at 5.42 % was the least of the proximate components tested in this study

Table 1: Proximate composition of tender cowpea leaves per 100 g (dry weight)

Nutrients	Mean Composition (%)
Moisture content	10.88
Protein	34.91
Fat	5.42
Carbohydrate	31.11
Ash	11.15
Crude fibre	19.46
LSD (0.05)	**0.05**

***Data are means of triplicate determinations from two separate experiments**

The results of the macro and micro elemental nutrients analyses presented in Fig. 2 showed that cowpea leaves contain a wide spectrum of useful micro and macro elemental nutrients. It indicated that potassium (k), sodium (Na), phosphorus (P), calcium (Ca), magnesium (Mg), iron (Fe), and zinc (Zn) were detected in the test samples. The results also showed that amongst the assayed elemental nutrients K (1.35 g) was the most abundant nutrient in the cowpea leaves being statistically ($P \leq 0.05$) superior in amount to every other elemental nutrient. This was followed by Na (2.22 g) and then Mg (1.66 g) and Ca (1.62 g) both of which were statistically ($P \geq 0.05$) at par with each other; and phosphorus (0.55 g) amongst the macronutrients. However iron recorded at 65.21 mg was the least ($P \leq 0.05$) abundant micro-nutrient in test cowpea leaves and it was not significantly ($P \geq 0.05$) different from the value of 121.84 mg obtained for Zn (Fig. 2).

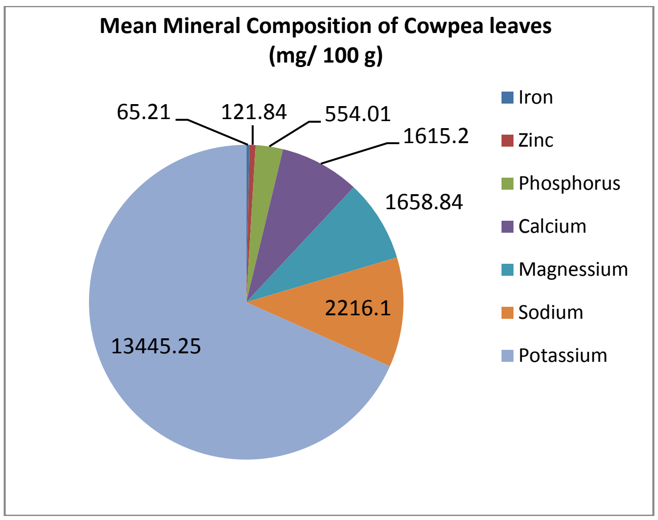

Figure 2: Mean micro and macronutrients composition of the test cowpea (Var. IAR-48) leaves

DISCUSSION

Micronutrients, minerals and protein-calorie deficiencies are primary public health concerns in sub-Saharan Africa (Okonya and Maas, 2014). Less utilized vegetables such as cowpea had contributed immensely to improving the food bank in Ethiopia (Alemu *et al.,* 2016) and provided cheap and easily available sources of protein, minerals and microelements that enhanced the nutritional and health status of resource-poor rural dwellers in the area (Okonya and Maas, 2014). In Nigeria, Afolabi *et al.* (2012) reported that leafy vegetables contribute significantly to supply the body with minerals, vitamins, fibre, and certain hormone precursors, energy and protein which could play roles in preventing cancers, reduce cardiovascular diseases and promote heart health. Findings from this study indicated a wide array of proximate and mineral nutrients in the leaves of cowpea.

Protein

Protein was detected at 34.91% in this study. This is in harmony with 39.24% reported by Chikwendu *et al*. (2014) for dry leaves of cowpea in Benue State, Nigeria. The findings from this study also shows that the protein value of cowpea leaves though lower however, compares well with those reported for water leaf (37.28%), scent leaf (47.17), and green amaranths (47.57) by Afolabi *et al.* (2012). However, it is superior to the protein values of 4.2% and 8.72 mg/100g on dry matter basis reported for fluted pumpkin (*Ugu*) (*Telferia occidentalis*) by Dike (2010) and Idris (2011) respectively. Protein is an important nutrient which plays roles in maintenance of body tissues, building enzymes and hormones (Afolabi *et al*., 2012). In comparative studies with cowpea grains, several workers found cowpea leaves to be richer in protein (34.91-39.24%) and essential amino acids than the seeds which contains 23.32% protein ((Nielsen *et al.,* 1997; Mamiro *et al.,* 2011; Chikwendu *et al.,* 2014). Therefore consumption of cowpea leaves could help heal and repair muscle tissues, improve immunity, prevents cold sores and depression and encourage mental wellbeing (Afolabi *et al*., 2012; HBT, 2016).

Carbohydrate

Findings in this study indicated that 31.11% of carbohydrate was detected in the leaves of cowpea which agrees with 30.39% reported by Chikwendu *et al*. (2014). This value however, is superior to 12.11-16.03% reported for green amaranths, sweet basil, waterleaf, and bitter leaf in western Nigeria by Afolabi *et al.* (2012). On the other hand, it compares well with 26.82% and 39.64 mg/100g carbohydrate on dry matter basis reported for fluted pumpkin (*ugu*) a commonly consumed vegetable in eastern Nigeria by Idris (2011) and Mohd *et al*. (2016) respectively. Carbohydrates are a rich source of metabolizable energy for cellular activities (Afolabi *et al*., 2012). Generally, they are broken down to glucose during tissue respiration and released into the blood stream. Persistently high blood sugar levels have been implicated in diabetes and to increase the threat of heart attacks. It has been reported that people with high blood sugar levels are 3 times more likely to get a heart attack (Best Health Magazine, 2016). However, carbohydrates sourced from fish, skinless chicken, walnut, olive, apples, berries, pulses and legumes including cowpea have been described as of low glycemic index (Rizkalla *et al*., 2002; Glatter, 2014; FAO, 2016). Low glycemic index carbohydrates breakdown and release glucose slowly into the blood stream and thereby increase the body's sensitivity to insulin; and therefore reduce the risk of heart disease and improve control of metabolic syndrome (diabetes) (Glatter, 2014; Body Building, 2016). In coronary heart disease patients, legumes and legumes-derived vegetables modulate glucose, insulin, homocysteine concentration and lipid peroxidation leading to 21% and 11% lower risks of coronary heart disease (CHD) and cardiovascular disease (CVD) respectively (Campos-Vega *et al*., 2010). This wellbeing advantage of pulses to consumers Rizkalla *et al*. (2002) maintained is reaped by both healthy and metabolism-compromised individuals. Though metabolic syndromes including diabetes have genetic undertones, excess body fat, lack of exercise and eating the wrong kind of carbohydrates could worsen the problem (BHM, 2016). According to this source, eating diets rich in pulses and leguminous vegetables up to 4 times a week could reduce the risk of developing Type-2 diabetes by 42% and the risk of coronary heart diseases by 29%. This happens through lowering cellular insulin, reducing tissue triglycerides levels and blood pressure better than low-fat meals. Reduction in triglycerides concentration is thought to result from inhibition of pancreatic activity (Campos-Vega *et al*., 2010). Hence, consumption of cowpea leaves could effectively play a role in preventing metabolic syndromes including diabetes and improve the health status for its consumers.

Prebiotics (Crude fibre)

Crude fibre was detected at 19.46% in this study. This value does not agree with 14.26% reported for cowpea leaves by Chikwendu *et al*. (2014). The differences in the values may be due to differences in cultivars or method of analyses adopted in the study. Compared to fluted pumpkin, it compares well with 20.17 mg/100g reported for the leafy vegetable by Mohd *et al.* (2016). Pulses are high in content of prebiotics which function to improve or better digestion (FAO, 2016). Dietary fibre provides substrate for bifidobacteria in the colon of the human gastrointestinal tract (GIT) which is antagonistic to harmful bacteria. Prebiotics absorb water, and reduce pouches in the colon and in addition functions to prevent haemorrhoids and cancer (Afolabi *et al*., 2012; HBT, 2016). It is also reported to lower the risk of endometrial cancer, breast cancer and colorectal adenoma. Prebiotics and the low fat content of cowpea leaves could contribute to glycemic control leading to reduction of obesity by lowering average body mass index, waist circumference and waist-to-hip ratio (Campos-Vega *et al*., 2010). It is reported that consuming up to 20 g of food-borne fibre leads to 63% reduction of inflammation in humans (Campos-Vega *et al*., 2010; BHM, 2016). Though the phytochemical contents of leaves of cowpea were not evaluated in this study, flavonoids and polyphenols had been detected previously at 26.72% and 32.56% respectively in the leaves of the crop (Olayiwole *et al.,* 2012; Chikwendu *et al*., 2014). These classes of plant-derived secondary metabolites possess antioxidant, stress fighting, anticancer and antibacterial activities (Okwu and Njoku, 2009; Enyiukwu and Awurum, 2013b). These antioxidant phytochemicals and prebiotics in conjuction with copper, potassium, folate and thiamine aid in the formation of acetylcholine and play significant roles in maintaining healthy metabolism and ventricular function (HBT, 2016).

Fat

Fat was detected at 5.2% in this study. This value is significantly higher than 1.31% reported by Chikwendu *et al.* (2014). Though generally vegetables have low fat content, however, unimproved varieties have much more lower fat contents (Mamiro *et al*., 2011); indicating that IAR-48 used in this study is improved compared with local varieties used by Chikwendu *et al*. (2014) in their study. Lipids are good sources of lipophilic vitamins. Unsaturated fatty acids as found in plant-obtained oils reduce risk of coronary cancer and contribute to heart health (Afolabi *et al*., 2012). They lower triglyerides and cholesterol and hence contribute to improving heart and bladder health and maintaining the wellbeing of the circulatory system (BHM, 2016; FAO, 2016). Legumes-derived vegetables could also contribute to lowering serum lipid level and reducing the risk associated with low-density lipoprotein and cholesterol (Campos-Vega *et al*., 2010). Besides fat soluble vitamins, cowpea leaves is also a cheap and rich source of folate and B-vitamins (FAO, 2016). Folate aid in DNA replication in fetuses and contributes to preventing anencephaly (lack of a major part of the brain, skull and scalp during embryonic development usually at 23-24 days after conception) and fight limb and heart malformation. Hence being low in unsaturated fatty acids, and containing fat-soluble vitamins makes cowpea leaves a vegetable of choice to prevent or combat heart attacks, anencephaly and improve health of its consumers.

Magnesium

Magnesium which is one of the six essential macro-minerals in the body was also detected at 1.66 g in this study. This is significantly superior to the range of 195-212 mg reported by Afolabi *et al*. (2012) in a similar evaluation in western Nigeria for sweet basil, water leaf, bitter leaf and green amaranths. Idris (2011) reported that the recommended daily intake (RDI) for the nutrient is 350 mg meaning that cowpea leaves could supply 3.5 times the RDI of the nutrient. Magnesium is an important nutrient which plays crucial role in the prevention of diabetes. In the metabolism of carbohydrates, magnesium functions in improving insulin production and sensitivity reducing Type-2 diabetes by 15% (Health Benefit Times, 2016). Magnesium thus aids in the regulation of blood sugar levels, and promote normal blood pressure. It also plays an important role in regulating vital energy processes, helps in protein synthesis and maintaining healthy cells and muscles and communicating nerve impulses. The nutrient also functions in keeping the heart rhythm steady, support healthy immune system and strong bones (Ancient-Minerals, 2016). High magnesium rich foods was found to be associated with reduced loss of bone mineral density, reduced risk of type-1 diabetes and lower risk of heart disease as well as contributes to cheerfulness. However, irrespective of age, gender, and sociostudies conducted by both Dike (2010) in Umudike, southeast Nigeria and Idris (2011) and Mohd *et al*. (2017) in Kano State in the northern region of the country.

The results of this evaluation showed that cowpea leaves therefore are rich in nutrients; and as vegetable has the potential to maintain and/or promote human health comparable to other tropical leafy vegetables like spinach, bitter leaf, taro, water leaf, sweet potato and lettuce. Hence, its consumption could be exploited and promoted especially in eastern Nigeria (Igbatim *et al.,* 2014).

CONCLUSION

Cowpea leaves are mild and good tasting vegetable that could be produced profusely in the rain-fed, low-input agriculture which characterize the humid southeast Nigeria. Outcome of the nutrient profiling of the leafy vegetable in this evaluation shows that it is rich in a wide array of nutrients such as iron, protein, carbohydrate, magnesium, potassium, zinc, sodium and calcium. With the exception of phosphorus (0.55 g) whose value is lower than the WHO recommended daily intake (RDI) of 700-1000 mg, the leafy vegetable, can effectively provide their WHO recommended daily averages in diets of its consumers. As a result of the significant heart, circulation, hormone, bone and muscle improving activities of the identified macro and micronutrients in the test cowpea leaves, their consumption could help to prevent or at least ameliorate iron, mineral and protein-calorie deficiencies reported to be endemic in Africa south of the Sahara. It could also reduce the risks of diabetes, high blood pressure, heart failure, cancer, depression, dementia and osteoporosis. Because the leaves are low in anti-nutrient factors; and have contributed immensely in improving the food baskets of other countries such as Tanzania, Uganda, Ethiopia and Southern Africa, it could be exploited as a rich source of health-promoting food-source in southeast Nigeria. Therefore, adoption, utilization and consumption of cowpea leaves as vegetables will be a step forward towards combating the protein-calorie malnutrition and iron deficiency in this part of the world.

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