X-ray diffraction and FTIR spectroscopic characterization of microcrystalline cellulose from agricultural residues

Obesity, a common disorder causing excess mortality due to the development of cardiovascular disease, hypertension, respiratory illness and diabetes is difficult to control by simple dieting techniques. Low calorie foods, which can facilitate newer weight reduction approaches such as behavior modification, often lack adequate palatability due to the absence of carbohydrate or fat. Various low calorie bulking agents that can replace the traditionally used carbohydrates and fats in terms of caloric value, utility are requirements of food industry. For fat, no really satisfactory replacement is currently available, although several promising are under development. Dietary fiber sources such as microcrystalline cellulose are receiving considerable attention as flour replacement.
Abstract

Microcrystalline cellulose (MCC) was prepared from agricultural residues namely, coffee husk and pepper spikes. The materials were extracted successively with n-hexane, ethanol and water in soxhlet apparatus and were dried at 60 degrees in hot air oven. Digestion, bleaching and hydrolysis of material were carried out using aqueous sodium hydroxide, sodium hydrochlorite and dilute hydrochloric acid respectively. MCC, so obtained were characterized through various techniques (bulk and tapping density, moisture content, soluble matter in water, pH value, sulphate ash content, X-ray powder diffraction and FTIR studies) and compared with a commercial MCC. The results obtained show that the prepared MCC have almost similar characteristics like that of commercial MCC.

Keywords: Microcrystalline cellulose; Coffee husk; Pepper spikes; XRD; FTIR

Carbohydrate polymers (Communicated)
V.I. Introduction

MCC has been widely used especially in food, cosmetic and medical industries as water-retainer, a suspension stabilizer, flow characteristics controller in the systems used for final products and as a reinforcing agent. MCC has relatively low chemical reactivity combined with excellent compatibility at low pressures. MCC was rated the most useful filler for direct compression tableting [1]. However, a number of limitations to the use of MCC have been reported [2], the most important of which were considered to be its low bulk density, high lubricant sensitivity, poor flow characteristics and the influence of moisture on the compression characteristics.

MCC is widely used in food industry as anti-caking agent and flavour carrier in grated and shredded cheese, stabilizes foams stabilizes emulsion, replaces fat and oils is used in low fat hot dogs, reduced fat ice cream, forms gels, improves adhesion (cling) of sauces, salads dressings, modify texture-thickens with favorable mouth feel improves quality of low - solids tomato sauces, freeze thaw stability, retards ice crystal growth, suspending agent in ice cream, extends starches vegetable fat whipped toppings - improves body, texture and stability. Other food uses includes; barbecue sauces, frozen cheese lasagna, frozen guacamole, marshmallow topping, liquid diet products, sandwich spreads, low calorie mayonnaise to mention a few [3-5].

Preparation of MCC from materials other than wood and cotton such as water hyacinth[6], coconut shells
X-ray diffraction residues

[7], sugar cane bagasse [8-12], ramie [13], wheat and rice straws [14,15], jute[16], flax fibers and flax straw and soybean husk [17] have been studied. However, for the best of our knowledge MCC from the proposed agricultural wastes has not been studied.

In the present work, MCC was prepared from coffee husk and pepper spikes. India is one of the major producer of coffee and black pepper in the world with approximate annual production of \(27.52 \times 10^3\) tones/year and \(70 \times 10^3\) tonnes/year respectively [18].

Coffee husk is a major agro-industrial residue obtained after removal of pulp and seed from fruit of *coffee arabica* and is estimated to yield 0.36 million tones/year [18]. Coffee husk has no fertilizer value as it contains mostly lignocellulose but it contributes to the problem of environmental pollution.

Black pepper (*Piper nigrum*) is the king of spices and has been used all over the world as a food additive. Pepper is picked from the spikes when one or few of the berries start turning yellow-orange and the berries become hard. In India the pepper berries are removed from the spikes before drying. It is estimated that the pepper spikes produced which is a waste is almost equal to that of the pepper. The large quantity of spikes produced is either incinerated or dumped in the open land thus contributing to the problem of environmental pollution.

The MCC prepared from above materials were characterized through bulk and tapping density, moisture

Dietary fiber is the part of a plant that provides and maintains the plant's structure. Cellulose, hemicellulose, polysaccharides, pectins, gums, mucilages, and lignins are dietary fibers. These fibers are unrelated chemically, however, they all have one thing in common -- they can't be digested by the human body. For this reason, they can help correct disorders of the large intestine (colon), and keep it functioning normally. Therefore, it is important to increase the amount of fiber in the diet.

www.endowsec.com/pat ed/edtgs01.htm
content, soluble matter in water, pH value, sulphate ash content, X-ray powder diffraction and FTIR studies and compared with commercial MCC with the objective of introducing new MCC source.

V.2. Experimental

V.2.1. Chemicals

Calcium hypochlorate, sodium hydroxide, (BDH India); potassium bromide (IR grade), MCC, EDTA (Sigma Aldrich) all other reagents used were of analytical grade chemicals unless specified other wise.

V.2.2. Raw materials

Coffee husk and pepper spikes were collected from plantation of Western Ghats in India. The samples were preextracted with n–hexane, ethanol and water subsequently in soxhlet apparatus and were dried at 60 degrees in hot air oven.

V.2.3. MCC isolation

Pretreated materials (coffee husk and pepper spikes) were digested with sodium hydroxide (5% w/v) under reflux for 2 h the liquor ratio was 1:10. The digested pulps were washed with distilled water. Sodium hypochlorite was added to the pulps mixed thoroughly and allowed for 1h to bleach. The bleached pulps were treated with EDTA to chelate the metal ions, if present. Finally, the pulps were hydrolyzed with 5N hydrochloric acid for 20 min. The hydrolyzed pulps were dried at 60°C to get MCC.

To improve your diet, add foods that contain more dietary fiber. You can include some or all of the following:

1. Whole-grain foods (such as bran cereals) and breads (those made with whole wheat grains).
2. Fresh fruits (including the skin and pulp).
3. Dried or stewed fruits (such as prunes, raisins, or apricots).
4. Root vegetables (such as carrots, turnips, or potatoes).
5. Raw or fresh vegetables, such as cabbage. (Lettuce is actually low in fiber.)

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**V.2.4. Bulk and tapping densities**

An appropriate amount of the sample was poured in a 50 ml tarred graduate cylinder. The cylinder was lightly tapped twice to collect all the powder sticking on the wall of the cylinder. The volume was then read directly from the cylinder and used to calculate the bulk density according to the relationship: mass/volume. For tapping density, the cylinder was tapped until no change in volume. The volume of the sample was then read and used in the calculation.

**V.2.5. Moisture content**

Wide mouthed glass weighing bottle was dried in an oven at 100 to 105°C and then it was cooled in a desiccator to room temperature and weighed accurately. 5 g of the MCC was weighed into the tarred weighing bottle. Weighing bottle was kept in an oven, partly removing the stopper and the sample was dried at 100 to 105°C to constant weight (about 4h drying is sufficient). Calculation.

\[
\text{Moisture content} = \frac{M - M_1}{M} \times 100 \%
\]

Where,

\( M = \text{Weight in g of the material taken for the test, and} \)

\( M_1 = \text{Weight in g of the material after drying.} \)

**V.2.6. Soluble matter in water**

Five grams of the material was taken with 80 ml of water and then it was mixed by shaking for 10 min. The mixture was filtered through whatman No.42 filter paper into a tarred beaker. Then it was evaporated to dryness on a
steam bath and dried at 105°C for 1h. The residue was weighed for a constant weight after dried in an oven at 100 to 105°C.

Calculation.

Soluble matter in water (% by weight on dry basis) = \( \frac{M_1 \times 100}{M_2 \times (100-M)} \times 100 \)

Where,

\( M_1 \) = Weight in g of the residue.

\( M_2 \) = Weight in g of the material taken for the test, and

\( M \) = Moisture content of the material (% by weight).

\( V.2.7. \) pH value

Five grams of the material with 50 ml of carbon dioxide free water was shaked for 20 min and centrifuged. The pH value of the supernatant liquid was determined with the help of pH meter.

\( V.2.8. \) Sulphate ash content

Five grams of the material was weighed accurately and taken in a tared silica crucible. It was ignited gently, until the substance thoroughly chars. The mixture was cooled, 1 ml of concentrated sulphuric acid was added and again it was ignited at 800 ± 25°C in a muffle furnace until all carbon consumed. It was cooled in a desiccator, weighed for constant weight and % residue was calculated.
Calculation.

\[
\text{Sulphate ash} \quad \text{(% by weight on dry basis)} = \frac{M_1 \times 100}{M_2 (100-M)} \times 100
\]

Where,

\[M_1 = \text{Weight in g of the ash}\]
\[M_2 = \text{Weight in g of the material taken for the test, and}\]
\[M = \text{Moisture content (% by weight)}\]

\[V.2.9. \text{X-ray powder diffraction studies}\]

Diffraction patterns were obtained using a Phillips X-ray diffractometer. The diffraction patterns were recorded using Cu-Kα radiation at 40 kV and 25 mA. The samples were pressed into pellets (25 mm in diameter) by compression of 0.25 g in a mold under a pressure of 50 MPa.

The crystallite size of MCC was measured using the half-height width of the \(I_{002}\) reflection and crystallinity index (CrI) was calculated as follows [19]:

\[\text{CrI} = \frac{(I_{002} - I_{am})}{I_{002}}\]

Where, \(I_{002}\) is the intensity of the 002 peak (at about \(2\theta = 26\)) and \(I_{am}\) is the intensity corresponds to the peak at about \(2\theta = 18\).

\[V.2.10. \text{FTIR Studies}\]

Coffee husk MCC, pepper spikes MCC and commercial MCC were analyzed by FTIR using Bomem MB100 instrument.

Cellulose is a linear polymer of \(\beta-(1 \rightarrow 4)-D\)-glucopyranose units in \(\beta-C_1\) conformation. The fully equatorial conformation of \(\beta\)-linked glucopyranose residues stabilizes the chair structure, minimizing its flexibility (e.g. relative to the slightly more flexible \(\alpha\)-linked glucopyranose residues in amylose). Cellulose preparations may contain trace amounts (~0.3%) of arabinoxylans.
For FTIR scans, KBr pellets containing 1% of the sample were prepared. For characterization of MCC samples three ratios of peak areas were analysed: the paracrystalline regularity index \( \Omega = \frac{A_{1730}}{A_{897}} \) which can be related with degree of ordering of the largest macromolecules [20] and the \( I_1 = \frac{A_{1430}}{A_{897}} \) and \( I_2 = \frac{A_{1370}}{A_{2900}} \) indices, which have been proposed as sensible to cellulose type I and type I and II crystallinity, respectively [21].

V.3. Result and discussions

Digestion is the process which involves cooking of raw material in alkaline liquor under pressure and at relatively high temperature which makes material free from non cellulose material substances. The active component of the cooking liquid is sodium hydroxide cooked at 90 degrees. As the temperature rises the various components commence to diffuse from the chips into the liquor [22]. It also helps to control the viscosity and reactivity of the pulp. Temperature above 70 degrees up to 190 degrees was necessary to yield better results.

Bleaching is one type of the chemical treatment which removes the color and other impurities by oxidation. It is a very important process in which all the important properties such as viscosity and reactivity are adjusted and also the color is removed. Important factors which govern bleaching operation are time, temperature, pH and concentration of bleaching agent. The active bleaching agents that were studied includes elemental chlorine, sodium
hypochlorite, chlorine dioxide, peroxides, permanganate, oxygen and ozone. The best results were obtained with sodium hypochlorate.

Metals if present in the proposed MCC material was removed by adding EDTA, other substitutes for EDTA were EGTA and Salicylic acid.

The glycosidic linkage in cellulose is susceptible to acid-catalyzed hydrolysis, which leads to high yield of D-glucose under suitable condition. The mechanism of the reaction is as follows. It comprises of three stages: (1) Rapid protonation of the glycosidic oxygen atom, (2) Slow transfer of the positive charge to C-1 with consequent formation of a carbonium ion and fission of the glycosidic bond and (3) Rapid attack on the carbonium ion by water to give the free sugar residue and to reform the hydroxinium ion [23]. The concentration of hydrochloric acid was studied form 2 N to 10 N, the best results were obtained with concentration ranging from 4N to 6N. Hence, 5N hydrochloric acid was used for both materials and 15 to 20 min were required for complete hydrolysis of the materials.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Coffee husk</th>
<th>Pepper spikes</th>
<th>Standards</th>
</tr>
</thead>
<tbody>
<tr>
<td>Loss on drying</td>
<td>4.38 %</td>
<td>3.73 %</td>
<td>≤5 %</td>
</tr>
<tr>
<td>Soluble matter in water</td>
<td>0.157 %</td>
<td>0.186 %</td>
<td>≤0.242 %</td>
</tr>
<tr>
<td>PH</td>
<td>6.8</td>
<td>6.7</td>
<td>7.0</td>
</tr>
<tr>
<td>Sulphate ash content</td>
<td>0.081 %</td>
<td>0.062 %</td>
<td>0.05 %</td>
</tr>
</tbody>
</table>

The characterization tests for the obtained microcrystalline powder as per the specifications of Food Chemical Codex standards of identity (1981).
Chapter V

X-ray diffraction residues

V.3.1 Crystallinity and crystallite size

The X-ray diffraction pattern of MCC samples prepared from coffee husk and pepper spikes along with that of the commercial sample is shown in Figure V.1. The calculated crystallinity index of the different MCC samples are given in Table V.1. As shown in Figure V.1 all samples have a typical crystal lattice for cellulose I [21]. Also, all MCC samples had similar CrI values with slightly lower values for coffee husk and pepper spikes.

Figure V.1.
X-ray diffraction pattern of commercial MCC, coffee husk MCC, pepper spikes MCC
Table V.1
Crystallinity index (Cri) of MCC samples

<table>
<thead>
<tr>
<th>MCC Sample</th>
<th>Cri</th>
</tr>
</thead>
<tbody>
<tr>
<td>Commercial MCC</td>
<td>0.78</td>
</tr>
<tr>
<td>Coffee husk</td>
<td>0.75</td>
</tr>
<tr>
<td>Pepper spikes</td>
<td>0.73</td>
</tr>
</tbody>
</table>

V.3.2. FTIR characterization

The FTIR spectra (Figure V.2) are very similar and are characteristic of cellulose type I. The $\Theta$, $I_1$ and $I_2$ parameters obtained were 2.42, 7.56 and 0.12 for the commercial MCC, 2.62, 7.82 and 0.11 for coffee husk and 2.18, 6.05 and 0.12 for pepper spikes. These results indicate similar crystallinities for all MCC samples.

Figure V.2.
Infrared spectroscopy (FTIR) spectra of commercial MCC, coffee husk MCC, pepper spikes MCC
V.4. Conclusion

MCC prepared in this work from agricultural residues (coffee husk and pepper spikes) are interesting alternatives as MCC source for several applications. Such materials are renewable and vastly available in many regions of the world and are generally burned or disposed for ambiental degradation.

A linear relationship between two FTIR parameters ($\Theta$ and $I_1$) was observed, which can be related to crystallinity of cellulose type I. Both the samples have a typical crystal lattice for cellulose I. Also, all MCC samples had similar CrI values with slightly lower values for coffee husk and pepper spikes.
References


