Hindawi
Journal of Food Quality
Volume 2018, Article ID 2936589, 6 pages
https://doi.org/10.1155/2018/2936589

Research Article

Ultrasound Improves Antimicrobial Effect of Sodium Hypochlorite and Instrumental Texture on Fresh-Cut Yellow Melon

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Received 30 August 2018; Revised 29 October 2018; Accepted 14 November 2018; Published 6 December 2018

Academic Editor: Efstathios Giaouris

Ultrasound combined with sanitizers is efficient for the reduction of microbiological contaminants in fruits and vegetables. However, the physicochemical changes remain to be elucidated. Therefore, the isolated and combined effect of ultrasound (40 kHz, 500 W) and sodium hypochlorite (NaOCl) (100 mg/L) for 5 min in the bacterial microbiota and physicochemical changes on yellow melon (*Cucumis melo* L.) were evaluated. Mesophilic aerobic bacteria (MAB), pH, total titratable acidity (TTA), and texture profile were performed. No changes in pH and TTA ($p > 0.05$) were obtained. Firmness, chewiness, cohesiveness, and gumminess increased ($p < 0.05$) after the ultrasound application. A synergistic effect between ultrasound and NaOCl in the MAB reduction was achieved. Therefore, ultrasound improves the antimicrobial effect of NaOCl and texture profile without undesirable chemical changes.

1. Introduction

Sanitation is the critical stage to reduce microbiological contaminants in fruits and vegetables. Several chemical compounds have been used with high efficiency at this stage. Sodium hypochlorite is the most commonly used sanitizer in the food industry [1, 2]. Its antimicrobial effect is due to penetration into the target cell followed by its dissociation into hypochlorous acid [3, 4]. This compound results in the occurrence of oxidation reactions and reduction of intracellular pH (hypochlorite ion (OCl^-) and proton (H^+)) that alter cellular metabolism. Essential cell compounds such as proteins, lipids, and mainly enzymes are damaged/inactivated and generate cell death [5]. To improve the antimicrobial effect of sanitizers, other preservation methods have been combined following the hurdle theory [6]. Among the nonthermal preservation technologies such as atmospheric cold plasma [7], high hydrostatic pressure [8], UV-C radiation [9], and ultrasound (US) [10] can be efficiently applied in the decontamination process with high potential to maintain food quality.

US is considered a green technology for not generating waste and being environmentally friendly [10, 11]. The antimicrobial effect of US is due cavitation generated by sound waves in the liquid medium [11]. During this process, the formation of bubbles occurs. Sound waves cause compression and expansion of the bubbles [12]. On the critical point, the bubbles implode and form microjets. The shear force generated by microjets can break or form pores in the membrane of microorganisms and lead to cell death [11, 13]. In addition, during bubble implosion, hot spots and free radicals are formed causing DNA fragmentation [13]. In this way, US has been applied to reduce pathogenic and spoilage microorganisms of fruits and vegetables.

Several fruits and vegetables have a high risk of causing diseases by being consumed fresh. Among the vegetables, melon has great global economic importance and good consumer acceptance [14, 15]. Melon is often consumed raw, and due to this, were recorded several outbreaks. In the USA, three deaths and 94 hospitalizations occurred due to consumption of cantaloupe contaminated with *Salmonella* serotype Typhimurium and *Salmonella* serotype Newport [16]. In addition, 33 deaths and 143 hospitalizations occurred due to contamination by *Listeria monocytogenes* [17]. Spoilage bacteria are also very important for fruits. The spoilage microbiota causes important physicochemical changes [10], and texture is one of the main physical factors that determine fruit quality [14, 18].

US combined with sanitizers is promising in the decontamination process. However, a long application period may cause undesirable physicochemical changes. In this way, the US effect on the physicochemical characteristics of fruits and vegetables remains to be elucidated. Therefore, the aim of this study was to evaluate the effects of US isolated and combined with sodium hypochlorite in natural microbiota reduction and physicochemical changes of fresh-cut yellow melon.

2. Materials and Methods

2.1. Sample Preparation. Melons (*Cucumis melo* L.) were obtained at the local supermarket and transported in isothermal boxes to the laboratory for analysis. Melons were washed in tap water. After this step, the melons were sliced in 5 × 5 × 20 cm (length × width × height). A total of 300 g of melon were submitted for each sanitization treatment. The experiment was conducted with three experimental and analytical replicates.

2.2. Sanitization Procedures. Treatments were applied using sterilized water (control, SW), sodium hypochlorite (NaOCl) at 100 mg/L (Alphatec, Rio de Janeiro, Brazil), and ultrasound (US) (a capacity of 12 L) (Model Soniclean 15, Sanders Medical®, Minas Gerais, Brazil) at 40 kHz and 500 W for 5 min according to Rosário et al. [10] and Duarte et al. [19]. The US power (P) dissipated into the liquid was calculated using equation (1), and 100.32 W (0.33 ± 0.01 W/g) was obtained. Acoustic energy density (AED) dissipated in the US bath was 0.10 KJ/g (equation (2)). The combination of ultrasound and sodium hypochlorite (US + NaOCl) was performed in the same conditions previously described. A total of 2 L of the sanitizer solution (0.15 g/mL, amount sufficient to avoid overlapping of the fresh cut in the US bath) at 7°C were used for each treatment. The temperature was maintained using ice cubes:

$$P = mC_{\mathrm{p}}\left(\frac{\partial T}{\partial t}\right), \quad (1)$$

where C_{p} is the water specific heat (4.18 J/g·K), m is the mass of water on the US bath (g), ∂T denotes the increase in the T (°C), and ∂t is the sonication time (s).

$$\mathrm{AED}\left(\frac{\mathrm{J}}{\mathrm{g}}\right) = \frac{\text{power (W)} \cdot \text{process time (s)}}{\text{sample weight (g)} + \text{NaOCl weight (g)}}. \quad (2)$$

2.3. Mesophilic Aerobic Bacteria (MAB) Quantification. After the sanitization procedures, 25 g of each treatment was homogenized in 225 mL of 0.1% peptone water using stomacher (Marconi®, Piracicaba, São Paulo) for 1 min [20]. Appropriate serial dilutions were performed, and aliquots were added in plate count agar (PCA) (HiMedia®, Mumbai, India) and incubated at 35 ± 0.1°C for 48 h [20]. The results were expressed in $\log_{10}$ of the colony-forming units per gram of melon (log·cfu/g). The decimal reduction (DR) was calculated (equation (3)). The value of N corresponds to the MAB (cfu/g) count after the sanitization treatment and the value of N_0 to the control (SW) treatment:

$$\mathrm{DR} = \log\left(\frac{N_0}{N}\right). \quad (3)$$

2.4. Physicochemical Analysis

2.4.1. pH. The pH values of the melons were measured using the digital pH meter (Model mPA-210, Tecnopon, São Paulo, Brazil). The electrode was immersed in 10 g of melon pulp diluted in 100 mL of distilled water [21].

2.4.2. Total Titratable Acidity (TTA). TTA was performed using the titrimetric method. A total of 10 g of sample pulp were homogenized with 100 mL of distilled water. Titration was performed using 0.1 N NaOH and 1% phenolphthalein indicator [21]. The results were expressed in grams of citric acid per 100 g of melon (percentage, %).

2.4.3. Texture Profile Analysis. Texture profile analysis (TPA) was performed using Texture Analyzer (Brookfield®, Model CT3, São Paulo, Brazil). The analyzer was equipped with a 10 kg load cell, and the TA44 probe was used. The analysis was performed following the conditions of a pretest speed of 5.0 mm/s, a test speed of 1.0 mm/s, a posttest speed of 8.0 mm/s, and a penetration distance of 4 mm [22]. The parameters of firmness, adhesiveness, springiness, cohesiveness, gumminess, and chewiness were obtained.

2.5. Statistical Analysis. To evaluate the significant effect of treatments on the reduction of bacterial microbiota and physicochemical characteristics, one-way ANOVA was performed using Software Assistant® [23]. To verify the difference between the treatments, Tukey's test was performed. The significance level used was 0.05.

3. Results and Discussion

3.1. Effect of Treatments on the Bacterial Microbiota. Sanitization is the critical step for reducing microbiological contaminants and the risk of food-borne diseases [24, 25]. In the present study, the initial contamination of MAB in the melon was 2.9 log·cfu/g (Figure 1). US + NaOCl reduced the microbial count to 2.5 log·cfu/g (0.4 log·cfu/g). A synergistic effect between US and NaOCl in natural microbiota reduction of the melon was obtained (Figure 1). São José et al. [26] found an improvement in the effect of lactic acid (1%) and acetic acid (1%) when combined with US (40 kHz) for 2 min in *Salmonella* serotype Enteritidis reduction on the melon surface. Park et al. [27] also found a synergistic effect between NaOCl at 100 mg/L and US (35 kHz, 380 W) applied for 5 min on lettuce. The occurrence of the synergistic effect in the present study is due to the cavitation process formed during the US application (Figure 2). The intense pressure generated by this phenomenon can propel the active principle of the sanitizer into the microbial cell [12, 13].

US and NaOCl reduced the microbial count to 2.8 and 2.7 log·cfu/g (0.1 and 0.2 log·cfu/g), respectively, and no significant difference ($p > 0.05$) was obtained. In addition, both treatments did not differ ($p > 0.05$) from the SW. This fact demonstrates the low efficiency of these isolated methods. The low chlorine efficiency in the microorganisms inactivation has been reported [13, 28]. Although some countries in Europe (Belgium, Denmark, Germany, and the Netherlands) have prohibited the use of chlorine, this sanitizer is still widely used by the food industry [28, 29]. Therefore, due to the chlorination's ability to cause selection of chlorine-resistant bacteria [30], the high use of this sanitizer may be causing its inefficiency in the inactivation of bacteria. Likewise, low ultrasonic efficiency was also observed in the natural microbiota inactivation of fruits and vegetables [19, 31, 32]. This effect is confirmed by the abundant presence of microbiological contaminants in the sanitizing solution after sanitization [10]. These phenomena state that US has the effect of removing surface microorganisms [33] and improving the antimicrobial effect of sanitizers.

3.2. Effect of Treatments on Physicochemical Characteristics. Sanitation is an important step for the reduction of microbiological contaminants. However, physicochemical alterations of fruits and vegetables may be a limiting factor for the application of this procedure. For pH and TTA, no significant difference ($p > 0.05$) between treatments (Table 1) and overall mean 6.46 and 0.13%, respectively, were obtained. Palekar et al. [34] found alteration of melon pH using a nonthermal preservation technology (electron beam irradiation, 1.5 kGy). pH and TTA unchanged at the present study are important results indicating that the oxidative effect of NaOCl (100 mg/L) and chemical as well as thermal effects of US are not able to change these values when applied for 5 min. This fact demonstrates that the US-assisted chemical sanitation is a promising combined preservation method of melons.

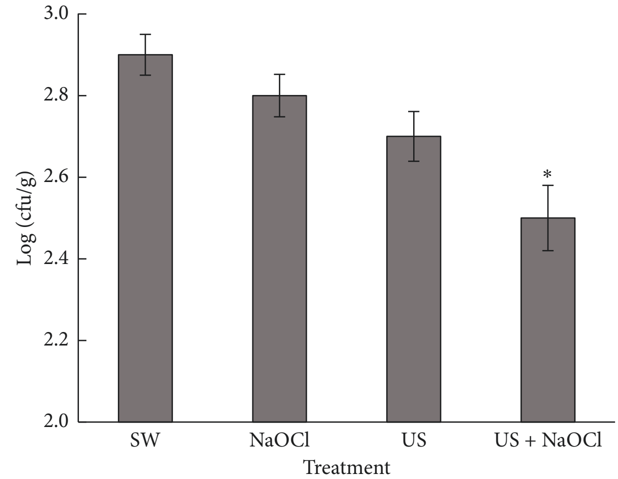

Figure 1: Mesophilic aerobic bacteria (MAB) count on sanitized and no sanitized melon. SW, sterilized water; US, ultrasound (40 kHz, 500 W); NaOCl, sodium hypochlorite (100 mg/L). *The difference is significant at $p < 0.05$ by Tukey's test.

Texture profile has great importance mainly due to the acceptance and shelf life of foods. Firmness can be described as cell wall resistance and intracellular adhesions [35, 36]. Significant difference of firmness ($p < 0.05$) between treatments was obtained and US increased this parameter (Table 1). Conversely, São José and Vanetti [32] evaluated firmness immediately after sanitization, and this parameter reduced with the US application (45 kHz) in isolated or combined with sodium dichloroisocyanurate (50 and 200 mg/L) or chlorine dioxide (10 mg/L) on strawberries for 10 min. In the present study, the time of 5 min was used. This fact demonstrates that the long period of US application can decrease firmness of fruits. Firmness reduction can be attributed to the mechanical effects of cavitation that break down plant tissues [37]. However, sanitization of 5 min improved firmness on melons. This effect is possible because the US has the ability to increase the diffusion of substances [38]. This process may have caused compounds homogenization in the melon such as water together with water soluble substances. In this way, US increased firmness without damage to the fruit cells. However, this phenomenon as well as its mechanisms of action need to be better understood.

Adhesiveness is the force required to remove the specimen from the sample [39] which indicates the adhesion properties of the sample. For this parameter, no significant difference ($p > 0.05$) between treatments and overall mean of 2685.09 g/mm were obtained. This result is important for the reduction of microbiological contaminants without physical alterations. Springiness indicates the elastic capacity of the

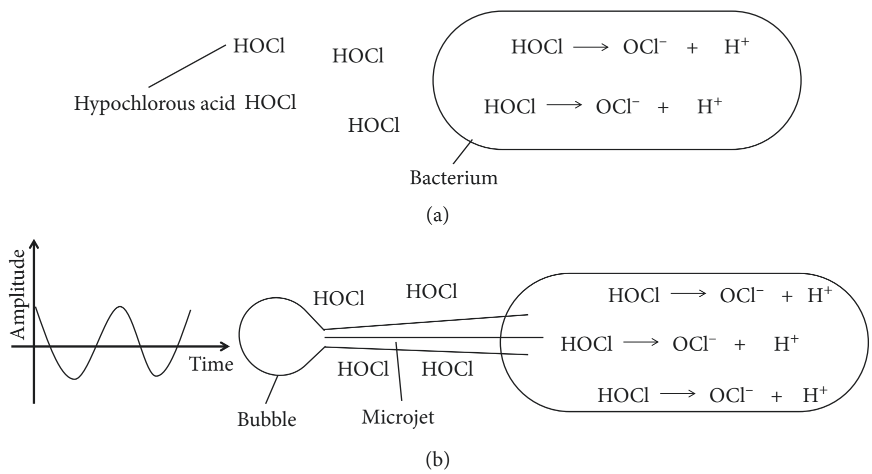

Figure 2: (a) Hypochlorous acid penetrates the bacteria and dissociates. (b) The bubble implosion generated by the cavitation process forms a microjet that impulses hypochlorous acid into the bacterial cell. Therefore, a greater amount of this compound is obtained in the cellular cytoplasm resulting in a greater antibacterial effect of sodium hypochlorite.

Table 1: Mean values of texture profile analysis and physicochemical parameters of melon.

Variables	Sanitation treatment			
	SW	NaOCl	US	US + NaOCl
pH	6.50 ± 0.13a	6.38 ± 0.07a	6.52 ± 0.03a	6.43 ± 0.14a
TTA	0.13 ± 0.01a	0.14 ± 0.03a	0.13 ± 0.01a	0.12 ± 0.01a
Firmness	8.09 ± 0.71b	7.99 ± 0.30b	9.52 ± 0.90a	9.42 ± 0.86a
Adhesiveness (g/mm)	2545.78 ± 211.48a	2400.78 ± 182.74a	3244.11 ± 653.43a	2549.67 ± 874.17a
Springiness	25.25 ± 2.39a	26.44 ± 1.23a	24.78 ± 4.93a	23,85 ± 5.63a
Cohesiveness	0.14 ± 0.06b	0.16 ± 0.02ab	0.22 ± 0.01a	0.22 ± 0.02a
Gumminess (N)	1.14 ± 0.19b	1.28 ± 0.12b	2.04 ± 0.22a	1.91 ± 0.02a
Chewiness (g/mm)	2932.78 ± 181.80b	3491.56 ± 452.49ab	5796.11 ± 404.75a	4705.06 ± 197.14ab

SW, sterilized water; US, ultrasound (40 kHz, 500 W); NaOCl, sodium hypochlorite (100 mg/L); TTA, total titratable acidity (%). Means followed by the same letter in the same column do not differ from each other ($p > 0.05$) by Tukey's test.

matrix [35], and no significant difference ($p > 0.05$) between treatments and overall mean of 25.08 g/mm was achieved. Garcia-Loredo et al. [40] found springiness reduction using ascorbic acid 1% and calcium chloride 0.1% in fresh-cut pear. The present study demonstrates that US application does not alter the elastic structure and adhesiveness of melon.

Cohesiveness is a parameter used to refer to the strength of the internal bonds of fruits [41]. Gumminess is a term used to represent the disintegration ability of the sample to be swallowed by the consumer [42]. In this study, greater values ($p < 0.05$) of cohesiveness and gumminess in melons treated with US when compared to the control were obtained (Table 1). The US has an ability to inactivate enzymes, remove oxygen trapped inside the fruits [13], and redistribution of water through cell substructures [43], causing this observed effect. Moreover, it is known that these parameters are naturally reduced with the start of spoilage [22]. Therefore, the US can maintain these texture parameters and the integrity of the fruit for a longer period after the sanitization.

Chewiness can be defined as the energy for chewing food until swallowing [44]. US used alone did not differ from control ($p > 0.05$) and caused an increase on chewiness (Table 1). In fruits, this parameter decreases with storage time [42]. Therefore, this fact makes it increase an important factor. The NaOCl treatments did not differ ($p > 0.05$) from the control. Therefore, it is possible to explain that the oxidative effect of this sanitizer does not cause undesirable changes in melon chewiness applied for 5 min.

4. Conclusion

US improved the sodium hypochlorite effect on reducing the bacterial microbiota of melon. A synergistic effect in US-assisted chemical sanitation was achieved. This hurdle effect did not affect physicochemical characteristics. Furthermore, texture parameters such as firmness, cohesiveness, gumminess, and chewiness have been improved with the US application. Therefore, to sanitize melon, US (40 kHz, 500 W) for 5 min (0.10 KJ/g) is appropriate. Finally, US has a potential of fruit industrial application combined to sodium hypochlorite in the decontamination process of fresh-cut yellow melon.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

All authors declare that there are no conflicts of interest.

Acknowledgments

The authors would like to thank the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES, Brazil) Finance Code 001 and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, Brazil) for the financial support to D. K. A. Rosario and Marina C. M. Madalão (postgraduate scholarship).

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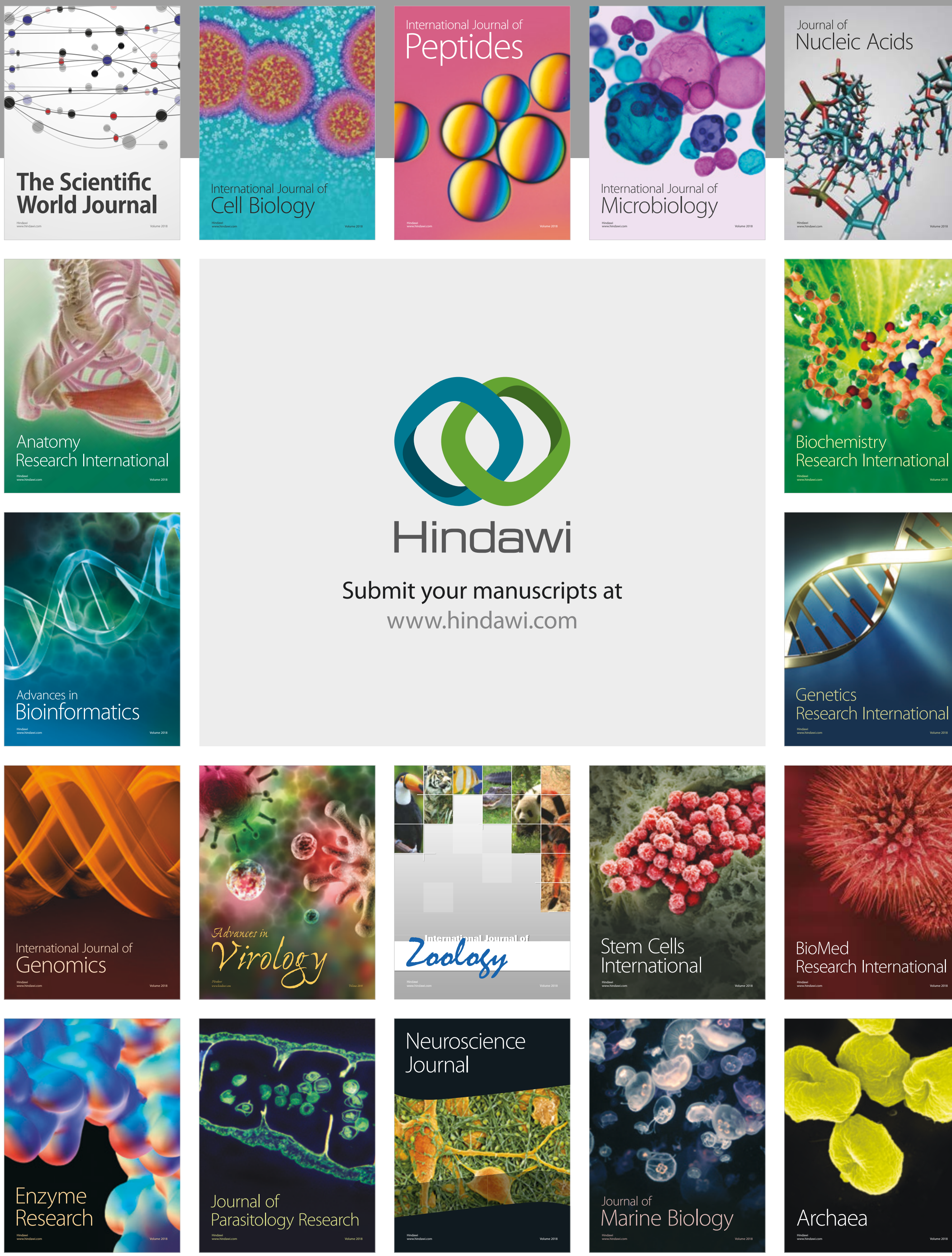
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