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Review

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Underground Coal Gasification Studies on Chakwal Coal, Punjab, Pakistan

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Abstract. Underground coal gasification (UCG) experimentation was carried out on low-rank lignite coal of Eastern Salt Range, Chakwal, Punjab Province, Pakistan. A simulation reactor was designed in laboratory environments and gas input volume, type of gas input, gasification linkage and mode of combustion were investigated. Geological characteristics of the coal were also studied. The composition of emitted gases was evaluated and the syngas having calorific value of 2.42 MJ/m^3 was produced.

Keywords: underground coal gasification, lignite, combustion, gas composition, Chakwal

Introduction

Coal is a valuable fuel resource and is far more abundant than oil or gas. It is burned to produce heat which is used for various purposes. The most significant uses are in electricity generation, steel production, manufacture of cement, fertilizers and paper, preparation of liquid fuels, synthetic natural gas, methane, ammonia and hydrogen gas in alumina refineries and in pharmaceutical industries (Prebstein and Hicks, 1982).

Coal gasification is a technology of converting coal into combustible gas by reacting it with controlled amount of air (oxygen) and water (steam) at high temperature. The resulting gas mixture, called synthesis gas or syngas, is itself a fuel which can be used for industrial heating. Currently a number of coal gasification technologies are being used in the world. However, underground coal gasification (UCG) has recently emerged as a technology for coal conversion and utilization (Kostur and Blistanova, 2008). It is carried out in non-mined coal seam, which is deep-underground, using injection of oxidants and bringing the product gas to surface through production wells, drilled from the surface. UCG can produce syngas at 1/2 to 1/4 of the cost compared to the surface gasifier (Khadse *et al*., 2010; Blinderman and Anderson, 2004).

Mining is the most common method for extraction of coal associated with constraints and disadvantages of mining. Surface mining is economical only when the coal seam is nearer to the surface. UCG offers an alternate technique to conventional methods and can be applied to deep and uneconomical resources to extract (Ghose and Paul, 2007). Compared to traditional coal mining and gasification, the UCG technology has the advantages of low plant cost, less environmental impact and absence of coal transport (Shuqin and Junhua, 2002). However, the presence of seam at a depth of 30 to 800 m having thickness of more than 5 m with minimal discontinuities are the basic requirements for UCG (Turner and Liu, 2004).

Siemens (1868) first suggested the underground gasification of waste coal left in the mine. Later on, many significant researchers took part in the development of this technology. In 1989, European Working Group recommended that a series of trials should be undertaken to evaluate the commercial feasibility of UCG. The trials were undertaken by the UK and Belgium, and were supported by the European Commission. The largest ongoing programme is being conducted by China, which includes 16 UCG trials. The successful demonstration of UCG during 1999-2003 at Chinchilla town in Australia resulted in gasification of around 35,000 tonnes of coal (Blinderman and Anderson, 2004).

Pakistan has large estimated deposits of over 185 billion tonnes of low quality lignite to sub-bituminous coal of tertiary age. Only the Punjab Province has 235 million tonnes of coal reserves located in the Eastern and the Central Salt Range and in Makerwal area of Surghar Range. Coal seams of economic value are present locally in Dandot area in the Eastern Salt Range and belong to the Patala Formation of late Paleocene (Shah, 1977).

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Main objective of the present study was to develop a viable process for exploitation and utilization of large unmineable coal reserves of lignite located in District Chakwal of Punjab Province, Pakistan. Hence, the potential of UCG process for clean coal technology of Chakwal lignite was investigated and experiments were carried out in laboratory environments with multiple options. A comprehensive account of experimentations and the results obtained are presented here.

Materials and Methods

The proximate analysis of coal and combustion products was performed in accordance with ASTM (2007) methods. Gross calorific value was determined by Parr Isoperibol Bomb Calorimeter (Model: 6200). Total and sulphate sulphur was determined gravimetrically while pyrite sulphur was estimated by stoichiometric combination with iron. Organic sulphur was calculated by subtracting the sum of sulphate and pyrite sulphur from the total sulphur. Chemical compositions of the representative sample of coal and the typical combustion products are presented in Table 1 and Table 2, respectively.

Laboratory scale UCG experiments were performed on lignite coal of Durmeyal area, Tehsil Choa Syden Shah, District Chakwal, Pakistan which is easily available. The size of coal used was 100% down 1" (2.54 cm) mesh sieve. A simulation rectangular shaped reactor was designed to cover almost all the conditions of underground coal gasification. A site having length 210 cm, width 90 cm and height 90 cm was prepared for UCG experiments within the boundary of the Mineral Processing Research Centre, PCSIR Laboratories Complex, Lahore. The coal bed of 90 cm thickness (200 kg) was placed in the first layer for installation of equipment for heating. Afterward, two adjacent stainless steel seamless pipes of 213 cm length and 2.5 cm diameter were inserted vertically into the bed as inlet pipes to pass oxygen and fresh air, simultaneously. Another pipe of similar length with 3.8 cm diameter was inserted on the other end at the distance of 150 cm from each other for outlet. The connectivity (channel) between inlet and the outlet pipes was made by passing pressurized oxidants (air ~700 kPa and oxygen ~250 kPa) into the coal bed. Two electrical heaters of 1200 Watt each were placed into the coal bed for initial ignition of coal; 50 cm long thermocouples with temperature range of 0-1600 °C were inserted between both the heaters to measure temperature inside the coal bed during experiments. As a second step, a layer of 30 cm thickness coal (800 kg) was again introduced to make a total coal bed of 120 cm thickness (1000 kg). The reactor was covered with clay and soil. The coal bed was ignited electrically under controlled conditions to start gasification. The initial ignition was set for 2 h using oxygen gas; and after coal starts burning itself, the gasification ran for 8 h using air injection.

Table 1. Proximate analysis of coal before burning

Constituents	Amount (%)
Moisture content	3.38
Volatile matter	24.50
Ash	44.93
Fixed carbon	21.20
Organic sulphur	2.63
Pyrite sulphur	1.89
Sulphate sulphur	1.34
Total sulphur	5.85
Gross calorific value	14.30 MJ/ kg

Table 2. Chemical composition of ash

Constituents	Quantity (%)
Silica (SiO_2)	55.76
Aluminum oxide (Al_2O_3)	10.10
Iron oxide (Fe_2O_3)	30.4
Sodium oxide (Na_2O)	0.25
Potassium oxide (K_2O)	0.77
Calcium oxide (CaO)	Nill
Magnesium oxide (MgO)	0.116
Phosphorous oxide (P_2O_5)	0.13
Sulphate (SO_4)	1.617

The temperature variation of coal was continuously measured. The reactor was connected with air compressor, which supplied air through the air inlet to reactor during the process. A gas flow meter (Model: SW 100) was used to measure the volume of air input flowing to the reactor. The air flow rate was maintained at ~70 dm^3 /min. The gasification was continued for total 10 h. Gas from the experiment was sampled by gravitation method using a glass made gas sampling tube (length 30 cm, diameter 5 cm). The sampling was repeated on hourly basis during the gasification process so that there were 8 gas samples for one experiment in

each reactor. The combustion gases were analyzed using electrochemical and IR sensing device (Eurotron 8000 Greek). The calorific value of gas was calculated based on the percentage of CO, H_2 and CH_4 contents.

Four experiments were conducted during this study. In the first experiment, only air was used and coal was moistened by water, whereas in the second experiment, a mixture of air and steam was applied to the coal. In the third experiment, the effect of linkage through the coal bed was investigated while in the fourth experiment, the steam and air flow direction was changed.

Results and Discussion

Proximate analysis of the representative sample of coal presented in Table 1 shows the ash content in coal is less than 45% which is sufficient to exploit it for UCG to produce syngas on commercial scale. Fixed carbon (21.20%), volatile matter (24.50%) and gross calorific value of coal (14.30 MJ/kg), show the coal, lignite in nature. However, the presence of 5.86% sulphur appeared to be the main objectionable impurity. Nature of sulphur in composite sample of coal shows that it contains 2.63% organic, 1.89% pyrite and 1.34% sulphate sulphur. The high iron oxide content in the ash also indicates the presence of considerable amount of inorganic sulphur mainly in the form of iron pyrite and sulphate (Table 2). Proximate analysis of coal, left after burning, presented in Table 3 shows that fixed carbon has been reduced from 19.20% to 2.74%, volatile matter from 24.50% to 3.72% and gross calorific value of coal from 14.30 MJ/kg to nil.

The coal used in this study is of Patala Formation which conformably overlies the Lockhart Limestone and transitionally overlain by the Nammal Formation in the Salt Range (Warwick *et al.,* 1990). Based on the sections and borehole data, the formation consists of shale and marl with subordinate limestone, sandstone and coal.

Table 3. Proximate analysis of coal after burning

Constituents	Amount (%)
Moisture content	1.05
Volatile matter	3.12
Ash	93.09
Fixed carbon	2.74
Total sulphur	4.05
Gross calorific value	No ignition

The shale is dark greenish to grey in colour, at some places it is carbonaceous and calcareous. The limestone is white to light grey and nodular. It occurs as interbeds. Subordinate interbeds of yellowish brown and calcareous sandstone are present in the upper part. The thickness of the seam varies and is generally the thickest in south towards anticlinal core and the thinnest in the north towards synclinal axis of the Potwar synclinorium. It is 27 m thick at Khewra Village and 90 m at Patala Nallah.

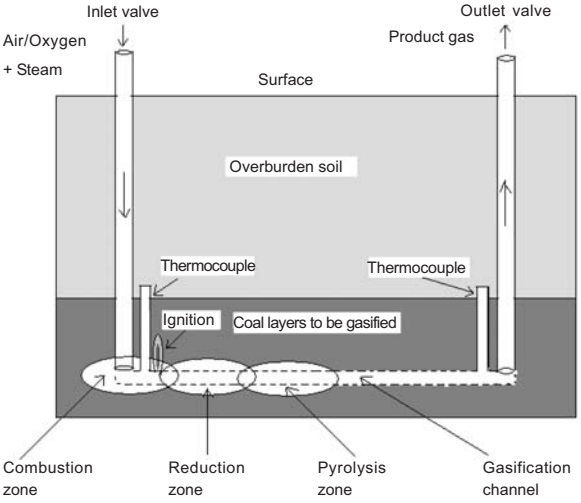

Fig. 1. Schematic representation of an underground coal gasification (UCG) reactor.

It has been observed the combustion is initiated at the bottom of the inlet hole by oxygen and then it is maintained by the continuous injection of air in between inlet and outlet holes, in the underground reaction zone (Fig. 1). As the coal face burns, the immediate area is depleted and the mixture of gases comes out from the outlet hole. Yang *et al.* (2002) explained that in the initial reaction zone (combustion zone), carbon dioxide is generated by the reaction of air with the coal. Afterward, carbon dioxide reacts with coal (reduction zone) to produce carbon monoxide. In addition, at high temperature (pyrolysis zone), moisture inherent in the coal bed also reacts with the coal to produce carbon monoxide and hydrogen:

$$C_{(coal)} + O_2 \rightarrow CO_2 \quad \text{(Combustion)}$$
$$C_{(coal)} + CO_2 \rightarrow 2\,CO \quad \text{(Reduction)}$$
$$C_{(coal)} + H_2O \rightarrow CO + H_2 \quad \text{(Pyrolysis)}$$

The results of first underground coal gasification experiment, conducted using air as oxidant and water

for moisture, have been summarized in Fig. 2. The quality of syngas produced in term of calorific value has been given in Fig. 6. It is obvious from this figure that the produced gases had a heating value of 1.19-1.56 MJ/m^3 which indicates the product gas belonged to low-Btu category. The reason is that the injection of air only raises the nitrogen content and reduces the heating value of the product gas through reduction in the percentage of combustible gases i.e. hydrogen (H_2), carbon monoxide (CO) and methane (CH_4) during the gasification process.

The results of second underground coal gasification experiment, performed through introducing the air along with super heated steam (400-450 ºC) in the inlet hole, are presented in Fig. 3. It is clear from this figure that the amount of gases produced in the second experiment is higher than the amount of gases obtained from the first experiment. Consequently, the calorific value of gases (1.34-2.01 MJ/m^3) obtained from the second experiment is better as compared to the calorific value of gases from the first experiment (Fig. 6). The reason is the injected gas used in this experiment is air and super heated steam. The injected steam reacts with carbon monoxide at > 800 ºC which is produced during the gasification process to form additional hydrogen. This step, also called as shift conversion, sets up the proper ratio of gases for the next step called methanation (Yip *et al*., 2007; Shuqin *et al*., 2003). The hot gases thus produced are passed through the coal bed which boosts up the percentage of methane in the resultant gases making a relatively high-Btu gas to the exit holes.

$$CO + H_2O \rightarrow CO_2 + H_2 \quad \text{(shift conversion)}$$
$$3H_2 + CO \rightarrow CH_4 + H_2O \quad \text{(methanation)}$$

The coal in natural state has generally insufficient permeability to enable air percolation necessary for efficient coal gasification. For successful coal gasification, a linkage to open up internal pathways in the coal bed was used. The result of gasification experiment conducted with gasification linkage inside the reactor shows that gasification process in the third experiment is better as compared with the gasification process in previous experiments (Fig. 4). The calorific value of the product ranges from 1.56-2.23 MJ/m^3. This is due to the fact that pyrolysis and gasification of coal occurs at lower degree in the first and second experiments due to unavailability of gasification linkage inside the reactor. The ash, produced after initial coal burning, covers unburned coal which lies at the bottom of reactor due to gravitation effect. This condition is unfavourable for gasification process and consequently some part of the coal remains unburned, so that there is a loss of heat.

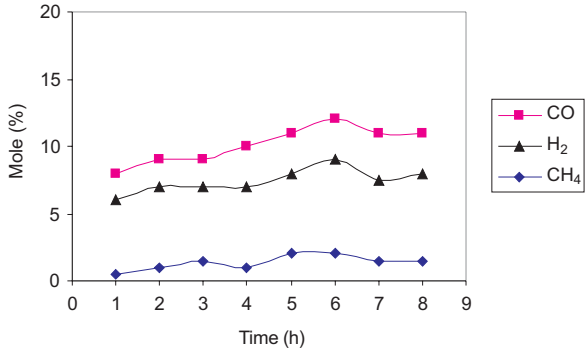

Fig. 2. The composition of gases obtained from UCG experiment with air (conducted without steam, channel and reverse combustion).

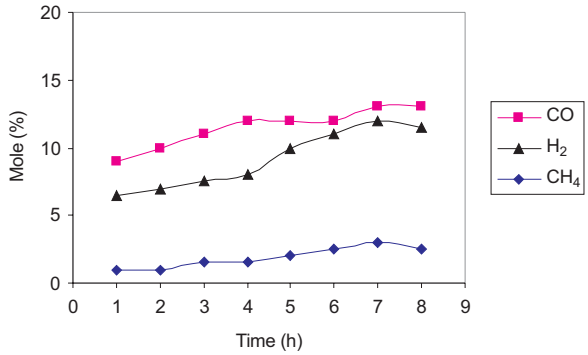

Fig. 3. The composition of gases obtained from UCG experiment conducted with air and steam (without channel and reverse combustion).

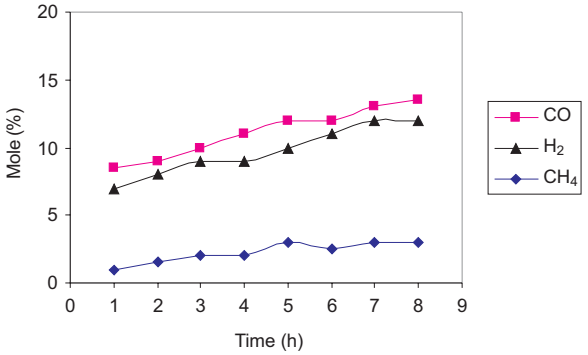

Fig. 4. The composition of gases obtained from UCG experiment conducted with air, steam and channel (without reverse combustion).

The temperature along the bed is not high enough to maintain the reactions. This reduction in temperature has negative effect as shown by the amount and quality of gases produced. However, in the third experiment, due to availability of channel at the bottom of reactor, the burning of coal continues in right track and ash settles down in the bottom of reactor without interference in gasification progress (Perkins and Sahajwalla, 2006).

It was also found that success of the technique was dependent upon location of linkage in coal bed thickness relative to the bottom of the bed. The positioning of linkage low in the bed is extremely important as it allows gasification front to undercut coal as it moves from back to front after completion of linkage. The gasification linkage at the bottom of coal bed allows a better reaction to take place and the coal at bottom is initially consumed and then progresses to the upper part of coal seam. The combustion moves along the bed and as the void grows, unburnt coal falls into it creating a bed of coal rubble that is relatively reactive because of large surface area present (Blinderman *et al.*, 2008; Jie, *et al*., 2008).

The results of UCG reverse combustion linkage experiment are shown in Fig. 5 and the quality of produced syngas in term of calorific value (1.71-2.42 MJ/m^3) is given in Fig. 6. Air at ~100 kPa pressure was injected at the ignition hole to sustain a combustion zone. Then air injection was switched to adjacent hole. The injected air percolates through coal bed to ignition hole and the combustion zone proceeds to ignition hole i.e. toward the source of oxygen. Due to counter-current movement of injected air and combustion zone, a localized highly permeable pathway of carbonized coal is left behind. When combustion zone reaches injection hole, gasification zone expands around the injection hole until the full bed thickness was gasified between two adjacent holes.

The product gases of typical UCG process with air injection may have calorific values ranging from 4.0 to 5.5 MJ/m^3, almost double of the value with oxygen injection, depending on the specific properties of coal and operating conditions (Yang, 2008). The calorific value of syngas produced at optimized conditions was found to be 2.42 MJ/m^3. The achieved value is slightly on the lower side due to low quality of Chakwal coal. The temperature patterns of gasification experiments also show that gasification temperatures "T" in the first, second, third and the fourth experiment are in the ranges of (302-285 °C), (305-540 °C), (373-635 °C) and (390-695 °C), respectively. These data indicate that temperature measured in the first experiment is lower than the temperature of second experiment, which in turn is lower than the third experiment and so on.

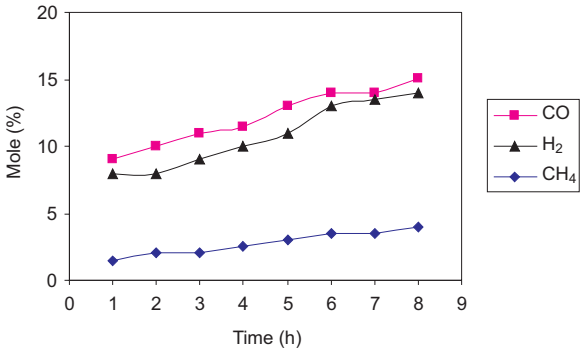

Fig. 5. The composition of gases obtained from UCG experiment conducted with air, steam, channel and reverse combustion.

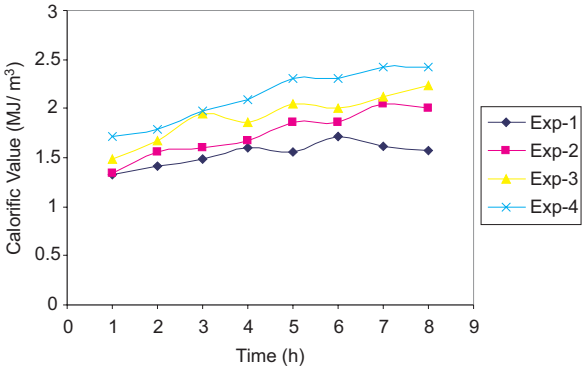

Fig. 6. The calorific value of gases obtained from different UCG experiments.

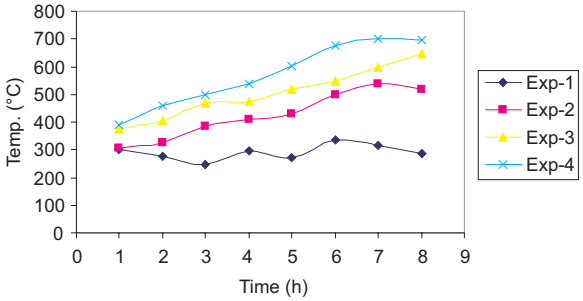

Fig. 7. The temperature pattern obtained from different UCG experiments.

Besides that, the pattern of change in temperature during the course of gasification in the first experiment is quite different from that in other experiments. It was observed that in the first experiment, the pattern was irregular during the progress of gasification (Fig. 7). On the other hand, the pattern of temperature in other experiments had tendency of increasing with the progress of gasification experiment.

Conclusions

The results of investigation show that during underground coal gasification, conducted in laboratory using a simulation reactor; flow rate, type of injected gases, gasification linkage and the mode of combustion affect the production and quality of gases. Results of the first UCG experiment show that without using steam, gasification linkage and reverse combustion, fewer amounts of gases are produced. The calorific value of the gases produced in the first experiment is in the range of 1.19-2.01 MJ/m^3, whereas in the second experiment - using steam along with air - it is in the range of 1.34-2.01 MJ/m^3. The gasification process in the third experiment with linkage is more effective as compared to that in second experiment without it, as is evident by its calorific value of 1.56-2.23 MJ/m^3. Similarly, the gasification process in the fourth experiment with reverse combustion linkage is better as compared to the process in third experiment without reverse combustion. The calorific value of gases produced in the fourth experiment ranged from 1.71-2.42 MJ/m^3.

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The Production of Activated Carbon from Nigerian Mineral Coal *via* Steam Activation

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Abstract. Activated carbon was produced from Okpara sub-bituminous coal and Ogwashi brown lignite coal of Nigeria through steam activation at 900 °C and 960 °C each for 30 min and 60 min. Okpara and Ogwashi precursor coals had carbon content of 67.41 and 64.47%, respectively, whereas the bulk density and the ash content were 0.59-0.68 g/mL and 2.56-9.91%, respectively. The former exhibited up to 901.0 mg/g iodine number and Brunauer Emmett Teller (BET) surface area of 604 m^2/g while the latter, iodine number of 998.0 mg/g and 669 m^2/g BET surface area. Both showed adequate porosity indicative of their potential for utilization for commercial production of active carbons.

Keywords: Nigerian coal, activated carbon, steam activation, adsorption capacity

Introduction

The advent of oil exploration in Nigeria as well as import and operation of heavy trunks and trains with diesel engines led to total neglect of large deposits of mineral coals found in the most parts of Nigeria. This state of affairs gave impetus to investigate the possible conversion of two Nigerian coals, namely Okpara sub-bituminous coal and Ogwashi lignite coal to useful products like activated carbon.

Total recoverable coal reserves around the world have been estimated to be 930 billion tonnes, about 76% of which are located in USA (28%), Russia (19%), China (14%), Australia (9%), India (7%), while the remaining 23% is distributed among other 65 countries (EIA, 2008; USDA, 2008; IEA, 2007). The only African country is South Africa that produces 5.75 quadrillion Btu and consumes 3.81 quadrillion Btu, (Coal Association, 2007; EIA, 2005).

Some of these imported coals are utilized by Japan and China in production of activated carbon. The global consumption of activated carbon has been put as over 350,000 tonnes per annum and due to its high market demand and applications related to the environmental policies worldwide, a projected (7%) annual increase has been forecasted. Thus, by the year 2020, a total of 600,000 metric tonnes of activated carbon might be needed globally (EMS Energy Institute, 2001). Activated carbons are used in industry, medicine, agriculture, and in almost all areas of human activities. No single active carbon has universal application. Thus, commercial active carbons could be classified into four groups on the basis of their physico-chemical properties, pore structure and applications (Choudhury *et al*., 1985; Hassler, 1963). (i) Decolourizing carbon grade which are soft, finely powdered with high porosity and large surface area; (ii) gas/vapour adsorbent grade which are granular with high density, porosity and strength, used for industrial gas/vapour adsorption; (iii) metal adsorbent grade used as catalyst and catalyst support and (iv) medicinal grade adsorbent.

Active carbon is versatile and indispensable adsorbent especially in removal of colour, taste and odour from municipal water, industrial waste water and from food products. It is also used for recovery of gold in mineral industries and recovery of toxic organic solvents in chemical industries. Their use in pharmaceutical industries and medicine cannot be over emphasized such as its use in kidney dialysis machine (Lozano-Castello *et al*., 2001; Zanzi *et al*., 2001; Teker *et al*., 1999). In gas applications, active carbon acts as gas filters, in general air conditioning and in storage of natural gases.

Coal and lignocellulosic materials are two main sources utilized for commercial production of active carbons. Low ash content is desirable in commercial active carbon and is prepared either by acid leaching or by a suitable selection of precursors (Bansal *et al*., 1998).

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Some well known low ash precursors are lignocellulosic materials (Rodriguez-Reinoso and Linares-Solano, 1988); some brown coals (Woskoboenko *et al.*, 1991) and peat (Wigmans, 1983). Some of these precursors have lower yield of char that originates during carbonization, while coals from low rank lignite to high rank anthracite exhibit higher carbon yield on carbonization but have higher ash level (Spreigh, 1994; Van Krevelen, 1993). Lozano-Castello *et al*. (2001) used Spanish anthracite to prepare active carbon *via* chemical activation process with the aid of KOH as activating agent and obtained a high micropore volume of 1.45 cm^3/g and BET surface area of 3290 m^2/g. Buczek *et al*. (2000) produced two types of active carbons by steam activation of carbonized hard coal and tar, allowing burn offs of the carbonized coals to be 41% in one instance and 54% in another. Carrasco-Marin *et al*. (1996) used demineralised Spanish bituminous coal as raw material using CO_2 activation and combination of phosphoric acid and steam activation processes yielding microporous carbon that exhibited Type 1 adsorption isotherm and BET surface area of 1074 m^2/g. Linares-Solano *et al*. (2000) compared the effect of mineral content in active carbon prepared from bituminous coal from Puertollano basin (Spain) on porosity development using steam activation and CO_2 activation. They observed that active carbon produced using CO_2 activation seemed to create new narrow micropores as well as widening the existing ones developed during carbonization process whereas steam appeared to widen only the narrow microporosity of the char, giving a more open microstructure.

In preliminary studies of inorganic constituents of Nigerian coals, Oderinde (1989) reported variability in the levels of concentration of inorganic metals present in Okaba, Okpara, Obi/Lafia coal mines. Earlier works on some Nigerian coals by Afonja (1975) and Oderinde (1989) showed that Nigerian coal produced large quantities of ash. Furthermore, Oderinde (1989) pointed out that the relatively large differences in elemental concentrations of the samples might be attributed to the areas, where the coals are mined or the size of material used. However, in a recent report, the Federal Ministry of Solid Mineral Development, Nigeria and the Raw Material Research Development Council of Nigeria (RMRDC, 2006) revealed Nigerian coal to be one of the most bituminous in the world owing to its low sulphur and ash content which makes it environmental friendly. Thus, the use of Nigerian coals as precursor for production of active carbons is of interest especially due to the large coal reserves (RMRDC, 2006). About 3 billion tonnes of coal reserves have been identified in 17 coal fields of Nigeria Federal Ministry of Solid Minerals Development, Nigeria, 2006 and the Raw Material Research Development Council of Nigeria (RMRDC, 2006). The Nigerian coals have mainly been used locally as fuel and underutilized. Though, some physico-chemical properties of Nigerian coals have been reported, studies are not available exploring the possibility of production of active carbon *via* steam activation of coals. The aim of this study is to examine the physico-chemical properties of two Nigerian coals *viz* Okpara sub-bituminous coal and Ogwashi brown lignite coal and prepare their corresponding active carbons using steam activation process and compare their performance with commercial active carbons (BDH).

Materials and Methods

Collection of materials. Okpara coal and Ogwashi coal samples were obtained from Enugu, Enugu State in the eastern Nigeria and Ogwashi, Delta State in mid-western Nigeria, respectively, through Nigerian Coal Cooperation, Enugu, Nigeria. They are designated as sub-bituminous and lignite brown coal, respectively.

Preparation of active carbon. Four steps were used for preparation of active carbon : pretreatment of precursor raw coals, oxidation, carbonization and activation processes. Figure 1 shows the flow chart for manufacture of active carbon from some Nigerian precursor coals.

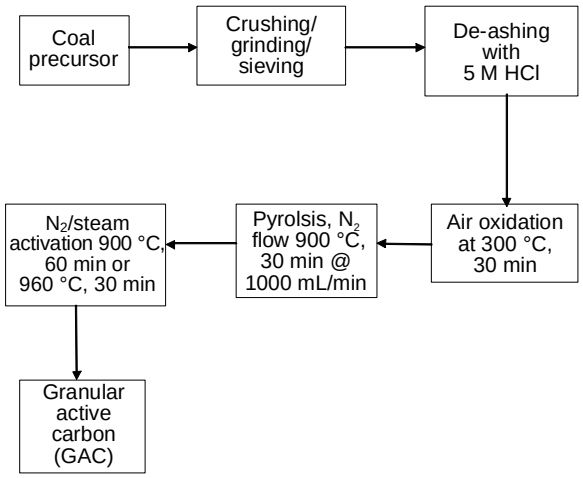

Fig. 1. Flow chart for manufacture of activated carbon from selected Nigerian coals.

Pretreatment of coal precursors. Each coal sample was ground and sieved (0.5-1.0 mm size), 50 g of each was treated with 150 mL of 5 M HCl and boiled in a 250 mL conical flask for 5 min. The solution was then filtered and the residue was washed several times with distilled-deionized water until the pH the filtrate was between 6.5 and 7.5. The samples were then dried at 60 °C in an air circulating oven for 2 h.

Oxidation, carbonization and activation processes. The coal samples were then individually heated at ambient temperature to 300 °C and oxidized with air for 30 min. Carrasco-Marin *et al*. (1996) method of activation of demineralised precursor was used. A known quantity (9.0-10.5 g) of the acid-treated oxidized coal was loaded in hollow quartz glass tube (100 × 2.5 cm) and placed in activation horizontal tubular reactor furnace (Carbolite tube furnace, CTF 12/65/550 Model, Italy). The reactor was first evacuated with the aid of N_2 flowing at 750 mL/min for 30 min. The treated-oxidized coal samples were then pyrolysed at 900 °C for 30 min in the presence of N_2 at flow rate of 1000 mL/min. The activation process involved separate heating of the coal samples in the presence of nitrogen/steam mixture that was achieved *via* nitrogen supply with flow rate of 750 mL/min through a heated water bath kept at constant temperature of 92 ± 2 °C to specified final temperatures of 900 or 960 °C for either of 30 min or 60 min. Figure 2 shows the steam activation laboratory reactor coupled with horizontal tube furnace. A heating belt was used to cover the quartz glass in order to avoid condensation of vapours and thus prevent breakage of the glass. The heating rate was maintained at 20 °C/min. The active carbon samples were allowed to cool down in the quartz tube in the presence of nitrogen/steam mixture. The yields of active carbons were determined gravimetrically, while those of volatile (burn off) were calculated by difference using the following equations:

$$\text{(i) Active carbon yield (\%)} = \frac{\text{mass of active carbon}}{\text{mass of char}} \times 100$$

$$\text{(ii) Volatile yield (\%)} = \frac{\text{Loss in mass of active carbon}}{\text{Initial mass of char}} \times 100$$

Physicochemical properties of precursor coals. The bulk density and moisture content of precursor coals were also determined following Ahmedna *et al*. (1997) and AOAC (1990), respectively, while the method of AWWA (1991) was used for determination of ash content. The ASTM (1996) was used for determination of pH and conductivity of the precursor coal samples.

Elemental analysis of coal samples. The precursor coal samples were subjected to elemental analysis to determine their carbon-hydrogen-nitrogen and sulphur (CHNS) composition. The analysis was carried out in duplicate by the Institute fur Organische Chemie (Universitat Tubingen, Germany) using a Carlo Erba elemental analyzer. The higher heating values (HHV) for each of the biomass types were calculated from the values of CHNS and ash content of the precursor materials (Graboski and Bain, 1981). The equation is given as:

HHV (kJ/kg) = 2.3236 [(141C + 615 H - 10.2 N + 39.95 S) - (1- Ash)(17244H/C) + 149]

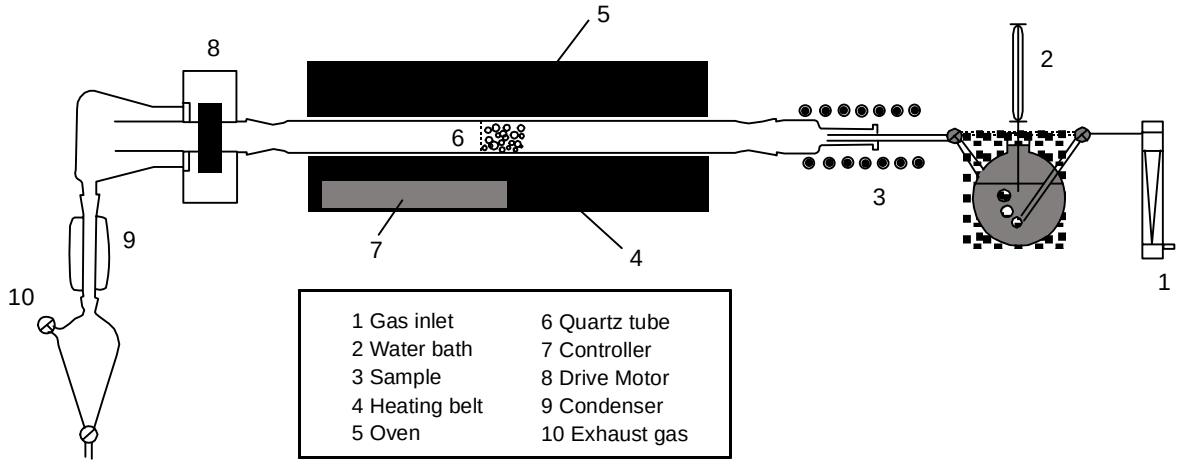

Fig. 2. Schematic diagram of the horizontal tube furnace for steam activation process.

Analysis of active carbon. The Brunauer Emmett Teller (BET) surface areas of steam activated carbons derived from the coal precursors were measured by nitrogen adsorption isotherm at 77 K using a Coulter SA 3100 surface analyser, U.K. The *n*-hexane adsorption method (Bayer *et al*., 1995) was adopted for determination of micropore and mesopore volumes of the prepared coal-based active carbons types.

Adsorption of iodine and methylene blue. The active carbon produced from the two coal types were characterized by iodine and methylene blue adsorption. Methylene blue number and iodine number are measures of adsorption capacity of any type of active carbon. Iodine number indicates the extent of micropore distribution in the carbon (Kirubakaran *et al*., 1991). It is a measure of iodine molecules adsorbed in the pores and indicates pore volume capacity. The iodine number, which gives an idea of the microporosity, was determined by the modified AWWA procedure (AWWA, 1974). The modification involves centrifugation of active carbon-iodine mixture for 5 min prior to titration with sodium thiosulphate solution in the presence of starch indicator. The methylene blue (MB) adsorption capacity of the active carbon, that depicts the extent of mesoporosity of the adsorbent, was measured in accordance with ASTM standards for active carbons (ASTM, 1989).

SEM analysis of active carbon. The physical surface morphology of the steam-based active carbon derived from Nigerian coal was conducted using scanning electron microscopy instrument (model DSM 982, Germany). A thin layer was mounted by a double sided tape. It was coated with Au/Pd to a thickness of about 30 nm. The scanning was carried out *in situ* at × 500 magnification.

Results and Discussion

The physico-chemical properties of selected Nigerian coals: Okpara sub-bituminous coal (OKP) and Ogwashi brown lignite coal (OGW), are presented in Table 1. The OKP coal OGW brown lignite coal exhibited, in order, bulk density, moisture content, ash content, pH values and conductivity values of 0.68 g/cm^3, 4.86%, 9.91%, 4.06 and 0.16 ms/sec, respectively, and 0.59 g/cm^3, 8.10%, 2.56%, 3.62, 0.39 ms/sec, respectively. The variability in the values of various parameters of the two coals could be attributed to different location of the sites, where they were found. This report agreed with the report of Energy Information Administration (EIA, 2010; 2006) about significant variability in heat content, ash and sulphur content by region and even at times within the same seam. The sulphur content range (0.59-0.66%) of OKP and OGW coals are lower than 0.9% of the United States coking coals. Table 1 also shows carbon content of precursor OKP and OGW coals as 67.41% and 64.47%, respectively, which are high enough for their utilization as raw material for production of active carbon. It had been noted that carbonization and controlled activation increased carbon content of the precursor materials (Adebowale and Adebowale, 2008); and consequently increase BET surface area of the active carbon. Low ash content in the precursor material is desirable in production of active carbon as high ash content does not contribute to surface area of the active carbon. The ash content of OKP and OGW Nigerian coals were 9.91 and 2.56%, respectively, which are low and desirable for production of active carbon.

Table 1. Physico-chemical properties of precursor Nigerian coals

	Okpara sub-bituminous coal (OKP)	Ogwashi lignite coal (OGW)
Proximate analysis		
Bulk density (g/cm^3)	0.67±0.01	0.59±0.01
Moisture (wt %)	4.86±0.27	7.43±1.20
Ash (wt %)	9.91±0.83	2.56±0.09
pH	4.06±0.01	3.62±0.01
Conductivity (ms/sec)	0.16±0.01	0.39±0.00
Ultimate analysis		
C	67.41±0.31	64.47±0.65
H	5.13±0.01	6.57±0.08
N	1.70±0.10	0.66±0.01
S	0.66±0.01	0.59±0.03
*O	14.88±0.23	25.15±0.76

*O = oxygen determined by difference.

The higher heating value (HHV) or heat content of precursor coals reported for various countries are comparable with the values of the two investigated Nigerian coals (Table 2), such as for United States coking coal (26.30 million Btu/ton), Greece lignite coal (4.4 million Btu/ton) and Canada lignite coal (12.4 million Btu/ton), which are higher than 0.31 and 0.19 million Btu/ton values for OKP and OGW precursor Nigerian coals, respectively. The heat content value is used for judging the capability of particular precursor coal for utilization for electricity production or

preparation of industrial product like active carbon. It is interesting to know that United States utilized about 90% of its coal to generate about 55% of the electricity and also produced active carbon from the unburnt carbon obtained as by-product of coal combustion (EMS-Energy Institute, 2001). From Table 3, it is evident that the yield and burn off values (volatile matter) resulting from separate activation of OKP coal at 900 °C for 60 min and 30 min are similar i.e. 47.39 % and 48.57%, respectively. However, when temperature of activation of OGW coal was raised from 900 °C to 960 °C, the yield was reduced from 27.84% to 22.15% 60 min. These findings are in line with the reports of Mameri *et al*. (2000) and Mc dougall (1991) that temperature affects yield of chars or active carbon more than variation in duration of activation process.

Total pore volumes of OKP coal active at 900 °C for 60 min and 30 min were 0.256 cm^3/g and 0.089 cm^3/g, respectively, while those of OGW active carbon prepared separately at 960 °C and 900 °C were 0.170 cm^3/g and 0.120 cm^3/g, respectively, for 60 min (Table 4). Thus the value of OKP active carbon is greater than that of 0.199 cm^3/g for commercial powder carbon (BDH, England) and 0.206 cm^3/g of commercial granular active carbon (BDH, England). The values, determined by *n*-hexane adsorption are ranked in increasing order of extent of porosity as follows:

OKP 900 °C, 30 min < OGW 960 °C, 60 min < active carbon (powder BDH) < active carbon (granular BDH) < OKP 900 °C, 60 min.

The micropore volume, Vmi ranges were found to be 0.048 - 0.066, 0.060 - 0.067 and 0.006 - 0.079 cm^3/g while mesopore volume, Vme fell within the range of 0.041 - 0.190, 0.060 - 0.103, 0.127 - 0.193 cm^3/g for OKP, OGW and commercial active carbons, respectively (Fig. 3). These values are lower than those reported elsewhere by N_2 adsorption method. It has, however, been recommended by IUPAC that N_2 should be used as standard adsorbate for pore volume measurement (Sing *et al*., 1985). The micropore filling nature, unusual shape and molecular size of N_2 gas makes its use as adsorbate in pore volume measurements to give higher values than values obtained through other molecular probes like *n*- hexane and benzene that exhibit higher reliability (Sing, 1989). In consideration of the foregoing explanation, the micropore volume range (0.048-0.066 cm^3/g) and mesopore range (0.041-0.190 cm^3/g) for OKP active carbon is lower than micropore volume range of 0.192-0.40 cm^3/g obtained for active carbon with 40-60% burn off (Linares-Solano *et al*., 2000) and 0.281 and 0.315 cm^3/g miropore volumes for 41% and

Table 2. Higher heating value (Heat content) of some coal from selected countries

Countries	Type of coal	Amount of higher heating value (HHV) units	
		(MJ/kg)	(Btu/ton) × 106
*United States	Coking coal	61170	26.30
*Greece	Lignite	10230	4.40
*Canada	Lignite	28840	12.40
Nigeria (OKP)	Sub-bituminous	711.74	0.31
Nigeria (OGW)	Lignite	430.57	0.19

* = values obtained from Energy Information Administration, DOE/EIA-0121(2006/4Q); Washington DC (2007).

Table 3. Activation time, temperature, and burn off of active carbons

Coal types	Activation temperature (°C)	Activation time (min)	Yield (wt %)*	Burn off (wt %)*
Okpara	900	60	47.39	52.61
subituminous	900	30	48.57	51.43
Ogwashi	900	60	27.84	72.16
lignite	960	60	22.15	77.85
Okaba lignite	900	60	33.10	66.90
	900	30	40.68	59.32

*standard error is within the 5% error limit.

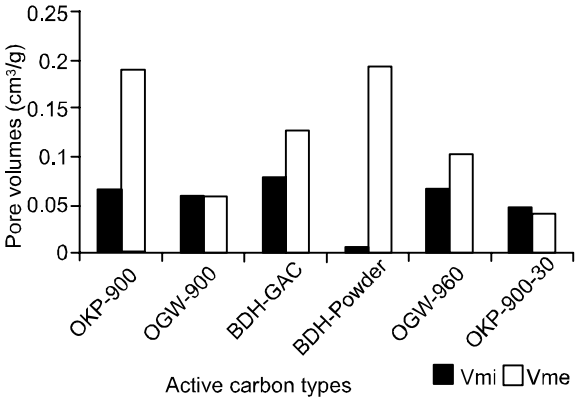

Fig. 3. Comparison of micopore/mesopore volumes of various active carbons with commercial active carbons.

Table 4. Textural characteristics of prepared active carbons from selected Nigerian coal

Coal types	Activation temperature (°C)	Activation time (min)	Iodine number (mg/g)	Methylene blue number (mg/g)	BET surface area (m^2/g)	*Vtotal (cm^3/g)
OKP-900-60	900	60	901	130.6	604	0.256
OKP-900-30	900	30	801	27.4	537	0.089
OGW- 900-60	900	60	998	< 0.1	669	0.120
OGW-960-60	960	60	920	70.7	616	0.170
Commercial coal powder (BDH)	_	_	785	54.3	526	0.199
Commercial coal granular (BDH)	_	_	873	70.1	751	0.206

*Vtotal = represents total pore volume.

54% burn off, respectively (Buczek *et al*., 2000) from granules prepared from hard coal and tar. The same applies to the micropore and mesopore volumes of OGW active carbons.

Total pore volume 0.256 cm^3/g for OKP-90-60 active carbon was higher than those of the commercial powder active carbons (0.199 cm^3/g) and granular active carbon, GAC (0.206 cm^3/g) (Table 4). The values of pore volumes and BET surface area for OKP-900 active carbon prepared *via* steam activation for 60 min was somewhat better than those prepared with contact time of only 30 min (OKP-900-30). Steam activation of oxidized pre-treated OGW precursor coal at 960 °C for 60 min resulted in lower yield of 22.15% and higher burn off percentage of 77.85% as compared to steam activation at 900 °C for the same contact time, which yielded 27.84% active carbon and 72.16% burn off. Porosity of the OGW active carbon derived from steam activation at higher temperature (960 °C) exhibited higher values (0.170 cm^3/g) than that activated at 900 °C with value of 0.120 cm^3/g. This result is in agreement with the reports of Buczek *et al*. (2000). The iodine number of 920 mg/g and 998 mg/g were obtained for OGW active carbons prepared for 60 min at temperature of 960 °C and 900 °C, respectively, while those for OKP carbons activated at 900 °C for 60 and 30 min are 901 and 801 mg/g, respectively. The BET surface area were found to be within 537-669 m^2/g for all the prepared active carbons and are comparable with the commercial BDH powder and granular active carbons (Table 4). These values are within the range of values obtained by Bacaoui *et al*. (2001) for adsorption of methylene blue (115-490 mg/g), adsorption of iodine (741-1495 mg/g) and BET surface area (514-1271 m^2/g) for series of active carbons prepared by physical activation with steam. Interestingly, the minimum molecular size of MB is 0.8 nm which could pass through minimum pore diameter of only about 1.3 nm and therefore, enters the largest micropore diameter. However, the most mesoporous carbons adsorb methylene blue, molecules while iodine molecules are greatly adsorbed into micropores due to its small size.

The FT- IR spectra (spectra not shown) show that there exists similarity in the functional groups present on the surface of OGW (900 °C, 60 min.), OKP (900 °C, 60 min.) and commercial BDH granular active carbons. The OH functional group, C=O found in COOH acids and C-O functional groups present in simple ethers, alcohols and acid anhydrides show stretch vibrations within the range of 3449-3456 cm^{-1}, 1635- 1637 cm^{-1} and 1050-1360 cm^{-1}, respectively (Petrov *et al*., 2000). These stretch vibrations are common to both the prepared active carbons and the commercial active carbon (BDH). The existence of 449-673 cm^{-1} band range in the active carbons depicts carbon-heteroatom bond (Duran-Valle *et al*., 2006). The presence of C=C functional group in the OKP-900-60 active carbon as well as the commercial granular active carbon (BDH) is additional similarity in functionality. However, it was observed that C=C functional group was conspicuously absent from the spectra of the OGW-960-60 active carbon. It might be that the carbon atoms burnt off and broke the C=C

double bond functional groups, widening the existing pores at the carbonization stage; the high burn off value may thus be a confirmation of destruction of C=C bond.

The SEM micrographs of Fig. 4a-4c revealed that there are pores and crevices all over the surfaces of the active carbons prepared from OKP and OGW Nigerian coals as well as that of the commercial powder active carbon. It was observed that OGW carbon active at 960 °C for 60 min exhibited the highest burn off percentage (77.85%) and also exhibited high methylene blue number (70.7 mg/g) and iodine number (920 mg/g) and thus could be utilized for de-colourization purposes as well as for treatment of municipal water while the OKP active carbon prepared *via* steam activation at 900 °C for 60 min with insignificant methylene blue number (< 0.1 mg/g) and high iodine number (901 mg/g) may be used for adsorption of gas molecules or small molecules of volatile organic compounds.

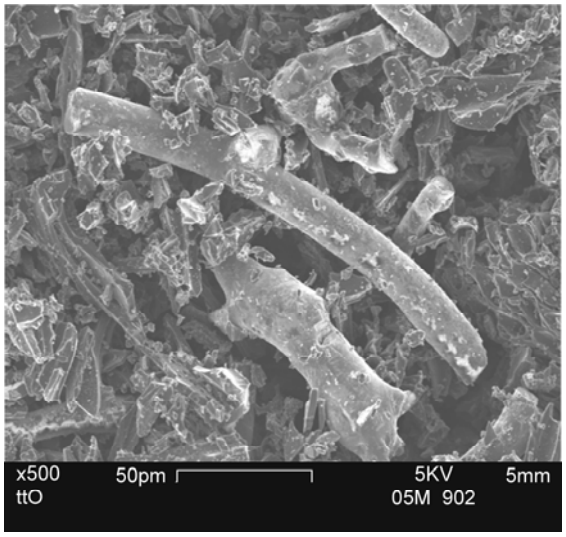

Fig. 4a. Micrograph of OGW active carbon prepared by activation at 900 °C for 60 mins.

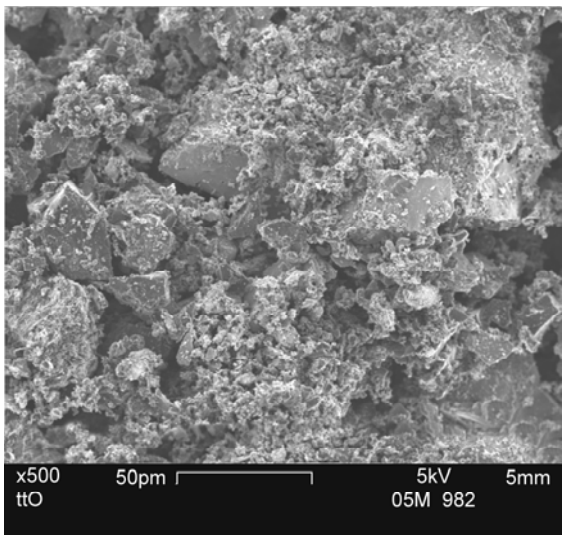

Fig. 4b. Micrograph of OKP active carbon prepared by activation at 900 °C for 60 mins.

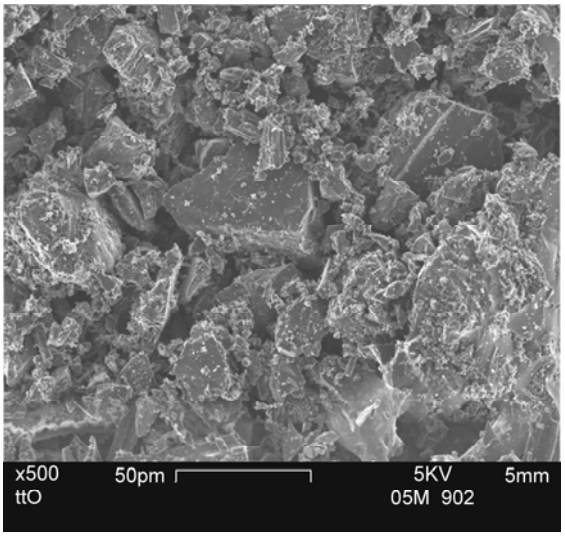

Fig. 4c. Micrograph of commercial powder active carbon.

Conclusion

The active carbon prepared *via* steam activation from Nigerian brown lignite and sub- bituminous coals compared favourably with commercial BDH active carbons in terms of porosity and surface area properties of active carbons. The effect of temperature on development of porous properties using steam activation process during production of active carbon is greater than the effects of duration of contact period. More so, the investigated Nigerian coals may contain less heat content compared to United States bituminous or Canada lignite coals and may only allow its full utilization in production of industrial product such as active carbon.

Acknowledgements

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Biosorptive Removal of Cadmium from Aqueous Solutions by *Pleurotus ferulae:* Equilibrium, Kinetic and Thermodynamic Studies

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Abstract. Equilibrium, kinetics and thermodynamic parameters were evaluated to establish the potential usefulness of the *Pleurotus ferulae* biomass for biosorption of cadmium from aqueous solutions. Maximum biosorption was observed at initial pH of 4.5, temperature of 30 °C and at the initial cadmium concentration of 100 mg/L. Pseudo- second order rate expression well fitted the experimental data for cadmium when compared to pseudo-first order kinetic model. Equilibrium analysis using Langmuir and Freundlich models showed that the biosorption process is Langmuir model. The process was exothermic and ΔG^{O} was negative showing spontaneity of the process within the studied temperature range. The possible functional groups on the dried *Pleurotus ferulae* biomass, responsible for the sorption of Cd (II), are: – OH, – NH, – COO^{-} and – C– O.

Keywords: biomass, biosorption, cadmium, *Pleurotus ferulae*

Introduction

Heavy metal pollution in wastewaters is an extremely important environmental problem. Discharge of heavy metals from various industrial operations such as mining, ore processing, smelting and metal plating can easily cause metal pollution and hazardous effects on humans, animals as well as environmental imbalance. Due to metal accumulation through food chain and persistence, it is necessary to remove such chemical agents from wastewater before discharging it to the environment. Cadmium is usually associated with some principal metal ores widely used in daily life such as zinc, copper, mercury, iron, lead etc. Cadmium is also a dangerous pollutant originating from metal plating, metallurgical alloying, mining, ceramics and other industrial operations. Chronic exposure to elevated levels of cadmium is known to cause renal dysfunction (Fanconi syndrome), bone degeneration ('Itai–itai' syndrome), liver damage and blood damage (ATSDR, 1993). The US Department of Health and Human Services has determined that cadmium and cadmium compounds may be carcinogens. Cadmium has been found in at least 388 of 1300 items of national priority list identified by the Environmental Protection Agency (ATSDR, 1993).

Though much efforts have been significantly put into curtail the amount of cadmium in the environment, these have not really yielded the results desired by the most world related health and environmental standard regulatory bodies possibly due to inefficient and uneconomical removal technology used in the past two to three decades (Roskill Information Services, 1995). Therefore, the release of Cd into the environment, and resultant risk of exposure at various trophic levels still remain substantial. Some of the bases of conventional technologies for removing metallic ions from wastewater are solvent extraction, ion exchange, chemical precipitation, adsorption and reverse osmosis techniques (Kapoor and Viraraghavan, 1995). Chemical precipitation, especially as metal hydroxide or sulphide, is widely practiced, being simple employing inexpensive chemicals. However, it is not effective to reduce toxic metal concentrations to the level of water quality standards; also the generation of voluminous toxic waste sludge is a major problem encountered. Therefore, during the last few decades, research yielded adsorption to be one of the attractive techniques for removing noxious substances and for water purification; it is fast becoming an alternative to conventional precipitation and other techniques, especially for wastewaters that contain low concentrations of metals (Sahu *et al.,* 2010; Jyotikusum *et al.,* 2009; Wahid, 2009; Sing *et al.,* 2008; Sheng *et al.,* 2004).

Activated carbon is the most widely used adsorbent in the wastewater treatment. Owing to high cost and

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ineffectiveness of activated carbon in the removal of aqueous soluble matter particularly heavy metals (Faust and Aly, 1987), research on low-cost and high affinity-bearing metal adsorbents such as biomass wastes and various other biological materials has been receiving much attention. Biomass has been advocated for removal of heavy metals for the following reasons: multiplicity of functional groups that have affinity for metals as ligands, lightness of the particles and so low bulk density, ability to be pulverized to very small particles and hence having large surface area per unit mass, low cost and easy availability, among other properties.

Biomass consists of polysaccharides and proteins carrying many functional groups, such as carboxylate, hydroxyl, sulphonate, phosphate and amino groups, which can bind metal ions. Different types of biomass have been investigated for biosorption of cadmium and other heavy metal ions. Those include bacteria (Bang *et al.*, 2000), fungi (Zafar *et al.*, 2007), wood bark (Al-Asheh and Duvnjak, 1998), red algae (Sarý and Tuzen, 2008), agricultural by-products (Krishnani *et al.*, 2008; Schiewer and Patil, 2008), marine algae (Freitas *et al.*, 2008; Lodeiro *et al.*, 2006) and aquatic mosses (Sarý and Tuzen, 2008; Herrero *et al.*, 2006). Literature review showed that little work had been reported on sorption potential of some notable basidiomycete, a fungi class mostly used as food supplement in Africa and some parts of Asia.

The characteristics of the biosorption behaviour are generally analyzed by means of both biosorption kinetics and equilibrium isotherms (Herrero *et al.*, 2006; Lodeiro *et al.*, 2006). The biosorption isotherm is an important tool for understanding the biosorption mechanism for theoretical evaluation as well. These basic data are useful in the design of suitable reactors for the adsorption process technology. The objective of this research was to investigate the removal of cadmium metal ions from simulated aqueous solutions by the fungus *Pleurotus ferulae*. It is widely available, growing on dead decaying mango trees and some other dead trees. In this study, it was chosen as a biosorbent material due to the lack of information on its biosorption capabilities and such basic data required for reactor design. The biosorption capacity was determined using various kinetic models; the well-known equations of Langmuir and Freundlich were used for the equilibrium analysis while the kinetic was analysed by using Lagergren expressions. The effect of biosorbent dosage, initial metal concentration, contact time, temperature and pH was examined. The thermodynamic parameters were also deduced from the adsorption measurements to further strengthen the useful sorbent potentials.

Materials and Methods

Reagents and equipments. The reagents used in this study are cadmium nitrate, nitric acid and sodium hydroxide procured from BDH Chemicals, England; they are all analytical grade reagents. Atomic absorption spectrophotometer (AAS Alpha 4 model) was used for analysis of metal ions and Fourier Transformation Infra-Red (FTIR Buck 500M) for determination of functional groups of the adsorbent.

Preparation of *Pleurotus ferulae* biosorbent and Cd (II) ion solution. *P. ferulae* was collected from an old cocoa plantation in Apatapiti area, off Federal University of Technology, Akure Road, Ondo State, Nigeria. The collected material was identified in the Department of Microbiology of the Federal University of Technology, Akure, Nigeria. The material was washed with deionised water to remove dirt particles. The washed material was first air dried and later oven dried at 70 °C for 48 h. Dry biomass was crushed into powder and sieved to 100 µm particle size. The stock solution containing 1000 mg/L of Cd (II) was prepared by dissolving 2.103 g of cadmium nitrate in 10 mL of concentrated nitric acid and then diluted up to 1000 mL in a volumetric flask with distilled water. The working solutions were prepared by diluting cadmium nitrate stock solution in accurate proportions to the required concentrations.

Batch biosorption experimental process. The experiments were conducted in 600 cm^3 Erlenmeyer flasks containing appropriate amount of dried *P. ferulae* and 50 mL of Cd (II) ion synthetic solutions having different concentrations and pH. At the end of the predetermined contact period, the mixtures were filtered and the filtrates were analysed for Cd (II) using atomic absorption spectroscopy and the equilibrium concentration were calculated (equation 1) while the percentage of the removed cadmium (R%) was obtained as shown in equation 2:

$$q = \frac{(C_0 - C_e)V}{M} \quad (1)$$

$$R\% = \frac{C_e}{C_0} \times 100 \quad (2)$$

where, M is the biosorbent mass (g), q the adsorbed metal ions (mg per gram of biosorbent at equilibrium), C_0 the initial metal concentration (mg/L), C_e the metal concentration at equilibrium (mg/L) and V is volume of the the working solution.

Spectrometric determination of the functional groups on dried *Pleurotus ferulae*. Infra-red spectroscopy was used for the determination of functional groups on the dried *P. ferulae*. The spectra were collected by using a Buck 500M FT-IR spectrometer within the range 400–4000 cm^{-1} using a KBr window. The background obtained from the scan of pure KBr was automatically subtracted from the sample spectra.

Results and Discussion

Effect of pH on biosorption of Cd (II) using *P. ferulae.* The effects of pH were investigated in the range of 1 to 7.5 at a constant contact time of 20 min, biosorbent dosage of 2.5 g, initial metal concentration of 100 mg/L. The percentage of the removed cadmium against pH of the solution, plotted in Fig. 1, showing that uptake of Cd (II) increases with pH.

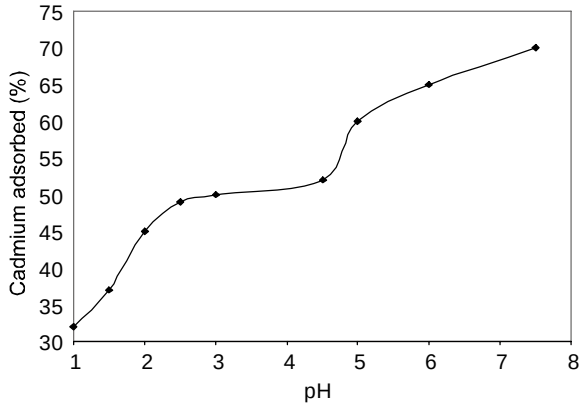

Fig. 1. Variation of pH with cadmium removed % by *P. ferulae*.

However, an inflection pH point was observed; the first region was between pH of 1 to 5 in which there was increase in uptake considerably between pH of 1 and 3 and, thereafter, almost remained constant till 4.5. Then another increase in the uptake was observed above pH of 5; in fact during the studies at this pH range (5 to 7.5), there was precipitate in the solution which most probably was due to formation of insoluble $Cd(OH)_2$. At pH between 1 and 4.5, the solution was obviously clear and no precipitate was observed. It could be deduced that true sorption took place between pH 1 and 4.5 whereas at pH between 5 and 7.5, there was precipitation in which removal of the metal is not really by adsorption alone. The removal of Cd (II) by *P. ferulae* as affected by the initial pH of the metal solution could probably be due to ionic attraction between the possible charges on the biomass and the metal ions, particularly at much lower pH. Earlier reports on heavy metal biosorption have shown that pH is one of the important parameters affecting most biosorption processes (Sarý and Tuzen, 2008; Bulut and Baysal, 2006; Sittig, 1973). The pH of medium affects the solubility of metals and the ionization state of the functional groups like carboxylate and amino groups of the biomass. The carboxylate groups carry negative charges that allow the biomass components to be potent scavengers of cations.

Effect of biosorbent dosage on biosorption of cadmium using *P. ferulae.* The effect of biosorbent dosage was monitored to attain the maximum uptake of Cd (II). The dosage was varied from 0.5 to 4 g at the predetermined initial Cd (II) concentration of 100 mg/L and 30 °C in 50 mL of the solution. Each dosage was monitored for a period of 1 h and 1 mL of the sample was taken at 10 min intervals, diluted to 25 mL with distilled water and analysed for cadmium. The percentage of cadmium removed was found to be increasing with increase in biosorbent dosage (Fig. 2). At low dosage, between 0.5 -1.5 g, the maximum removal at saturation was 20% for 1.5 g, which increased to 52% for 4 g after 20 min of contact. The removal of Cd (II) with sorbent between 2.5-4 g does not change appreciably thus 2.5 g of biosorbent dosage was taken as optimum value for the subsequent experiments. Various reasons have been suggested to explain the increased biosorption of metal with increasing biosorbent dosage including availability of more surfaces, electrostatic interactions and interference between binding sites (Sittig, 1973).

Effect of contact time on biosorption of cadmium using *P. ferulae.* The effect of contact time from 0 to 60 min on the removal of Cd (II) was investigated at a

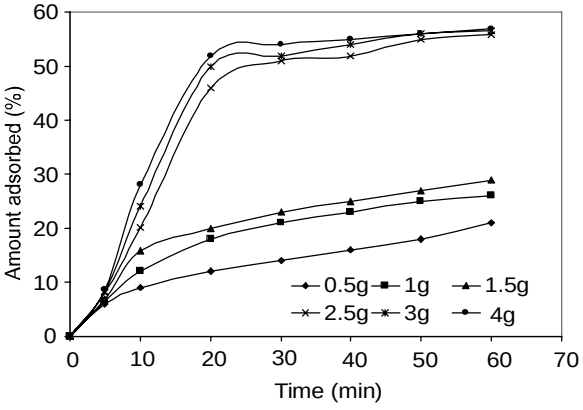

Fig. 2. Effect of adsorbent biomass on adsorption of Cadmium.

fixed predetermined initial Cd (II) concentration of 100 mg/L at 30 °C in 50 mL solution. The metal biosorption increased rapidly during the first 20 min and attained equilibrium as shown in Fig. 3.

After attaining the equilibrium, the amount of biosorbed metal ions did not appreciably changed with time. The removal of Cadmium increased to about 50% during 20 min and was less than 60% in 60 min of continuous contact. This might be due to saturation of the surface of the adsorbent in 20 min.

Effect of initial metal concentration on removal of Cd (II) using *P. ferulae*. The effect of initial metal concentration on the removal of Cd (II) was conducted using *P. ferulae* at different Cd (II) concentrations of 20 to 300 mg/L for contact time of 20 min, 2.5 g biosorbent material at 30 °C. It was observed that the amount of removed cadmium initially increased linearly with the Cd (II) concentration and then finally attained saturation after 20 min. (Fig. 4).

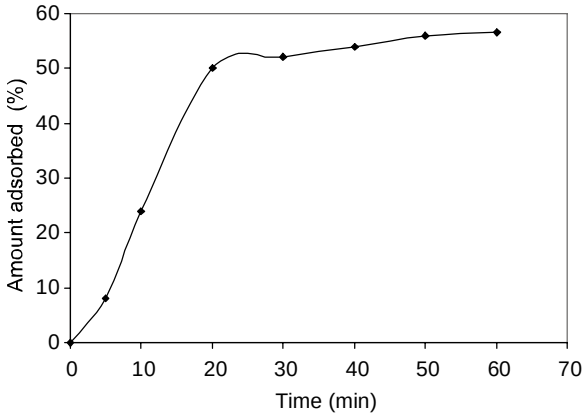

Fig. 3. Effect of contact time on removal of cadmium using *P. ferulae*.

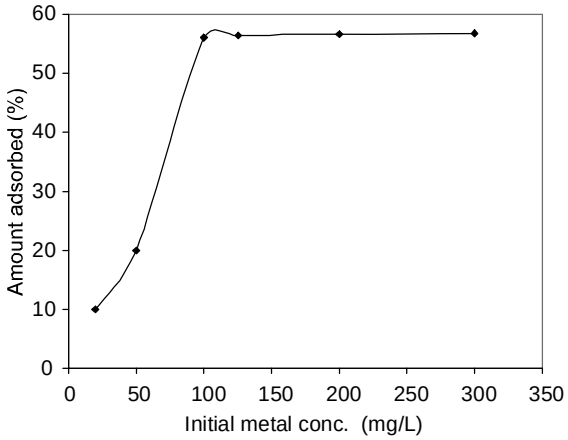

Fig. 4. Effect of initial metal concentration on removal of cadmium using. *P. ferulae*.

The saturation concentration was observed at 100 mg/L of the metal solution from which only 56% was removed. There are many factors which can contribute to the sorbate concentration effect. The first and important one is that adsorption sites remain unsaturated during the adsorption process. The second cause can be the aggregation/ agglomeration of sorbent particles at higher concentrations. Such aggregation would lead to a decrease in the total surface area of the sorbent particles available for metal adsorption and an increase in the diffusional path length. The particle interaction brought about at high sorbent concentrations may also desorb some of the metal ions, which are loosely and reversibly bound to the sorbent surface (Bulut and Baysal, 2006).

Effect of temperature and thermodynamic consideration of biosorption of Cd (II) on *P. ferulae*. The effect of temperature on the equilibrium sorption capacity of dried *P. ferulae* biomass was investigated in the temperature range of 30-50 °C. It was observed that biosorption capacity of the biomass decreased with increasing temperature. The reduction of the metal removal in this temperature range means that the process of cadmium sorption by *P. ferulae* is exothermic as it is the case for the majority of gas adsorption processes. Similar results have been reported for the sorption of lead by *Cephalosporium aphidicola* from aqueous solution at temperature 20-40 °C (Tunali *et al.,* 2006) and biosorption of lead and copper with dried activated sludge (Katsumata *et al.,* 2003).

The effect of temperature in the range of 30-50 °C was used to determine the effect of some important thermodynamic parameters on the biosorption of Cd (II). The values of equilibrium constants (K_d) at 30, 40 and 50 °C were calculated from the relation in Equation 3:

$$k_d = \frac{C_s}{C_e} \qquad (3)$$

where, C_e and C_s are the equilibrium concentrations (mg/L) of Cd (II) on the biosorbent and in solution, respectively.

$$\Delta G^o = - RT \ln k_d \qquad (4)$$

where, T is the absolute temperature, R gas constant and ΔG^o is the standard free energy change. The values of enthalpy change (ΔH^o) and entropy change (ΔS^o) were calculated from the following relation in equation 5:

$$\ln k_d = \frac{\Delta S^o}{R} - \frac{\Delta H^o}{RT} \qquad (5)$$

where, ΔS^o and ΔH^o were calculated from the slope and intercept of Van't Hoff plot of $\ln k_d$ versus $1/T$ (Fig. 5).

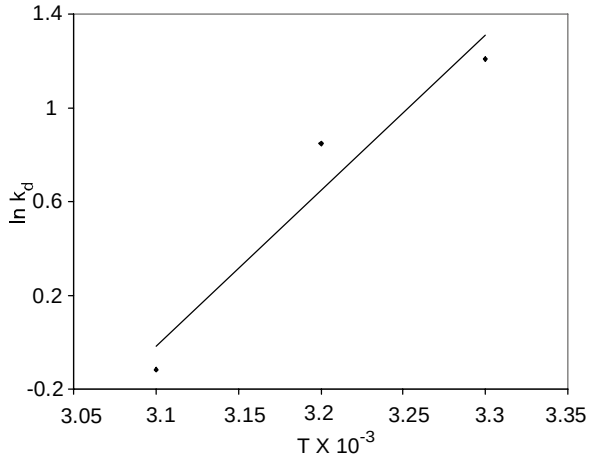

Fig. 5. Plot of ln k_d against 1/T.

Table 1 shows the values of k_d, ΔH^o, ΔS^o and ΔG^o obtained from eq. 4. Thermodynamic parameters are quite useful for evaluation of feasibility of any process.

Table 1. Thermodynamic parameters for biosorption of Cd (II) on *P. ferulae*

Temperature (K)	k_d	ΔG° (kJ/mol)	ΔH° (kJ/mol)	ΔS° (kJ/mol)
303	3.35	-3.046	-0.0551	0.1711
318	2.33	-2.236	-0.0551	0.0171
323	0.89	0.313	-0.0551	0.1711

The negative values of ΔG^oshow that the process is spontaneous and the spontaneity decreases with increase in temperature within the studied range. The positive value of ΔS^o suggests an increase in randomness at the solid–liquid interface during biosorption. The negative values of ΔH^oconfirm that the process under study is exothermic while the values of k_d indicate favoured process at 303 K but decreasing with increase in temperature. These parameters show that the process is favourable within the temperature range but could best be performed at lower temperatures.

Biosorption kinetic of Cd (II) on *P. ferulae.* The biosorption mechanism and potential rate controlling steps have been investigated, using the Pseudo-first and pseudo-second order kinetic models. The Pseudo-first order (Lodeiro *et al.*, 2006) rate expression of Lagergren is

$$\frac{dq}{dt} = k_{1,ad}(q_e - q), \qquad (6)$$

where, q is the amount of metal adsorbed on the biosorbent at time t and $k_{1,\,ad}$ /min is the rate constant for pseudo-first order biosorption. The integral form of equation 6 is

$$\ln (q_e - q) = \text{In } q_e - k_{1,ad}\, t \qquad (7)$$

A linear fit of ln (q_e - q) versus t shows the applicability of this kinetic model. Expression for the pseudo-second order (Herrero *et al.*, 2006) kinetic model is

$$\frac{dq}{dt} = k_{2,ad}(q_e - q)^2 \qquad (8)$$

where, $k_{2,\,ad}$ (g/mg/min) is the rate constant of the pseudo-second order biosorption. The integrated linear form of equation 8 is

$$\frac{t}{q} = \frac{1}{k_{2,ad}\, q_e^2} + \frac{1}{q_e}\, t \qquad (9)$$

If the experimental data fits the plot of t/q versus t as linear relationship, the pseudo-second order kinetic model is valid.

From the slopes and intercepts of plots of log (q_e - q) against time t, the pseudo-first-order rate constants (k_1, ad) and theoretical $q_{eq,\,\text{cal}}$ values were determined. The pseudo-second order biosorption rate constant ($k_{2,\,ad}$) and theoretical q_e values were determined from the slope and intercept of the plots of t/q against t. The values of the parameters ($k_{2,\,\text{ad}}$), q_e calculated and q_e experimental together with correlation coefficients are presented in Table 2.

The linearized form of the pseudo-first and pseudo-second order kinetic model at different initial metal concentrations for a period of 60 min was analysed. The correlation coefficients of the pseudo-first-order kinetic model obtained for metal using *P. ferulae* were found to be less as compared to the pseudo-second order kinetic model studied in the present investigation (Table 2.).

Biosorption Equilibrium of Cd (II) on *P. ferulae.* The biosorption of metal can be quantitatively evaluated by experimental equilibrium isotherms. The graphical expression of isotherm is a plot of the metal uptake by per unit weight of biosorbent against the residual metal ion concentration in the biosorption medium. There are two widely accepted and easily linearized adsorption isotherm models used in the literature, namely Freundlich and Langmuir models.

The Freundlich model based on the relationship between the metal uptake capacity "q" (mg/g) of biomass and the equilibrium metal ion concentration "C_e" (mg/L). The general Freundlich equation is as follows:

$$q = k_f C_e^{1/n} \qquad (10)$$

Table 2. The Pseudo-first and second order rate constants of Cd(II) using *P. ferulae*

C_o (mg/L)	$q_{e,exp}$ (mg/g)	Pseudo-first order			Pseudo-second order		
		$q_{e,cal}$ (mg/g)	k_1 (1/min)	r^2	$q_{e,cal}$ (mg/g)	k_2(g/mg/min)	r^2
20	4.65	1.79	0.068	0.971	2.72	0.166	0.992
50	6.31	2.17	0.082	0.990	5.89	0.116	0.998
100	7.51	2.05	0.069	0.963	7.54	0.118	1.000
125	7.80	2.29	0.107	0.972	7.07	0.119	0.999
200	7.95	2.07	0.084	0.935	7.39	0.116	0.999
300	8.10	2.03	0.084	0.934	8.03	0.115	0.999

r^2, calculated and experimental; q_{eq},values for the biosorption of Cd(II).

and linearized form of the model is

$$\ln q_e = \ln k_f + \frac{1}{n} \ln C_e \qquad (11)$$

where intercept, $\ln k_f$, is a measure of adsorbent capacity, and the slope, $1/n$, is the intensity of adsorption.

The general Langmuir equation is commonly presented as:

$$q_e = \frac{Q_o b C_e}{1 + bC_e} \qquad (12)$$

and the equation may be linearized as follows:

$$\frac{C_e}{q_e} = \frac{1}{Q_o b} + \frac{C_e}{Q_o} \qquad (13)$$

where, q_e is the amount of metal ion removed (mg/g), C_e the equilibrium concentration (mg/L), Q_o and b are the Langmuir constants related to adsorption capacity and affinity, respectively. Experimental data fitted both the Freundlich and the Langmuir isotherms. But the correlation coefficients of Langmuir adsorption isotherm showed that the Langmuir isotherm yielded the best fit to experimental data. The necessary equilibrium constants were calculated from the corresponding plots (figures not shown) for the biosorption of Cd (II) on the biosorbent; the results are presented in Table 3.

Hence, the biosorption process in this study may be interpreted as monolayer adsorption. The values of *b* indicate the affinity of biosorbent to the investigated metals and imply strong binding of metal ions.

Fourier transform infrared analysis (FTIR). The FTIR spectra of dried *P. ferulae* biomass (Fig. 6) in the range of 400-4000/ cm were used to obtain information on the nature of possible functional groups that could be responsible for biomass-metal ion interactions. The broad stretching absorption band at 3441 cm^{-1} is assigned to both –NH and bonded – OH groups.

The band observed at 2926 cm^{-1} is an indication of symmetric/asymmetric stretching vibration of the $–CH_3$ and $–CH_2$ groups and their bending vibrations are 1458 and 1467 cm^{-1} for dried biomass. Carbonyl stretching band of un-ionized carboxylates was observed at 1792 cm^{-1}. The band at 1947 cm^{-1} is a consequence of –C–O stretching vibration conjugated to a –NH deformation and is indicative of amide-I band. The other band referred to amide group, namely amide-II, is present at 1541 cm^{-1} assigned to -NH deformation conjugated to –CN=N deformation for dried biomass. The 1077 cm^{-1} band was due to C–O stretching of carboxyl groups and the bending vibration band of hydroxyl groups. The functional

Table 3. Langmuir and Freundlich constants from Cd (II) isotherms

C_o (mg/L)	Langmuir			Freundlich		
	q_{max} (mg/g)	b (g/mg/min)	r^2	k_f	1/n	r^2
20	2.79	5.68	0.992	0.72	0.166	0.912
50	5.17	5.82	0.990	0.89	0.116	0.928
100	7.54	5.69	1.000	1.54	0.118	0.870
125	7.09	5.70	0.999	1.07	0.119	0.919
200	7.37	5.84	0.999	1.39	0.116	0.909
300	8.03	5.84	0.999	1.03	0.115	0.919

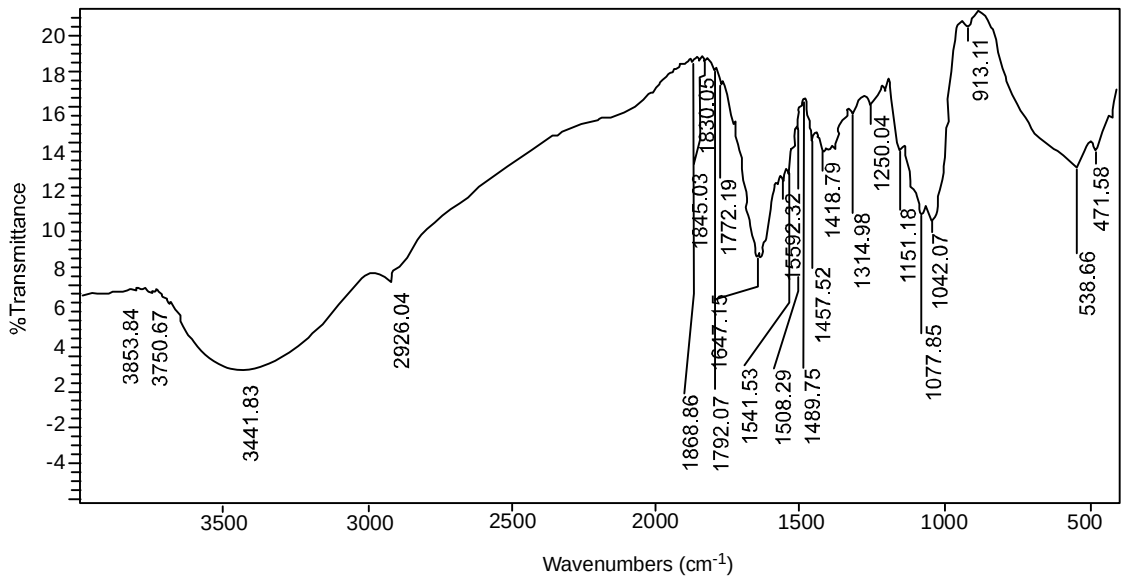

Fig. 6. FTIR of dried biomass.

groups which can adsorb Cd (II) ions are of the type–OH, –NH, $–COO^-$ and –C–O present on the biomass (Xuejiang *et al.*, 2006).

Conclusion

In this study, a dried powdered *Pleurotus ferulae* biomass was used for investigating the effect of contact time, biosorbent dosage, initial metal concentration, initial pH and temperature on the biosorption of Cd (II) from simulated solution. From these variables, kinetic, equilibrium and thermodynamic parameters were determined and conclusions were drawn. There was rapid biosorption of Cd (II) within the first 20 min with biosorption capacity of 6.5 mg/g and thereafter became very slow. There was increase in sorption of Cd^{2+} with increase in biosorbent dosage from 0.5 to 2.5 g and thereafter did not show appreciable sorption. Biosorption of the metal increased with increase in pH up to 3.0 and thereafter reached a plateau. Though removal of Cd (II) was still observed up to pH 7.5, but this was strongly assumed to be due to precipitation. Rapid biosorption was observed upto initial Cd (II) concentration of 100 mg/L but thereafter did not show appreciable sorption. Increase in temperature lead to decrease in sorption of Cd (II) on to *P. ferulae* biomass indicating an exothermic process. Kinetic analysis using rate expressions of Lagergren shows that the process is pseudo-second order reaction model. Initial biosorption rate increased with the increase in initial Cd (II) concentration and decreased with increase in temperature. Equilibrium analysis using Freundlich and Langmuir models showed that the biosorption process follows Langmuir model. Thermodynamic consideration shows that equilibrium constant (K_d) decreases with temperature; decrease in Gibb's free energy (ΔG^o) with increase in temperature shows that the biosorption of Cd (II) by *P. ferulae* is better performed at 30 °C. The possible functional groups on the dried *P. ferulae* responsible for the sorption of Cd (II) are: – OH, –NH, $– COO^-$ and –C–O.

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Fatty Acids, Phospholipids and Sterols Levels of the Skin and Muscle of Tongue Sole Fish

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Abstract. The levels of fatty acids, phospholipids and sterols were determined in the skin and muscle of Tongue sole fish on dry weight basis. Results showed crude fat varied from 0.027-0.360 g/100 g; SFA varied from 35.0-36.9% of total fatty acids, total unsaturated fatty acids varied from 42.6-47.3%, PUFA ranged from 0.068-0.149. In the phospholipids, phosphatidylcholine was highest in both skin and muscle, with respective values of 12.7 and 16.1 mg/100 g. The sterols level in the skin varied between 6.86-6.94 (6.90±0.04) mg/100 g and muscle was n.d.-0.961 mg/100 g. Samples had low levels of n^{-6} fatty acids [4.20% (skin) and 0.140% (muscle)] and n^{-3} fatty acids [1.20% (skin) and 2.36% (muscle)].

Keywords: lipid profiles, skin, muscle, tongue sole fish

Introduction

Fish and meat from wild animals are the chief source of animal protein in the diets of the rural communities, especially in the southern states of Nigeria (Petrides, 1962). The FAO calculation for apparent annual per capita consumption of fish and shellfish for human food, by region and country (2001-2008) put the expected estimate for 2008 as 26.6 kg or 58.8 pounds in Nigeria (Adeyeye, 2009). Hence work on the determination of the chemical composition of fish should be an important part of aquaculture research.

Fish are widely recognized as a nutrition source, due to their high content of proteins, phospholipids and polyunsaturated fatty acids, as well as essential minerals (Simopoulos, 2002). In particular, fish are an important source of essential polyunsaturated fatty acids, which contribute to the reduction of cardiovascular disease (Kris- Etherton *et al*., 2003), inflammatory diseases (Tapiero *et al*., 2002), colon cancer (Roynettle *et al*., 2004), and disorders of the immune system (Belluzi, 2001).

Sole is the common name for various species of flatfish. Generally speaking, they are the members of the family Soleidae, but outside Europe, the name 'sole' is also applied to various other similar flatfish. The main aim of this paper was to investigate the lipid composition (fatty acids, phospholipids and sterols) of Tongue sole fish (Cynoglossidae), commonly found in the fish markets of Nigeria. These fish are sold after drying and with their skin peeled off for storage, hence the skin and the muscle of the fish in this study were separately evaluated, to determine the potential loss of nutrition from the consumption of fish without their skin.

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Materials and Methods

Sample collection and treatment. Five Tongue sole fish were purchased from the local fish market and brought to the laboratory; all bones and viscera were carefully removed and oven-dried at 55 °C for 5 h. The cooled dried samples were further separated into the skin and muscle, ground using mortar and pestle into a fine powder. The ground portions were kept in plastic rubbers in the freezer (-4 °C) pending analysis.

Determination of ether extract. An aliquot (0.25 g) of each part was weighed in an extraction thimble and 200 mL of petroleum ether (40-60 °C boiling range) was added. The covered porous thimble containing the sample was extracted for 5 h using a Soxhlet extractor. The extraction flask was removed from the heating mantle when it was almost free of petroleum ether, oven dried at 105 °C for 1 h, cooled in a desiccator and the weight of dried oil was determined.

Preparation of fatty acid methyl esters and analysis. A 50 mg aliquot of the dried oil was saponified for 5 min at 95 °C with 3.4 mL of 0.5 M KOH in dry methanol. The mixture was neutralized by 0.7 M HCl and 3 mL of 14% boron triflouride in methanol was

added. This mixture was heated for 5 min at 90 °C to achieve complete methylation. The fatty acid methyl esters were thrice extracted from the mixture with redistilled *n*-hexane and concentrated to 1 mL for analysis. The fatty acid methyl esters were analysed using an HP 5890 gas chromatograph (GMI, Inc., Minnesota, USA) fitted with a flame ionization detector and using ChemStation software. Nitrogen was used as the carrier gas with a flow rate of 20-60 mL/min. The oven program was: initial temperature at 60 °C, ramping at 10 °C/min for 20 min, held for 4 min, with a second ramping at 15 °C/min for 4 min and held for 10 min. The injection temperature was 250 °C and the detector temperature was 320 °C. A polar (HP INNOWAX) capillary column (30 m × 0.25 mm × 0.25 µm) was used to separate the esters. A split injection was used with a split ratio of 20:1. The peaks were identified by their relative retention time compared with known standards.

Sterols analysis. Aliquots of the dried oil were added to screw-capped test tubes. The sample was saponified at 95 °C for 30 min, using 3 mL of 10% KOH in ethanol, to which 0.20 mL of benzene was added to ensure miscibility. Deionised water (3 mL) was added and 2 mL of hexane was used in extracting the non-saponifiable materials. Three extractions, each with 2 mL of hexane, were carried out for 1 h, 30 min and 30 min respectively to achieve complete extraction of the sterols. Hexane was concentrated to 1 mL for gas chromatographic analysis.

Phospholipids analysis. Using a modified method of Raheja *et al*. (1973), 0.01 g of the dried oil was added to test tubes. Any remaining solvent was removed by passing a stream of nitrogen gas over the oil. Then 0.40 mL of chloroform was added, followed by addition of 0.10 mL of the chromogenic solution. The tube was heated to 100 °C in a water bath for 1 min 20 sec., cooled to room temperature, 5 mL of hexane was added and the tube was shaken gently several times. After separation of the solvent and aqueous layers, the hexane layer was recovered and concentrated to 1.0 mL for analysis. Analysis was performed using the gas chromatograph with a polar (HP5) capillary column (30 m × 0.25 mm × 0.25 µm). The oven programme was: initially at 50 °C, ramping at 10 °C/min for 20 min, held for 4 min, a second ramping at 15 °C/min for 4 min and held for 5 min. The injection temperature was 250 °C, and the detector temperature was 320 °C. As previously described, a split injection type was used having a split ratio of 20:1. Peaks were identified by comparison with the known standards.

Quality assurance. Standard chromatograms were prepared for cholesterol, phospholipids and fatty acid methyl esters which were then compared with respective analytical results; calibration curves were prepared for all the standard mixtures and correlation coefficient was determined for each fatty acid (28), sterol (4) and phospholipid (5). Correlation coefficient > 0.95 was considered acceptable.

Fatty acid values were also subjected to the calculation of uncertainty interval percentage. Some CRMs values were available for sterols and phospholipids but none in food samples relevant to this study. The CRMs used here were from Wolf (1993).

Calculation of fatty acid per 100 g in samples. Crude fat level was multiplied by a conversion factor of 0.70 to convert it to total fatty acids (Paul and Southgate, 1978). For fatty acids expressed in g per 100 g total fatty acids, precision is best limited to the 0.1 g/100 g level, with trace being set at < 0.06 g/100 g to fatty acids (Greenfield and Southgate, 2003).

Statistical analysis. Statistical analysis (Oloyo, 2001) was carried out to determine the mean, standard deviation, coefficient of variation in percent. Also calculated were linear correlation coefficient (r_{xy}), coefficient of determination (r_{xy}^2), linear regression coefficient (R_{xy}) and coefficient of alienation (C_A) in percent and index of forecasting efficiency (IFE) in percent. The r_{xy} was subjected to the table (critical) value at r=0.05 to see if significant differences existed in the values of fatty acids, sterol and phospholipids between the skin and muscle of Tongue sole.

Results and Discussion

Fatty acids. Table 1 depicts the crude fat and the calculated total acid levels of the fish parts on dry weight basis. The values between the skin and muscle were wide spread with the coefficient of variation of 122 and a ratio of skin: muscle as 13.3:1 (crude fat) and 13.3:1 (total fatty acids), showing that virtually all the fat was concentrated in the skin of the Tongue sole. The crude fat in the skin was lower than the values reported for three different types of land snails found in Nigeria with values of 1.12-1.42 g/100 g (wet weight basis) (Adeyeye, 1996) and also lower than all parts of male and female common West African fresh water crab *Sudananautes africanus africanus* with values of

Table 1. Crude fat and total fatty acid levels of skin and muscle (g/100 g) of Tongue sole fish

Parameter	Skin	Muscle	Mean	SD	CV %
Crude fat	0.360	0.027	0.194	0.235	122
Total fatty acid*	0.252	0.019	0.136	0.165	122

*Crude fat × 0.70; SD = standard deviation; CV % = coefficient of variation.

1.69-8.88 g/100 g (dry weight basis) (Adeyeye, 2002), lower than in insects: 52.7 g/100 g (dry weight) in winged termites (Adeyeye, 2005) and 13.3 g/100 g (dry weight) in grasshopper (Olaofe *et al*., 1998). The concentration of fat in the skin was similar to the observation in the exoskeleton of *Penaeus notabilis* where the value was greater than in the muscle (54.0-40.4 g/100 g dry weight) (Adeyeye and Adubiaro, 2004); the epicarp of *Chrysophyllum albidum* than its mesocarp (15.6-0.7 g/100 g wet weight) (Adeyeye and Agesin, 1999) and in the hull of two varieties of African yam bean than their cotyledons (17.0-8.15 and 16.5-10.8 g/100 g dry weight respectively) (Adeyeye and Agesin, 2007). The energy density from the skin (due to fat) was 14.0 kJ/100 g whilst it was 1.05 kJ/100 g from the muscle.

Table 2 shows the saturated fats (SFA) and the monounsaturated fats (MUFA) of the samples. The following members were found in traces: C22:0, C24:0, C14:1 n^{-5}, *cis*, C20:1 n^{-9}, *cis*, C22:1 n^{-9}, *cis*, C24:1 n^{-9}, *cis* and C18:1 n^{-11}, *trans*. Both SFA from skin and muscle was with coefficient of variation (CV %) of 3.74. C16:0 was the most concentrated fatty acid in the two samples; whilst C18:0 level was the second most concentrated in both samples. SFA with C12:0, C14:0 and C16:0 are the primary contributors to elevated blood cholesterol, and so contribute to cardiovascular diseases; C14:0 is the main culprit. SFA with 12, 14, or 16 carbons generally constitute about 25% - 50% of the total fat in animal foods. C18:0 is also thought to increase the risk of cardiovascular disease.

Like in SFA, C16:1n^{-7}, *cis* was the most concentrated fatty acid in the group of monounsaturated fatty acid (MUFA) in both skin and muscle. It was followed by C18:1 n^{-9}, *cis* in both samples with a value of CV % of 13.8. In the *trans* MUFA group, C18:1 n^{-9}, *trans* was the most concentrated in both samples; all *trans* MUFA value was 10.8% (skin) and 11.4% (muscle) but the total [MUFA (*cis*) + MUFA (*trans*)] was 42.1% (skin) and 45.2% (muscle) and CV% of 3.44 which are very close in the present results. The natural trans fatty acids in butter are said not to be harmful and may even have health-promoting properties, such as preventing certain forms of cancer (Wardlaw, 2003).

Table 2. Saturated and monounsaturated fatty acid composition of the skin and muscle of Tongue sole fish (% total fatty acid)

Fatty acid	Skin	Muscle	Mean	SD	CV %
C12:0	1.12	1.05	1.09	0.049	4.56
C14:0	4.60	5.45	5.03	0.601	12.0
C16:0	20.3	21.2	20.8	0.64	3.07
C18:0	8.96	9.17	9.07	0.148	1.64
C20:0	Tr	Tr	-	-	-
C24:0	-	-	-	-	-
SFA	35.0	36.9	36.0	1.34	3.74
C14:1 n^{-5}, *cis*	Tr	Tr	-	-	-
C16:1 n^{-7}, *cis*	21.7	19.1	20.4	1.84	9.01
C18:1 n^{-6}, *cis*	3.17	1.81	2.49	0.96	38.6
C18:1 n^{-9}, *cis*	6.44	7.83	7.14	0.98	13.8
C20:1 n^{-9}, *cis*	Tr	Tr	-	-	-
C22:1 n^{-9}, *cis*	-	Tr	-	-	-
C24:1 n^{-9}, *cis*	Tr	-	-	-	-
MUFA (*cis*)	31.3	28.7	30.0	1.84	6.13
C18:1 n^{-6} *trans*	1.19	1.80	1.50	0.43	28.8
C18:1 n^{-9}, *trans*	9.59	9.57	9.58	0.014	0.15
C18:1 n^{-11}, *trans*	Tr	Tr	-	-	-
MUFA (*trans*)	10.8	11.4	11.1	0.424	3.82
MUFA (totals)	42.1	40.1	41.1	1.41	3.44

Tr = trace; - = not detected; SFA = saturated fatty acid; MUFA = monounsaturated fatty acid.

Table 3 contains the polyunsaturated fatty acids (PUFA) composition of n^{-6} and n^{-3} of the samples. Among the n^{-6} family, C20:2 n^{-6}, *cis* was the most concentrated with a value of 3.86% of the total fatty acids in the skin but not detected in the muscle. Whilst total PUFA n^{-6}, *cis* was 4.20% in the skin, it was 0.140% in the muscle. The C18:2 n^{-6}, *trans* was in traces. C18:2 n^{-6}, *trans* is known as conjugated linoleic acid (CLA) which occurs naturally. The bacteria that live in the rumens of some animals, for example produce trans fatty acids that eventually appear in foods such as beef, milk and butter (Wardlaw and Smith, 2009), this may have happened in case of Tongue sole. The n^{-3} that was observed in the sample was only C22:6 n^{-3} in both samples: 1.20% (skin) and 2.36% (muscle); this brought the total PUFA (*cis* + *trans*) in skin as 17.8% and 12.1% in the muscle. These results showed that the eicocanoids in the samples were only in traces (less than 0.06% each). The relative values of PUFA in both the skin and muscle made the two parts important in the fish flesh. However, C20:2

n^{-6}, *cis* constituted the highest levels of PUFA in both the samples. The eicosanoids help regulate blood clot formulation, blood pressure, blood lipid (including cholesterol) concentrations, the immune response, the inflammation response to injury and infection and many other body functions (Whitney *et al*., 1994). A deficiency of n^{-6} fatty acids in the diet leads to skin lesions. A deficiency of n^{-3} fatty acids leads to subtle neurological and visual problems. Deficiencies in PUFA produce growth retardation, reproductive failure, skin abnormalities and kidney and liver disorders. However, people are rarely deficient in these fatty acids (Tapiero *et al*., 2002). Both the skin and muscle of the fish were good sources of the PUFA (in combination).

Total unsaturated fatty acids in the skin were 47.3% and 42.6% in the muscle; these were made up by MUFA and PUFA. The essential fatty acids (EFA) are not unique in their ability to supply energy. The ß-oxidation of fatty acids in fish is basically the same as in mammals. The EFA, SFA and monoenoic fatty acids are all equally utilized for energy production. The relative amounts of PUFA and SFA in oils is important in nutrition and health. The ratio of PUFA/SFA (P/S ratio) is therefore important in determining the detrimental effects of dietary fats. The higher the P/S ratio the more nutritionally useful is the oil. This is because the severity of atherosclerosis is closely associated with the proportion of the total energy supplied by saturated fats and polyunsaturated fats (Honatra, 1974; Keys, 1972). The present PUFA/SFA in skin was 0.149 and 0.068 in the muscle, the value of P/S in the skin was poor to discourage atherosclerotic tendency whilst the muscle would support the skin in this action. N^{-3} fatty acids were all in traces in the samples. The n^{-6} and n^{-3} fatty acids have critical roles in the membrane structure (Kinsella, 1990; Lynch and Thompson, 1984) and as precursors of eicosanoids, which are potent and highly reactive compounds. Since they compete for the same enzymes and have different biological roles, the balance between the n^{-6} and the n^{-3} fatty acids in the diet can be of considerable importance (WHO/FAO, 1994). The ratio of n^{-6} to n^{-3} in the diet should be between 5:1 and 10:1 (WHO/FAO, 1994) or 4-10 g of n^{-6} fatty acids to 1.0 g of n^{-3} fatty acids (Canadian Government Publishing Centre, 1990; Nestel, 1987). However, strictly speaking the C18 polyunsaturated fatty acids, linoleic or *cis* -9, *cis*-12-octadecadienoic acid [18:2(n^{-6})] and α-linolenic or *cis*-9, *cis*-12, *cis*-15-octadecatrienoic acid [18:3(n^{-3})], are the main essential fatty acids in that they cannot be synthesized in animal tissues. On the other hand, as linoleic is almost always present in foods, it tends to be relatively abundant in animal tissues. This is supported in the present report as follows: C18:2 (n^{-6}) in skin 0.06% and in muscle it was 0.14% whereas C18:3 (n^{-3}) in skin and muscle was in traces (Tr). In turn, these fatty acids are the biosynthetic precursors in animal systems of C20 and C22 polyunsaturated fatty acids, with three to six double bonds, *via* sequential desaturation and chain -elongation steps (desaturases in animal tissues can only insert a double bond on the carboxyl side of an existing double bond) (Steyer *et al*., 2007). Whilst it would be easy for the body to synthesize arachidonic acid [20:4 (n^{-6})] from [18:2 (n^{-6})], it would be a bit difficult to synthesize the n^{-3} PUFA series: especially eicosapentaenoic acid [20:5 (n^{-3}) or EPA] because of the low level of C18:3 (n^{-3}) and so the diet must be enhanced in this PUFA if this fish is to serve as the only dietary oil source. However, docosahexaenoic acid [22:6 (n^{-3}) or DHA], was present in both the samples.

Table 3. PUFA n^{-6} and n^{-3} fatty acid composition of the skin and muscle of Tongue sole fish (% total fatty acids)

Fatty acid	Skin	Muscle	Mean	SD	CV %
C18:2 n^{-6}, *cis*	0.06	0.14	0.10	0.06	56.6
C18:3 n^{-6}, *cis*	Tr	Tr	-	-	-
C20:2 n^{-6}, *cis*	Tr	Tr	-	-	-
C20:3 n-6, *cis*	-	Tr	-	-	-
C20:4 n^{-6}, *cis*	0.283	Tr	-	-	-
C22:2 n^{-6}, *cis*	3.86	-	-	-	-
n^{-6} PUFA (*cis*)	4.20	0.140	2.17	2.87	132
C18:2 n^{-6}, *trans*	Tr	Tr	-	-	-
n^{-6} PUFA (totals)	4.20	0.14	2.17	2.87	132
C18:3 n^{-3}	Tr	Tr	-	-	-
C18:5 n^{-3}	Tr	-	-	-	-
C22:6 n^{-3}	1.20	2.36	1.78	0.82	46.1
n^{-6} + n^{-3} (PUFA)	5.20	2.50	3.85	1.91	49.6
Totals (SFA+MUFA + PUFA	82.3	79.5	80.9	1.98	2.45
Totals (MUFA+PUFA)	47.3	42.6	45.0	3.32	7.39
PUFA/SFA	0.149	0.068	0.109	0.06	52.8
2 n^{-6}/3 n^{-3}	-	-	-	-	-
Ratio	1.03:1		-	-	-

PUFA = unsaturated fatty acid (essential fatty acid).

The results in Tables 2 and 3 were further subjected to statistical analysis (Table 4). Results showed highly positive and significant linear correlation coefficient (r_{xy}) at r=0.05 and n^{-2} degrees of freedom. The coefficient

Table 4. Statistical analysis of the results from Table 2 and 3

Parameter	Skin(X)	Muscle(Y)	r_{xy}	r_{xy}^2	C_A	R_{xy}	IFE	Remark
SFA	35.0	36.9	-	-	-	-	-	-
MUFA (totals)	42.1	40.1	-	-	-	-	-	-
$n^{-6} + n^{-3}$PUFA	5.20	2.51	-	-	-	-	-	-
Totals	82.3	79.5	0.9973	0.99	0.05	-0.81	0.95	*
MUFA+PUFA	47.3	42.6	-	-	-	-	-	-
PUFA/SFA	0.15	0.07	-	-	-	-	-	-

r_{xy} = correlation coefficient; r_{xy2} = coefficient of determination; C_A = coefficient of alienation; R_{xy} = regression coefficient; IFE = index of forecasting efficiency; * = result significantly different at n^{-2} and r = 0.05.

of determination (r_{xy}^2) was also high showing that 99.0% of variance in the muscle (Y) was associated with the variance in the skin (X). The linear regression coefficient (R_{xy}) showed that for every unit increase in the skin fatty acid, there was a corresponding decrease of 0.81 in the fatty acid of the muscle. The coefficient of alienation (C_A) was low at 5.0% with a corresponding high value of index of forecasting efficiency (IFE) with a value of 95.0%. The IFE is actually a value of reduction in the error of prediction of relationship between the skin and muscle fatty acids; this meant that the error in the prediction of relationship was just 5.0%. The implication of this was that the skin fatty acids could carry out adequately the functions of the muscle fatty acids of Tongue sole.

Table 5 shows the values of fatty acids per 100 g of skin and muscle distribution in Tongue sole as food. The values in the skin were consistently higher than the corresponding values for the muscle; this was mainly due to the total fatty acids (calculated) which were more in the skin than in the muscle. This calculation accounted for 0.209 g/100 g or 79.5% in skin and 0.015 g/100 g or 75.5% in muscle, the balance being due to trace levels of other fatty acids.

Phospholipids. Table 6 shows the levels of various phospholipids in the samples. Phospholipids are not essential nutrients; they are just another lipid and, as such, contribute 9 kcalories per gram of energy. Cephalin (phosphatidylethanolamine, PE) was the second largest concentrated entity in muscle and in skin. PE is found

Table 5. Fatty acids level in the Tongue sole fish per 100 g skin and muscle samples as food

Fatty acid	Skin	Muscle	Mean	SD	CV %
C12:0	0.003	0.0002	0.0016	0.002	124
C14:0	0.012	0.001	0.0065	0.008	120
C16:0	0.051	0.004	0.028	0.033	119
C18:0	0.023	0.002	0.013	0.015	114
C16:1n^{-7}, *cis*	0.055	0.004	0.03	0.036	122
C18:1n^{-6}, *cis*	0.008	0.0003	0.004	0.005	131
C18:1n^{-9}, *cis*	0.016	0.001	0.009	0.011	125
C22:1n^{-9}, *cis*	-	-	-	-	-
C18:1n^{-6}, *trans*	0.003	0.0003	0.002	0.002	116
C18:1n^{-9}, *trans*	0.024	0.002	0.013	0.016	120
C18:2n^{-6}, *cis*	0.0002	0.00003	0.0001	0.0001	105
C18:2n^{-6}, *trans*	-	-	-	-	-
C18:3n^{-6}, *cis*	-	-	-	-	-
C20:2n^{-6}, *cis*	-	-	-	-	-
C20:4n^{-6}, *cis*	0.001	-	-	-	-
C22: 2n^{-6}, *cis*	0.10	-	-	-	-
C20:5n^{-3}, *cis*	-	-	-	-	-
C22:6n^{-3}, *cis*	0.003	0.0004	0.002	0.002	108
Totals	0.209	0.015	0.112	0.137	122
Difference	0.043(20.5%)	0.004(24.3%)	0.023	0.03	117

in all living cells, although in human physiology it is found particularly in nervous tissue such as the white matter of brain, nerves, neural tissue and in spinal cord (Adeyeye, 2011).

Phosphatidylserine (Ptd-L-Ser or PS) is a phospholipid usually kept on the inner-leaflet, the cytosolic side, of cell membranes by an enzyme called flippase. When a cell undergoes apoptotic cell death, PS is no longer restricted to the cytosolic part of the membrane, but becomes exposed on the surface of the cell. PS has been demonstrated to speed up recovery, prevent muscle soreness, improve well-being, and might possess ergogenic properties in athletes involved in cycling, weight training and endurance running. PS supplementation promotes a desirable hormonal balance for athletes and might attenuate the physiological deterioration that accompanies overtraining and/or overstretching (Starks *et al*., 2008). In recent studies, PS has been shown to enhance mood in a cohort of young people during mental stress and to improve accuracy during tee-off by increasing the stress resistance of golfers (Alter, 2006). The US Food and Drug Administration (USFDA) had stated that consumption of PS may reduce the risk of dementia and cognitive dysfunction in elder perons (Adeyeye, 2011). PS can be found in meat, but most abundant in the brain and innards such as liver and kidney. The present results recorded 6.33 mg/100 g in the skin, and 3.49 mg/100 g in the muscle which were lower then the value in beef (69) and pork (57); but both were also lower than the value in European pilchard (sardine) of 16.0 mg/100 g (Alter, 2006). Phosphatidylcholine (lecithin) is usually the most abundant phospholipid in animal and plants, often amounting to almost 50% of the total, and as such it is the key building block of membrane bilayers. This observation is true for lecithin value in the muscle (16.1 mg/100 g or 66.8%), and in the skin (12.7 mg/100 g or 46.7%). Phosphoinositides (P1, P2, P3) play important role in lipid signaling, cell signaling and membrane trafficking (Adeyeye, 2011). PI was of minor concentration in both samples. Partial hydrolysis of lecithin with removal of only one fatty acid yields a lysophosphatidylcholine (White *et al*., 1973). An example of alterations in enzymic activity related to association of a membrane -bound protein with lipid is that of phenylalanine hydroxylase, which catalyzes the conversion of phenylalanine to tyrosine. The activity of this enzyme, which is attached to the endoplasmic reticulum, is enhanced fifty fold in the presence of lysophosphstidylcholine, with which it is probably complexed in the hepatic cell (White *et al*., 1973). Lysophosphatidylcholine was of low level in both samples. Table 7 depicts the statistical analysis of the results from Table 6. Both r_{xy}, r_{xy}^2 and IFE were low. The R_{xy} was high and negative. The r_{xy} was significant at r = 0.05 and n^{-2} degrees of freedom.

Table 6. Phospholipids level (mg/100 g) of skin and muscle of Tongue sole fish

Phospholipids	Skin	Muscle	Mean	SD	CV %
Cephalin (PE)	8.23 (30.3)	4.48 (18.6)	6.36	2.65	41.7
Lecithin	12.7 (46.7)	16.1 (66.8)	14.4	2.40	16.7
Ptd-L-Ser (PS)	6.33 (23.3)	3.49 (14.5)	4.91	2.01	40.9
Lysophosphatidylcholine	0.06 (0.221)	0.007 (0.029)	0.034	0.04	112
PtdIns (PI)	0.002 (0.007)	0.011 (0.046)	0.007	0.006	97.9
Totals	27.2	24.1	25.7	2.19	8.55
Ratio	1.13:1				

PE = phosphatidylethanolamine; Lecithin = phosphatidylcholine; PS = phosphatidylserine; PI = phosphatidylinosotol; Values in parentheses are in percentages.

Table 7. Statistical analysis of the results from Table 6

Phospholipids	Skin(X)	Muscle(Y)	r_{xy}	r_{xy}^2	C_A	R_{xy}	IFE	Remark
PE	8.23	4.48	-	-	-	-	-	-
Lecithin	12.7	16.1	-	-	-	-	-	-
PS	6.33	3.49	0.9092	0.83	0.42	-1.19	0.58	*
Lysophosphatidylcholine	0.06	0.007	-	-	-	-	-	-
PI	0.002	0.011	-	-	-	-	-	-

Sterols. The sterol levels are shown in Table 8. The values in the cholesterol, cholestanol, stig-masterol and sitosterol range were close in both samples as: 6.86-6.94 mg/100 g (6.90 ± 0.04 mg/100 g) in skin. Cholestanol was not detected in the muscle. The skin predominantly had higher levels of all the sterols detected than in the muscle. On the whole the total sterol ratio in the skin to the muscle was 14.1:1 or 7.60 mg/100 g to 1.96 mg/100 g. This showed that the skin could be discarded to have lower sterol levels; however this might not be necessary since both samples contained high PUFA levels. The total dietary fats and oils range from 0.01-2% (Itoh *et al.*, 1973); the present levels were 0.360% in the skin and 0.027% in the muscle which were within the literature values. Stig-masterol shared first position in the two samples with respective values of 6.93 mg/100 g (skin) and 0.961 mg/100 g in the muscle. Stigmasterol is used as a precursor in the manufacture of synthetic progesterone, a valuable human hormone that plays an important physiological role in the regulatory and tissue rebuilding mechanisms related to estrogen effects, as well as acting as an intermediate in the biosynthesis of androgens, estrogens and corticoids. Research has indicated that stigmasterol may be useful in prevention of certain cancers, including ovarian, prostate, breast and colon cancers. Studies have also indicated that a diet high in phytosterols may inhibit the absorption of cholesterol and lower serum cholesterol levels by competing for intestinal absorption. Studies with laboratory animals fed stigmasterol found that both cholesterol and sitosterol absorption decreased 23% and 30% respectively over a 6 week period (Adeyeye, 2011). Stigmasterol is also known as Wulzen antistiffness factor. The levels of cholestanol in the skin and muscle could have come from cholesterol breakdown or to both cholesterol breakdown and liver transformation of cholestenone. Both cholestanol and sitosterol shared close positions in the skin, cholestanol was not detected in the muscle but sitosterol occupied the second position in the muscle. Results from Table 8 were analyzed statistically and shown in Table 9. The r_{xy}, r_{xy}^2 and IFE were high. The r_{xy}^2 showed that 75.0% variance in the muscle was related to the variance in the skin. R_{xy} was high and negative, C_A was high and the r_{xy} was lower than the critical value (table value) at $r_{=0.05}$ and n^{-2}, showing no significant difference existed in the samples.

Quality assurance. Table 10 shows the uncertainty interval percent (UIP) for the fatty acids. Most of the literature Table UIP levels were correspondingly higher than the present results in both skin and muscle. Also the correlation determined for all the standards: fatty acids, phospholipids and sterols, all had values ranging as follows: 0.99833-0.99997 (fatty acids), 0.99909-0.99999 (phospholipids) and 0.99920-0.99994 (sterols); all the correlation values were greater than 0.95 which is the critical correlation for acceptance of these types of analytical results. Both the correlation values and the UIP values attested to the quality assurance of the determinations.

Table 8. Sterols level (mg/100 g) of skin and muscle of Tongue sole fish

Sterols	Skin	Muscle	Mean	SD	CV %
Cholesterol	6.86 (24.9)	0.303 (15.5)	3.58	4.64	129
Cholestanol	6.87 (24.9)	-	-	-	-
Stigmasterol	6.93 (25.1)	0.961 (49.0)	3.95	4.22	107
Sitosterol	6.94 (25.1)	0.698 (35.6)	3.82	4.41	116
Totals	27.6	1.96	14.8	18.1	123
Ratio	14.1:1				

Table 9. Statistical analysis of the results from Table 8

Sterols	Skin (X)	Muscle (Y)	r_{xy}	r_{xy}^2	R_{xy}	CA	IFE	Remark
Cholesterol	6.86	0.303	-	-	-	-	-	-
Cholestanol	6.87	-	-	-	-	-	-	-
Stig-masterol	6.93	0.961	0.8662	0.75	-44.8	0.50	0.50	NS
Sitosterol	6.94	0.698	-	-	-	-	-	-

NS = results not significantly different at n^{-2} and r = 0.05.

Table 10. Uncertainty intervals as percent of analytical results

Fatty acid	UIP (table)	UIP (skin)	UIP (muscle)
C12:0	3.0	0.585	0.624
C14:0	2.8	6.87	5.80
C16:0	3.3	6.01	5.75
C18:0	4.2	2.94	2.87
C20:0	12	-	-
C16:1	3.2	3.76	4.27
C18:1	3.0	-	-
C18:1n^{-6}			
-cis	-	-	-
-trans	-	-	-
C18:1n^{-9}	10.2	-	-
-cis	-	0.152	0.125
- trans	-	0.102	0.102
C18:1n^{-11}	1.3	-	-
C20:1n^{-9}	-	-	-
C22:1	22.6	-	-
C22:1n^{-9}	-	-	-
C24:1	-	-	-
C18:2	6.6	-	-
-cis	-	-	-
-trans	-	-	-
C18:3	11.3	-	-
C18:3n^{-6}	-	6.44	4.97
C18:3n^{-3}	-	-	-
C20:4	9	-	-
C20:4n^{-6}	-	-	-
C22:2	3	-	-
C22:2n^{-6}	-	23.8	-
C22:6n^{-3}	-	-	-

UIP = uncertainty interval in percent.

Conclusion

The findings of this study showed that the samples contained unequal distribution of all the parameters determined. Both samples were high in n^{-6} fatty acids but low in n^{-3} fatty acids. Both samples had unsaturated acids as the predominant fatty acids. Significant difference occurred in the fatty acid levels. Both samples could serve as average source of lecithin but are much lower in sterols particularly in the muscle. Quality assurances of the determinations were highly satisfactory.

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Short Communication

Elemental Composition of Date Palm (*Phoenix dactylifera* L.) Using Energy Dispersive X-Rays Spectrometry

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Abstract. In the present study, date palm (*Phoenix dactylifera* L.) including fruit and pit (Mashkeel variety) was collected from a local market in Peshawar, Pakistan and analyzed using EDX spectrometry. The results showed the presence of various important elements such as O, C, K, Cl, Ca, S, Mg, Fe, Co and Si in date plam fruit and pit. Sufficient amount of oxygen (>54 wt.%) and carbon (>42 wt.%) were determined in the date palm, which is the evidence of the presence of nutritionally enriched constituents. Similarly, the mineral elements including Na, Ca, Mg, K, Ni, Si, Co, Fe and Mo were present in sufficient quantities.

Keywords: date palm, EDX spectrometry, elemental composition, essential nutrients, Pakistan

The date palm (*Phoenix dactylifera* L.) is usually grown in hot, arid and desert regions of the world. At present, more than 2000 different cultivars of date palm are known to exist all over the world, but only a few important ones have been evaluated for their agronomic performance and fruit quality. The fruit of date palm is composed of fleshy pericarp and seed and is well known as staple food. The fruit undergoes several changes during various stages of growth and development. Different varieties of dates growing in various countries have been studied for chemical composition and nutritional quality (Mohammadzai *et al.,* 2010a & b; Williams *et al.,* 2005; Al-Hooti *et al.,* 1997; Al-Showiman, 1990; Sawaya *et al.,* 1983).

The date is considered an important cash crop and a good source of foreign exchange earnings. Pakistan is the fourth largest dates producing country in the world which grows different varieties of dates. Total cultivated area of all types of dates in the country exceeds 78.1 thousand hectares, which produce over 630 thousand tones dates annually (Mohammadzai *et al.,* 2010a & b; Ihsanullah *et al.,* 2005). The effect of gamma irradiation, and colourless and coloured polyethylene packing on the quality and shelf life of Pakistani dates were studied by Mohammadzai *et al.* (2010a) and Ihsanullah *et al.* (2005). They found that irradiation caused minor losses in the tested parameters (moisture, proteins, fibres and fats) of controlled and irradiated packed samples. Similarly, Baloch and co-workers improved the quality of Dhakki dates of Pakistan during its various growing stages using the different physio-chemical processes (Saleem *et al.,* 2005; Baloch *et al.,* 2003). Recently, mineral composition of Pakistani dates was also reported using atomic absorption spectrophotometry (Mohammadzai *et al.,* 2010b). The Mashkeel variety of dates grown in Pakistan have not been subjected to elemental analysis using EDX spectrometry so far. The current study, therefore, was aimed at assessing the level of various elements present in the date palm fruit and pit.

Among various analytical techniques, EDX spectrometry is highly proficient for the elemental analyses of samples of diverse nature. The method is non-destructive and is more advantageous in multi-elementary analysis as compared to other existing methods, in the ease of sample preparation and analysis as it requires no chemical treatment or separation of the sample constituents. Although, this technique has been extensively used for elemental analyses of samples of biological and environmental importance (Khan *et al.,* 2009; Khan *et al.,* 2006), however, in the present study, this modern and rousted analytical technique has been applied probably for the first time, to determine the elemental composition of date palm fruit and pit.

Date palm (Mashkeel variety) was collected from a local market of Peshawar, Pakistan, in the same form as marketed. For each sample, ten date palm fruits and pits were collected in properly cleaned polyethylene

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bags in triplicate (Mohammadzai *et al.,* 2010 a & b) and brought to the laboratory. During the sampling processes and transportation and storage, all the precautionary measures were observed to avoid contamination. EDX spectrometry was performed using energy dispersive X-rays spectrometer (INCA-200, Oxford Instruments, UK). For this purpose, the dried powder samples were mounted on the sample stubs and coated with gold foil using gold coating machine (JSM-420, JEOL, Japan). The samples were then analyzed by EDX spectrometer (Khan *et al.,* 2009; Khan *et al.,* 2006). Each sample was analyzed in triplicate and reported as the mean ± S.D. (standard deviation) on dry weight basis, in wt.%.

The important elements identified in date palm fruit were O, C, K, Cl, Ca, S, Mg and Si (Fig. 1, Table 1) while O, C, K, Fe, Cl, Si Ca, S, and Co were found in date palm pit (Fig. 2, Table 1). The elements were also determined quantitatively. In the fruit, among all the investigated elements, O was in the largest quantity, followed by C. The rest of the elements were in lower concentrations (Table 1).

In the date palm pit, the amounts of O and C were the largest. Next were the concentrations of K, Fe, Cl, and Si while Ca, S, and Co were in traces. Thus both in the date palm fruit and pit, higher and comparable amounts of O and C were recorded (Table 1). However, the amount of K in the fruit samples was smaller than the pit samples while the levels of the Cl, Ca, and S were almost the same in both the samples. The concentration of Si in the pit samples was slightly higher than the fruit samples. Mg was found only in the fruit while Fe and Co were only in the pit samples. This means that date palm fruit and pit accumulate different quantities of some mineral elements such as K, Si, Mg, Fe and Co. Similar trend was reported in the previous study (Mohammadzai *et al.,* 2010b).

It is well-known that health depends upon the organized state of elements in the body and their imbalance causes disease (Khan *et al.,* 2009; Khan *et al.,* 2008). Due to the presence of higher contents of C and O, the date palm shows abundance of various nutritive constituents such as carbohydrates, proteins, fats, fibres, vitamins, etc. as reported previously (Mohammadzai *et al.,* 2010a; Ihsanullah *et al.,* 2005; Al-Hooti *et al.,* 1997). Hydrogen was not found in both samples, as this element cannot be detected with EDX spectrometry (Khan *et al.,* 2009; Khan *et al.,* 2006). Thus it is concluded that the date palm possesses good nutritional qualities. In addition, the presence of various macro-and microelements (K, Ca, Mg, Fe and Co) further increases its nutritional importance. The results are in good agreement with the previous findings (Mohammadzai *et al.,* 2010b; Al-Hooti *et al.,* 1997). It was also reported that the

Table 1. Elemental composition of date palm fruit and pit

Elements	Concentration (wt. %) in fruit	Concentration (wt. %) in pit
O	54.84±1.98	55.77±1.35
C	43.83±1.01	42.23±1.10
K	0.66±0.08	0.81±0.02
Fe	-	0.49±0.01
Cl	0.28±0.01	0.32±0.01
Ca	0.10±0.001	0.09±0.001
S	0.10±0.003	0.09±0.00
Mg	0.09±0.00	-
Si	0.09±0.00	0.13±0.005
Co	-	0.05±0.00

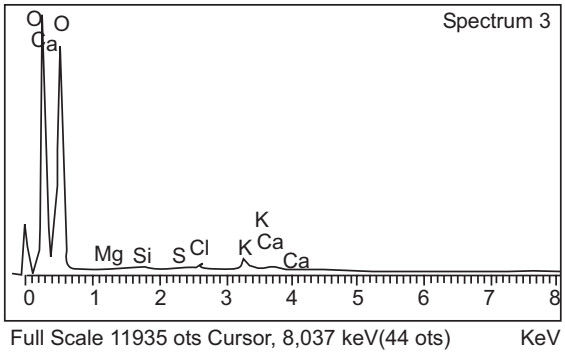

Fig. 1. A representative EDX spectrum of date palm fruit (Full scan).

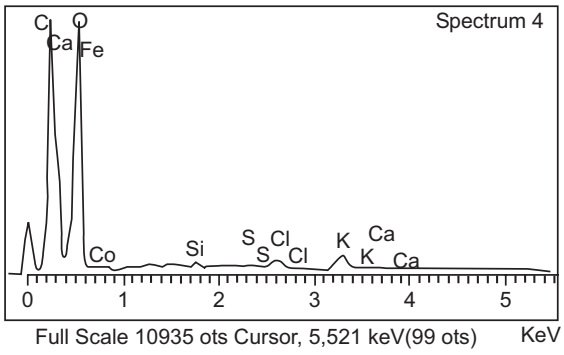

Fig. 2. A representative EDX spectrum of date palm pit (Full scan).

mineral contents of dates grown in the United Arab Emirates decreased progressively as the fruit matured (Al-Hooti *et al.,* 1997). Williams *et al.,* (2005) determined the levels of selected metals (Ag, Al, Ba, Be, Ga, La, Mo, Se, Si, Tl, and V) in the soil, fruit, and leaves of date palm (Fard cultivar) grown in the Sultanate of Oman. Similarly, Al-Showiman (1990) found highly significant amount of Ca, while K, Na, and Mg comes in to second place in the Saudi Arabian dates. Sawaya *et al.* (1983) have also reported a wide variation in the mineral contents of Saudi Arabian date cultivars. These studies show that the mineral contents of the date fruits may be influenced by the level of soil fertility and the amount of fertilizers applied to the trees. Comparison of the present results with the previous studies is quite complicated due to varietal differences and diverse ecological conditions.

The results showed that the date palm (Mashkeel variety) grown in Pakistan could be a good source of nutritionally important constituents and vital mineral elements. The current work is the first of its kind, which will provide useful data as baseline for further studies. These findings may be helpful for the concerned government parties and public sector regarding the nutritional potentials of Pakistani dates.

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Short Communication

Production and Characterization of Activated Carbon Using Indigenous Waste Materials

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Abstract. Activated carbon was produced from shisham wood and coconut shell through chemical activation, using phosphoric acid and low temperature carbonization. Proximate analysis and characterization of the product were carried out and Brunauer Emmett Teller (BET) surface area, total ash content, moisture content, pH value and iodine number were determined. The product characteristics were well comparable with those of the commercially available activated carbon.

Keywords: waste material, activated carbon, chemical activation, carbonization

Activated carbon can be produced from different raw carbon sources such as lignite, peat, coal, wood, sawdust, bagasse, and coconut shells. Earlier researchers utilized eucalyptus bark (Patnukao and Pavasant, 2008), flax shive (Marshall *et al*., 2007), date stone (Haimour and Emeish, 2006), hardwood (Lima *et al*., 2004), almond shell, pecan shell (Bansode *et al*., 2003) and coal (Jagtoyen *et al*., 1992) as precursor for production of activated carbon. Despite many related studies, there is little information available on the preparation of activated carbon using shisham wood as the precursor.

In principle, the methods for preparing activated carbon can be divided into two categories: physical activation and chemical activation (Narbaitz and Karimi-Jashni, 2009). In the physical activation, the raw material is first carbonized and then activated by steam or carbon dioxide, air or their mixture. The carbonization temperature ranges between 400 and 850 ºC, and the upper limit being sometimes 1000 ºC, whereas the activation temperature ranges between 600 and 900 ºC. In the chemical activation method, the raw material is impregnated with an activating agent and then heat-treated under inert atmosphere. The carbonization step and activation step are carried out simultaneously in the chemical activation process, with the precursor being mixed with chemical activating agents as dehydrating agents and oxidants (Moreno-Piraján *et al*., 2010). Investigations have been extensively conducted to elucidate the mechanism of phosphoric acid activation (Al-Qaessi and Abu-Farah, 2010; Lim *et al*., 2010). In the present study, activated carbon was produced from locally available waste materials i.e., coconut shell and shisham wood through chemical activation method. The product was characterized and characteristics were compared with the activated carbon commercially available in the market.

Initially, raw samples were washed with the hot water (50-60 ºC), dried in oven and then soaked in 30% phosphoric acid (H_3PO_4) overnight (Saleem *et al*., 2010; Masood-ur-Rehman, 2008). Later the samples were again dried in oven, poured in steel cylinders and placed in furnace for 45 min. The resulting material was cooled, washed with hot water to neutralize, dried in oven and stored in air-tight bottles for further characterization.

Characterization was carried out on the basis of volatile matter,fixed carbon, total ash content (ASTM D2866-94), moisture content (ASTM D4933-99), pH value (ASTM D3838-05) and iodine number (ASTM D4607-94). Surface area of the activated carbon was characterized by a physical technique involving nitrogen adsorption at 195.6 ºC, Brunauer Emmett Teller (BET) surface area.

Results of the proximate analysis of both the types of activated carbon are presented in Table 1, which show that properties of both are comparable with those supplied commercially at international level (Zakwan, 2010). Especially, low values of moisture content and volatile matter endorse the good quality of the produced activated carbon. The results of the product relating to BET surface area, Iodine number, moisture content, ash content and pH value and typical values of powdered activated carbon (PAC) and granular activated carbon (GAC) are also shown in Table 1 and are compared with the characteristics of activated carbon reported in

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Table 1. Characteristics and comparison of produced activated carbon (AC) with commercially available activated carbon and with typical values available in literature

Parameters	Proximate analysis (dry basis)			Detailed analysis			
	Shisham wood AC	Coconut shell AC	Commercial coconut AC	Shisham shell[a] wood AC	Coconut shell AC	Typical values[b] PAC	GAC
Volatile mater (%)	0.4	1.9	12.0	-	-	-	-
Fixed carbon (%)	88.4	82.8	75.0	-	-	-	-
Ash content (%)	7.1	8.7	3.0	-	-	≤ 6	≤ 8
Moisture content (%)	4.1	6.6	12.0	-	-	3-10	2-8
BET surface area (m^2/gm)	-	-	-	812	735	800-1800	700-1300
Iodine number (mg/g)	-	-	-	940.7	819.7	800-1200	600-1100
pH value	-	-	-	7.9	8.3	6 - 8	6 - 8

[a] = CV. Zakwan, 2010; [b] = specific values will depend on the source material used for the production of activated carbon (Metcalf and Eddy, 2003).

Table 2. Comparison of indigenous coconut shell GAC with commercial coconut shell AC

Parameters	Indigenous coconut shell AC	Commercial coconut shell AC[a]				
		QAC-400	QAC-600	QAC-800	QAC-1000	QAC-1200
BET surface area (m^2/g)	735	400	600	800	1000	1200
Iodine number (mg/g)	819.7	400	600	800	1000	1200
pH	8.3	9-10	9-10	9-10	9-10	9-10
Ash content (%)	8.7	6	6	5	5	5
Moisture content (%)	6.6	5	5	5	5	5

[a] = Quantum Activated Carbon Pvt Limited (manufacturer and exporters of activated carbon), New Delhi, India.

the literature. A detailed comparison of GAC produced from indigenous coconut shell is also made with the five commercially produced activated carbon in Table 2 (a commercial product of Quantum Activated Carbon Pvt. Limited, India).

BET surface areas of coconut shell and shisham wood was found to be 735 m^2/g and 812 m^2/g, respectively. The values are comparable with those reported in the literature. Activated carbon produced from shisham wood and coconut shell had ash content of 7.1% and 8.7%, respectively (Table 1), which are slightly higher than the typical values reported in the literature. This may be attributed to higher heating rate (i.e. 600 ºC) and impregnation ratio (i.e. 1:1.7), as depolymerization reactions between the volatile materials and phosphoric acid during the carbonization are affected. However, this parameter may be improved by lowering the heating rate and adjusting the impregnation ratio (Masood-ur-Rehman, 2008). The moisture content of shiham wood and coconut shell samples (i.e. 4.1% and 6.6%, respectively) are comparable with the typical values of PAC and GAC found in the literature. Furthermore, these values are also comparable with the PAC available in the market (Table 2). The final pH of the products was within the range of pH of commercially available activated carbon (i.e. 8.3 and 7.9). Similarly values of iodine number in the present study for coconut shell and shisham wood activated carbon were 819.7 mg/g and 940.7 mg/g, respectively, as compared to the typical values mentioned in Table 2 (600 to 1200 mg/g) and with the values of commercial grade activated carbon (400 to 1200 mg/g).

Thus both the products meet the typical values for powdered and granular activated carbon. Moreover, values of the indigenous coconut shell activated carbon compare well with the commercial coconut shell activated carbon available in the market. The results suggest that shisham wood is a suitable precursor for activated carbon production with higher BET surface area than that from the coconut shell. The conversion of shisham wood and coconut shell to activated carbons offers significant potential for reducing the cost and the environmental damage, resulting from uncontrolled disposal of these residues.

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Review

Morphological, Hydrolytic and Thermal Properties of Legume Starches

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Abstract. Legumes are an excellent source of carbohydrate and provide an inexpensive source of protein. With the exception of beach pea (12.3%), the percentage yields of extracted legume starches fall within the range of 18.0-45.0% on a whole seed basis. The total lipid contents of legume starches range from 0.01-0.87%. Legume starches have variable granule diameters, generally between 4 and 80 µm. Granule shape may be oval, spherical, elliptical or irregular, depending on the source. Legume starches exhibit a two-stage solubilization pattern; the rates of hydrolysis for the first and second stages are identical in some legume starches but differ in others. Most legume starches exhibit C-type X-ray diffraction patterns. The degrees of crystallinity of most legume starches are similar to, or slightly lower than, those of cereal starches. Most legume starches exhibit nearly identical gelatinization transition temperatures and enthalpies. However, their gelatinization temperature ranges ($T_C - T_O$) differ. Legume starches easily retrograde due to their relatively high amylose contents, although long term retrogradation is attributed to short chains of amylopectin.

Keywords: legume, starch, granule morphology, hydrolysis, gelatinization, retrogradation, thermal properties, granule crystallinity

Introduction

The legume fruit is formed from a single carpel, which splits along the dorsal and the ventral sutures, and usually contains a row of seeds borne on the inner side of the ventral suture. Grain legumes are dicotyledonous seeds of plants that belong to the family Leguminosae having 16,000-19,000 species in approximately 750 genera (Allen and Allen, 1981). They rank fifth in terms of annual world grain production (171 million metric tons) after wheat, rice, corn and barley (FAO, 2003; Deshpande and Damodaran,1990). Approximately 12 species of the Leguminosae, which is the third largest family of flowering plants, are widely used as food (Chavan *et al*., 1999). Examples include lima bean, garbanzo bean, lentil bean, mung bean, pinto bean, adzuki bean, red kidney bean, smooth pea, wrinkled pea, and the two oilseed legumes, soybean and groundnut. The food legumes are rich in starch, protein, dietary fibre, minerals and water-soluble vitamins. Legumes constitute an important source of carbohydrates for a large part of human population, mainly in the developing world. India is the largest producer and consumer of legumes in the world (Singh et al.,2008). The total carbohydrate contents of food legumes vary from 24% (winged bean) to 68% (cowpea) (Ratnayake *et al.*, 2001). Starch is the most abundant carbohydrate in the seed (22-45%; Hoover and Sosulski, 1991). Legumes are used as food and feed (Leon *et al*., 1991) as the seed is a good source of both starch and protein, 36.7-50% (Leon *et al*., 1989; Duke, 1981) and 29.7% (Clemente *et al.*, 2000; Menkov, 2000; Kessler, 1985), respectively. But lentil seeds contain more protein than other legume seeds, the protein content ranges from 24.3% to 30.2% for different cultivars (Wang and Daun, 2006). Variations in the values for the starch and protein contents of legumes reported in the literature may be attributed, in part, to differences in the methods of analyses.

A major factor which has an adverse effect on the widespread utilization of legume starches in the food industry is their relatively high amylose contents (Hoover and Sosulski, 1985). The association between amylose molecules and the outer branches of amylopectin in

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cooked starch pastes leads to extensive retrogradation, which results in cloudiness and syneresis, especially when legume starch gels are subjected to repeated freeze-thaw cycles (Hoover *et al*., 1988). The degree of syneresis seen in native legume starch gels would be unacceptable in most food products (Hoover *et al*., 1988). The physico-chemical properties and functional characteristics that are imparted by starches to aqueous systems and their uniqueness in various food applications vary with the biological origin (Svegmark and Hermansson, 1993). Starches contribute greatly to the textural properties of many foods and have many industrial applications as thickeners, colloidal stabilizers, gelling agents, bulking agents, water retention agents and adhesives (Singh *et al*., 2003). Methods used in the chemical analysis of legume starches are applicable to starches from other botanical sources.

Legume starches are usually extracted from the source using a procedure similar to that of Hoover and Sosulski (1985). Quantitative estimations of moisture, ash, nitrogen and damaged starch are performed by standard American Association of Cereal Chemists International (AACCI, 1984) or Association of Official Analytical Chemists International (AOACI, 1990) methods. Many methods of characterizing starch have been developed which could be used for screening large number of genotypes for unique properties (Kim *et al*., 1995). A large number of techniques, such as differential scanning calorimetry (DSC) (Donovan, 1979), X-ray diffraction (Zobel *et al*., 1988), small angle neutron scattering (Jenkins, 1994) and Kofler hot stage microscopy (Watson, 1964), have been used to study the gelatinization behavior of starches. Additionally, DSC is well suited to investigate the phase transitions of starch/water systems, for it allows the study of starch gelatinization over a wide range of starch/water ratios, determination of gelatinization temperatures above 100°C and estimation of transition enthalpies (Biliaderis *et al*., 1980). DSC has been used to study starch phase transitions from a physico-chemical approach (Donovan, 1979; Marchant and Blanshard, 1978; Lelievre, 1973). Biliaderis *et al*. (1980) studied legume starches specifically using DSC. Polarizing light microscopy had been used to determine the size, shape, and position of the hilum of common starches (McCrone and Delly, 1973; Schoch and Maywald, 1967; Reichert, 1913). The scanning electron microscope (SEM) is superior to the polarizing light microscope for the study of starch granule morphology. Advantages of SEM include a greater depth of focus and much higher resolution and magnification (Jane *et al*., 1994). SEM has been used to relate paste structures to paste properties (Fannon and BeMiller, 1992; Fannon *et al*., 1992a) and also to relate granule morphology to starch genotype (Fannon *et al*., 1992b). Many other studies involving scanning electron microscopy of starch granules have been reported in the literature (Fannon *et al*., 1990; Fitt and Snyder, 1984; Banks and Greenwood, 1975; Schoch and Maywald, 1967).

Wide angle X-ray diffraction has been applied to the study of legume starches (Davydova *et al*., 1995; Gernat *et al*., 1990; Hoover and Sosulski, 1985; Colonna *et al*., 1982). From DSC and X-ray studies, Bogracheva *et al*. (1998) were able to deduce a relationship between the 'A' and 'B' polymorphs of gelatinized legume starches. Identical methods were utilized for the calculation of the composition of 'A' and 'B' polymorphs of legume starches (Davydova *et al*., 1995). Other X-ray diffraction studies of starches have shown the dependence of starch crystallinity on amylose content, average chain length (CL) of amylopectin and the mole percentage of short chain fractions of amylopectin (Cheetham and Tao, 1997). The pasting and viscometric properties of starches have been studied with the Brabender visco-amylograph, the rapid visco-analyzer (RVA) and rotational viscometers (Wiesenborn *et al*., 1994).

Many researchers have used the dynamic rheometer for studying the viscoelastic or rheological properties of starches (Hsu *et al*., 2000; Tsai *et al*., 1997; Lii *et al*., 1996). Laser light scattering has been used to characterize granule diameter based on the assumption that granules are spherical (Wiesenborn *et al*., 1994). Unlike wide angle X-ray scattering (WAXS) which quantifies crystalline order throughout starch granules, small angle X-ray scattering (SAXS) quantifies differences (periodicity) at the level of amorphous-crystalline lamellae radiating from the hilum to the periphery of starch granules (Tester *et al*., 2000). More detailed discussion regarding the application of this technique to the investigation of structural, gelatinization and hydrothermal mechanisms of starches can be found in the literature.

In this review, information is presented on the yield, composition, swelling, morphological and thermal characteristics of legume starches.

Yield, composition and crystallinity. Data on the yield and composition of legume starches is presented in Table 1. The purity of legume starches has been judged

on the basis of composition and microscopic observation. The low nitrogen and ash contents and the absence of any adhering protein is related to the purity of the starches. With the exception of beach pea (12.3%; Chavan *et al*., 1999), the yields fell within the range (18-45%) reported by Hoover and Sosulski (1991) for most legume starches. The yield of great northern bean starch has been reported to be 18.2% (Sathe and Salunkhe, 1981). Naivikul and D'Appolonia (1979) reported yields of 40.3, 38.3, 39.9, 42.5 and 34.5% for navy bean, pinto bean, faba bean, lentil, and mung bean starches, respectively. Lineback and Ke (1975) obtained a starch yield of 37% from horse bean flour. Schoch and Maywald (1968) reported starch yields of 27, 38, and 37% from navy bean, lentil and mung bean, respectively. Differences in legume starch yield can be attributed, in part, to differences in the method of isolation. Difficulties in the isolation of starches from legumes have been attributed to the presence of a highly hydrated fine fiber fraction (Vose, 1977) which is derived from the cell wall enclosing the starch granules (Schoch and Maywald, 1968).Recently,the importance of lentil starches were re-emphasised and this had led to studies been conducted on them (Chung *et al.,* 2009; Chung *et al.,* 2008a; Lee *et al.,*2007).

Starch is one of the most abundant organic chemicals in the world. It is synthesized in the form of granules within cellular organelles (amyloplasts) and also found in the leaves of green plants in the plastids. The major polysaccharide of legume plants is starch. Starch consists of two polymers within its granules: amylose and amylopectin. Debranching (Takeda *et al*., 1992) and chain length (CL) studies (Shibanuma *et al.,* 1994) on amylose have shown that the α-(1-4)-linked glucose polymer is actually a mixture of linear and randomly limited branched polymers.

In contrast, amylopectin is a branched polymer with one of the highest molecular weights known among naturally occurring polymers (Abd Karim *et al*., 2000). Starch is semi-crystalline in nature with varying levels of crystallinity (Singh *et al*., 2003). The crystallinity is solely associated with the amylopectin component, while the amorphous regions mainly represent amylose (Zobel, 1988a, 1988b). Crystalline lamellae are made up of amylopectin double helices, which are packed in a parallel fashion, whereas the amylopectin branch points are in the amorphous zones (Jacobs and Delcour, 1998). Using SAXS and neutron scattering, a periodicity of 9 – 11 nm has been found for starches from various botanical sources (Jenkins *et al*., 1993; Cameron and Donald, 1992; Oostergetel and Van Bruggen, 1989; Blanshard *et al*., 1984;Muhr *et al*., 1984; Sterling, 1962). Kassenbeck (1978) and Yamaguchi *et al*. (1979) attributed the periodicity to the repeat distances of crystalline and amorphous lamellae.

The amylose content of starch varies with the botanical source (Table 1) and is affected by the climatic conditions and soil type during growth (Morrison and Azudin, 1987; Asaoka *et al*., 1985; Morrison *et al*., 1984; Inatsu *et al*., 1974; Juliano *et al*., 1964). Apart from Table 1, recent studies show that apparent amylose content of field pea,kidney bean,chickpea,blackgram,pigeon pea and mung bean starches to be 39.9%, 36.0%, 34.4-35.5%, 32.9-35.6%, 38.0-41.5% and 31.7-33.8% respectively (Chung *et al.,* 2008b; Nishinari,2008; Kim *et al*.,2007;Tan *et al*.,2006). In contrast,the apparent amylose concentration of chickpea starches varies from 28% to 40% (Hughes *et al.,* 2009; Singh *et al.,* 2004) and that of smooth pea and wrinkled pea starches vary from 30-40% and 60-76%, respectively (Ratnayake *et al.,* 2002). Limited co-crystallization between amylose and amylopectin has been suggested by Blanshard (1987) and Jenkins and Donald (1995). WAXS has revealed three forms of packing of amylopectin double helices, A, B, and C crystal types, and the features of starch ^{13}C CP/MAS (solid state ^{13}C cross polarization/magic angle spinning) spectra are consistent with starch being a combination of amorphous (single chain) and ordered (double-helix components) material (Gidley and Bociek, 1985). Legume starches exhibit the typical mixed-state pattern 'C'. Many studies tend to show that the 'C-type' pattern is characteristic of all legume starches. Beach pea, green pea and grass pea starches (Hoover *et al*., 1997; Hoover and Manuel, 1996; Gernat *et al*., 1990; Hoover and Sosulski, 1985; Colonna *et al*., 1981), field pea starches (Davydova *et al*., 1995; Gernat *et al*., 1990; Hoover and Sosulski, 1985; Colonna *et al*., 1982), mung bean starches (Tan *et al.,* 2009), lentil starches (Sodhi *et al.,* 2009), chickpea starches (Polesi *et al.,* 2011) and black gram starches (Singh *et al.,* 2004) all showed the characteristic 'C-type' pattern of legume starches. However, these X-ray patterns of legume starches were characterized by different intensities. In general, most legume starches exhibit C-type X-ray diffraction patterns characterized by two very distinct intensity lines at 17.2 and 18.1° (2□) angles (Table 2). The differences in X-ray intensities were attributed to the manner in which the double helices

are arranged within the crystalline domains of the granule (Chavan *et al*., 1999). According to Gernat *et al*. (1990), the legume starch 'C' crystalline polymorph is a mixture of 'A' and 'B' unit cells, and that these starches contain pure 'A' and 'B' polymorphs in varying proportions. Both 'A' and 'B' type starches are based on parallel stranded double helices, in which the helices are closely packed in the 'A' type starch but loosely packed in the 'B' type starch (Ratnayake *et al*., 2001). Bogracheva *et al*. (1998) reported from studies of gelatinized pea starch that the 'A' and 'B' polymorphs are present in the same granule and that the 'B' polymorph is situated in the centre of all granules surrounded by the 'A' polymorph.

Table 2 shows some of the X-ray diffraction behaviours of some legume and other starches. Legume starches generally have higher amylose content than non-legume starches (Hoover and Manuel, 1995; Gernat *et al.*, 1990; Hoover and Sosulski, 1985; Colonna *et al.*, 1981). The degrees of crystallinity of most legume starches, such as broad bean (18.5%), smooth pea (18.9%) and wrinkled pea (15.4-16.0%) are similar to, or slightly lower than, those of cereal starches (Table 2). The degrees of crystallinity of wheat, maize, waxy maize and amylomaize starches are 19.5, 21.8, 31.0 and 17.0%, respectively (Gernat *et al.*,1993; Gernat *et al.*, 1990).

Navy bean, pinto bean, faba bean, lentil and mung bean starch granules exhibited similar birefringence characteristics in polarized-light photomicrographs (Naivikal and D' Appolonia, 1979).

Starch paste behavior in aqueous systems depends on the chemical and physical characteristics of the starch granules, such as mean granule size, granule size distribution, amylose/amylopectin ratio and mineral content (Madsen and Christensen, 1996). Morrison *et al.* (1993a,b) reported the presence in starches of two amorphous forms of amylose, namely lipid-free amylose and lipid-complexed amylose. Both forms of amylose are found in legume starches. The amylose content of native legume starches (Table 1) is generally higher than that of unmodified cereal and tuber starches. This association of legume starches with high amylose content explains their higher degree of retrogradation and syneresis compared to either cereal or tuber starches. Ratnayake *et al*. (2001) reported the amylose contents of four cultivars of field pea (*Pisum sativum* L.) to be in the range of 48.8 – 49.6% (Table 1). These values were much higher than those reported by Chavan *et al*. (1999) for beach pea (29.0%), green pea (36.7%), grass pea (36.0%) starches (Table 1), and lower than those of smooth pea (52.6-57.0%) and wrinkled pea (94.0%) (Czuchajowska *et al*., 1998), but comparable to that of mung bean starch (45.3%; Hoover *et al*., 1997) (a different sample than that described in Table 1).More contradicting is that amylose content of 40.69% was proposed for mung bean starch (MBS) by Thao and Noomhorm (2011) while Li and Gao (2010) indicated a different value of 27.73% for MBS. Biliaderis *et al.* (1980) reported the amylose content of MBS to be

Table1. Chemical composition of some legume starches.

Legume	Phosphorus	Amylose	Fat	Yield	Ash	Nitrogen
			(%)			
Adzuki bean	0.013[a]	34.9[a]	0.60[h]	21.5[i]	-	-
Smooth pea	0.006[a]	33.1[a]	-	-	-	-
Garbanzo bean	0.010[a]	34.1[a]	0.11-0.12[g]	38.0-40.0[j]	0.042-0.053[j]	0.044-0.047[j]
Red kidney bean	0.016[a]	35.0[a]	-	-	-	-
Lentil	0.008[a]	45.5[a]	0.27-0.38[f]	42.5[i]	0.054-0.060[j]	0.031[j]
Navy bean	0.011[a]	36.0[a]	0.11[g]	40.3[i]	0.051[j]	0.041-0.046
Mung bean	0.016[a]	34.9[a]	0.32[e]	34.5[i]	-	-
Faba bean	0.010[a]	32.5[a]	-	39.9[i]	-	-
Lima bean	-	32.6[b]	0.54[b]	23.0-30.0[j]	0.14[b]	0.036-0.070
Field pea	-	48.8-49.6[c]	0.28-0.34[c]	32.7-33.5[c]	0.03-0.14[c]	0.04-0.07[c]
Beach pea	-	29.02[d]	0.16[d]	12.3[d]	0.22[d]	0.08[d]
Green pea	-	36.70[d]	0.19[d]	30.0[d]	0.07[d]	0.09[d]
Grass pea	-	36.37[d]	0.12[d]	26.0[d]	0.05[d]	0.07[d]

[a] Biliaderis *et al*., 1980; [b] Betancur-Ancona *et al*., 2003; [c] Ratnayake *et al*., 2001; [d] Chavan *et al*., 1999; [e] Hoover *et al*., 1997; [f] Hoover and Manuel, 1995; [g] Hoover *et al*., 1988; [h] Tjahjadi and Breene, 1984; [i] Naivikul and D'Appolonia, 1979; [j] Schoch and May wald, 1968.

34.9% (Table 1). Tjahjadi and Breene (1984) reported a rather low value of 28.8% for the amylose content of adzuki bean. Other researchers (Biliaderis *et al*., 1980; 1979) reported a value of 34.9% for the amylose content of adzuki bean. The different values for the amylose content of the same starch (e.g. mung bean starch and adzuki bean starch) show that the literature is replete with conflicting information with respect to the amylose contents of legume starches. The reported variations in amylose content have been attributed to differences in variety and to the use of different starch isolation procedures and to different methods of analysis, i.e., colorimetry versus potentiometry (Kim *et al.*, 1995; Naivikul and D'Appolonia, 1979). The activity of the enzymes involved in starch biosynthesis may also be responsible for the variations in amylose content among starches (Krossmann and Lloyd, 2000).

Minor constituents commonly found in starch include lipids, proteins, phosphorus and other minerals (Ca, K, Mg and Zn) (Ellis *et al*., 1998). Although the proportion of amylose and amylopectin and their properties are paramount in determining the characteristics of the starch, minor constituents of the starch granule seem to affect the properties relevant to its use in food and non-food applications. These minor constituents are materials that are associated with the surface of the granule or are true internal components.Protein has been classified as either surface protein or integral protein, and its association with starch granules varies in amount between and within species (Ellis *et al*., 1998). One of the granule surface proteins, friablin, has been linked with kernel hardness in wheat (Anjum and Walker, 1991). Lipids associated with legume starch granules have been found to occur on the surface, as well as inside the granule (Morrison, 1981). The surface lipids are principally triglycerides, but also include free fatty acids, glycolipids and phospholipids (Vasanthan and Hoover, 1992; Galliard and Bowler, 1987; Morrison, 1981). Vasanthan and Hoover (1992), Morrison (1981) and Hargin and Morrison (1980) each reported that the internal lipids were predominantly monoacyl lipids with the major components being lysophospholipids and free fatty acids. The total lipid contents of legume starches were found to be in the range of 0.01-0.87% (Hoover and Sosulski, 1991). All of the values shown in Table 1 for the total lipid contents of different legume starches fall within the stated range. Morrison (1981) and Mikus *et al*. (1946) postulated that starch lipids may be present in the free state or bound to starch components, either linked via ionic or hydrogen bonding to hydroxyl groups of the starch components or in the form of amylose inclusion complexes in which the ligand resides within the central hydrophobic core of the helix. Total lipid contents of 0.28-0.34% have been ascribed to field pea starches (Table 1; Ratnayake *et al*., 2001) and this is similar to the range reported for mung bean (0.32%; Hoover *et al*., 1997), and lentil (0.27-0.38%; Hoover and Manuel, 1995) starches, but higher than that reported for beach pea (0.16%), green pea (0.19%) grass pea (0.12%) starches (Chavan *et al*., 1999) and chickpea (0.01%) starches (Hoover and Ratnayake,2002). The discrepancies in the lipid contents of the legume starches described above may be due to various reasons. Many researchers (Kawano *et al*., 1989; Goshima *et al*., 1985; Maningat and Juliano, 1980; Melvin, 1979; Lorenz, 1976; Goering *et al*., 1975; Medcalf *et al*., 1968) have used different lipid extractants, which differ in their ability to extract firmly bound lipids (Vasanthan and Hoover, 1992; Morrison and Coventry, 1985; Morrison, 1981). Because of the utilization of different solvents for the extraction of total lipids (both surface and internal) from legume starches, it is difficult to compare results from different published data. Additionally, some solvent systems utilized have proved ineffective in the removal of internal starch lipids. The same is true for bound lipids, especially those complexed with amylose. Controversy still exists with regard to lipid binding ability to the short linear (15-20 glucose units) portions of the outer branches of amylopectin (Eliasson and Ljunger, 1988a; Gidley and Bociek, 1988; Biliaderis and Vaughan, 1987; Hahn and Hood, 1987; Evans, 1986; Destefanis *et al*., 1977; Goering *et al*., 1975; Krog, 1971; Lagendijk and Pennings, 1970). Internal lipid content increases with amylose content, and unless the granule integrity is disrupted, the lipids remain inaccessible to normal fat solvents, suggesting that they are present as an amylose inclusion complex. There is limited information in the literature on minor constituents of legume starches as compared to what is available regarding cereal and tuber starches.

Swelling, solubility and hydrolysis. Starch granule swelling is known to begin in the bulk, relatively mobile, amorphous fraction and in the more restrained amorphous regions immediately adjacent to the crystalline region (Donovan, 1979). Leach *et al*. (1959) postulated that the bonding forces within the starch granule influence the extent of swelling. Thus, highly associated starch granules should be relatively resistant to swelling and amylose

leaching (Vasanthan and Hoover, 1992). Furthermore, the swelling factor (SF) has been shown to be influenced by amylose-lipid complexes (Hoover and Manuel, 1995, 1996; Tester *et al*., 1993). The swelling power and amylose leaching (AML) of pea starches increased with increasing temperature (Table 3). Chavan *et al*. (1999) and Ratnayake *et al*. (2001) investigated the SF and AML of pea starches over the temperature range of 50-95°C. Ratnayake *et al*. (2001) found no significant differences in the SF of starches from four cultivars (Curneval, Carrera, Grande and Keoma) of field pea (*Pisum sativum* L.) (Table 3).

These authors showed that the SFs (at 95°C) of field pea starches were lower than those reported for beach pea (30.72), green pea (34.1) (Chavan *et al*., 1999), mung bean (43.6) and gold lentil (31.0) starches, but was comparable to that of Laird lentil starch (26.0) (Hoover and Manuel, 1995). They also reported that the AML values of field pea cultivars at 95°C (Table 4) were much higher than those reported by (Chavan *et al*., 1999) for beach pea (12.94), green pea (17.08), grass pea (19.07), but lower than those reported for gold lentil (35.5) and Laird lentil (38.5) starches (Hoover and Manuel, 1995).

In all four starches studied by Ratnayake *et al*. (2001), SF and AML increased dramatically between 60 and 85°C (Table 4); thereafter, the increase were gradual. An identical trend has been observed for other legume starches (Chavan *et al*., 1999; Hoover and Manuel,

Table 2. X-ray diffraction patterns of some legume and other starches

Starch source	Moisture content (%)		Diffraction angle (□)				Source
Maize starch (Type A)	N.A.	23.0^{vs}	18.0^{m}	17.2^{m}	15.1^{s}		(Gernat *et al.*, 1990)
Potato starch (Type B)	N.A.	24.0^{m}	22.0^{m}	17.2^{vs}	13.5^{m}	5.4^{vs}	(Gernat *et al.*, 1990)
Kidney bean	10.3	23.0^{s}	21.4^{w}	17.8^{vs}	17.2^{vs}		(Hoover and Sosulski, 1985)
Northern bean	10.5	22.9^{s}	-	17.2^{vs}	15.3^{m}		(Hoover and Sosulski, 1985)
Pinto bean	10.4	23.0^{vs}	18.0^{vs}	17.2^{vs}	15.1^{m}		(Hoover and Sosulski, 1985)
Navy bean	10.7	22.9^{s}	17.8^{vs}	17.2^{vs}	15.3^{w}		(Hoover and Sosulski, 1985)
Black bean	10.4	23.1^{s}	17.8^{vs}	17.2^{vs}	15.3^{w}		(Hoover and Sosulski, 1985)
Broad bean	14.0	24.0^{s}	-	17.0^{vs}	15.1^{m}		(Colonna *et al.*, 1981)
Smooth pea	15.9	23.0^{w}	-	17.0^{vs}	15.1^{m}	5.5^{m}	(Colonna *et al.*, 1981)
Lentil	9.7	23.0^{m}	-	17.2^{m}	13.4^{w}		(Hoover and Sosulski, 1986; Hoover and Manuel, 1995)
Field pea	9.8	23.0^{s}	-	17.2^{s}	13.4^{w}		(Hoover and Sosulski, 1986)
Adzuki bean	N.A.	24.0^{s}	18.0^{vs}	17.0^{vs}	15.0^{s}		(Biliaderis *et al*., 1981)
Wrinkled pea	N.A.	24.0^{m}	20.0^{m}	17.0^{vs}	15.0^{m}	5.5^{m}	(Biliaderis *et al*., 1981)

^{vs} = very strong intensity; ^{s} = strong intensity; ^{m} = medium intensity; and ^{w} = weak intensity.

Table 3. Swelling factors (SF) and amylose leaching (AML) values for beach pea, green pea and grass pea starches at different temperatures[1,2] (Chavan *et al.*, 1999)

Temp.	SF			AML		
(°C)	Beach pea	Green pea	Grass pea	Beach pea	Green pea	Grass pea
50	7.33± 0.17^{a}	7.52 ± 1.01^{a}	1.42±0.14^{b}	*	*	*
60	8.55± 0.05^{a}	8.94 ± 1.08^{a}	1.56±0.14^{b}	*	*	*
70	16.73±0.18^{a}	17.9± 1.06^{a}	10.02±0.08^{b}	3.43 ± 0.03^{b}	6.16± 1.09^{a}	6.25±0.09^{a}
80	18.43±0.10^{b}	21.11±1.07^{a}	13.03±0.07^{c}	7.54 ± 0.08^{b}	14.33±1.03^{a}	15.07±0.54^{a}
85	19.61±0.13^{b}	22.41±1.03^{a}	14.90±0.07^{c}	9.84 ± 0.29^{b}	15.08±1.10^{a}	15.66±0.53^{a}
90	24.92±0.21^{b}	28.01±0.37^{a}	19.58±0.13^{c}	11.55± 0.52^{b}	16.69±1.02^{a}	17.68±0.10^{a}
95	30.72±0.82^{b}	34.13±0.24^{a}	26.01±0.07^{c}	12.94± 0.18^{b}	17.08±1.59^{a}	19.07±0.13^{a}

[1] = The data represent means of four determinations ± SD. Means in each row with different superscripts are significantly different ($p<0.05$); [2] = Swelling factor is ratio of volumes of wet to dry granules; * = Amylose leaching was not observed at these temperatures.

Table 4. Swelling factors (SF) and amylose leaching (AML) values for field pea starches at different temperatures (Ratnayake *et al.*, 2001)

Starch source	Temperature (°C)						
	50	60	70	80	85	90	95
Carneval SF	4.2 ± 0.21	8.5 ± 0.25	13.7 ± 0.16	19.4 ± 0.11	24.3 ± 0.04	26.5 ± 0.03	26.7 ± 0.21
AML	0.0 ± 0.00	10.5 ± 0.23^{q}	16.3 ± 0.17^{q,r}	19.6 ± 0.12^{q}	25.1 ± 0.03	26.3 ± 0.22^{q}	26.6 ± 0.16^{q,r}
Carrera SF	4.2 ± 0.22	8.6 ± 0.21	13.8 ± 0.22^{p}	19.4 ± 0.05	24.2 ± 0.05	26.4 ± 0.21	26.7 ± 0.24
AML	0.0 ± 0.00	10.1 ± 0.22^{p}	15.1 ± 0.12^{p}	18.1 ± 0.25^{p}	24.8 ± 0.24^{p}	25.1 ± 0.19^{p}	25.2 ± 0.10^{p}
Grande SF	4.1 ± 0.21	8.4 ± 0.22	13.8 ± 0.11	19.4 ± 0.10	24.2 ± 0.05	26.5 ± 0.16	26.7 ± 0.23
AML	0.0 ± 0.00	10.5 ± 0.23^{q}	16.0 ± 0.10^{q}	20.2 ± 0.08^{r}	25.7 ± 0.10^{r}	26.0 ± 0.12^{q}	26.2 ± 0.09^{q}
Keoma SF	4.1 ± 0.18	8.4 ± 0.22	13.3 ± 0.11	19.2 ± 0.20	24.1 ± 0.20	26.4 ± 0.20	26.5 ± 0.05
AML	0.0 ± 0.00	10.7 ± 0.15^{q}	16.6 ± 0.20^{r}	20.3 ± 0.06^{r}	25.5 ± 0.16^{r}	26.6 ± 0.20^{r}	26.8 ± 0.02^{r}

Values for AML followed by the same letter, in the same column, are not significantly different (P<0.05) by Tukey's HSD test. No significant differences (P<0.05) were observed among values for SF within the same column by Tukey's HSD test (Ratnayak*e et al.*, 2001).

1995; Hoover and Sosulski, 1985; Tolmasquim *et al*., 1971; Schoch and Maywald, 1968). Ratnayake *et al*., (2001) suggested that the rapid increases in SF and AML of four cultivars of field pea starches, between 60 and 85°C (Table 4), were probably due to an increase in molecular mobility of the amorphous region, which causes unraveling and melting of the double helices present within the amorphous and crystalline domains. Other authors (Chavan *et al*., 1999) suggested that SF was determined by interactions between amylose chains within the amorphous domains of the granule and suggested that AML in these legume starches (beach pea, green pea and grass pea) (Table 3) is influenced by the interplay between differences in amylose content and bound lipid content and by the magnitude of the interactions between amylose chains within the native granule. When starch molecules are heated in excess water, the crystalline structure is disrupted and water molecules become linked by hydrogen bonding to the exposed hydroxyl groups of amylose and amylopectin, which causes an increase in granule swelling and solubility (Singh *et al*., 2003). The presence of lipids in starch may have a reducing effect on the swelling of the individual granules (Galliard and Bowler, 1987). Swelling power and solubility provide evidence of the magnitude of the interactions between starch chains within the amorphous and crystalline domains. The extent of this interaction is influenced by the amylose to amylopectin ratio and by the characteristics of amylose and amylopectin in terms of molecular weight distribution, degree and length of branching, and conformation (Hoover, 2001). Differences in the swelling and solubility behaviour of starches from different botanical sources, and of starches from different cultivars of a botanical source, are caused by differences in amylose and lipid content, as well as in granule organization (Singh *et al*., 2003).

Many researchers (Chavan *et al*., 2010; Kevate *et al*., 2010; Chavan *et al*., 2009; Ratnayake *et al*., 2001; Chavan *et al*., 1999; Biliaderis *et al*., 1980) hydrolysed legume starches (smooth pea, wrinkled pea, adzuki bean, mung bean, red kidney bean, green lentil, field pea, beach pea, green pea, grass pea,moth bean,rice bean and horse gram) with 2.2N HCl and found that they all exhibited a two-stage solubilization pattern. The same two-stage hydrolysis pattern has also been reported for corn, waxy corn, wheat, potato and rice starches (Robin *et al*., 1974, 1975). These authors reported a relatively fast rate of hydrolysis during the first eight or ten days, followed by a slower rate between ten and twenty days. The faster first stage corresponds to the hydrolysis or degradation of the more amorphous parts of the starch granule (Cairns *et al*., 1990; Biliaderis *et al*., 1981; Kainuma and French, 1971). The slow degradation during the second stage has been attributed to the erosion of the crystalline material (Robin *et al*., 1974; Kainuma and French, 1971). Despite the two-stage solubilization pattern, the rates of hydrolysis for the first and second stages were identical in some legume starches but differed in others. Ratnayake *et al*. (2001) investigated the acid hydrolysis of starch from four cultivars (Carneval, Carrera, Grande and Keoma) of field pea. They observed no significant differences in the extent of hydrolysis among the starches during the first eigt days and further showed that at the end of 20 days, the legume starches were hydrolyzed to the same

extent. Identical rates of hydrolysis of starch in the first solubilization stage has been attributed to similar degrees of packing and orientation of the starch chains in the amorphous regions (Ratnayake *et al*., 2001). Chavan *et al*. (1999) and Hoover and Manuel (1995) reported comparable extents of hydrolysis for other legume starches.

The identical kinetics of the second solubilization stage (from 10 to 20 days) has been attributed to similar amounts of double helices within the crystalline region, and similar crystallite size, in all four starches. In contrast, Chavan *et al.* (1999) hydrolyzed legume (beach pea, grass pea and green pea) starches with 2.2N HCl and reported differences in the two-stage solubilization pattern.

It seems the differences in the susceptibility towards acid hydrolysis during the first 10 days is influenced by the interplay of bound-lipid content and amylose chain associations within the amorphous domains of the starch granule (Chavan *et al*., 1999). Morrison *et al*. (1993) have shown by studies on lintnerized barley starches that lipid-complexed amylose chains are resistant to acid hydrolysis. To account for the slower hydrolysis rate of the crystalline parts of the starch granule, several hypotheses have been proposed (Hoover and Manuel, 1996; Kainuma and French 1971; BeMiller, 1965). Firstly, it has been suggested that the dense packing of starch chains within the starch crystallites does not readily allow the penetration of H_3O^+ into these regions. Secondly, a change in conformation of D-glucopyranose units (from chair to half chair) is a pre-requisite for hydrolysis of glucosidic bonds by H_3O^+. Additionally, these transformations in conformation could be more difficult in amylose-complexed lipid chains, due to a decrease in chain flexibility. The crystalline regions (consisting basically of double helices of external A and B chains of amylopectin) are generally less accessible than the amorphous regions to attack by hydrated protons (Cairns *et al*., 1990; Robin *et al*., 1974; Kainuma and French, 1971) due to dense packing of starch chains within the starch crystallites and to the high energy of activation (Wu and Sarko, 1978) required to cause the conformational change of the glucose units (within the starch crystallites) from chair to half chair (a pre-requisite for acid hydrolysis).

Morphological properties. Amylose and amylopectin molecules are arranged together in a relatively water-insoluble granule of definitive size, shape and morphological characteristics peculiar to a particular plant source (Jane *et al*., 1994). Variation in the size and shape of starch granules is attributed to differences in biological origin (Svegmark and Harmansson, 1993), genotype and cultural practices (Singh *et al*., 2003), and maturity (Manners, 1974). The morphology of starch granules depends on the biochemistry of the chloroplast or amyloplast, as well as the physiology of the plant (Badenhuizen, 1969). The shape of the starch granule is also influenced by the growth environment (Hizukuri, 1969).

Many researchers (Fannon *et al*., 1990; Fitt and Synder, 1984; Banks and Greenwood, 1975; Schoch and Maywald, 1967) have studied and identified starch granules with SEM, and other researchers (McCrone and Delly, 1973; Reichert, 1913) did the same with light microscopy. The size, shape, and position of the hilum of legume starch granules has been observed most often using polarized light microscopy or scanning electron microscopy (SEM). SEM showed field pea starch granules from four cultivars (Carneval, Carrea, Grande and Keoma) to have irregular shapes, which varied from round (5-7 µm) to elliptical (shorter diameter, 10 µm; longer diameter, 25 µm; Ratnayake *et al*., 2001). These values were lower than those reported for other legume starches (Czuchajowska *et al*., 1998; Hoover and Sosulski, 1991). Microscopic examination showed that beach pea, green pea and grass pea starch granules had irregular shapes, which varied from round (6-33 µm) to elliptical (shorter diameter, 11-22 µm; longer diameter, 17-35 µm; Chavan *et al*.,1999). The surfaces of pea starch granules appeared smooth and showed no evidence of fissures when viewed by SEM (Miao *et al*.,2009; Ratnayake *et al*., 2001; Chavan *et al*., 1999). Other researchers (Liu and Shen,2007 ; Tan *et al.*,2007) indicated that MBS granule ranged from 6.5 to 43.4µm in dimension and 14-15µm in width, 18-21µm in length with oblong or kidney-like shape (Liu and Shen, 2007). According to Tjahjadi and Breene (1984), the granules of adzuki bean starch were mostly oval to kidney shaped, although some were irregular in shape, when viewed under the light microscope. Scanning electron micrographs of these granules revealed that the fissures extended to the surfaces of the granules (Tjahjadi and Breene, 1984). These surface irregularities appeared to be characteristic of adzuki bean starch granules and presumably caused by the way the granules are packed within the protein matrix of the cotyledon (Lineback and Ke, 1975). The size of the adzuki starch granules

ranged from 15-45 µm with an average size of 32 µm (Tjahjadi and Breene, 1984). These authors also reported that the granules possessed striae and centric positioned hila. Jane *et al*. (1994) extensively studied starch granule morphology using SEM. These authors used a magnification of x1500 and emphasized the importance of identical magnification with SEM for the purpose of comparing observations of starch granules from different studies. Bean and pea starches are characterized as thick disks with a 'cut' around the middle or at the end and an indentation at one end. The actual cause for the individual characteristics and morphologies are not known, but obvious factors are genetical control, types and amounts of synthetic enzymes in the biosynthesis of the starch molecules, membranous structure of the amyloplast organelle, and arrangement and association of starch molecules (Jane *et al*., 1994). Physicochemical properties, such as percent light transmittance, amylose content, swelling power and water-binding capacity, were significantly correlated with the average granule size of starches separated from different plant sources (Kaur *et al*., 2002; Singh and Singh, 2001; Zhou *et al*., 1998).

In general, starches isolated from legumes have variable granule dimensions, ranging from 4-80 µm (Table 5). The shape of the granules varies from oval, spherical, round and elliptical to irregularly shaped, depending on the source of the starch (Hoover and Sosulski, 1991)**.** Mung bean and black bean have relatively small starch granules (Table 5).

Gelatinization and retrogradation properties. When the starch granule is heated up to the gelatinization temperature in excess water, heat transfer and moisture transfer phenomena occur (Lii *et al*., 1996). The term gelatinization has become established in connection with starch and refers to irreversible physical changes taking place upon the heating of starch in water involving the loss of molecular order, the melting of crystallites, granular swelling and disruption and starch solubilization (Biliaderis, 1998; Atwell *et al*., 1988). The degree of gelatinization can be determined qualitatively and quantitatively by physical, chemical and biochemical methods such as loss of birefringence (Liu *et al*., 2002), increase in viscosity (Wiesenborn *et al*., 1994), decrease in enthalpy (Steven and Elton, 1971), proton magnetic resonance (Cooke and Gidley, 1992; Gidley and Bociek, 1988, 1985), loss of X-ray diffraction pattern (Collison, 1968a,b), and differential scanning calorimetry (Marshall *et al*., 1993).

Table 5. Physical dimensions of granules of some legume starches (Hoover and Sosulski, 1991)

Starch source	Range (diameter)			Shape
	Width (µm)	Length (µm)	Unspecified (µm)	
Kidney bean	16-42	16-60	-	Elliptical, oval
Northern bean	12-40	12-62	-	Oval, irregular, round
Black bean	8-34	8-55	-	Oval, spherical
Mung bean	7-20	10-32	-	Oval, irregular, round
Smooth pea	-	-	20-40	Oval, round
Wrinkle pea	-	-	6-80	Round
Chick pea	-	-	8-54	Oval, spherical
Faba bean	12-24	20-48	-	Oval, spherical
Lentil	15-30	10-36	-	Oval, round, ellipsoid

Gelatinization starts at the hilum of the granule and progresses rapidly to the periphery (Singh *et al*., 2003). It occurs initially in the amorphous regions as opposed to the crystalline regions of the granule, because hydrogen bonding is weaker in the amorphous areas (Singh *et al*., 2003). Waxy starches swell more than starches having a normal amylose content (Tester and Debon, 2000). Tester and Morrison (1990) stated that the swelling behaviour of starch is primarily a property of its amylopectin content, and amylose acts as both a diluent and an inhibitor of swelling, especially in the presence of lipid. They also reported that maximal swelling might also be related to the molecular weight and the shape of the amylopectin molecules.Juhasz and Salgo (2008) concluded in their work that amylopectin was primarily responsible for uptake of water and associated low viscosities and restricted swelling of most legume starch granules to their high amylose content.

Ghiasi *et al*. (1982) indicated that starches with high amylopectin content, e.g., waxy starches have higher gelatinization temperatures than those with a higher amylose content because of the increased levels of crystalline structure associated with amylopectin. Because amylopectin plays a major role in starch granule crystallinity, the presence of amylose lowers the melting point of crystalline regions and the energy for initiation of gelatinization (Flipse *et al*., 1996).

Kreuger *et al*. (1987) postulated that more energy is needed to initiate melting in the absence of amylose-rich amorphous regions. This correlation is clearly seen in Table 6 which is derived from a study of the gelatinization of legume and non-legume starches using

DSC (Biliaderis *et al.*, 1980), and which indicates that starches with higher amylose contents have more amorphous regions and less crystalline regions, which thus lowers their gelatinization temperatures (Sasaki *et al.*, 2000). Hence, legume starches, which tend to have higher amylose contents, would be expected to have lower gelatinization temperature (Table 6). However, Vandeputte and Delcour (2004) indicated that whether amylopectin chains have a positive or negative influence on gelatinization temperature depends on the way they are packed into the lamellar structure of the starch granules. They also proposed that the short amylopectin chains may destabilize the lamellar structure in several ways. Chang *et al.* (2006) reported that the higher average chain length of amylopectin or lower proportion of its short chains might contribute to higher gelatinization temperature of starch granules. Tester (1997) has postulated that gelatinization and swelling properties are controlled, in part, by the molecular structure of amylopectin (unit chain length, extent of branching, molecular weight and polydispersity), starch composition (amylose to amylopectin ratio and phosphorus content), and granule architecture (crystalline to amorphous ratio). Amylopectin from cereals has also been shown to retrograde to a less extent than pea, potato and canna amylopectin, which has been attributed to shorter average chain length in the cereal amylopectin (Kalichevsky *et al.*, 1990; Orford *et al*., 1987).

During DSC analysis of starch, single or double endothermic peaks are obtained depending on the water concentration during starch gelatinization. Starch gelatinization in excess water exhibits a single endothermic transition, whereas, when a starch-water dispersion is heated in the presence of a limited amount of water, two endothermic transitions are observed (Maaruf *et al.*, 2001; Donovan, 1979). In an extensive study carried out by Biliaderis *et al.* (1980) to investigate the influence of water content on the appearance of these two endotherms with smooth pea, adzuki bean and lentil starches, similar results were obtained for the three legume starches. When the starches were heated at high water concentrations, single endothermic transitions were observed at approximately 64°C, 75°C and 56°C for smooth pea, adzuki bean and lentil starch, respectively (Table 6). As the ratio of starch to water increased for each of the starches, the second endotherm started to develop at higher temperatures and became predominant at low water contents. This concentration

Table 6. Thermal characteristics and other physico-chemical properties of various starches (Biliaderis *et al.*, 1980)

Starch source	Phos-phorus content (%)	Amylose content (%)	Initial pasting temp (°C)	Gelatini-zation temp (°C)	Starch conc. for DSC exp. (%, w/w)	Tp_0	Tp_1	Tp_2	Tm	ΔH (cal/g)
						°C				
Adzuki bean	0.013	34.9	78	83-(85)-89	47.7	69	75	89	112	4.4
Smooth pea	0.006	33.1	73	65-(67)-69	47.5	56	64	87	101	3.5
Acid-modified, 5.1[a]	-	26.8	-	-	47.8	60	73	95	103	2.4
Acid-modified, 9.6[a]	-	23.0	-	-	47.9	60	72	96	109	2.2
Garbanzo bean	0.010	34.1	75	65-(68)-71	45.6	68	72	96	108	3.1
Red kidney bean	0.016	35.0	73	64-(66)-68	46.3	61	68	86	100	2.6
Lentil	0.008	45.5	66	58-(59)-61	47.5	48	56	80	95	2.6
Navy bean	0.011	36.0	75	68-(71)-74	46.1	59	67	83	99	3.5
Mung bean	0.016	34.9	73	63-(65)-69	47.5	57	65	83	99	3.9
Faba bean	0.010	32.5	72	61-(63)-66	46.6	56	65	83	97	3.3
Potato, commercial	0.075	20.0	51	64-(65)-67	46.3	55	60	68	85	4.4
Corn, commercial	0.019	22.6	74	63-(65)-68	46.4	60	67	78	89	3.3[b]
Corn, lab prepared	0.012	22.4	73	62-(65)-67	47.3	53	63	75[c]	86	2.7
Acid-modified, 6.5[a]	-	22.6	-	-	47.9	54	73	99[c]	89	2.4
High-amylose corn comm.	0.029	50.3	96	82-(86)-99	48.2	71	82	105	114	4.2[b]
Waxy corn, commercial	0.002	00.0	72	64-(68)-70	47.6	64	71	88	97	4.0[b]

[a]Numbers represent percent lintnerization; [b]For calculation of ΔH values only p_1 and P_2 were used; [c]shoulder.

dependent shift and differences in the melting points of the three legume starches were proposed to be related to various factors, of which the granular organization and its inherent crystallinity are probably the most important (Biliaderis *et al*., 1980). The authors also identified factors such as differences in the degree of branching among the amylopectins of the starches. The order of increasing degree of branching was adzuki bean < smooth pea < lentil (Biliaderis *et al*., 1980). The branching is detrimental to crystallization and hence broadens the melting temperature range and lowers the melting temperature, as found in the field of synthetic polymers (Cowie, 1973). One would expect that the higher the degree of branching, the wider the melting temperature range and the less resistant the starch is to gelatinization (Biliaderis *et al*., 1980).

Ratnayake *et al*. (2001) studied the gelatinization transition temperatures [T_o (onset); T_p (midpoint); T_c (conclusion)] and the enthalpies of gelatinization (ΔH) of starches from four cultivars (Carneval, Carrera, Grande and Keoma) of field pea. They reported that the T_o, T_p, T_c and $\Delta H/\Delta P$ (enthalpy calculated on the basis of amylopectin content) did not vary significantly among the starches. In contrast, they indicated that the gelatinization temperature range ($T_c - T_o$) followed the order: Grande ~ Keoma > Carneval > Carrera. The T_o, T_p, T_c and ΔH of the field pea starches were within the range reported for other legume starches (Hoover and Sosulski, 1991).Additionally,Sandhu and Lim (2008) separated starches from pigeon pea,chick pea, field pea,kidney bean and black gram and reported their gelatinization temperature to be in the range of 68.3 to 69.3%.Abu *et al.* (2006) also reported gelatinization temperature of cowpea starch to range from 67.0-78.0°C. Nearly identical nature of the gelatinization transition temperatures and enthalpies indicates that the numbers of double helices (in the amorphous and crystalline domains) that unraveled and melted during gelatinization were nearly similar in the four starches (Ratnayake *et al*., 2001). However, the gelatinization temperature range ($T_c - T_o$) differed due to differences in the degree of heterogeneity of the starch crystallites within the granules (Ratnayake *et al*., 2001).

When gelatinized starch cools, the molecules begin to reassociate into an ordered structure, in a process called retrogradation (Orford *et al*., 1987). During retrogradation, amylose forms double helical associations of 40-70 glucose units (Jane and Robyt, 1984) whereas amylopectin crystallization occurs by association of the outermost short branches (Ring *et al*., 1987). The extent of reassociation (or retrogradation) depends on the botanical source of the starch (Gudmundsson, 1992; Gudmundsson *et al*., 1991; Kalichevsky *et al*., 1990; Roulet *et al*., 1990; 6.Orford *et al*., 1987; Gudmundsson and Eliasson, 1989, 1991, 1992, 1993), the fine structure of amylopectin (Ward *et al*., 1994; Kalichevsky *et al*., 1990), water content (Gudmundsson, 1994; Zeleznak and Hoseney, 1986; Longton and LeGrys, 1981), storage temperature (Colwel *et al*., 1969, Mclver *et al*., 1968), and the presence of lipids and surfactants (Gudmundsson, 1992; Gudmundsson and Eliason, 1990; Eliasson and Ljunger, 1988a,b; Slade and Levine, 1987; Batres and White, 1986; Evans, 1986). A greater amount of amylose has traditionally been linked to a greater retrogradation tendency in starches (Whistler and BeMiller, 1996), but amylopectin and intermediate materials also play an important role in starch retrogradation during refrigerated storage (Yamin *et al*., 1999). In non-mutant-genotype starches, the amylose is responsible for short changes (Goodfellow and Wilson, 1990). The amylopectin molecule is responsible for longer term rheological and structural changes of starch gels (Gudmundsson, 1994). The roles of amylose and amylopectin depend on the composite nature of the starch gels where swollen gelatinized starch granules are embedded within an amylose-gel matrix (Steeneken, 1989; Russell, 1987; Christianson and Bagley, 1983; Eliasson and Bohlin, 1982; Ring and Stainsby, 1982).

The impacts of retrogradation in starch-based products can be beneficial or, more commonly, undesirable. There is general consensus that starch retrogradation contributes significantly to staling or undesirable firming of bread and other starch products (Del Nobile *et al*., 2003; Abd karim *et al*., 2000;Liu and Thompson, 1998a,1998b). Similarly, the vulnerability of legume starch (high amylose content, Table 1) gels to retrogradation and syneresis makes these types of starches unacceptable for products requiring low-temperature storage. In contrast, retrogradation is sometimes promoted to modify the structural, mechanical or organoleptic properties of certain starch-based products, for example; jam, gels, sauce, jelly, gravy, extruded snacks, vermicelli, soup, biscuit, and creamy desserts (Morikawa and Nishinari, 2000; Perera and Hoover, 1999; Yoshimura *et al*., 1999). Of considerable interest from a food point of view is that retrograded starch is resistant to the action of α-amylase in the ileum and is therefore not a source of blood glucose (Crapo *et al*., 1977) but passes into the colon where it is acted upon

by gut bacteria (Roder *et al.*, 2005). The retrograded starch shows a B-type X-ray diffraction pattern (Zobel, 1988b). Because starch retrogradation is a kinetically controlled process (Slade and Levine, 1987), the alteration of time, temperature and water content during processing can produce a variety of end products.

Starch retrogradation enthalpies are usually 60-80°C lower than gelatinization enthalpies, and transition temperatures are 10-26°C lower than those for gelatinization of starch granules (Baker and Rayas-Duarte, 1998; Yuan *et al*., 1993;White *et al*., 1989). The crystalline forms of retrograded starches are different in nature from those present in the native starch granules (Abd Karim *et al*., 2000). Retrograded starches show lower gelatinization temperatures and enthalpy than native starches because they have weaker starch crystallinity (Sasaki *et al*., 2000).

Tjahjadi and Breen (1984) reported that the degree of retrogradation, as measured by syneresis of Adzuki bean starch was greater than that of corn, wheat or potato starch gels. The authors also observed that the degree of syneresis decreased with increasing starch concentration. This behavior agreed with the results earlier obtained by Lii and Chang (1981) who reported that this pattern is characteristic of many legume starches. Ratnayake *et al*. (2001) studied the extent of retrogradation during gel storage and monitored it by determining changes in retrogradation enthalpy and in freeze-thaw stability. They indicated that in four field pea starches, T_o, T_p and T_c of retrograded gels were lower than those for the gelatinization endotherm, and $T_c - T_o$ for retrogradation was broader than for the gelatinization endotherm. These authors reported that the magnitude of ΔH_R (enthalpy of retrogradation) followed the order: Carneval > Carrera > Grande > Keoma, whereas, $T_c - T_o$ followed the order: Keoma > Grande > Carneval > Carrera. They implied that the wide melting temperature range ($T_c - T_o$) might be due to a large variation in the quantity and heterogeneity of the recrystallized amylopectin and explained differences in ΔH_R among starches on the basis of amylopectin unit chain length distribution (Lai *et al*., 2000; Fredriksson *et al*., 1998; Lu *et al*., 1997; Ward *et al*., 1994; Shi and Seib, 1992; Kalichevsky *et al*., 1990).

Generally, legume starches retrograde significantly due to their relatively high amylose contents, although long term retrogradation, which has been blamed for deterioration in the quality of starch-based product, is attributed to short chains of amylopectin (Robin *et al*., 1974). Amylopectin has high water-binding capacity and slowly undergoes retrogradation, thus forming clear gels that are soft and flow well (Yuan *et al*., 1993).

Summary and conclusions. Inexpensive legumes are the major sources of dietary proteins, as animal proteins are expensive and beyond the reach of the poor. They are also rich in other nutrients such as starch, dietary fibre, vitamins, oils, phytochemicals and mineral elements.

Greater attention has been given to the protein component of legume seeds, despite the fact that the major component is starch. The protein found in this legumes is rich in lysine yet deficient in sulphur containing amino acids, hence the need to consume the products with cereal products to improve the quality of the protein. However, utilization of the starch fraction will be economically important if the proteins are used as food. Despite the current low production and utilization of legume starches in comparison with cereal starches, the former play important roles in the food industries because they affect the physical properties of many foods and are used as gelling agents, thickners, emulsion stabilizers and water binders. Legume starches differ in granule morphology, gelatinization temperature range and amylose content. They generally exhibit C-type X-ray patterns (mixture of A- and B- type X-ray patterns). The retrogadation and syneresis associated with legume starches can be reduced by physically or chemically modifying the native starches to make them more acceptable in food and non-food applications.

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