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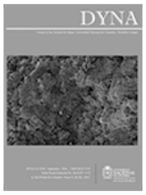

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Effect of amplitude and pulse in low frequency ultrasound on oil/water emulsions

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Abstract
The application of ultrasound within advanced or emerging technologies requires selecting parameters that depend on the target application. This study evaluated pulse and amplitude parameters of oil/water emulsions (20:80% w/w) using low frequency probe ultrasound equipment (20 KHz). A categorical multilevel factorial design was used with Design Expert® in which the following pulse treatments were defined: continuous, pulse 20:20 (on:off) and pulse 30:30 (on:off), for five minutes. Six amplitudes (30, 36, 42, 48, 54 and 60 µm) were evaluated for the following response variables: separation of phases in emulsion, temperature and accumulated power. The results showed that the best condition to obtain an emulsion with less phase separation was the 20:20 (on:off) treatment with an amplitude of 42 µm. The ultrasound probe application parameters that were obtained will enable the design of stable products from low-fat emulsions.

Keywords: Ultrasound probe, low frequency; emulsion; pulse; amplitude; phase separation.

Efecto de la amplitud y pulsación en ultrasonido de sonda a baja frecuencia sobre emulsiones aceite/agua

Resumen
La aplicación de ultrasonido dentro del concepto de tecnologías avanzadas o emergentes, requiere de la selección de parámetros según sea el objetivo de su aplicación. En esta investigación fueron evaluados parámetros de pulsación y amplitud para mezclas aceite/agua (20:80 % p/p), empleando un equipo de ultrasonido de sonda de baja frecuencia (20KHz). Los tratamientos de pulsación utilizados fueron: continuo, pulsación 20:20 (on:off) y pulsación 30:30 (on:off) durante cinco minutos; se evaluaron seis amplitudes (30, 36, 42, 48, 54 y 60 µm) sobre las variables de respuesta: separación de fases en la mezcla, temperatura y potencia acumulada. Los resultados obtenidos analizados por Design Expert® por medio de un diseño factorial multinivel categórico mostraron que la mejor condición para obtener una mezcla con menor separación, fue el tratamiento 20:20 (on:off), con una amplitud de 42 µm.Lo anterior evidencia el uso de ultrasonido de sonda como una metodología potencial para la homogenización de mezclas.

Palabras clave: Sonda de ultrasonido, baja frecuencia; emulsión; pulsación; amplitud; separación de fases.

1. Introduction

Ultrasound is characterized by the generation of acoustic waves, which require a medium to propagate at a rate that is characteristic to the nature of the wave and the medium through which it propagates [1]. Usually ultrasonic waves are classified according to the human hearing limit by frequency (~20 kHz) [2–6]. Ultrasound parameters include 1) the frequency, which is defined as the number of cycles completed by the wave per unit time ($\boldsymbol{f = 1/T}$) , and 2) the intensity, which is defined as the average energy transmitted through unit area that is perpendicular to the direction of wave propagation ($\boldsymbol{I = (P\_A{}^\wedge 2)/2\rho C}$) , where I corresponds to the acoustic intensity (W/m2), PA is the

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maximum pressure (atm), ρ is the density (kg/m3), and c is the wave velocity in the medium (m/s). The acoustic power is the total energy irradiated by a source per unit time ($\boldsymbol{W} = \boldsymbol{IS}$) where S represents the surface radiant area (m2), and W represents the acoustic power (W). [7].

The effect of high power ultrasound (frequency >20 kHz) is due to wave propagation through materials of different natures, inducing compressions and decompressions of the propagation medium that generate the acoustic cavitation phenomenon. This phenomenon is transmitted through waves that compress and extend the molecular structure of the medium through which waves pass, and this cavitation generates high temperatures and pressures in the medium, generating bubbles [5,8]. Cavitation has been identified as transitory at low ultrasonic frequencies (<100 kHz) where cavitation causes the rapid growth of bubbles, leading them to collapse [7].

Low frequency and high power ultrasound (<20 kHz) is known in the food industry because it changes the physical and chemical properties of food. Different applications of ultrasound in food matrices were studied by [9–12], among which emulsification, an anti-foaming effect, microbiological inactivation, extraction, colour change, and lipid oxidation, among others, have been found. Thus, power ultrasound applications have been considered to be emerging technologies, and they are considered a green technology that offers great potential for a variety of processes [13]. One of the main uses of high-power ultrasound is the application in designing emulsions with a minimum amount of surfactant, wherein the effect depends on the characteristics of the matrix on which it is applied. For example, in the case of two immiscible liquids, if a bubble collapses near the phase boundary of the liquid, the resulting shock wave can provide a very effective mixing of the layers, causing them to require fewer surfactants and producing emulsions, with smaller drop sizes within a size distribution, i.e., producing micro or nanoemulsions, compared to other methods [6,9].

The mixing of two insoluble substances, generally oil in water, can produce emulsions as liquid-liquid dispersing systems. However, these type of emulsions constitute thermodynamically unstable systems and show phase separation or degradation due to temperature changes with phenomena such as flocculation, creaming and coalescence. The above characteristics mean that the system requires energy to disperse a liquid phase (dispersed phase) as droplets into a second phase (continuous phase) [14–17]. However, the use of ultrasound in mixtures as acoustic emulsification systems has been described by [13], in which stable particles were produced in the submicron range with a very narrow particle size distribution, permitting the use of a suitable emulsifier ratio with less energy. Thus, for the food industry, ultrasonic emulsification is of interest for the treatment of products such as fruit juices, mayonnaise, sauces, and salad dressings and for the encapsulation of aromas [6]. The effect of the power of the ultrasound on the

emulsification process was explained by M.A.T.J. Mason [8] as a process of successive disintegration that consists of two stages. In the first stage, the instability of the oil-water interface appears, as illustrated by large oil drops with a diameter of approximately 70 microns, and in the second stage, these large drops breakdown via shockwaves that are produced by cavitation bubbles. Chemat, Zill-E-Huma, and Khan [24] stated that the energy required to produce emulsions via acoustic waves is less than that needed via conventional methods that are used in mechanical emulsification, such as rotor-stator systems and high-pressure homogenizers [14].

Cárcel [7] and Peshkovsky and Bystryak [18] studied the importance of selecting different parameters, such as the frequency, wavelength, intensity, amplitude and power, for ultrasound applications to achieve more stable emulsions over time to preserve their properties and physical characteristics to scale up to the industrial level. The probe size was found to affect the intensity of the emitted wave, and this is a challenge for this technology scaling. Thus, food processing is constantly evolving, which is represented by the different challenges that the food industry should address. The evaluation of ultrasound application parameters, depending on the stability and accumulated power, is necessary to obtain stable emulsions for food applications. Thus, the aim of this study was to choose the amplitude and pulse conditions in a probe-type system for applying continuous or pulsed power ultrasound, depending on oil/water (O/W) emulsion stability, which was defined by the phase separation time.

2. Materials and methods

2.1. Materials

Borges® extra virgin olive oil, which was purchased at a local supermarket in Bogota, and type I ultrapure deionised water that had a resistance of 18.2 MΩ-cm (PURELAB option-Q) were used to prepare the O/W emulsions. The samples were placed in 50 ml falcon tubes.

2.2. Preparation of the O/W emulsion

The O/W emulsion (w/w) was prepared using 80% water and 20% olive oil.

2.3. Ultrasound application

Ultrasound was applied to the O/W emulsion in an ultrasound device (Qsónica®, Q700 sonicator, 700 W RMS, USA) at 20 kHz for 5 minutes via a continuous and pulsed process, this time was selected in order to avoid the overheating [19]. The ultrasound probe was placed inside a sound-reduction box whose interior walls were coated with water-resistant acoustic foam (Fig 1). A 25.4 mm titanium alloy probe with a cylindrical diameter and geometry was used. The probe was placed 10 mm below the surface of the O/W emulsion with a height of 100 mm, and the amplitudes levels were changed between 30, 36, 42, 48, 54 and 60 µm (50%, 60%, 70%, 80%, 90% and 100%, respectively) in the continuous and pulsed processes. Two pulsed treatments were applied: one 20:20 on/off and another 30:30 on/off. These treatments were performed in triplicate.

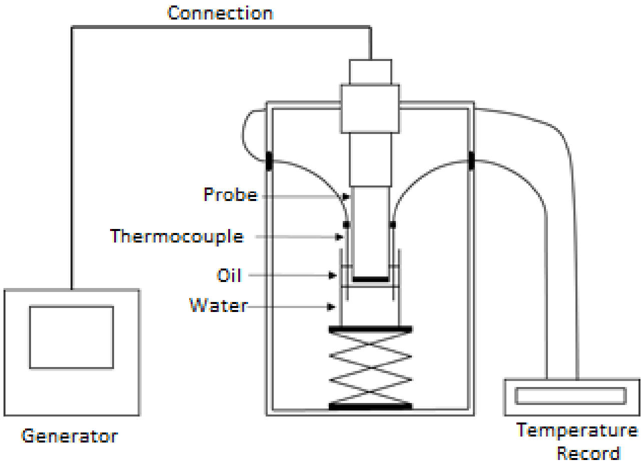

Figure 1. Experimental setup for ultrasound preparation of the emulsion O/W.
Source: Authors

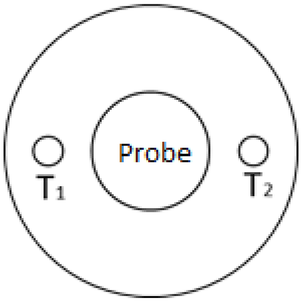

Figure 2. Schematic location of Thermocouples. Source: Authors

The temperature increase for the sonication time for each of the pulse and amplitude conditions was determined using two thermocouples (Digisense®) at a distance of 1 cm from the probe (Fig 1). The following fixed variables were defined: (i) Two types of pulses – continuous pulses in which the wave was transmitted without intermittence, and 20:20 and 30:30 on/off pulses in which the wave was intermittent in time. In the case of the 20:20 (on/off) pulsation, intermittency occurred for 20 seconds during which the wave propagated through the medium and for 20 seconds in which the wave stopped its propagation through the medium. For the 30:30 (on/off) condition, intermittency occurred for 30 seconds during which the wave propagated through the medium and for 30 seconds during which the wave propagation stopped [3]. (ii) Six amplitude levels (30, 36, 42, 48, 54 and 60 µm) were used for the three evaluated pulses. (iii) A sonication time of five minutes was defined as a fixed factor [20–23]

2.4. *Application of ultrasound treatments*

The O/W emulsion was subjected to continuous ultrasound treatment for five minutes for each amplitude. Pulse treatments of 20:20 and 30:30 on-off were performed. Each treatment was evaluated for five minutes for the chosen amplitudes. The temperature (°C) and energy (J) values were recorded for every minute.

After the pulsed or continuous sonication time, the emulsions were kept at room temperature (19 °C) for the next three hours for the phase separation measurement.

2.5. *Response variables*

2.5.1. Phase separation

The time required for the phase separation was evaluated for each treatment during the three hours after sonication. A visible mark on the outer surface of the falcon tubes that contained the samples was labelled every 30 minutes, and an image was captured with a 13 Mpx camera. The images were analysed using ImageJ®.

2.5.2. Temperature record

The temperature was measured using a Digi-Sense Scanning Thermometer (model 92000-0). The temperature was recorded every minute for the continuous ultrasound, after 20 seconds in the on position for the 20:20 pulsed ultrasound, and after 30 seconds in the on position for the 30:30 pulsed ultrasound.

2.5.3. Accumulated power

The power (P) was calculated based on the power dissipated in all of the samples according to the different evaluated amplitude and pulse conditions. The calorimetric method was used for the P calculation using the ratio presented in equation (1):

$$P = mCp\left(\frac{\Delta T}{\Delta t}\right) \quad (t = 0) \tag{1}$$

where “m” is the mass of the sample (kg), Cp is the specific heat of the emulsion (kJ/kg °C), and ΔT/Δt is the temperature change over time (°C/s) [19]. The Cp was calculated using the method by Choi and Okos (1986). The accumulated power was calculated as the sum of the power that dissipated at each measurement point.

2.5.4. Experimental design

The effects of different pulses (continuous, 20:20 and 30:30) and amplitudes (30, 36,42,48,54 and 60 µm) were evaluated in the O/W emulsion. A multilevel categorical factorial design was performed using the statistical software Design Expert Version 9 (Statease Inc., Minneapolis, USA) to process the data. The pulsation and amplitude were the two categorical factors that were defined at different levels. Table 1 shows the experimental design in which analysis of variance (ANOVA) was performed to find significant differences for the pulses and amplitudes in the results of the phase separation. The fit of the model was evaluated using the R2 and adjusted R2 values.

Table 1.
Experimental Design

Run	**Pulse**	**Amplitude** μm
1	Continuous	48
2	20:20	54
3	20:20	48
4	20:20	42
5	30:30	30
6	30:30	60
7	30:30	48
8	Continuous	30
9	30:30	36
10	30:30	54
11	20:20	60
12	Continuous	54
13	Continuous	36
14	20:20	30
15	Continuous	42
16	20:20	36
17	30:30	42
18	Continuous	60

Source: Authors

3. Results and discussion

The selection of parameters as power, frequency and pulse type, helps to define the different effects that are produced by high-power ultrasound when it passes through a medium, which demonstrates their significance depending on the features of the material [6]. Therefore, the density difference between both phases under the influence of gravity leads to phase separation [25]. The following effects are described in our study: (1) the effect of amplitude on the medium temperature and phase separation, (2) the accumulated power during the sonication time, and (3) the effect of the treatment on the phase separation. As stated by Gaikwad and Pandit [26], the use of ultrasonic emulsification can be described using four characteristics: (1) a minimum intensity is required to start the emulsification process, (2) an increase in the power emitted to the matrix enhances the emulsion stability, (3) an increase in the sonication time decreases the dispersed phase droplet size, favouring the formation of microjets, and (4) the forces responsible for the emulsification are the ratio of the force acting on the droplets and the surface tension, which represent the physicochemical properties of the system.

3.1. *Effect* of amplitude and pulse type on temperature

The effect of different amplitudes on the temperature for each ultrasound pulse treatment was evaluated for the three types of pulse. Thus, Fig 3 shows the temperature increase during the 5 minutes of treatment for each type of pulse. This increase has been widely described by Shanmugam, Chandrapala, and Ashokkumar [26] and Pingret, Fabiano-Tixier, and Chemat [27] as being caused by the cavitation phenomenon. Thus, the ultrasound generates a strong resonance in the pulses that are represented in bubbles in the form of micro-jets, which significantly influences the acoustic environment of the liquid [29]. However, amplitude conditions are directly related to temperature increases; thus, the highest temperature was obtained in the range of 60 μm for the pulse treatments in continuous 62°C, 20:20 pulse 55.1°C and 30:30 pulse 57.7°C. This allowed some of the acoustic energy to be degraded as heat, leading to an increase in medium temperature due to the direct relation between the temperature and the solubility of water and oil [7,30]. The pulse and amplitude were found to be significant regarding the effect of the pulses for each amplitude on the temperature (P<0.05).

Figure 3 shows that the 20:20 and 30:30 pulsed treatments have a lower temperature increase compared to the continuous treatment due to the off period of the pulsed wave effect.

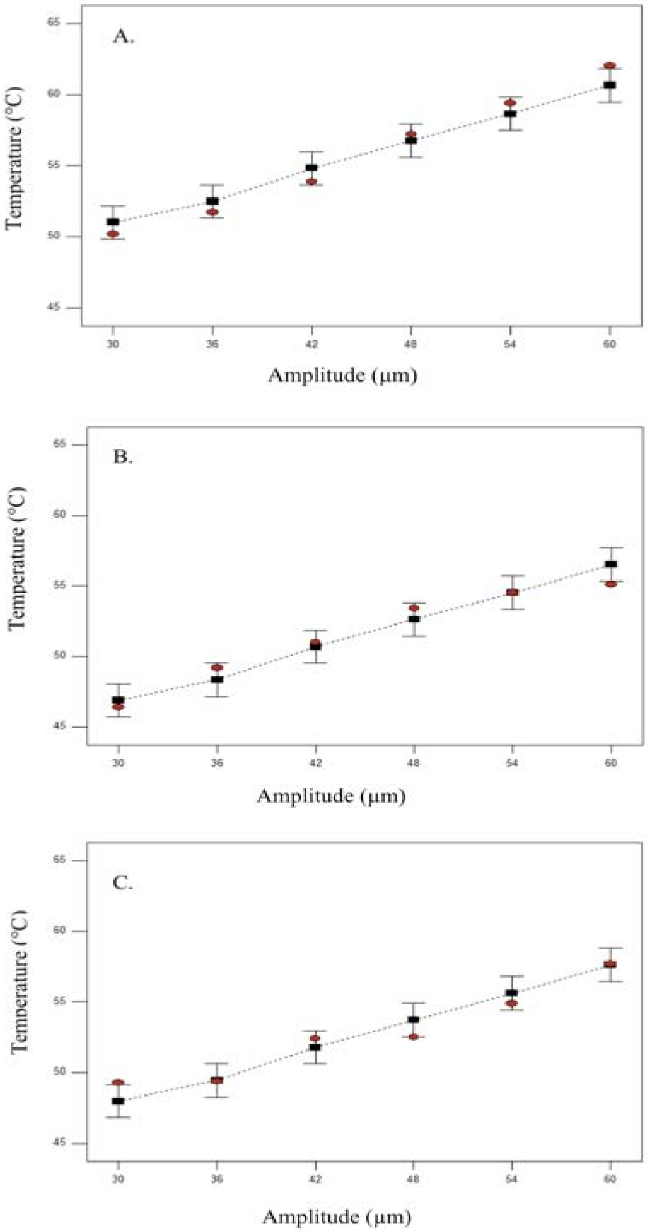

Figure 3. Temperature (°C) during the five minutes of sonication A. Continuous process, B. 20:20 pulse and C. 30:30 pulse.
Source: Authors

Table 2.
Specific heat and accumulated power for continuous process, 20:20 pulse and 30:30 pulse

Amplitude	Continuous		20:20		30:30	
	Cp (kJ/kg°C)	**Aaccumulated Power (W)**	**Cp (kJ/kg°C)**	**Aaccumulated Power (W)**	**Cp (kJ/kg°C)**	**Aaccumulated Power (W)**
30 µm	3.756	101.75	3.754	268.77	3.749	188.06
36 µm	3.759	105.42	3.757	295.65	3.756	193.19
42 µm	3.757	113.26	3.758	312.77	3.758	210.73
48 µm	3.760	122.97	3.755	327.98	3.758	218.42
54 µm	3.762	129.92	3.759	337.85	3.752	230.58
60 µm	3.763	137.10	3.759	341.17	3.761	247.77

Source: Authors

3.2. Power accumulated during the sonication time

Table 2 shows the accumulated power, as calculated from equation (1). An increased accumulated power in relation to the increase in amplitude for all the pulses showed significant differences ($P< 0.05$) between the treatments. The 20:20 pulsed treatment with the amplitude of 60 µm accumulated the largest power during the five minutes of experimentation, which is explained by the stronger and more violent cavitation of bubble collapse in the on:off pulsation moments that generated more power. Anihouvi, Danthine, Kegelaers, Dombree, and Blecker [31] reported that cavitation is the most significant mechanism of power dissipation in a low frequency ultrasound system; thus, changes in cavitation intensity can be related directly to changes in power.

3.3. Effect of treatments on phase separation

The homogenization technology in emulsions has a relevant role in the stability of its phase, and this can be measurement as the minima distance of separation. As shown in Fig 4, the lowest phase separation occurs in the continuous treatment followed by the 30:30 pulsed treatment to 60 µm, with significant differences between them ($P<0.05$).

However, in relationship with the temperature smaller increases benefit O/W emulsion stability over time; nevertheless an increase in O/W emulsion temperature leads to a cavitation decrease. Therefore, it is necessary to maintain the lowest temperature possible for O/W emulsification processes, which is why the 20:20 pulsed treatment in this experiment exhibited a smaller increase in temperature of 27.4°C, 30.2°C, 32°C, 34.4°C, 35.5°C, and 36.1°C for each of the evaluated amplitudes.

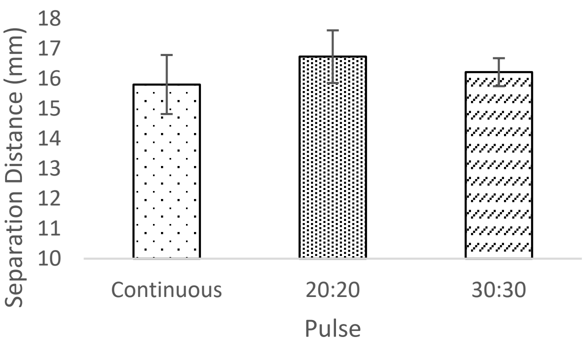

Figure 4. Phase Separation, amplitude 60 µm.
Source: Authors

Table 3.
Correlation of temperature, accumulated power and separation distance

	Temperature	**Accumulated Power (W)**	**Separation Distance (mm)**
Std. Dev.	1.12	8.45	0.29
Mean	53.34	215.74	16.39
R^2	0.9536	0.9942	0.6942
R^2 ajusted	0.9212	0.9901	0.6535

Source: Authors

3.4. Results of the correlation

Table 3 shows the correlation of the response variables, temperature, accumulated power and separation distance. We observed a high correlation with accumulated power, followed by a correlation with temperature and, finally, with the separation distance.

4. Conclusions

The use of technologies such as ultrasound to obtain O/W emulsions requires analysis of the parameters for improved application. Transient cavitation that is generated by the pulse that is related to the temperature during the emulsification process is highlighted. The relevance of the cavitation that is generated by low frequency ultrasonic waves contributes to the degradation of the acoustic energy into heat, favouring the increase in the emulsion temperature. However, smaller temperature increases that do not favour emulsion stability were found due to the accumulation of more power; thus, emulsions exhibiting less separation were obtained with continuous pulsing. This study helped to define the application parameters of ultrasound at a frequency of 20 kHz with a continuous pulse with an amplitude of 60 µm for a tip of 25.4 mm on O/W (20:80) emulsions. These results will allow the application of an advanced and emerging technology with less use of surfactants for low oil-content emulsions that can be the basis for low-fat food products. The aim of this work was to select the best conditions for emulsification, but it must be considered the formulation and the process time because shelf-life of emulsions is determined by lipid oxidation; fat/oil has an important role in determining the properties of emulsions, it influences the appearance (optical properties), sensory evaluation, texture (rheology) and stability

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