Ultrasonic-assisted extraction and automated determination of catalase and lipase activities in bovine and poultry livers using a digital movie-based flow-batch analyzer

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ABSTRACT

An ultrasonic reactor (UR) was developed and coupled to a digital movie-based flow-batch analyzer (DM-FBA) for the ultrasonic-assisted extraction (UAE) and fast determination of catalase and lipase activities in bovine and poultry livers. The lab-made UR mainly consisted of a borosilicate glass container and a piezoelectric disc. The DM-FBA mainly consisted of a webcam, an ultrasonic actuator controller, a peristaltic pump, six solenoid valves, a valve driver, a mixing chamber, a magnetic stirrer, an Arduino Mega 2560, and a personal computer. This setup, named UR-DM-FBA, was controlled by custom software. Ultrasound (US) frequency, US power, sonication time, and concentration of extraction agent were optimized using the Taguchi method. Experiments at silent conditions (mechanical stirring at 1500 rpm) were carried out to evaluate extraction efficiency. Optimized parameters for the UAE of catalase were US frequency of 30 kHz, 2.0 mL of Triton X-100, sonication time of 270 s, and US power of 10.8 W. For the UAE of lipase, the optimized parameters were US frequency of 20 kHz, 0.30 mL of triethanolamine, sonication time of 270 s, and US power of 18 W. Catalase and lipase activities obtained with the UR were, on average, 1.9 \times 10^3\% and 2.0 \times 10^3\% higher than those obtained at silent conditions, respectively, which indicates that that the lab-made UR was capable of extracting these enzymes more efficiently. Determinations using the UR-DM-FBA were highly accurate (relative error ranging from 1.98% to 1.96% for bovine catalase, 0.65% to 0.76% for bovine lipase, 2.03 to 2.08% for poultry catalase, and 0.55% to 0.64% for poultry lipase) and precise (overall coefficient of variation <0.02% for bovine and poultry catalase and <0.2% for bovine and poultry lipase). Results obtained with the proposed system and reference methods were in good agreement according to the paired t-test (95% confidence level). High sampling rates (>69 h⁻¹) and low sample/reagent consumption (<1.6 mL) were also obtained. Due to the highly efficient UAE, the proposed system can be applied for fast and accurate quantification of lipase and catalase in biological samples with low waste generation.

1. Introduction

Bovine and poultry livers are sources of proteins, enzymes, and vitamins, which are nutrients that play a vital role in living organisms. Among the enzymes present in bovine and chicken livers, catalases and lipases are especially important for humans. Catalase is one of the first studied enzymes [1], and its catalytic function involves the degradation or reduction of hydrogen peroxide to water and molecular oxygen. Being a preventive antioxidant, catalase helps the enzymatic defense systems to neutralize free radicals before they can damage cellular and intracellular membranes. Oxidative damage can change the quaternary structure of proteins, leading to cataract, atherosclerosis, ischemic injury/brain reperfusion, arthritis, neurodegenerative diseases, nutritional deficiency, and early aging [2–4]. Furthermore, the literature highlights the importance of catalase in mitigating oxidative stress, which can favor the occurrence of cancer, diabetes, and Alzheimer’s disease [5–7].

Lipases are very important enzymes since they control blood fat levels by catalyzing the hydrolysis of triglycerides into fatty acids and glycerol. Although they are filtered through the renal glomeruli before
being excreted, most of them are reabsorbed by the tubules near the kidneys and metabolized in the pancreas. Like amylases, lipases are released in the bloodstream by lesioned pancreatic acinar cells, which can cause an intestinal infarction, acute cholangitis, and small bowel obstruction [8,9].

In Brazil, meat products are defined as the edible parts of slaughtered animals that are sold in butcher shops. They should be handled with proper hygiene under the supervision of the Ministry of Agriculture, Livestock, and Supply. The animals need to be in good health at the time of slaughter, which should be certified by a veterinarian. Other factors evaluated are the presence of preservatives and the physicochemical, microscopic, microbiological, and sensory characteristics of the meat [10].

Catalase activity in biological samples can be assessed through a variety of methods [11–14], and the most popular of these involves UV spectrophotometric determinations of hydrogen peroxide at 240 nm. Likewise, lipase activity can be determined through [15–17]. However, these methods present some drawbacks such as low analytical frequency, high operational costs, and high waste generation. In this context, ultrasound (US) is a key versatile technology capable of minimizing or solving problems related to the extraction process in biological samples while maintaining the principles of sustainable “green” chemistry [18].

Ultrasound-assisted extraction (UAE) has been considered an attractive alternative to the conventional methods for a variety of food samples [19–22]. Power ultrasound, which is characterized by low frequencies (from 20 kHz to 100 kHz) and high ultrasonic power, induces the occurrence of physical and chemical effects by favoring sufficient interaction between ultrasonic waves and the elastic medium. The most accepted theory about the US action in a liquid medium involves the phenomenon of acoustic cavitation produced by power ultrasound. The collapsing of cavitation bubbles generates shockwaves, inter-particle collisions, and local shear forces within the liquid and on the surface of solid materials or membrane cells [23]. Some processes as fragmentation, micro-jetting, and streaming/microstreaming result from the evolution of cavitation bubbles (oscillation-collapse cycles) and they play an important role in extraction processes involving the mixing and release of soluble substances [24]. Fragmentation associated with micro-jetting increases the surface area of solid particles, which, in turn, increases mass transfer and extraction rates [24]. In biological samples, disrupted cell wall structures cause proteins, enzymes, and other substances to be released in the extraction solution through diffusion [25]. Thus, considering the advantages of UAE, this study aimed at developing a lab-made ultrasonic reactor (UR) for sonochemical extraction of catalase and lipase from biological samples and coupling it to a digital movie-based flow-batch analyzer (DM-FBA) for quantification of their enzymatic activities. The complete setup, named UR-DM-FBA, was applied in the analysis of samples of bovine and poultry liver. US frequency, US power, sonication time, and concentration of extraction agent were optimized using the Taguchi method. Additional experiments using mechanical stirring (silent condition) were performed to compare the efficiency of the UAE process. Finally, catalase and lipase activities in bovine and poultry samples were quantified using the proposed system.

2. Experimental

2.1. Reagents, solutions, and samples

Five samples of bovine liver purchased in markets of João Pessoa city, Paraíba State, Brazil, and four samples bought in Bahia Blanca city, Buenos Aires Province, Argentina, were used for the optimization of enzyme extraction. For quantification of enzyme activity, 15 samples of poultry liver and 15 samples of bovine liver were acquired in markets of João Pessoa city. All samples were stored for a maximum period of three days and kept under refrigeration at −18 °C.

All reagents used in this work were of analytical grade and all solutions were prepared with freshly distilled and deionized water (>18 MΩ cm), purified in a Milli-Q Millipore system. For catalase activity determinations, the following solutions were prepared according to the literature [12,26,27]: 0.5 mmol L−1 Na2HPO4 (Synth, Brazil) buffer at pH 7.0; 0.3 mmol L−1 H2O2 (Merck KGaA, Germany), 0.33 mmol L−1 Triton X100 (Synth, Brazil), 0.01 mol L−1 NH4VO3 (Merck KGaA, Germany), and 0.5 mol L−1 H2SO4 (Dinâmica, Brazil). For total protein content determinations, the following solutions were prepared according to the reference methodology [27]: 10 mg L−1 Coomassie Bright Blue (Merck KGaA, Germany, USA), 0.4889 mol L−1 NaOH (Dinâmica, Brazil), and 0.5000 mol L−1 potassium biphthalate (Synth, Brazil). For lipase activity determinations, the following solutions and solvents were prepared according to the reference methodology [28]: 0.050 mol L−1 NaOH (Dinâmica, Brazil), 0.005 mol L−1 triethanolamine (Merck KGaA, Germany), 10 mg L−1 phenolphthalein (Synth, Brazil), acetone (Synth, Brazil), and absolute ethyl alcohol (Dinâmica, Brazil).

2.2. Flow-batch system

The UR-DM-FBA mainly consisted of a personal computer (PC), a peristaltic pump (PP), a webcam, six solenoid valves, a valve controller (VC), an ultrasonic actuator controller (URC), a webcam, a mixing chamber (MC), a magnetic stirrer, and an Arduino Mega 2560 board. The use of a webcam allowed the monitoring of color changes from chemical reactions occurring inside the MC. The schematic diagram of the analyzer used for the determination of catalase activity can be seen in Fig. 1.

However, only five solenoid valves were required for the determination of lipase activity, as illustrated in Fig. 2.

2.3. UAE system

As shown in Fig. 3, the UR consisted of a borosilicate-made glass container suitable for enzyme extraction, a piezoelectric ceramic disk (Lidit Electronics Co. Ltd, China) with 18 mm diameter, a coaxial input jack connected to the piezoelectric actuator controller, and an electrical isolating system. The glass container was also equipped with an axial outlet able to connect the UR to the DM-FBA.

However, enzymatic extracts were manually inserted into the MC because preliminary tests showed that this connection favored the occurrence of clogging problems.
The reference method was carried out as described in the literature [12,26]. Spectrophotometric measurements were performed at 240 nm in quadruplicate with a spectrophotometer (Instrutherm, model UV-2000A). For each measurement, a kinetic assay was performed for 2 min with cycles of 0.1 min, resulting in a curve of hydrogen peroxide decomposition by catalase. Since hydrogen peroxide decomposition (µmol min⁻¹) is directly proportional to catalase concentration (mg L⁻¹), Eq. (1) was used to determine its enzymatic activity (kU) [29]:

\[
kU = \frac{(A_0 - A_t) \cdot V_t}{\varepsilon_{240} \cdot d \cdot V_s \cdot C_t}
\]

where \(A_0\) is the initial absorbance; \(A_t\) is the final absorbance; \(V_t\) is the total volume (mL); \(kU\) is the catalase activity (U mL⁻¹); \(\varepsilon_{240}\) is the molar absorptivity coefficient of catalase at 240 nm; \(d\) is the optical path; \(V_s\) is the sample volume (mL); \(C_t\) is the total protein concentration (mg mL⁻¹).

For all catalase activity determinations, \(V_s\), \(\varepsilon_{240}\), \(d\), and \(V_s\) values were 2.0 mL, 34.9 mol cm⁻¹, 1.0 cm, and 0.02 mL, respectively.

### 2.5. Reference method for total protein determination

Total protein determination was performed using the Bradford method, which is based on the interaction between Coomassie bright blue dye (CBB) BG-250 and protein macromolecules containing amino acids with basic or aromatic side chains [27]. After the extraction procedure, 0.6 mL of the filtrate was mixed with 2.4 mL of the Bradford reagent. After allowing the mixture to stand for 5 min, spectrophotometric measurements were carried out at 590 nm and 460 nm. It is worth noting that these measurements should be performed within 20 min after the addition of the Bradford reagent to avoid interference of the complex formed between the proteins and CBB. Eq. (2) was used for the determination of total proteins [27]:

\[
\frac{A_{590}}{A_{460}} = \frac{\varepsilon_{590} \cdot k \cdot f_b \cdot n}{\varepsilon_{460}} \cdot C_t + \frac{\varepsilon_{590}}{\varepsilon_{460}}
\]

where \(A_{590}/A_{460}\) is the ratio of the absorbances measured at 590 and 460 nm; \(k\) is the dilution factor; \(f_b\) is the fraction of the reactive blue dye form (0.053); \(n\) is the sample mass ratio; \(\varepsilon_{590}/\varepsilon_{460}\) is the ratio of the molar absorptivity coefficients of blue dye at 590 and 460 nm obtained by the analytical curve using albumin; \(\varepsilon_{590}\) is the molar absorptivity coefficient of the dye-protein complex; and \(C_t\) is the total protein concentration (mg mL⁻¹).

### 2.6. Analytical procedure for determination of catalase activity using ammonium metavanadate

The colorimetric method used in this work for catalase determination is based on the reaction of ammonium metavanadate with hydrogen peroxide in an acidic medium to form ammonium peroxovanadate (AP):
Catalase activity is directly proportional to the decline in absorbance due to the reduction of the peroxovanadium complex, and its value can be calculated according to Eq. (3) [30]:

\[ kU = \frac{2.303}{t} \log_{10} \left( \frac{S}{S'} \right) \]  

(3)

where \( t \) is the time (s), \( S' \) is the absorbance of the standard solution, \( S \) is the absorbance of the sample, and \( kU \) is the catalase activity (U mL\(^{-1}\)).

### 2.7. Reference method for determination of lipase activity

The method for extracting lipase from liver samples involved the formation of an emulsion between olive oil and triethanolamine in an aqueous buffered medium (pH = 7). This emulsion was used as a substrate and kept under constant stirring at 180 rpm for 30 min. Then, 0.5 mL of distilled water was added to a 5.0 mL aliquot of the substrate, and the resulting solution, labeled as “E1 solution”, was incubated for 10 min [28]. While the E1 solution was being prepared, the enzymatic extract obtained by lyophilization of 0.5 g of liver sample in the ultrasonic processor using 5.0 mL of phosphate buffer. After that, another solution, labeled “E2 solution”, was prepared by adding 0.5 mL of the enzymatic extract and 16.0 mL of an acetone/ethanol solution (1:1 v/v) to the E1 solution. After titrating E2 solution with 0.050 mol L\(^{-1}\) NaOH using phenolphthalein as an indicator [28,30], lipase activity was calculated according to Eq. (4) [28]:

\[ A = \frac{(V_a \cdot N \cdot 1000)}{V_s} \]  

(4)

where \( A \) is the lipase activity (U mL\(^{-1}\)), \( V_a \) is the volume of titrant used (mL), \( N \) is the normality of the NaOH solution (mol L\(^{-1}\)), and \( V_s \) is the sample volume (mL).

### 2.8. Control software

The UR-DM-FBA was controlled by software written in ActionScript 3.0. The software was able to control the on/off times of the solenoid valves, process the digital images captured by the webcam at 30 fps, calculate Pearson’s correlation coefficients for the RGB (red–green–blue) channels, and export results to a spreadsheet. Other parameters such as number of replicates, cleaning time, stirring time, US frequency, and US power could be set by the analyst.

### 2.9. Experimental design

The Taguchi method is based on the design of orthogonal arrays (OAs) to optimize experiments under various circumstances aiming to decrease errors and improve reproducibility and efficiency. Since this methodology minimizes noise during the optimization process, it has been considered a robust experimental design [31]. For catalase extraction, the following parameters were studied: power, frequency, sonication time, and volume of the surfactant Triton X-100. Regarding lipase extraction, triethanolamine was studied instead of the surfactant.

### Table 1

| Factor code | Name | Range of variables |
|-------------|------|--------------------|
| A           | Volume of Triton X-100 (mL)* | 0, 1.00, 2.00 |
|             | Volume of triethanolamine (mL) | 0.25, 0.30, 0.35 |
| B           | Power (W) | 10.8, 14.4, 18 |
| C           | Sonication time (s) | 90, 180, 270 |
| D           | Frequency (kHz) | 20, 30, 40 |

*for catalase extraction; **for lipase extraction.

All parameters were examined at three levels (Table 1) and measurements were carried out in triplicate, resulting in the OA shown in Table S1. It is important to note that catalase and lipase extraction was studied at two noise levels (4 and 25° C).

According to the Taguchi method, the optimal conditions are evaluated based on the signal-to-noise (S/N) ratio, which indicates the deviation between experimental results and ideal performance values [32]. In other words, the S/N ratio indicates how close actual performance is to the ideal one. The S/N ratios can be calculated by means of three different approaches: “bigger is better”, “nominal is better”, and “smaller is better” [33]. In this study, the “bigger is better” approach was used to calculate S/N ratios [34] according to Eq. (5) [35]:

\[ \frac{S}{N} = -10 \log \left( \frac{1}{n} \sum_{i=1}^{n} y_i \right) \]  

(5)

where \( n \) is the number of measurements under the same experimental conditions and \( y_i \) is the measured response variable.

### 2.10. Study of volume accuracy

Using an analytical balance (S cientech, model SA2010), the accuracy of the volumes inserted into the MC was investigated by weighing different amounts of deionized water pumped through the solenoid valves. The following on/off times were studied: 1, 3, 5, 7, and 10 s. For each time, water was pumped at 30 rpm and 25 °C, and its mass was measured 10 times. Using the water density at 25 °C, the volume flow rate through each solenoid valve was estimated by linear regression using the least-squares method.

### 2.11. Flow-batch procedure

Before the essays, the solutions in each channel were pumped and recirculated to their respective containers. In addition, all solenoid valves except \( V_{WB} \) and \( V_W \) were turned on so that the solutions could be pumped to the MC and the channels between it and the valves could be filled. Then, \( V_{WB} \) was turned on for 20 s to aspirate any liquid inside the MC to the waste container. The magnetic bar was kept on constant stirring to ensure solution homogenization. Cleaning of the MC was performed as follows: \( V_{WB} \) was turned on for 12 s, then \( V_{WB} \) was turned on to aspirate all liquid inside the chamber to waste. This procedure was repeated twice whenever necessary. All waste was treated after the determinations.

#### 2.11.1. Determination of catalase activity

The valve actuation sequences for the determination of catalase activity are described in Table 2. Signal measurement, disposal of solutions, and cleaning of the mixing chamber were carried out after this sequence.

Blank analysis was carried out by turning on \( V_{PB} \) and \( V_{AM} \) for 3.0 s and 8.0 s so that 0.35 mL of phosphate buffer solution and 0.28 mL of ammonium metavanadate solution could be transferred to the MC,

### Table 2

| Step | Valve |
|------|-------|
|      | \( V_{PB} \) | \( V_{SC} \) | \( V_{WP} \) | \( V_{AM} \) | \( V_W \) | \( V_{WB} \) |
| Blank analysis | 1 | 0 | 0 | 0 | 0 | 0 |
| Actuation time (s) | 3.0 | – | – | 8.0 | – | – |
| Calibration solution analysis | 1 | 0 | 1 | 1 | 0 | 0 |
| Actuation time (s) | 3.0 | – | 4.0 | 8.0 | – | – |
| Sample analysis | 0 | 1 | 1 | 1 | 0 | 0 |
| Actuation time (s) | 3.0 | 4.0 | 8.0 | – | – | – |
| Cleaning of the MC | 0 | 0 | 0 | 0 | 1 | 1 |
| Actuation time (s) | – | – | – | – | 12.0 | 20.0 |

The values 0 and 1 indicated that the corresponding device was switched off or on, respectively.
respectively. After that, the webcam was allowed to capture digital images of the resulting solution inside de MC for 8.0 s, which were saved on the PC.

Calibration solution analysis was carried out by turning on $V_{PB}$, $V_{HP}$, and $V_{AM}$ for 3.0 s, 4.0 s, and 8.0 s so that 0.35 mL of phosphate buffer solution, 0.26 mL of hydrogen peroxide solution, and 0.28 mL of ammonium metavanadate solution could be transferred to the MC, respectively. After that, the webcam was allowed to capture digital images of the solution inside de MC. Blank analysis and calibration were carried out only once.

To perform sample analysis, $V_{s}$, $V_{HP}$, and $V_{AM}$ were turned on for 3.0 s, 4.0 s, and 8.0 s so that 0.29 mL of the sample, 0.26 mL of hydrogen peroxide solution, and 0.28 mL of ammonium metavanadate solution could be transferred to the MC, respectively. After that, the webcam was allowed to capture digital images of the resulting solution inside de MC. After this procedure, the MC was cleaned.

The developed software extracted RGB data from the digital images and calculated Pearson’s correlation coefficients. However, only the G component was used to quantify catalase activity.

### 2.11.2. Determination of lipase activity

Lipase activity was determined using a digital movie-based titration method reported elsewhere [28]. The valve actuation sequence for the determination of lipase activity is shown in Table 3.

The titration process was carried out by initially turning on $V_{s}$ and $V_{1}$ for 10.0 s and 1.0 s so that 1.06 mL of the sample and 0.01 mL of the indicator solution could be transferred to the MC, respectively. After that, the webcam was allowed to capture digital images of the resulting solution inside de MC to perform blank analysis. Then, $V_{1}$ was turned on for 5.0 s while the webcam was allowed to capture digital images of the solution inside de MC as the titration proceeded.

The developed software extracted RGB data from the digital images and calculated Pearson’s correlation coefficients. However, only the G component was used to determine lipase activity [28].

### 2.12. Catalase and lipase extraction at silent conditions

Optimal extraction conditions obtained using the UR were compared with extraction at silent conditions (mechanical stirring at 1500 rpm). In this particular study, catalase and lipase were extracted from five samples of bovine liver, and their activities were determined in triplicate with the DM-FBA.

### 3. Results and discussion

#### 3.1. Optimisation of catalase and lipase extraction using the UR

The average values of the S/N ratios of catalase and lipase activities for each parameter level are shown in Table S2. The rank and delta values specified the most significant parameter. The delta values for each parameter, which indicates the difference between the highest and lowest S/N ratios, were used to assign their appropriate ranks in terms of significance. According to the study results, US frequency (D) was found to be the most significant parameter affecting the extraction of catalase.

| Step                  | Valve | $V_{s}$ | $V_{1}$ | $V_{t}$ | $V_{w}$ | $V_{rs}$ |
|-----------------------|-------|---------|---------|---------|---------|----------|
| Titrating             | 1     | 1       | 1       | 0       | 0       |
| Actuation time (s)    | 10.0  | 1.0     | 5.0     | 1       | 1       |
| Cleaning of the MC    | 0     | 0       | 0       | 16.0    | 20.0    |
| Actuation time (s)    | –     | –       | –       | 16.0    | 20.0    |

The values 0 and 1 indicated that the corresponding device was switched off or on, respectively.

#### Table 4

|             | Optimum level | Lipase                         | Optimum level |
|-------------|---------------|--------------------------------|---------------|
| Parameter   |               |                                |               |
| Volume of Triton X-100 (mL) | 2.0            | Volume of triethanolamine (mL) | 0.30          |
| Ultrasound power (W)     | 10.8           | Ultrasound power (W)            | 18.0          |
| Sonication time (s)      | 270            | Sonication time (s)             | 270           |
| Frequency (kHz)          | 30             | Frequency (kHz)                 | 20            |

#### Table 5

| Catalase activity (U mL$^{-1}$) | RE (%) | Lipase activity (U mL$^{-1}$) | RE (%) |
|---------------------------------|--------|-------------------------------|--------|
| Ultrasonic processor            |        | Ultrasonic processor          |        |
| 0.157 ± 0.0001                  | 0.64   | 0.489 ± 0.002                 | 1.99   |
| 0.118 ± 0.001                   |        | 0.452 ± 0.001                 |        |
| 0.198 ± 0.0004                  |        | 0.189 ± 0.007                 | 1.59   |
| 0.155 ± 0.0002                  |        | 0.360 ± 0.005                 |        |

UR: ultrasonic reactor; RE: relative error.

followed by volume of Triton X-100 (A), sonication time (C), and US power (B). For the extraction of lipase, the most significant parameter was US power (B), followed by volume of triethanolamine (A), sonication time (C), and US frequency (D). The optimal levels were those that produced the highest S/N ratios (Table 4).

A selected number of experiments were performed under optimal conditions to validate the viability and reproducibility of the Taguchi method employed in this study. Catalase and lipase activities in bovine livers were determined using the commercial ultrasonic processor and the lab-made UR (Table 5). The relative errors (between $–2.58\%$ and $0.64\%$ for catalase and between $–1.42\%$ and $1.99\%$ for lipase) indicate that the extraction efficiencies of the PR and the commercial ultrasonic processor were similar. Also, the paired t-test (95% confidence level) did not show significant differences between these results.

#### 3.2. Extraction efficiency

Table 6 shows the lipase and catalase activities obtained using the lab-made UR and mechanical stirring. Catalase and lipase activities obtained with the UR were, on average, $1.9 \times 10^{5}\%$ and $2.0 \times 10^{5}\%$ higher than those obtained at silent conditions, respectively, which indicates that the lab-made UR was capable of extracting these enzymes more efficiently. In addition to improving extraction efficiency and mass transfer, the succession of compression and rarefaction phases induced by US creates cavitation bubbles, which asymmetrically collapse onto the surface of cell walls producing high shear stresses, while transient cavitation does the main damage in liver cell membranes through high temperatures and pressures [36]. The high pressure and temperature released during this event dissociate molecular bonds [37] and generate microjets directed towards the cell’s membrane, destroying it and releasing the enzymes from the matrix. Other processes attributed to cavitation inside the cells, such as dispersal tissue, cell destruction, and intensive blending may also contribute to improving mass transfer through the destruction of boundary diffusional layers [38–41]. The US parameters (frequency, intensity, and duration of irradiation) were carefully optimized to prevent denaturation/inactivation of catalase and lipase [42].
3.3. Determination of catalase and lipase activities

All determinations were performed in triplicate. As shown in Fig. 4, catalase activity is directly proportional to the rate of decomposition of hydrogen peroxide, which causes the color of the titrate to vary slightly.

According to Fig. 4 (a and b), there was a gradual decrease in Pearson’s correlation coefficient for the B component over time. This result indicates that the reaction between ammonium metavanadate and hydrogen peroxide obeyed the rules of a first-order kinetic reaction. The slopes of these curves were calculated and consequently inserted in Equation (3) to determine the catalase activity values.

On the other hand, Fig. 4 (c and d) showed a sharp decline in Pearson’s correlation coefficient for the G component over time. The second derivatives of the titration curves were used to estimate their endpoints, which allowed the determination of lipase activity with the help of Eq. (4).

The results for the determination of catalase and lipase activities in bovine livers are given in Table 7. The UR-DM-FBA was capable of achieving low relative errors (between 1.98% and 1.96% for bovine catalase and between 0.65% and 0.76% for bovine lipase) and overall coefficients of variation (<0.02% for bovine catalase and < 0.2% for bovine lipase), suggesting that the proposed methods present high accuracy and precision. The paired t-test (95% confidence level) did not show significant differences between the proposed methods and the reference methods.

Table 8 shows the results for the determination of catalase and lipase activities in poultry livers. Low relative errors (between 2.03 and 2.08% for poultry catalase and between 0.55% and 0.64% for poultry lipase) and overall coefficients of variation (<0.02 for poultry catalase and <0.2% for poultry lipase) were also achieved with the proposed method. The paired t-test (95% confidence level) did not show significant differences between the proposed methods and the reference methods.

Comparison of analytical frequency and waste volume of the proposed analytical system and reference methods revealed significant differences. While the reference method for determination of catalase activity has an analytical frequency less than 0.042 h

-1 with a waste volume of 10.0 mL [12], the UR-DM-FBA achieved an analytical frequency of 11.2 h

-1 (approximately 267 times faster) with a waste volume of only 1.2 mL. Regarding determination of lipase activity, the reference method has an analytical frequency of 1 h

-1 with a waste volume of 27 mL [28]. In this case, the UR-DM-FBA also achieved an analytical frequency of 11.2 h

-1 with a waste volume of only 1.6 mL.

Table 6

| Sample | Catalase activity (U mL

-1) | Percentage increase (%) | Lipase activity (U mL

-1) | Percentage increase (%) |
|--------|----------------|-----------------|----------------|-----------------|
|        | Mechanical stirring | UR | Mechanical stirring | UR |
| 1      | 0.0053 ± 0.002 | 0.096 ± 0.001 | 1.7 × 10

3 | 0.10 ± 0.01 | 1.0 × 10

3 |
| 2      | 0.0047 ± 0.003 | 0.097 ± 0.004 | 1.9 × 10

3 | 0.10 ± 0.01 | 1.2 × 10

3 |
| 3      | 0.0043 ± 0.003 | 0.10 ± 0.001 | 2.2 × 10

3 | 0.21 ± 0.01 | 1.6 × 10

3 |
| 4      | 0.0070 ± 0.002 | 0.11 ± 0.001 | 1.5 × 10

3 | 0.63 ± 0.01 | 0.47 × 10

3 |
| 5      | 0.0050 ± 0.001 | 0.11 ± 0.001 | 2.1 × 10

3 | 0.97 ± 0.01 | 0.50 × 10

3 |

UR: ultrasonic reactor.

Fig. 4. Kinetic curves obtained for the determination of catalase in (a) bovine and (b) poultry livers. Titration curves for the determination of lipase in (c) bovine and (d) poultry livers.

Accuracy and precision of the UR-DM-FBA and reference methods did not show significant differences between the proposed methods and the reference methods.

Table 8 shows the results for the determination of catalase and lipase activities in poultry livers. Low relative errors (between 2.03 and 2.08% for poultry catalase and between 0.55% and 0.64% for poultry lipase) and overall coefficients of variation (<0.02 for poultry catalase and <0.2% for poultry lipase) were also achieved with the proposed method. The paired t-test (95% confidence level) did not show significant differences between the proposed methods and the reference methods.
lipase, which are important enzymes for humans. The UAE process using optimized conditions, catalase activities obtained with the UR were, on average, 1.9 ± 0.01× higher than those obtained at silent conditions. As for lipase, the obtained activities with the UR were, on average, 2.0 ± 0.02× higher than those obtained at silent conditions. As for lipase, the obtained activities with the UR were, on average, 2.0 ± 0.02× higher than those obtained at silent conditions. As for lipase, the obtained activities with the UR were, on average, 2.0 ± 0.02× higher than those obtained at silent conditions.

4. Conclusions

Bovine and poultry livers are important sources of catalase and lipase, which are important enzymes for humans. The UAE process using the lab-made UR proved to be an attractive alternative for the removal of these enzymes from biological samples such as animal livers. Optimized parameters for the UAE of catalase were US frequency of 30 kHz, 2.0 mL of triethanolamine, sonication time of 270 s, and US power of 100 W. For the UAE of lipase, the optimized parameters were US frequency of 20 kHz, 0.30 mL of triethanolamine, sonication time of 270 s, and US power of 18 W. Extraction of these enzymes was considerably more efficient than that performed with mechanical stirring (1500 rpm). Under optimized conditions, catalase activities obtained with the UR were, on average, 1.9 × 10⁻⁵% higher than those obtained at silent conditions. As for lipase, the obtained activities with the UR were, on average, 2.0 × 10⁻⁵% higher. Also, the UAE using the UR performed better than the reference methods. Thus, these results showed the suitability of US in extracting catalase and lipase from bovine and poultry livers and the fast, reliable, and inexpensive determination of their activities with little waste generation.

CRediT authorship contribution statement

Lucas A. Siqueira: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Software, Writing – original draft. Luciano F. Almeida: Conceptualization, Validation, Formal analysis, Investigation, Supervision, Writing – original draft. Julys Pablo Atayde Fernandes: Conceptualization, Validation, Writing – original draft, Investigation. Mario Cesar U. Araújo: Conceptualization, Investigation, Validation, Formal analysis, Writing – original draft, Resources. Ricardo Alexandre C. Lima: Conceptualization, Investigation, Validation, Formal analysis, Supervision, Writing – original draft, Resources.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.
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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ultsonch.2021.105774.

References

[1] N. Prieto, R. Roche, P. Lavin, G. Batten, S. Andréis, Application of near infrared reflectance spectroscopy to predict meat and meat products quality: a review, Meat Sci. 83 (2) (2009) 175–186, https://doi.org/10.1016/j.meatsci.2009.04.016.

[2] A.C.O. Mafra, W. Kopp, M.B. Beltrame, R. de Lima Camargo Giordano, M.P. de Aurruda Ribeiro, P.W. Tardiolli, Diffusion effects of bovine serum albumin on cross-linked aggregates of catalase, J. Mol. Catal. B Enzym. 133 (2016) 107–116. doi:10.1016/j.molcatb.2016.08.002.

[3] A. Karich, K. Scheibenreiter, R. Ulrich, M. Hofrichter, Exploring the catalase activity of unspcific peroxgenases and the mechanism of peroxide-dependent heme destruction, J. Mol. Catal. B Enzym. 134 (2016) 238–246, https://doi.org/10.1016/j.molcatb.2016.10.014.

[4] C. Gloorieu, M. Zamoeky, J.M. Sandoval, J. Verras, P.B. Calderon, Regulation of catalase expression in healthy and cancerous cells, Free Radic. Biol. Med. 87 (2015) 84–97, https://doi.org/10.1016/j.freeradbiomed.2015.06.017.

[5] G.F. Gaetani, H.N. Kirkman, R. Mangerini, A.M. Ferraris, Importance of catalase in the disposal of hydrogen peroxide within human erythrocytes, Blood 84 (1994) 925–330, https://doi.org/10.1182/blood.V84.7.925.

[6] S. Izawa, Y. Inoue, A. Kimura, Importance of catalase in the adaptive response to hydrogen peroxide: analysis of ataxicalsea Saccharomyces cerevisiae, Biochem. J. 325 (1996) 61–67.

[7] C. Michiels, M. Raes, O. Toussaint, J. Remacle, Importance of SE-glutathione peroxidase, catalase, and CN-SOD for cell survival against oxidative stress, Free Radic. Biol. Med. 17 (3) (1994) 235–248, https://doi.org/10.1016/0891-5849(94)90079-5.

[8] D. Bharathi, G. Rajalakshmi, Microbial lipases: an overview of screening, correlation analysis, Clin. Radiol. 75 (397) (2020) e1 10.1016/j.crad.2020.11.027.

[9] H.-J. Jiang, H. Yang, D.L. Akins, Direct electrochemistry and electrocatalysis of hydrogen peroxide: analysis of acatalasaemic Saccharomyces cerevisiae, Biochem. Eng. J. 143 (2019) 179–189, https://doi.org/10.1016/j.bej.2019.01.006.

[10] J. Wang, B. Sun, Y. Cao, Y. Tian, X. Li, Optimization of ultrasound-assisted extraction of phenolic compounds from wheat bran, Food Chem. 106 (2) (2008) 804–810, https://doi.org/10.1016/j.foodchem.2007.06.062.

[11] F. Chemat, N. Rombaut, A.-G. Sicaire, A. Meulemiste, A-S. Fabiano-Tixier, M. Abert-Vian, Ultrasound assisted extraction of food and natural products. Mechanisms, techniques, combinations, protocols and applications. A review, Ultrason. Sonochem. 34 (2017) 540–560, https://doi.org/10.1016/j.ultsonch.2016.06.035.

[12] K. Vilkh, R. Manasseh, R. Mawson, M. Ashokkumar, Ultrasonic recovery and modification of food ingredients, in: Ultrason. Tech. Food Bioprocess., Springer, 2011: pp. 345–368.

[13] H. Faliri, R. Koutri, M.-E. Luchenci, C. Abdelt, C. Magne, Ultrasonic-assisted extraction: effect of extraction time and solvent power on the levels of polyphenols and antioxidant activity of Mesembryanthemum edule L. Aizoaceae shoots, Trop. J. Pharm. Res. 11 (2012) 243–249.

[14] H. Aebi, (13) Catalase in vitro, Methods Enzymol. 105 (1984) 121–126.

[15] T. Zer, Z. Selinger, Linearization of the Bradford protein assay increases its sensitivity: theoretical and experimental studies, Anal. Biochem. 236 (2) (1996) 302–308.

[16] V.M.G. Lima, N. Krieger, D.A. Mitchell, D.J. Fontana, Activity and stability of a crude lipase from Penicillium aurantiogriseum in aqueous media and organic solvents, Biochem. Eng. J. 18 (1) (2004) 65–71, https://doi.org/10.1016/S1369-703X(03)00165-7.

[17] E. Orta-Zavalza, M. Briones-Marin-del-Campo, I. Castano, A. De Las Penas, Catalase activity assay in Candida glabrata, Bio-Protoc. 4 (2014) e1072–e1072.

[18] W. Stuer, K.E. Jaeger, U.K. Winkler, Purification of extracellular lipase from Pseudomonas aeruginosa, J. Bacteriol. 168 (3) (1986) 1070–1074.

[19] T.C. Hiang, K. Dehnad, Quality control, robust design, and the Taguchi method, J. Am. Stat. Assoc. 85 (410) (1990) 601, https://doi.org/10.1080/0041000096842222.

[20] R. Sathish Kumar, K. Sureshkumar, R. Velraj, Optimization of biodiesel production from Manilkara zapota (L) seed oil using Taguchi method, Fuel. 140 (2015) 90–96, https://doi.org/10.1016/j.fuel.2014.10.010.

[21] V. Ayhan, Ç. Cangal, I. Cesur, A. Coban, G. Ergen, Y. Cay, A. Kolip, I. Oseß, Optimization of the factoring affecting performance and emissions in a diesel engine using biodiesel and EGR with Taguchi method, Fuel. 261 (2020) 116371, https://doi.org/10.1016/j.fuel.2020.116371.

[22] S.A. Alavi-Borazjani, L.A. da C. Tarello, M.I. Capela, Parametric optimization of the dark fermentation process for enhanced biobiodiesel production from the organic fraction of municipal solid waste using the Taguchi method, Bioresource Technol. 219 (2018) 1302–1309.

[23] A. Henglein, Sonoschemie: historical developments and modern aspects, Ultrasonics. 25 (1) (1987) 6–16, https://doi.org/10.1016/0041-624X(87)90003-5.

[24] E.L. Carstensen, Biological effects of acoustic cavitation, Ultrason. Med. Biol. 12 (9) (1986) 703–704, https://doi.org/10.1016/0301-5629(86)90267-5.

[25] J. Zayas, Effect of ultrasound treatment on the extraction of insulin, Biotechnol. Bioeng. 27 (8) (1985) 1223–1228.

[26] S.D. Jayasooriya, R.R. Bhanderi, P. Torley, B.R. D'Arcy, Effect of high power ultrasound waves on properties of meat: a review, Int. J. Food Prop. 7 (2) (2004) 301–319.

[27] J.F. Zayas, Effect of ultrasonic treatment on the extraction of chymosin, J. Dairy Sci. 69 (7) (1986) 1767–1775.

[28] C.A. Bizzi, R.C. Zanatta, D. Santos, K. Giacobe, R.M. Dallago, P.A. Mello, E.M. M. Flores, Ultrasonic-assisted extraction of chromium from residual tanned leather: An innovative strategy for the reuse of waste in tanning industry, Ultrason. Sonochem. 64 (2020) 104682, https://doi.org/10.1016/j.ultsonch.2019.104682.

[29] H. Alligari, Ultrasonic disruption, Am. Lab. (1975) 75–85.