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MOLECULAR STRUCTURE EVALUATION OF BEESWAX AND PARAFFIN WAX BY SOLID-STATE ^{13}C CP/MAS NMR

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ABSTRACT

In this study, beeswax and petroleum wax are used in the laboratory analysis to compare the natural and processed substances based on their molecular structure. ^{13}C solid-state Nuclear magnetic resonance (NMR) experiments were conducted to investigate the structure of beeswax and paraffin wax. The carbon resonance region was comprehensively analyzed in terms of NMR chemical shift. Experimental results of the ^{13}C CP/MAS NMR studies revealed that the molecular structures of paraffin wax and beeswax were very similar. Both waxes consisted of long chains of carbon components and CH_2 carbons were predominantly present (≈ 90 %). The line shape of the int-(CH_2) carbon resonance region was comprehensively analyzed in terms of NMR chemical shift. The ^{13}C broad peak component covering from 31 to 35 ppm corresponds to int-(CH_2) carbons with trans conformation in crystalline domains, whereas the sharp signal at 30.3 ppm corresponds to gauche conformation in the non-crystalline domain.

Keywords: NMR test; beeswax; paraffin wax; physical and chemical properties.

1. INTRODUCTION

The uses of natural and synthetic waxes make them important in our daily life style. Waxes are normally used in producing candles and polishing materials. A recent research article (Chhetri et al., 2007) showed a non-toxic shoe polish can be produced from beeswax.

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They pointed out that beeswax is inherently non-toxic as opposed to paraffin wax that is inherently toxic, when long-term is considered. This latter point has eluded most researchers in the past. However, with increased awareness of the benefit of natural products, there is a need for investigating possible means of replacing artificial chemicals with natural substitutes. It is known that waxes form important materials in the manufacturing of products such as ceramics, food, rubber, timber, textiles, tobacco, paints, plastics, cosmetics, pharmaceuticals, engineering and industry (Basson and Reynhardt, 1988, Kameda, 2005). In order to eventually replace artificial wax with natural wax (such as beeswax), it is important to understand material characteristics of beeswax. The structural and dynamic characterization of beeswax is necessary for us to understand the relationships between its properties and structure and thus design new applications for beeswax. Therefore, the fundamentals, structural and chemical analyses of waxy materials are prime objectives of the industry. Natural waxes can be classifies as animal waxes, vegetable waxes and mineral waxes.

Paraffin waxes, a by-product of petroleum refinery have numerous applications. Their use includes candle production, packaging materials, construction and cosmetic industries (Sperber et al., 2005; Hossain et al., 2009). Deparaffination of lubricating oils gives the raw materials for the macrocrystalline waxes which are loose waxes. However, the vacuum residue of crude oil distillation produces the microcrystalline paraffin waxes (Sperber et al., 2005).

Basson and Reynhardt (1988) investigated the combwax of African bee *Apis Mellifera Adansonii* with NMR experiments supported by differential scanning calorimetry (DSC) and x-ray diffraction measurements. They measured the proton spin-lattice relaxation times and proton spin-spin relaxation time as a function of temperature (333 K > T > 110K). The use of pulse technique was completed for the liquid content of the wax as a function of temperature. They compared the results with Fischer-Tropsch waxes. They found that beeswax is branched to a much higher degree than Fischer-Tropsch waxes including oxidizes waxes. Their findings include: 1) a higher percentage of carbon atoms is involved in ester groups in beeswax; 2) oxidized hard wax contains a higher fraction of carbon atoms with double carbon-carbon bonds; and 3) the average chain length in beeswax, determined by ebullioscopic methods, is 40 carbon atoms. Morgan et al. (2002) studied the mechanical properties of beeswax and measured these properties as a function of temperature. They used a variety of techniques and compared with each other. They measured the coefficient of friction of beeswax and compared with that of plasticine and Nylon 6–6. They found that the frictional behavior of beeswax departs from Amonton's laws and behaves instead as a classic soft, elastic polymer.

Kameda (2004) studied solid-state ^{13}C NMR experiments to investigate the structure of beeswax. He analyzed the quantitative direct polarization ^{13}C magic angle spinning (MAS) NMR spectrum and found that the fraction of internal-chain methylene [*int*-(CH_2)] component compared to other components of crude beeswax is over 95%. They also analyzed the chemical shift analysis based on ppm of composition. He concluded that over 85% of the *int*-(CH_2) has a crystal structure in the aliphatic region. There also exist at least three of several kinds of molecular packing for *int*-(CH_2) in the crystalline domain. Kameda (2005) investigated the molecular structure of natural wax from Japanese bees, *Apis cerana japonica* in its native state by ^{13}C and ^{1}H solid-state NMR. They noticed that two strong ^{13}C peaks were qualified at 32.9 and 34.0 ppm where signals from internal-chain methylene carbons [int-(CH2)] in two types of crystal form. The peak at 32.9 ppm was known to an orthorhombic crystal form, and that at 34.0 ppm was assigned to a triclinic or monoclinic form. They observed bi-exponential decay of ^{13}C spin-lattice relaxation for the crystalline peaks due to chain diffusion in both crystalline regions. Tsunenori (2005) did an experiment on beeswax by ^{13}C solid-state NMR for investigating the structure of beeswax in the native state for the first time. They found that the ^{13}C broad peak component covering from 31 to 35 ppm corresponds to internal-chain methylene (int-(CH_2)) carbons with trans conformation in crystalline domains. They also pointed out that the sharp signal at 30.3 ppm corresponds to gauche conformation in the non-crystalline domain.

Tsunenori (2006) performed a series of experiments, ^{13}C ID-MAS, WISE, 2D-MAS exchange, and several relaxation-times NMR to investigate the molecular structure and dynamics of beeswax. He found out that int-(CH_2) carbon accounts for over 95% of all carbons from DP-MAS spectrum. He also noticed that beeswax contains int-(CH_2) from which 15% of the int-(CH_2) is amorphous and the remainder is present in two kinds of crystal form. To determine the molecular structure and dynamics of beeswax and hornet silk, a series of experiments, ^{13}C ID-MAS, WISE, 2D-MAS exchange, and several relaxation-times NMR were completed by Tsunenori and Yasushi (2006). They focused that the crystalline peak at 34.0 ppm is considered to consist of free acids and alcohols, the other crystalline peak at 34.0 ppm of hydrocarbons and esters. A sharp and strong ^{13}C NMR peak at 32.9 ppm was detected from hornet cocoon at room temperature which attributable to the signal from int-(CH_2) carbon in the orthorhombic form in the wax.

Ruffier-Meray et al. (1998) developed a low resolution NMR method for measuring the crystallized fraction as a function of temperature for paraffin wax. Kumar et al. (2005) investigated the structural composition of paraffin waxes and soft wax fraction derived from microcrystalline wax. Multistage solvent crystallization technique at different temperatures was used for wax separation. ^{1}H and ^{13}C NMR spectroscopy determine the average structural parameters of parent waxes, their fractions. They also used DSC for the thermal parameters viz. phase transition temperature and the associated energy during phase transitions. Sperber et al. (2005) studied composition of macro and microcrystalline paraffin waxes. They focused on the structure of these paraffin waxes. Their analytical technique gives substantial information about the structure of the paraffin waxes where they used a special ^{13}C-NMR spectroscopy method. They indicated that microcrystalline waxes are very complex mixtures. They identified more than 95% of the signals and quantified based on the chemical shifts of characteristic structure groups. In the investigation of waxes, the signals of the straight hydrocarbons dominate only a few signals of branched hydrocarbons.

Lu and Redelius (2006) separated bitumen waxes from different bitumens with respect to their chemical compositions and structural characteristics. They used distillation method for separation of waxes. Different techniques such as DSC, HTGC, GC-MS, FIMS, NMR, and WAXD were used to characterize the waxes. Their study showed that bitumen waxes are complex mixtures of hydrocarbons structured as *n*-alkanes (C_{15}-C_{57}) and isoalkanes, cycloalkanes, and aromatics, which could be larger than C_{57}. They concluded that the waxes with a predominance of *n*-alkanes generally melt (and crystallized) over a narrower temperature range. The sharpness of wax crystallization/melting peaks decrease with increasing nonnormal alkanes, particularly cycloalkanes and aromatics. Hossain et al. (2009) did a through investigation of beeswax and paraffin wax based on SEM technique. They have summarized the properties of beeswax and paraffin wax based on available literature review.

Numerous attempts have been made by diffraction analysis to explain the structure of beeswax. However, no detailed structural analysis was carried out through ^{13}C chemical shifts. This is probably due to the poor separation of peaks from the CH_2 region. As a result, little progress has been made to understand the relationship between the structure of beeswax and its corresponding NMR chemical shifts. This study is an attempt to clarify these issues. To obtain information about the structures and molecular dynamics of the natural waxes and synthetic waxes, a detailed investigation has been completed using high-resolution NMR in the solid state. This study uses ^{13}C CP/MAS solid state NMR on beeswax and paraffin wax. The question to answer by solid state NMR was the determination of functional groups. The dependence of the peak intensities on the cross-polarization time was also identified.

2. NMR TEST

To obtain physical, chemical, electronic and structural information about a molecule, NMR spectroscopy is used. It is the only technique that can provide one with detailed information on the exact three-dimensional structure of biological molecules in solution. The most often-used nuclei are hydrogen-1 (^{1}H) and carbon-13 (^{13}C), although certain isotopes of many other elements nuclei can also be observed. In this study, ^{13}C nuclei is used to determine the molecular structure. ^{13}C solid-state NMR experiment under magic angle spinning (MAS) is particularly useful for the elucidation of heterogeneous structure, because each component in the heterogeneous system can be discriminated by its distinct ^{13}C peaks (Kameda, 2004; Kameda et al., 2004; Kameda, 2005).

Waxes have unique characteristics and now it is being used in the development of new products in various fields such as cosmetics, foods, pharmaceuticals, engineering and industry (Dorset, 1999; Koga, 2000; Mariya and Nikolay, 2002; Al-Waili, 2003). Waxes consists of long-chain carbon components, including alkanes that contain 21 to 33 carbon atoms, acids that contain 22 to 30 carbons and esters that contain 40 to 52 carbons.

3. EXPERIMENTAL CONDITIONS

The ^{13}C NMR experiments were conducted on a Bruker Avance DSX NMR spectrometer with a 9.4 T magnet (proton Larmor frequency 400 MHz) using a HX probe head with rotors of 4mm diameter. The solid samples were studied by ^{13}C cross polarization (CP) magic angle spinning (MAS) NMR using TPPM proton decoupling and linearly varying proton contact powers. The conditions for the pulse sequence were optimized on glycine, which also serves as external chemical shift reference with its carbonyl peak at 176.06 ppm. The proton frequency for the CP and the proton decoupling was measured as the center frequency of the proton spectrum using single pulse excitation. Proton T_1 inversion/recovery measurements were used to determine the fastest possible repetition time for the ^{13}C cross-polarization experiments. The derived repetition times for the ^{13}C CP/MAS spectra were 3s for the paraffin and 3.5s for the beeswax. NMR Spectra were taken at 8.0 sample spinning speeds. No significant spinning sidebands were found. The cross polarization time was varied.

4. RESULTS AND DISCUSSION

The beeswax and paraffin wax samples were pulverized prior to NMR analysis. The solid state of the beeswax and paraffin wax samples were examined by ^{13}C CP/MAS NMR and chemical shifts were calculated relative to TMS (Tetramethylsilane) for NMR control. In order to gain insight into the resonance frequencies of the NMR spectra, ACD Spectrum Generator program was applied to predict the NMR spectra of some well known components of beeswax and paraffin wax.

Figure 1 exhibits the ^{13}C CP/MAS of beeswax at numerous contact times. From Figure 2, it is observed that the beeswax consists of long-chain carbon components including alkanes that contain 21-33 carbon atoms although the beeswax is known to be composed of multiple components, such as hydrocarbons, alcohols, free acids, esters, and other materials (Garnier 2002, Kimpe 2002, Tulloch 1972). The peak at 32.355 ppm on beeswax spectrum (see Figure 2) reveals that the fraction of int-(CH_2) units with the other units is over 89.55 %.

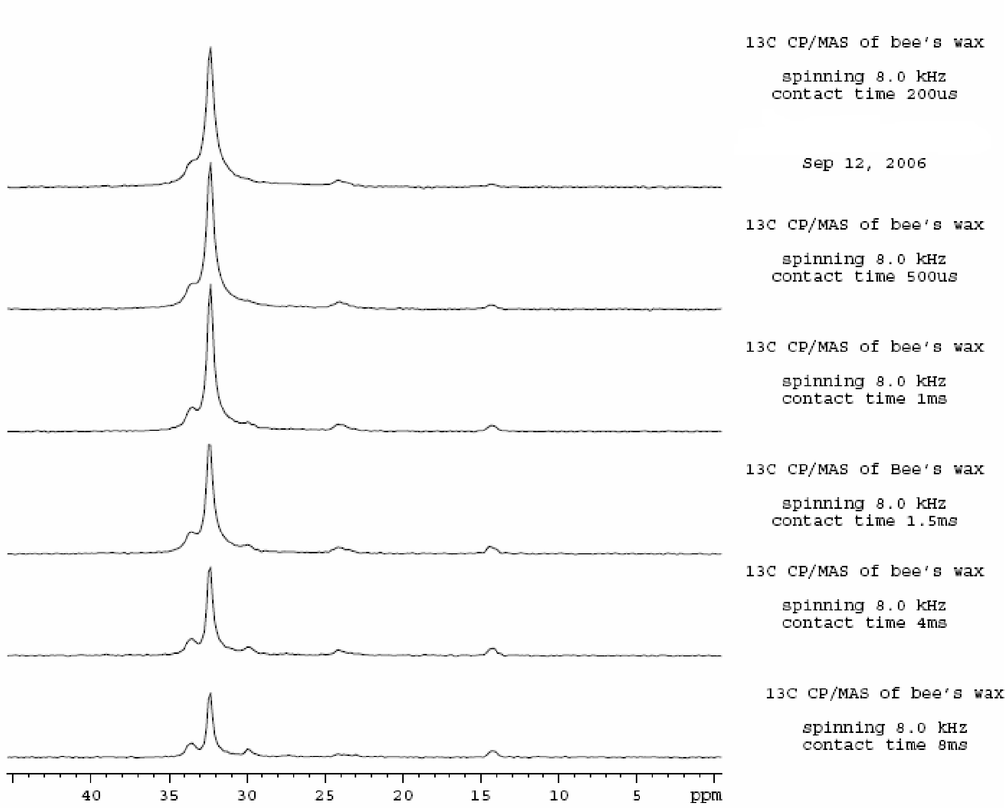

Figure 1. ^{13}C CP/MAS of beeswax at various contact times.

Figure 3 shows ^{13}C CP/MAS of paraffin at various contact times. From Figure 4, three identifiable peaks on the high-resolution ^{13}C spectrum were shown. The peak at 14.328 ppm (5%) was assigned to methyl carbons at the termini of alkyl chains by comparison with references (Basson and Reynhardt, 1988). The spectrum of paraffin wax showed a peak at 24.219 ppm (4.09 %) that belongs to straight-chain alkanes of carbons (Wu et al., 2004).

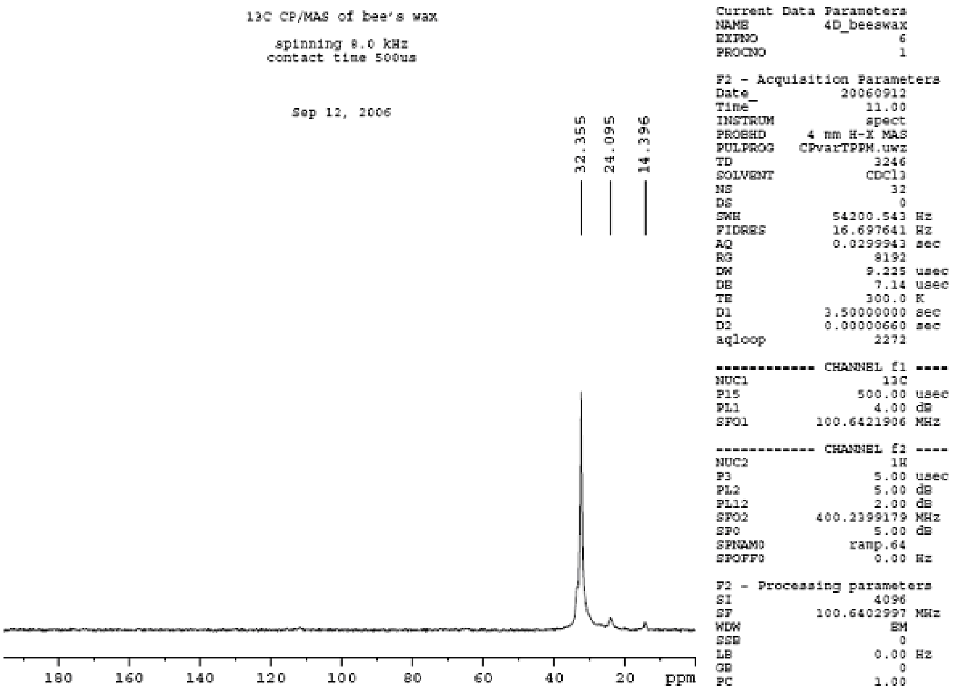

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Figure 2. ^{13}C CP/MAS NMR spectrum of beeswax.

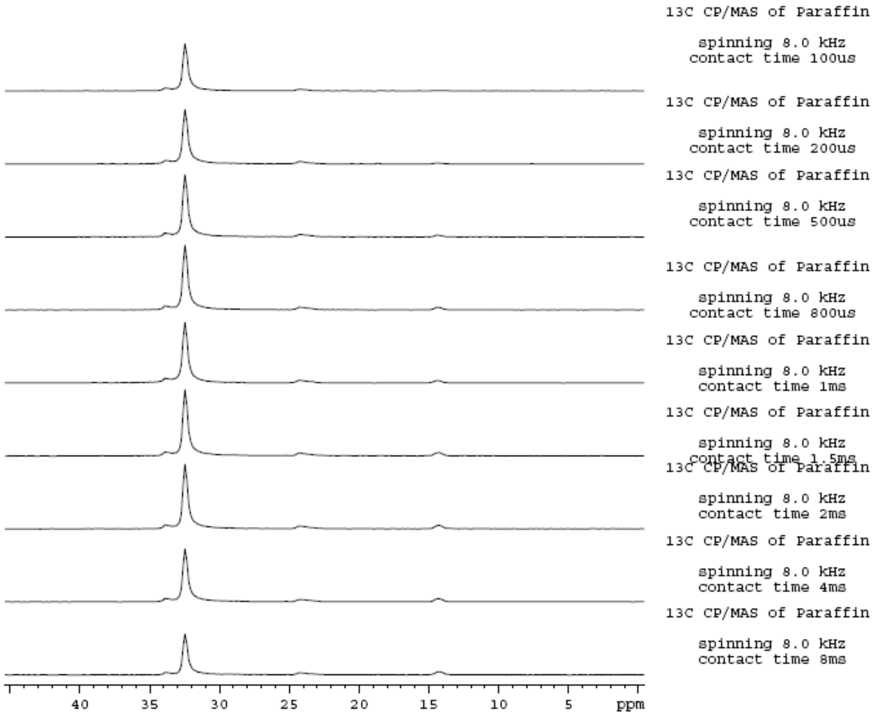
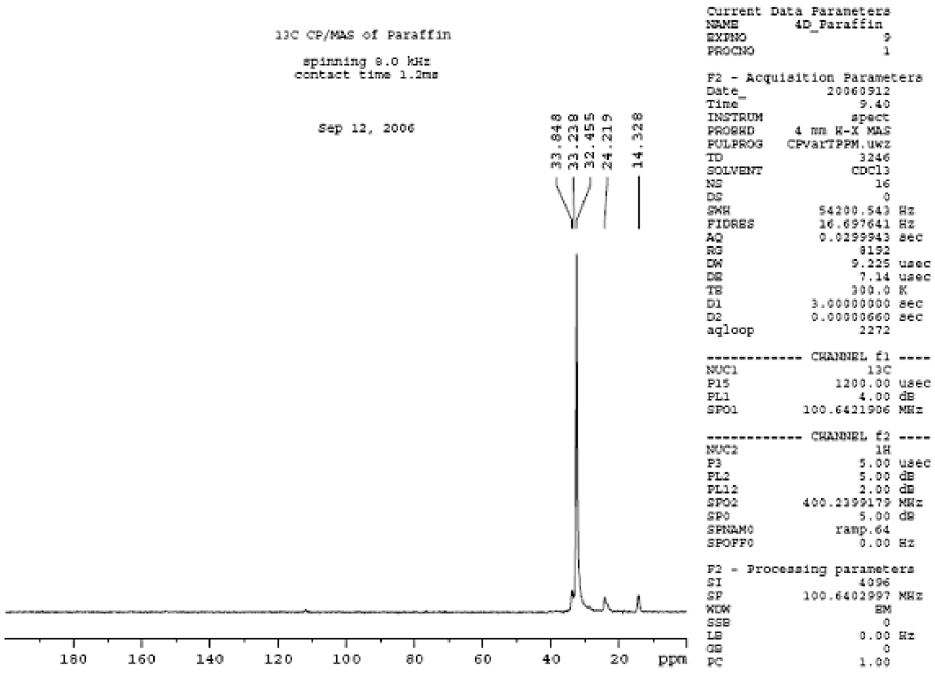


Figure 3. ^{13}C CP/MAS NMR spectra of paraffin wax at various contact times.

The strongest resonances were centered at 32.455, 33.238 and 33.848 ppm, which were typical chemical shifts for int-(CH_2) carbons (Basson and Reynhardt, 1988). At a quantitative consideration all peaks must be integrated and set to 100 %. These percentages should be considered as broad estimates because it is expected that the intensities of the peaks depend to some extent on operating conditions and the environment and the degree of mobility of the different carbon atoms (Basson and Reynhardt, 1988).

From Figure 4, it was found that the int-(CH_2) carbon accounted for 90.91 % of all carbons although microcrystalline waxes are complex mixtures, in which a great number of characteristic structure could be identified. The n-paraffins are known to have various crystallographic forms, such as orthorhombic, triclinic, monoclinic and hexagonal, under certain conditions, in which the conformation is always the same all-trans zigzag. Ishikawa et al. (1991) reported that the ^{13}C NMR chemical shift of n-paraffin depends on the crystal structure.

The ^{13}C NMR spectrum of the beeswax and paraffin wax are shown in Figures 1 and 3. The peaks of the ^{13}C spectrums were assigned to specific functional groups (Rahman, 2007). Each of the features in the spectra is a functional group that resonated at the found ppm value, based on typical ^{13}C chemical shift tables (Hesse et al., 1979).



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SWH           54200.543 Hz
FIDRES        16.697641 Hz
AQ            0.0299943 sec
RG                 8192
DW                9.225 usec
DE                 7.14 usec
TE                300.0 K
D1           3.00000000 sec
D2           0.00000660 sec
aqloop             2272

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P15             1200.00 usec
PL1                4.00 dB
SFO1        100.6421906 MHz

============ CHANNEL f2 ====
NUC2                 1H
P3                 5.00 usec
PL2                5.00 dB
PL12               2.00 dB
SFO2        400.2399179 MHz
SP0                5.00 dB
SPNAM0          ramp.64
SPOFF0             0.00 Hz

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LB                 0.00 Hz
GB                    0
PC                 1.00
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Figure 4. ^{13}C CP/MAS NMR spectrum of paraffin wax.

On the other hand, chemical shifts were also determined by analyzing standard substances (Sperber, 2005). Figure 5 shows that both waxes have a similar structure including the same functional groups.

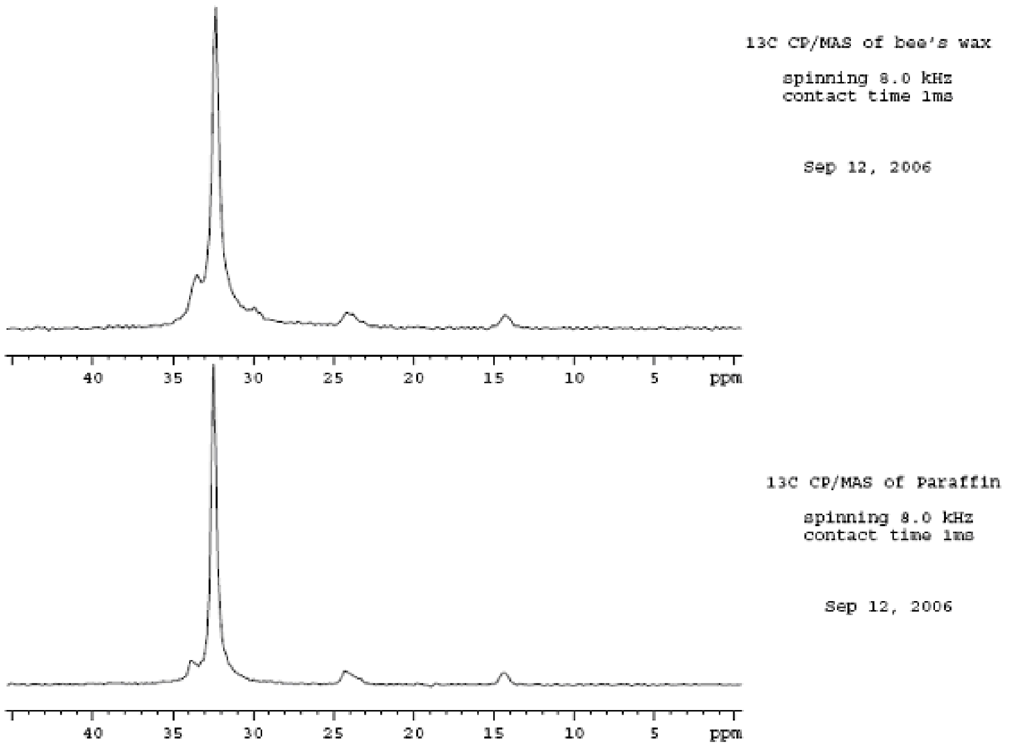

Figure 5. ^{13}C CP/MAS NMR beeswax and paraffin wax spectra.

5. REMARKS ON NATURAL BEESWAX AND PROCESSED PARAFFIN WAX

It is important here to mention that beeswax is natural whereas the paraffin wax underwent various processing stages, which altered its natural structure. Regarding the NMR spectra, remarkable in the natural product of beeswax is the absence of any signal other than the aliphatic groups, generally in the range from about 50 to 10 ppm. It is expected to find additional peaks from esters, acids and double-bonded carbon nuclei. Indications of those peaks are in the beeswax spectra for longer contact times, but barely above the noise level.

CONCLUSIONS

Comparison between the natural and processed substances based on their molecular structure has been shown in this study using ^{13}C NMR experiments. The ^{13}C CP/MAS NMR spectra of beeswax and paraffin wax, together with the experimental parameters and peak frequencies were shown. ^{13}C NMR spectra comparison between the two materials is analyzed and discussed. For each sample, a stack plot was presented as function of the cross-polarization contact time. In addition, the peak intensities were evaluated for the different peaks in the spectra. This ^{13}C CP/MAS NMR study revealed that the molecular structures of paraffin wax and beeswax were similar. Both waxes consisted of long chains of carbon components and CH_2 carbons were predominantly present in both wax types.

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