RAPID DETERMINATION OF FOOD QUALITY USING STEADY STATE FREE PRECESSION SEQUENCES IN TD-NMR SPECTROSCOPY

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1 INTRODUCTION

The use of time-domain NMR spectroscopy (TD-NMR) in food science began more than 40 years ago with the introduction of the small benchtop NMR analyzer.[1] Since then, TD-NMR has become one of the most robust, rapid, cost-effective and versatile tools in the food industry. Earlier TD-NMR applications were primarily based on quantitative analysis using the intensity of free induction decay (FID) and/or spin echo signals.[1-3] In the last two decades, the use of relaxometry and/or diffusometry methods have expanded the application TD-NMR in food science exponentially.[2,3]

The majority of these applications use the Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence.[1-3] This sequence is very robust[4], rapid and yields an exponential decay that is dependent upon the transverse relaxation time (T_2). [1-3] Therefore, CPMG has been used as an all-purpose sequence in TD-NMR applications and is a standard pulse sequence present in commercial and homemade TD-NMR spectrometers. CPMG has been used to study food products such as oilseeds, fresh meat, fish, and fruit, as well as industrialized and packaged food products.[1,5,6]

The longitudinal relaxation time (T_1) measurements using inversion-recovery (IR) or progressive saturation pulse sequences have rarely been used in food analysis due to the length of experiment time.[2,6] Pulsed field gradient spin-echo (PFGSE) pulse sequences are the second most used pulse sequence in TD-NMR applications.[2] PFGSE has been used to measure the water self-diffusion coefficient, water mobility, and droplet size in several food products. However, PFGSE requires an additional hardware accessory that is not available for all TD-NMR spectrometers. Thus, there is an effort towards the development and implementation of rapid TD-NMR analytical methods that meet the growing demand for tools of quality assessment.

Accordingly, we have been developing steady-state free precession (SSFP) pulse sequences for TD-NMR spectroscopy since 2000.[7] SSFP sequences have been used in quantitative analysis similarly to analyses performed with FID or spin echo.[7,8] However, the signal-to-noise ratio (SNR) with SSFP is much higher than that obtained with FID or echo in the same

average time.[7,8] Moreover, SSFP sequences can also be used in fast flow (online) quantitative measurements of liquid or solid samples.[9,10] The theory for quantitative analysis using the amplitude of an SSFP signal is presented in section 2.1.
Further advantages of SSFP sequences are: the dependence of the transient signals on two relaxation times (T_1 and T_2), the data are collected in a length of time similar to CPMG and it does not require special hardware and therefore can be implemented on any modern TD-NMR spectrometer. [1,6,8,11] The theory for the evolution of the NMR signal submitted to a train of pulses (SSFP sequence) is presented in section 2.2.

2 THEORY

2.1 Amplitude of the NMR signal in the SSFP regime

SSFP sequences have been used to improve the SNR in pulsed NMR spectroscopy since 1958.[12] It is a simple pulse sequence consisting of a train of radiofrequency pulses (*rf*) with the same phase and flip angle (θ), and the time between pulses (T_p) is shorter than T_2 ($T_p < T_2$) (Figure 1).

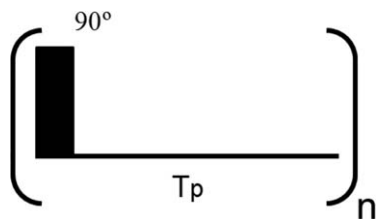

Figure 1 *Diagram of the SSFP pulse sequence, where n is number of rf pulses.*

In 1966, Ernst and Anderson derived the analytical solution for the SSFP regime.[13] They showed that the SSFP signal is composed of FID and echo signals. The echo component (M-) immediately preceding the pulse is given by equations 1 through 3, and the FID (M+) component is given by equations 4 through 6.

$$M_x^- = \frac{M_0(1-E_1)[E_2 \sin\theta \sin\Phi]}{D} \quad (1)$$

$$M_y^- = \frac{M_0(1-E_1)[E_2 \sin\theta \cos\Phi - E_2^2 \sin\theta]}{D} \quad (2)$$

$$M_z^- = \frac{M_0(1-E_1)[1-E_2 \cos\Phi - E_2 \cos\theta(\cos\theta - E_2)]}{D} \quad (3)$$

$$M_x^+ = M_x^- \quad (4)$$

$$M_y^+ = \frac{M_0(1-E_1)[(1-E_2 \cos\Phi)\sin\theta]}{D} \quad (5)$$

$$M_z^+ = \frac{M_0(1-E_1)[E_2(E_2 \cos\Phi) + (1-E_2 \cos\Phi)\cos\theta]}{D} \quad (6)$$

where $D=[(1-E_1\cos\theta)(1-E_2\cos\Phi)]-[(E_1-\cos\theta)(E_2-\cos\Phi)E_2]$, with the precession angle $\Phi=\Omega t$, offset frequency $\Omega=\varpi_{ref}-\varpi_0$, and relaxation components $E_1=\exp(-T_p/T_1)$ and $E_2=\exp(-T_p/T_2)$.

With these equations it is possible to calculate the magnitude of the magnetization in the *xy* plane after the nth *rf* pulse, assuming $T_p << T_1$.[14]

$$|\vec{M}|=\frac{M_0\,|\sin(\theta)|\sqrt{2-2\cos\Phi}}{(1+\cos\theta)(1-\cos\Phi)+(1-\cos\theta)2T_1/T_2} \tag{7}$$

Therefore, the amplitude of the SSFP signal is dependent upon the flip angle θ, precession angle $\Phi=\Omega T_p$ and T_1/T_2 ratio.[7,14] The magnetization goes to null $|M|\to 0$, when $\sqrt{2-2\cos\Phi}\to 0$ or

$$\Phi=n2\pi \tag{8}$$

where *n* is an integer.

Figure 2 shows the dependence of the NMR signal amplitude upon the precession angle Φ and frequency offset for θ = 45^o and 90^o, T_p = 0.3 ms, T_1 = 150 ms and T_2 = 50 ms, according to equation 7.

For $\Phi=n2\pi$, the magnetization is minimal because the FID and echo components are dephased by 180º, resulting in destructive interference. For $\Phi=(2n+1)\pi$, the FID and echo are in phase and the constructive interaction creates a maximum signal intensity when θ = 90^o.[7]

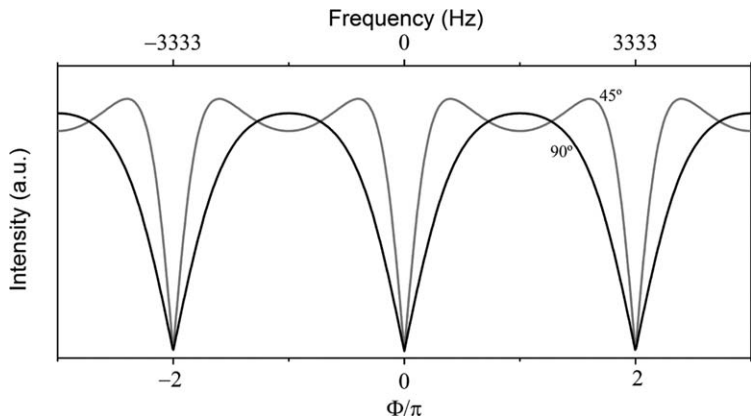

Figure 2 *Dependence of the normalized SSFP signal amplitude upon the precession angle Φ and frequency offset when θ = 45° and 90°, T_p = 0.3 ms, T_1 = 150 ms and T_2 = 50 ms.*

Equations 1 through 6 show that the behavior of the magnetization in the SSFP regime is complex and depends on a series of experimental parameters, such as Φ, θ, Ω, T_p, T_1 and T_2. However, these analytical descriptions do not include the effect of other parameters on the SSFP signal, such as T_p variation and phase alternation.

To fully describe the SSFP phenomenon we have numerically simulated (Matlab)[15] the

influence of the all the above parameters based on the rotation matrix and the method of the sum of isocromats[16], in which the Lorentzian distribution was assumed[9].

$$g(\varpi 0) = \frac{(T_2^* / \pi)}{1 + (\varpi - \varpi_0)^2 T_2^{*2}} \tag{9}$$

and

$$\frac{1}{T_2^*} = \frac{1}{T_2} + \frac{1}{T_{2inom}} \tag{10}$$

where $T_{2inom} = 1/\gamma \Delta B_0$.

Figure 3 shows the numerical simulations for the TD-NMR signals after reaching the steady state regime. The time necessary to reach the steady state is discussed in section 2.2. The steady state signals were simulated using T_1 = 100 ms, T_2 = 50 ms, T_2* = 0.5 ms, θ = 90°, a frequency offset of 8.333 (Figures 3A to D) and 6.666 (Figure 3E) KHz and various T_p values. In Figure 3, the pulse is observed in the center of the window (t = 0). The FID component after the pulse is on the right side of t = 0, and the echo component is on the left side of the pulse.

Figure 3A shows the NMR signal for $T_p = 5T_1$. This figure shows an FID signal with maximum amplitude. With this pulse repetition rate, the echo signals are not observed.

Figure 3B shows the NMR signal for $T_p = T_2$. In this condition the NMR signal is in the SSFP regime and is composed of an FID and an echo between the pulses. The FID signal has higher amplitude than the echo signal. The FID amplitude in Figure 3B is lower than that of the FID in Figure 3A because $T_p < 5T_1$, which does not allow the return of the magnetization to thermal equilibrium.

Figures 3C to E depicts more than one period between the pulses, in the interval of -1.5 to 1.5 ms. Figure 3C shows two periods for $T_p = 2.9T_2$* in which the FID and echo signals have similar amplitudes, and the FID decays faster than T_2* compared to the FID decay in Figures 3A and B. This faster decay is due to the partial destructive interaction between the FID and echo in the center of the SSFP signals.

Figures 3D and E show the SSFP signals for T_p = 0.3 ms < T_2*. In this condition the overlap between the FID and the echo signal is maximal, yielding a special SSFP regime, known as Continuous Wave Free Precession (CWFP).[6,10] The amplitude of CWFP signal is strongly dependent upon $\Phi = \Omega T_p$, as shown in Figure 2. Figure 3D depicts the maximum CWFP signal when Φ=5π with a frequency offset of 8.333 KHz (constructive interference) and Figure 3E depicts the minimal CWFP signal when Φ=4π with a frequency offset of 6.666 KHz (destructive interaction).

According to equation 11, the magnitude of the CWFP signal when θ = 90° and Φ = 5π is dependent upon the T_1/T_2 ratio. [1,6,11]

$$|M_{ss}| = \frac{M_0}{1 + \frac{T_1}{T_2}} \tag{11}$$

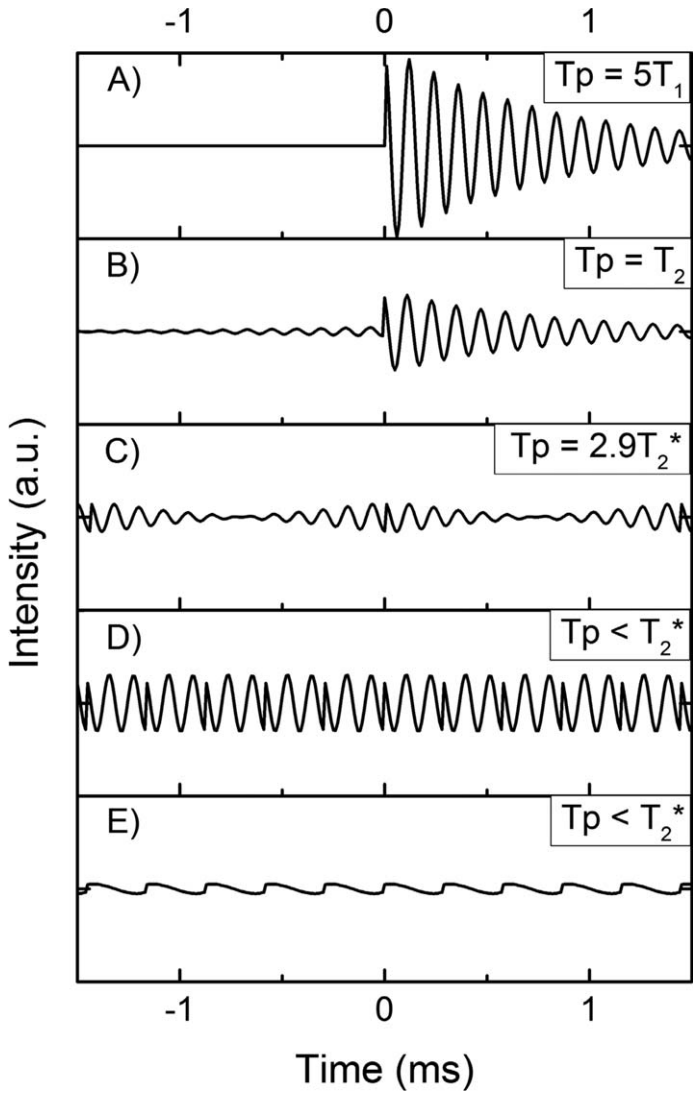

Figure 3 *NMR signals simulated numerically using T_1 = 150 ms, T_2 = 50 ms, T_2* = 0.5 ms and several T_p values. A) $T_p = 5T_1$, B) $T_p = T_2$, C) $T_p = 2.9T_2$*, D) and E) $T_p < T_2$*. The frequency offset is 8.333 KHz (A to D) and 6.666 KHz (E).*

The magnitude of the CWFP signal is not dependent on the pulse repetition rate, as in conventional pulse sequences (Figure 3A and B). Instead, it depends on the T_1/T_2 ratio (equation 11), and the repetition time can be short ($T_p << T_1$, $T_2 < T_2$*) and without saturation (Figure 3D). Therefore, thousands of CWFP signals can be averaged during one T_1 period, thereby enhancing the SNR by one order of magnitude in the same average time used for FID or echo signals.[7,8] The magnitude of CWFP signals has been used in quantitative analysis, in conventional benchtop spectrometers and in online measurements using long Halbach and superconducting magnets[1,6,9].

2.2 Transient SSFP signal

The evolution of the NMR signal, submitted to a train of pulses (SSFP sequence) has been used in several applications in TD-NMR. In 1977, Kronenbitter and Schwenk proposed the use of the transient SSFP signal to measure T_1 and T_2.[17] The method consists of two steps: First, the measurements of the T_1/T_2 ratio, by measuring the maximum amplitude of the SSFP signal as a function of the flip angle (θ) to obtain the optimum θ (θ_{opt}), and second, the use of θ_{opt} to measure the time constant (T*), equation 12, for the evolution of the SSFP signal, yielding the T_1+T_2 value. With these two measurements it is possible to determine both relaxation times.[17]

$$T^* = \frac{2T_1T_2}{T_1(1-\cos\theta)+T_2(1+\cos\theta)} \tag{12}$$

In 2006, Venâncio T. et al reported that is not necessary to use two steps to measure both relaxation times when the transient SSFP signal is obtained with $\theta = 90^o$ and $\Phi = (2n+1)\pi$.[11]

Figure 4 shows the evolution of the magnitude of the CWFP signal from the first pulse to the stationary regime ($|M_{ss}|$). This signal undergoes two transient regimes before the steady state is reached. The first transient regime (dark grey) shows an alternation of the amplitude between even and odd pulses followed by signal decay, with the time constant of T_2*. When the alternations subside, the signal reaches a quasi-stationary state (light grey). The decay of the quasi-stationary state to the stationary state (white) is governed by the time constant T*. For $\theta = 90^o$, equation 12 is reduced to:

$$T^* = \frac{2T_1T_2}{T_1+T_2} \tag{13}$$

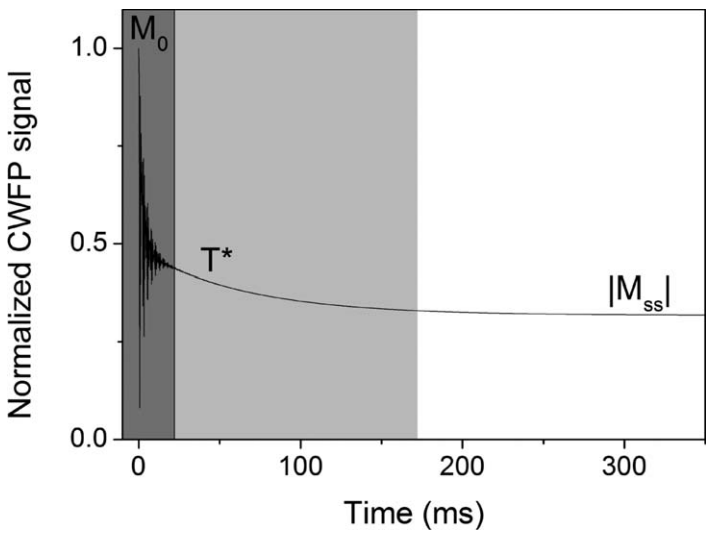

Figure 4 *Evolution of the CWFP signal magnitude from the first pulse to the stationary state (M_{ss}).*

Upon rewriting equations 11 and 13, we obtain the following:

$$T_1 = \frac{T^*/2}{|M_{ss}|/M_0} \quad \text{and} \quad T_2 = \frac{T^*/2}{1 - |M_{ss}|/M_0} \tag{14}$$

Therefore, upon measuring the magnitude of the signal after the first pulse $|M_0|$, the magnitude of the CWFP signal $|M_{ss}|$ and T*, it is possible to calculate the relaxation times in a single scan experiment using equation 14.[11] The T* value is calculated by fitting the T* decay with an exponential decay function. The T_1 and T_2 values are obtained with single CWFP experiments are similar to those obtained by Inversion recovery (T_1) and CPMG (T_2) pulse sequences.[11]

When $T_1 \sim T_2$ there is only a small difference in amplitude between the quasi-stationary state and the stationary state of the CWFP signal. This might yields, a T* with large error when the CWFP signal has low SNR.[4]

To solve this problem we proposed the use of a Carr-Purcell sequence, using 90°-refocusing pulses, also known as CP-CWFP (Figure 5).[4] The only difference between CWFP and CP-CWFP sequences is the addition of a pulse, which separates the CWFP pulse train (Figure 1) by the time interval $T_p/2$. The effect of this modification is shown in Figure 6. The CP-CWFP signal intensity decays to a minimum value (quasi-stationary state) and then increases to the same amplitude observed in the CWFP regime. As shown in Figure 6A, the amplitude variation during T* is much more pronounced in CP-CWFP than in the CWFP sequence. This results in improved fitting of T* for a sample when $T_1 \sim T_2$.

Conversely, CP-CWFP signals yield a small difference in amplitude during T* when $T_1 >> T_2$ (Figure 6B). In this case, T* of CWFP can be fitted with minimal error. When $T_1 > T_2$ (Figure 6C) CWFP and CP-CWFP have similar amplitude variations during T*.

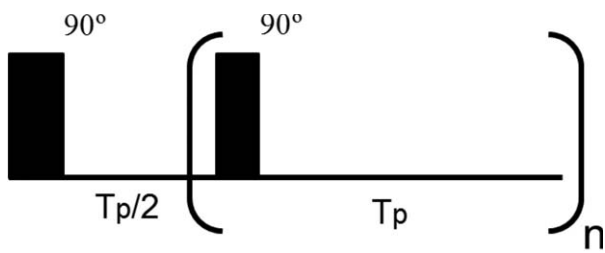

Figure 5 *Diagram of CP-CWFP pulse sequence.*

In addition to measuring the relaxation times, the CWFP or CP-CWFP signals have been used to obtain qualitative and quantitative information from food products using uni- and multivariate analyses.[1,5,18-20]

The ratio $|M_{ss}|/M_0$ of the CWFP signal has a higher correlation with intramuscular fat content and water loss during cooking (cooking loss) in beef than T_2 measured by CPMG.[18,19] $|M_{ss}|/M_0$ also has a higher univariate correlation with animal sex and genetics than CPMG.[20] However, the multivariate analysis results in similar beef classification (sex and genetics) using the full CPMG and CWFP data sets.[20]

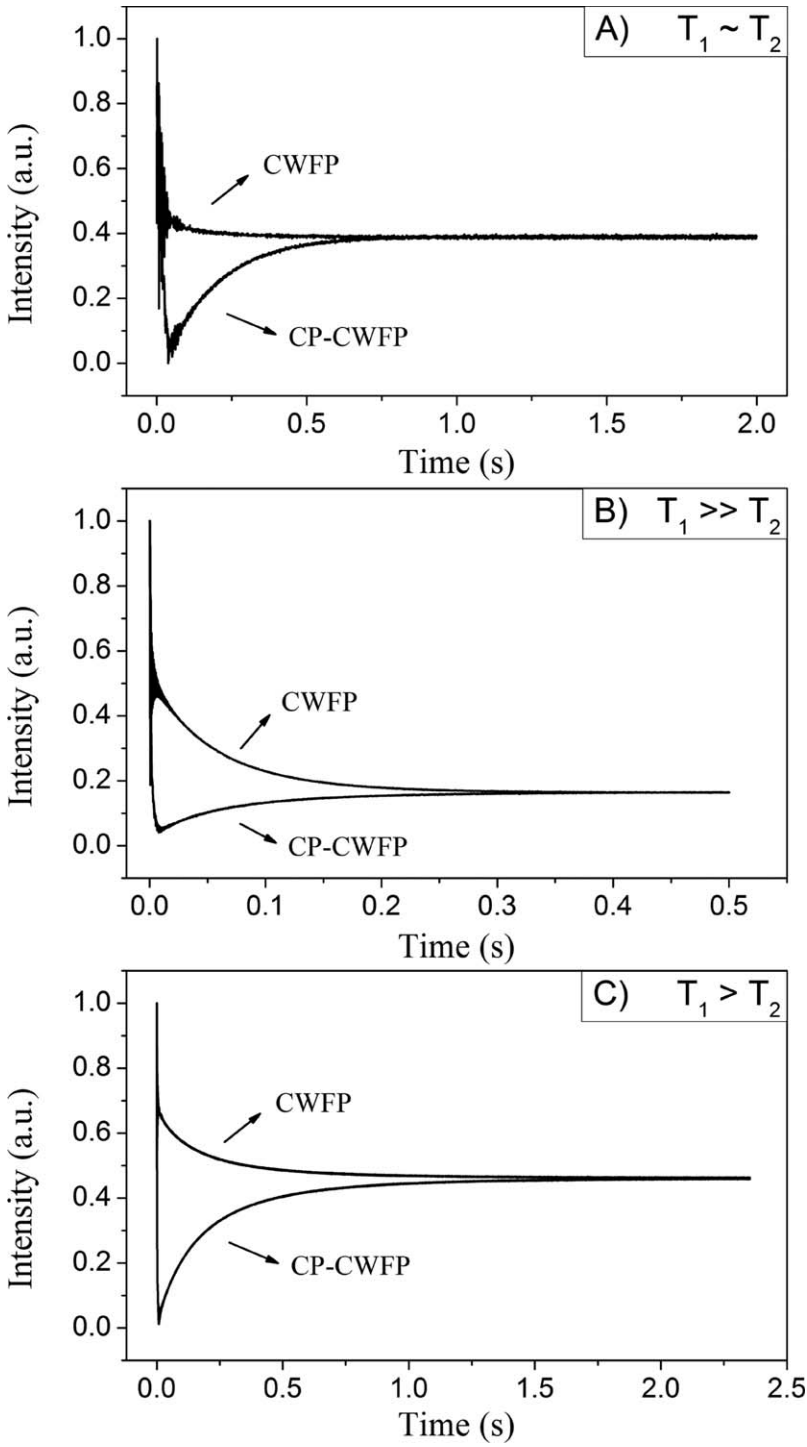

Figure 6 *Experimental CWFP and CP-CWFP signal intensities for tap water (A), hotdog sausage (B) and mayonnaise (C) samples.*

CWFP and CP-CWFP sequences are a useful alternative to CPMG because the analysis can be performed in the same amount time, they do not require special hardware and, therefore, the sequences can be implemented in any modern TD-NMR spectrometer. The scripts for the CWFP and CP-CWFP sequences for the Minispec spectrometer (BRUKER) are described in the appendix I.

3 COMPARISON BETWEEN CPMG, CWFP AND CP-CWFP ANALYSES OF FRESH AND PROCESSED FOOD PRODUCTS

To demonstrate the potential of CWFP and CP-CWFP analyses in food science we compared them with CPMG, using a benchtop spectrometer, SLK 100, Spinlock (Córdoba, Argentine).
Figure 7 shows the CPMG, CWFP and CP-CWFP signals for ripe and unripe grapes and bananas. Figures 7A and B show the CPMG and CWFP/CP-CWFP signals of ripe (brix = 18) and unripe (brix = 10) grapes. The CPMG decays for both grapes were similar. However, the ripe grapes, with a high sugar content, shows a more rapid CPMG decay (1.0 s) than the unripe grapes (1.2 s), with low sugar content, as expected.[21] Similar results for ripe and unripe grapes were also observed for CWFP and CP-CWFP signals (7B). The small and large variations in the amplitude during T* for CWFP and CP-CWFP, respectively, and similar $|M_{ss}|$ amplitudes (0.37 and 0.4, respectively) indicate that $T_1 \sim T_2$ for both grapes.
Conversely, the CPMG, CWFP and CP-CWFP signals (Figures 7C and D) of ripe and unripe bananas are much more distinct. The decay of the CPMG signal (7C) of unripe bananas (0.20 s) is longer than the decay of ripe bananas (0.09 s). This is observed elsewhere and is related to the large difference between the consistencies of bananas in these two conditions.[22]
This difference between ripe and unripe bananas was also observed in CWFP and CP-CWFP signals (Figure 7D). The variation in the amplitude of T* decay and $|M_{ss}|$ (0.40 and 0.24, respectively) indicates that $T_1 > T_2$ in ripe bananas and that $T_1 >> T_2$ in unripe bananas.
Similar results are also observed for processed food products. Figure 8 shows the CPMG, CWFP and CP-CWFP signals for hotdog sausages (two brands) (Figures 8A and B) and regular and light mayonnaises (Figures 8C and D). The CPMG, CWFP and CP-CWFP signals (Figures 8A and B) of the two hotdog sausages were similar. The duration of the CPMG signal is longer for brand I sausage (0.062 s) than brand II (0.056 s), indicating a minimal difference in the composition of the two sausages. The T* and $|M_{ss}|$ amplitude (0.16) of the two sausages indicate that $T_1 >> T_2$.
Figures 8C and D depict the CPMG, CWFP and CP-CWFP signals of the regular (33% fat) and light (24% fat) mayonnaise samples from the same brand. Although the fat content in the light mayonnaise is 30% lower, the duration of the CPMG signal is only slightly longer (0.15 s) than in regular mayonnaise (0.14 s).[7] However, the T* and M_{ss} of CWFP and CP-CWFP analyses are more sensitive to the fat content in mayonnaise. The $|M_{ss}|$ of regular mayonnaise is lower (0.22) than the light mayonnaise (0.28), thus, reflecting the difference in fat content.
It is possible to process the full CWFP and CP-CWFP data using multivariate analysis; this would provide more information about the products and improved calibration and classification models compared to univariate analysis.[5,7,20]

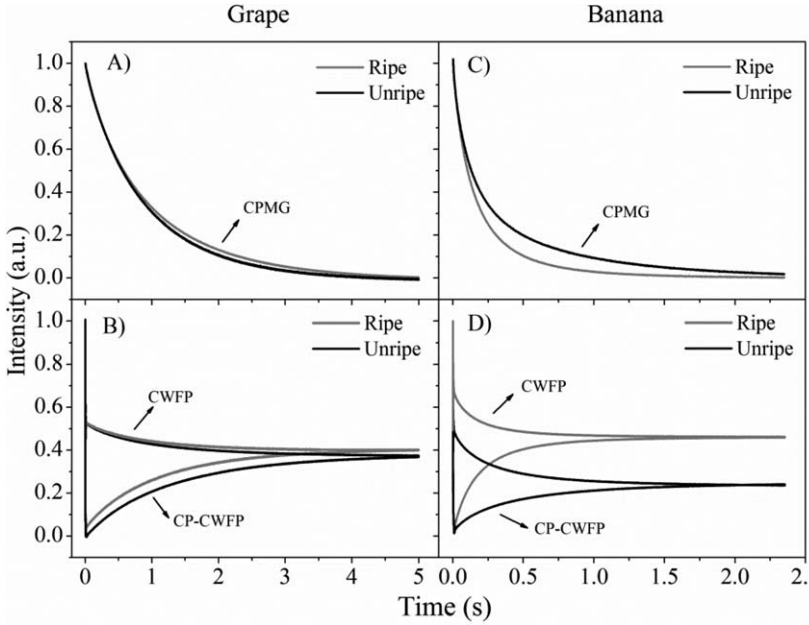

Figure 7 *NMR signals intensities obtained for ripe and unripe grapes (A and B) and bananas (C and D) using CPMG, CWFP and CP-CWFP pulse sequences.*

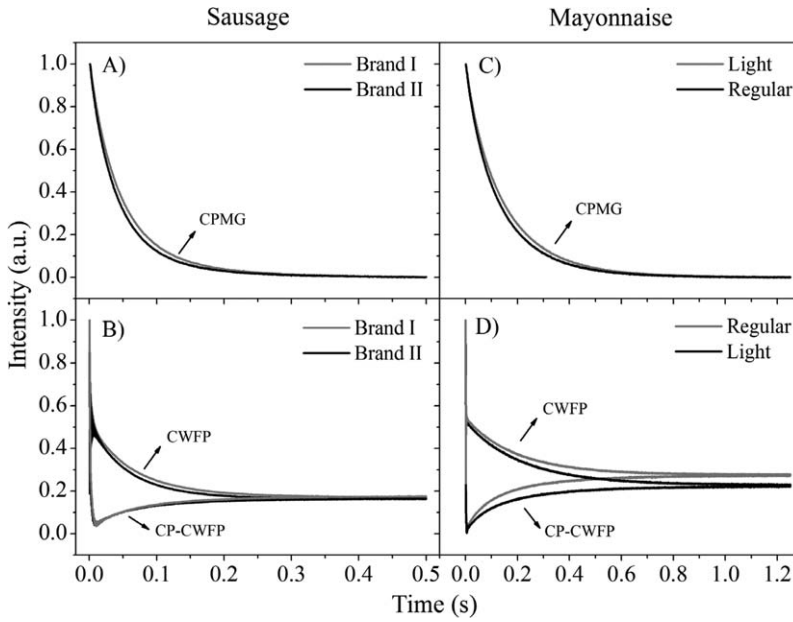

Figure 8 *NMR signal intensities obtained from two brands of hotdog sausages (A and B), and regular and light mayonnaise (C and D) using CPMG, CWFP and CP-CWFP pulse sequences.*

4 CONCLUSION

Given the results presented, we conclude that the SSFP pulse sequences, using CWFP and/or CP-CWFP regimes, are alternatives and/or complementary methods to CPMG. The SSFP sequences are more efficient than CPMG for samples in which the change in T_1 is greater than T_2. Furthermore, these sequences can be implemented in modern TD-NMR spectrometers.

Acknowledgements

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APPENDIX I

```
#-CWFP final version- uses signal averaging for processing data#
#Eduardo Ribeiro de Azevedo: azevedo@ifsc.usp.br#

program setup();

#----------------------Get main parameter from parameter table------------#

par;                              # starting parameter definition     #
  scans (16);
  rd    (1.000000000);
  gain      (66);
  dbw   (20000.000000);
  abw   ("broad");
  off_comp ("off");
  det_mode ("complex");
  magn_mode ( "PSD" );                  # phase sensitive magnitude
detection       #
  dig_res ("fast");
endpar;                           # end of parameter definition   #

return(TRUE);

#----------------------Application configuration table--------------------#

program config();

int temp_int;
real temp_real;
char temp_string[128];

strcpy( temp_string, get_text(CALIBRATION_FILE,"fname" ));
if(ERROR) set_conf (CI_INPUT,TRUE,"File
Name","F:\usuarios\Eduardo\Sequencias");
else set_conf (CI_INPUT,TRUE,"File Name",temp_string); endif;

temp_real = get_real(CALIBRATION_FILE,"tauus");
if(ERROR) set_conf (CI_INPUT,TRUE,"Delay tau [us]: ","300");
else set_conf (CI_INPUT,TRUE,"Delay tau [us]",temp_real); endif;

temp_int = get_int(CALIBRATION_FILE,"npi");
if(ERROR) set_conf (CI_INPUT,TRUE,"Number of 90 deg. pulses in
cwfp","100");
else set_conf (CI_INPUT,TRUE,"Number of 90 deg. pulses in cwfp",temp_int);
endif;

temp_int = get_int(CALIBRATION_FILE,"flag_cpcwfp");
if(ERROR) set_conf (CI_SELECT,TRUE,"Activate CP-CWFP",FALSE);
else if(temp_int==1) set_conf(CI_SELECT,TRUE,"Activate CP-CWFP",TRUE);
    else set_conf(CI_SELECT,TRUE,"Activate CP-CWFP",FALSE); endif;
endif;

temp_int = get_int(CALIBRATION_FILE,"savesig");
if(ERROR) set_conf (CI_SELECT,TRUE,"Save Signal",FALSE);
else if(temp_int==1) set_conf(CI_SELECT,TRUE,"Save Signal",TRUE);
    else set_conf(CI_SELECT,TRUE,"Save Signal",FALSE); endif;
```

```
endif;

set_conf (CI_TEXT,TRUE,"File Name and Delays");
get_conf ("Options","FID/ECHO aquisition",0);
if (ESC) goto escape; endif;

print_line (CALIBRATION_FILE,"fname", tst_conf (CI_INPUT,0));
print_line (CALIBRATION_FILE,"tauus", ator ( tst_conf (CI_INPUT,1) ));
print_line (CALIBRATION_FILE,"npi", atoi (tst_conf (CI_INPUT,2) ));
print_line (CALIBRATION_FILE,"flag_cpcwfp", tst_conf (CI_SELECT,0));
print_line (CALIBRATION_FILE,"savesig", tst_conf (CI_SELECT,1));

label escape;
return(TRUE);

#--------------------Initialize parameters for pulse sequence-------------#

program measure();
real acq, tau, tauus, pw, pw1, rdt,d1,gainr;
real x_array[3204800], y_array[3204800],yi_array[3204800];
int npi, cnt, nsc, ndp,flag_cpcwfp,save_flag;
int ph90[20], ph90cp[20],phrc[20];
charhlp fname[256], name[256], name1[256];

#---specific parameters ------------#

strcpy ( fname, get_text(CALIBRATION_FILE,"fname" ));
acq = 20/1000.0; # fixed acq time of 20 us with asd#
tauus = get_real(CALIBRATION_FILE,"tauus");
npi = get_int(CALIBRATION_FILE,"npi");
flag_cpcwfp = get_int(CALIBRATION_FILE,"flag_cpcwfp");
save_flag = get_int(CALIBRATION_FILE,"savesig");

#---global parameters ------------#

pw     = get("90P");
rdt = get ("RDT");
d1 =  get_rd;
gainr = get_gain;
nsc=get_scans;

print_line(RESULTBOX, "acq =  ", acq, " ms. " );

#--- Experiment Messages------------#

print_line( RESULTBOX, "------------------------" );
print_line( RESULTBOX, "  " );

if(flag_cpcwfp==1)

    print_line( RESULTBOX, "(CP-CWFP is activated (CP-CWFP)");

else

    print_line( RESULTBOX, "Doing Standard CWFP experiment");

endif

print_line( RESULTBOX, "  " );

print_line( RESULTBOX, "------------------------" );
```

```
# ------------convert variables units for calculatations---------------- #
tau=tauus/1000;      # convert to ms #
pw1 = pw/1000;       # convert to ms #

#-----------------initialize counters-----------------------------------#
cnt = 0;

#----Check for time limits------#

if ((tau - pw1/2.0 - acq/2 ) < rdt)
beep;

print_line(RESULTLINE, "Error, aquisition starts inside dead time. Please,
increase tau to be greater than: ", round((pw1/2.0 + acq/2 + rdt)*1000), "
us. " );
goto stopseq;
endif;

if ((pw > 30))
beep;
print_line(RESULTLINE, "Pulse lenght exceed the maximum limit. Please,
decrease de pulse length to less than 30 us");
goto stopseq;
endif;

if (npi > 30000)
beep;

print_line(RESULTLINE, "Too many pulses, please reduce npi to less than
30000");
goto stopseq;
endif;

#------------------  phase cycling ---------------------------------------#

ph90[0]  = 0;   ph90cp[0]  = 0;    phrc[0] = 0;
ph90[1]  = 90;  ph90cp[1]  = 90;   phrc[1] = 90;
ph90[2]  = 180; ph90cp[2]  = 180;  phrc[2] = 180;
ph90[3]  = 270; ph90cp[3]  = 270;  phrc[3] = 270;
ph90[4] = REDO; ph90cp[4] = REDO;  phrc[4] = REDO;

#-------------------Start Actual pulse sequence--------------------------#

pulses;

    sd (1000e-3);           # first delay for minimum time durantion of the
sequence #
    cta;

if(flag_cpcwfp==1)

    ssp ( pw, ph90cp);            # 90 degree pulse for CPCWFP #
    sd(rdt);
    asd (acq, phrc);                    # acq/2 delay is added to
compensate for an extra acq/2 delay in the first pulse of the cwfp loop #
    sd ( tau/2.0 - pw1/2. - rdt - acq );
else

    ssp ( pw, ph90);              # 90 degree pulse for CWFP #
    sd(rdt);
    asd (acq, phrc);            # data acquisition #
```

```
    sd ( tau - pw1/2. - rdt - acq );                # acq/2 delay is added
to compensate for an extra acq/2 delay in the first pulse of the cwfp loop
#

endif

   ploop(npi-1)                  # CWFP loop#

        ssp ( pw, ph90);       # 90 degree pulse for CWFP #
        sd ( tau/2.0 - pw1/2. - acq/2.);
        asd (acq, phrc);             # data acquisition #
        sd ( tau/2.0- pw1/2. - acq/2.);

 cnt=cnt+1; # loop counter #

 endploop;

endpulses;                        # end of pulse sequence     #

measure;                        # begin of data evaluation    #

#---------------------------------Saving echoes am-------------------------#

sig_abscissa(-1,-1,x_array);
sig_ordinate(-1,-1,y_array);   # write real part of the echoes amplitudes
in y_array#
sig_swap;
sig_ordinate(-1,-1,yi_array);  # write imaginary part of the echoes
amplitudes in yi_array#
sig_swap;

#-------------------Saving decay curve-----------------------------------#

if (save_flag == 1)

    strcpy (name,fname);
    strcat(name, ".dat");
    file_name (ASCII_FILE, name);
    ndp = data_points( -1, -1 );
    if(flag_cpcwfp==1)

        ndp=ndp-1;

    endif
   print_table(ASCII_FILE, x_array, y_array, yi_array, ndp);

#-------------------Saving acquisition parameters------------------------#

# Show in Resultbox#

    print_line( RESULTBOX, "-------------------------------------------"
);
    print_line( RESULTBOX, "Data file: ",fname);
    print_line( RESULTBOX, "-------------------------------------------"
);
    if(flag_cpcwfp==1)
        print_line( RESULTBOX, " Sequence: ","CP-CWFP");
    else
        print_line( RESULTBOX, " Sequence: ","Standard CWFP");
    endif
```

```
    print_line( RESULTBOX, "-------------------------------------------"
);
    print_line( RESULTBOX, "                Parameters" );
    print_line( RESULTBOX, "-------------------------------------------"
);
    print_line( RESULTBOX, "  " );
    print_line( RESULTBOX, "pw = ",  pw," us");

    print_line( RESULTBOX, "acq = ",  acq," ms");
    print_line( RESULTBOX, "tau = ",  tauus," us");
    print_line( RESULTBOX, "d1 = ",  d1," s");
    print_line( RESULTBOX, "number of scans = ",  nsc);
    print_line( RESULTBOX, "number of echoes = ",  npi);
    print_line( RESULTBOX, "receiver gain = ",  gainr);

# save in name_parameters name_parameters.txt file#

    strcpy (name1,fname);
    strcat(name1, "_parameters.txt");
    file_name (ASCII_FILE, name1);
    print_line( ASCII_FILE, "-------------------------------------------"
);
    print_line( ASCII_FILE, "Data file: ",fname);
    print_line( ASCII_FILE, "-------------------------------------------"
);
    if(flag_cpcwfp==1)
        print_line( ASCII_FILE, " Sequence: ","CP-CWFP");
    else
        print_line( ASCII_FILE, " Sequence: ","Standard CWFP");
    endif
    print_line( ASCII_FILE, "-------------------------------------------"
);
    print_line( ASCII_FILE, "                Parameters" );
    print_line( ASCII_FILE, "-------------------------------------------"
);
    print_line( ASCII_FILE, "  " );
    print_line( ASCII_FILE, "pw = ",  pw," us");

    print_line( ASCII_FILE, "acq = ",  acq," ms");
    print_line( ASCII_FILE, "tau = ",  tauus," us");
    print_line( ASCII_FILE, "d1 = ",  d1," s");
    print_line( ASCII_FILE, "number of scans = ",  nsc);
    print_line( ASCII_FILE, "number of echoes = ",  npi);
    print_line( ASCII_FILE, "receiver gain = ",  gainr);

endif

#--------------------finishing-------------------------------------------#
beep;
if ( ESC ) print_line( CONFIRMBOX, "USER INTERRUPT !" ); return( FALSE );
endif;
label stopseq;
return( TRUE );
```