Pig DNA Extraction Using the Ultrasound Method for Halal Product Authentication

Pramuja Baydillah[1], Ulfah Lailatul Khoiriah[1], Livya Safira Islam[1], Fadlilatul Taufany[1], Nurul Jadid[2], Andi Yudha Sasongko[3], and Setiyo Gunawan[1,4] *

[1] Chemical Engineering Department, Institut Teknologi Sepuluh Nopember, Surabaya, Indonesia
[2] Biology Department, Institut Teknologi Sepuluh Nopember, Surabaya, Indonesia
[3] PT Biotek Prima Indoplus, Sidoarjo, Indonesia
[4] Halal Study Center, Institut Teknologi Sepuluh Nopember, Surabaya, Indonesia
setiyo.gunawan@its.ac.id

Abstract. Deoxyribonucleic acid (DNA) extraction is the process of isolating DNA from cells or tissues of living organisms for further analysis. DNA extraction must be performed in matrix analysis of a sample to identify nucleic acids. This study evaluated the effectiveness of ultrasound in extracting pig DNA, with variations in sample type, power, and extraction time as independent variables. The results of the study showed the highest crude DNA pellets in the three samples (processed food, medicine, and cosmetics), which were 40.20 ± 1.10 ng, 0.69 ± 0.18 ng, and 0.10 ± 0.015 ng, respectively. These results were obtained from a combination of high power (450 W), and extraction time (40 min). This indicates that using the ultrasound method with the correct parameters can increase the efficiency of the extraction results through the cavitation mechanism. Ultrasound method is an efficient and environmentally friendly extraction technique for molecular analysis process.

Keywords: Cosmetics, Deoxyribonucleic Acid Extraction, Medicine, Processed Food, Ultrasound.

1 Introduction

Food is one of the basic needs that must be met by humans to perform daily activities [1]. In addition to food, medicinal products and cosmetics are also important for improving human health and confidence. Indonesia's demographic population, which is dominated by Muslims, makes the halalness of a product a significant concern [2]. Halal products meet religious requirements and must reflect cleanliness, safety, and high quality [3]. In fact, many processed foods, medicines, and cosmetic products still contain non-halal elements that are circulating in the market, such as pork and its derivatives [4]. Therefore, Law Number 8 of 1999 concerning Consumer Protection, which mandates compliance with halal production standards, provides legal protection for Muslim consumers [5]. However, this obligation applies only to business actors who

© The Author(s) 2026
V. D. S. Handayani et al. (eds.), *Proceedings of the 6th International Conference on Bioinformatics, Biotechnology, and Biomedical Engineering (BIOMIC 2025)*, Advances in Health Sciences Research 94,
https://doi.org/10.2991/978-94-6239-762-0_17

affix halal labels to their products. As a result, if a business labels its products as halal, it must comply with halal production guidelines, and proof of compliance is demonstrated through the possession of halal certificates [6].

Deoxyribonucleic acid (DNA) is a more robust and stable biomarker than ribonucleic acid (RNA). As a result, DNA can remain detectable even after a product has undergone intense heating or extensive processing. In addition, DNA offers high specificity, enabling the direct identification of a particular species, such as *Sus scrofa* (pig). Therefore, DNA-based detection is considered more reliable for processed products, as it allows contamination to be identified with greater specificity and accuracy [7]. Ultrasound has been shown to significantly improve the efficiency of enzyme extraction from the pig liver. This process can extract more target components than conventional methods and is said to "intensify" the release of active substances in complex tissue matrices. Thus, it is necessary to detect the presence of pork and its derivatives in products to protect consumers and maintain the integrity of halal products. The halal nature of a product can not only be determined through organoleptic examination, but laboratory testing is also required to detect the pork content in a product [8].

DNA extraction aims to isolate DNA from various cell contents, including proteins, lipids, carbohydrates, and other cellular components [9]. In general, the DNA extraction process has three main steps: (i) cell lysis, which aims to destroy the cell membrane and wall to release DNA into the solution; (ii) separation of DNA from other cell components, such as proteins, lipids, RNA, and polysaccharides; and (iii) final purification to remove reagent residues and contaminants so that pure DNA is obtained ready for molecular analysis.

Conventional methods, including polymerase chain reaction (PCR) and chemical-based analyses, are widely used to detect pork adulteration. However, their performance may be limited in highly processed products and complex food matrices, where target analytes can be degraded, masked, or present at low concentrations [9,10,11]. Therefore, more sensitive, selective, and reliable analytical approaches are needed to ensure accurate halal authentication.

DNA-based analysis is considered one of the most reliable methods for authentication because of its high specificity and its ability to detect species-specific markers even in processed foods. In this context, ultrasound-assisted extraction has emerged as a promising approach for improving DNA recovery from pork-containing samples. Although some variation in DNA yield may occur, this technique has demonstrated considerable potential for disrupting tissue structure and releasing amplifiable genetic material, thereby supporting accurate species identification for food safety and halal verification.

Ultrasonic-Assisted Extraction (UAE) is based on acoustic cavitation, in which ultrasonic waves generate microscopic bubbles that rapidly form and collapse within the liquid medium. This process creates localized high pressure and temperature, leading to cell disruption, enhanced solvent penetration, and improved release of intracellular components, including DNA [12, 13]. Consequently, UAE offers a rapid and efficient alternative for DNA extraction and represents a promising tool for halal authentication studies. Ultrasound methods have been successfully used to extract

biomolecules, such as proteins, lipids, polysaccharides, and DNA from animal and plant tissue samples [14]. In addition to its high efficiency, this technique can maintain the stability of the DNA structure and increase the purity of the obtained DNA.

Based on the above description, this study aimed to assess the effectiveness of ultrasound techniques in extracting DNA from pig tissue and analyze the quality of the resulting DNA by measuring its concentration, purity, and suitability for PCR applications. The results of this study are expected to provide alternative solutions in the form of more efficient and faster DNA extraction methods to support efforts to detect non-halal materials in the halal industry.

2 Materials and Method

2.1 Materials

The types of samples used in this study were processed foods (pork sausages, Sulawesi, Indonesia), cosmetics (Elizavecca Green Piggy Collagen Jella Pack, Elizavecca Co., South Korea), and medicine (Detusitrol, Pfizer Inc., USA). Samples were stored at a freezer temperature of -20 °C for further analysis. The chemical used were Maxwell®RSC Pure Food GMO and authentication kit (Promega), Wizard® Genomic DNA Purification Kit (Promega), mixture solvent consisting of phenol, chloroform and isoamyl alcohol with a ratio of (24:25:1) (Himedia), ammonium acetate 10 M, ethanol 70 %, isopropanol (Sigma Aldrich), and aquadest.

2.2 DNA extraction using a Commercial Kit

DNA extraction was carried out by using commercial kit as described by previous work [15].

2.3 DNA extraction using ultrasound

A total of 200 mg of the sample was dissolved in 500 µL of water in a 1.5 µL Eppendorf tube. After three seconds, the mixture was separated and was placed in an ultrasonic chamber. Before running the ultrasound process, the Eppendorf tube was welded by a cool tube rack, then set for power (9 W and 450 W) and 25 °C during a specific extraction time (10 min and 40 min) with a duration of 2 s (on) and 2 s (off). After cell lysis by ultrasonication, the sample was mixed, and the supernatant was transferred into a new 1.5 µL Eppendorf tube. 300 µL of Phenol, Chloroform, and Isoamyl Alcohol (25:24:1) was added to the supernatant and mixed by pipetting. The mixture was centrifuged at 16,000 ×g for 20 min, and the upper aqueous phase was transferred to a new microtube for further analysis. Subsequently, 300 µL of 10 M ammonium acetate was added and centrifuged 16,000 × g for 10 min. The supernatant was mixed with 600 µL of isopropanol and centrifuged at 16,000 × g for 10 min. The supernatant was discarded, and the resulting pellet was washed with 300 µL of 70 % ethanol and then dried.

2.4 Determination of Crude DNA pellet

The dried pellet was dissolved in 25 µL of TE buffer. Finally, the DNA concentration of the sample was determined using a QuantusTM Fluorometer. The crude DNA pellet was calculated using Equation 1.

$$Crude\ DNA\ Pellet\ (ng) = DNA\ Concentration\ \frac{(ng)}{(\mu L)} \times Volume\ (\mu L) \quad (1)$$

2.5 Determination of Effectiveness

The method effectiveness (%) was defined as the comparison of crude DNA pellet between ultrasound and commercial kits (Equation 2).

$$Effectiveness\ = \left(\frac{Crude\ DNA\ Pellet\ by\ Ultrasound\ (ng)}{Crude\ DNA\ Pellet\ \ by\ Commercial\ Kit\ (ng)}\right) \times 100\% \quad (2)$$

2.6 Statistical Analysis

This study used a Full Factorial experimental design method for pig DNA extraction, which combines factors of several levels of comprehensive data regarding variables that affect the extraction results, namely, sample type, extraction power, and time.

3 Results and Discussion

3.1 DNA extraction by commercial kit

Table 1. Comparison of Crude DNA Pellets between the three sample types using commercial kits

Sample Types	Crude DNA Pellets (ng)	Commercial Kit
Pork Sausage	160.00	Maxwell® RSC PureFood GMO and Authentication Kit
Cosmetics	0.0704	Wizard® Genomic DNA Purification Kit
Medicine	0.0465	Wizard® Genomic DNA Purification Kit

Table 1 shows a comparison of the crude DNA Pellets between the three sample types using commercial kits. The Maxwell RSC PureFood GMO and Authentication Kit were used for the analysis of the pork sausage samples [15]. The data produced by the Maxwell Pure Food Kit had a DNA concentration of 16.00 ± 1.41 ng/µL with a total DNA pellet of 160.00 ± 14.14 ng. This shows that the Maxwell® RSC PureFood GMO and Authentication Kit effectively purifies DNA from complex samples, such as processed meat. Table 1 also presents the results of DNA extraction from cosmetic and medicine samples using the Wizard® Genomic DNA Purification Kit [16]. The Wizard

Genomic Kit can extract DNA even in small amounts because of its intensive production processes. The resulting DNA concentration was 0.0704 ng/µL, and the total DNA pellet was 0.704 ng. In the medicine sample, the Genomic Wizard kit successfully extracted DNA from the medicine sample at a concentration of 0.0465 ng/µL and DNA pellet at 0.465 ng. Although the value is very low compared to the results extracted from food samples, it still shows that the kit has a better ability to extract DNA from complex and dense chemical matrices, such as medicines. Therefore, the Wizard result was the only one detected, with a percentage of 100% as a relative comparison.

3.2 DNA extraction by Ultrasound

DNA extraction results were evaluated by measuring the concentration of crude DNA and the percentage of DNA extracted using the ultrasound lysis method with different independent variables of power and time for each test sample. The results of measuring the crude DNA pellet and the effectiveness of extraction obtained from the extraction process are presented in **Error! Reference source not found.** (pork sausage sample), Table (medicine sample), and
Table (cosmetic sample).

A 10 min extraction time was chosen to represent a short extraction time, which is one of the advantages of the Ultrasonic-Assisted Extraction (UAE) method. This short time is expected to prevent DNA degradation due to high temperatures and maintain the quality and integrity of the targeted DNA. However, extraction times above 40 min increase the risk of DNA degradation.

Table 2. Crude DNA measurement results in pork sausage samples using the ultrasound lysis method

Power (Watt)	Extraction Time (min)	Crude DNA Pellets (ng)	Effectiveness (%)
9	10	15.40± 1.80	9.63%
9	40	19.55 ± 1.95	12.22%
450	10	29.80 ± 3.20	18.63%
450	40	40.20 ± 1.10	25.13%

Table 3. Crude DNA measurement results in drug samples using the ultrasound lysis method

Power (Watt)	Extraction Time (min)	Crude DNA Pellets (ng)	Effectiveness (%)
9	10	2.2 ± 0.055	463.44%
9	40	3.1 ± 0.41	664.52%
450	10	5.65 ± 1.50	1,213.98%
450	40	6.87 ± 1.77	1,477.42%

Table 4. Crude DNA measurement results in cosmetic samples using the ultrasound lysis method

Power (Watt)	Extraction Time (min)	Crude DNA Pellets (ng)	Emffectiveness (%)
9	10	0.61 ± 0.05	86.86 %
9	40	0.67 ± 0.06	94.53 %
450	10	0.76 ± 0.04	107.24 %
450	40	0.98 ± 0.15	138.49 %

Based on the measurement results, a higher crude DNA pellet from the extraction process was found in the pork sausage sample with a value of 40.20 ± 1.10 ng, with a power of 450 watts for 40 min. When compared to the effectiveness value, the effectiveness value in the pork sausage sample was the lowest compared to other samples, which was 25.13 %. It can be concluded that although DNA can be extracted in large quantities, most of the DNA comes from the total complex biomass and not from the specific DNA target (pork DNA). This is in line with the previous research, which stated that thermal processing of food, such as cooking, smoking, or drying, can reduce nucleic acids, the detection ability, and the proportion of target DNA in processed meat products [17]. An effectiveness value exceeding 100 % indicates that ultrasound is more effective in deciphering complex matrices, resulting in more target DNA than commonly used methods. Comparative studies were not available in literature; this is the first study to compare ultrasound extraction with commercial kits

The relationship between power, extraction time, and extraction results can be seen in the extraction results table. The crude DNA pellets with a power of 90 and 450 W, and an extraction time between 10 min and 40 min, produced the highest crude DNA pellet value at high power and longer extraction time. It can be concluded that the extraction time affects the extraction results of the analysis. The short contact time of the material when the solvent is exposed to ultrasonic waves with the produced heat energy results in few broken cells, so that the compounds extracted by the solvent from the cell matrix are few, and the extract mass is high [18]. Thus, if the extraction time is longer, the number of cells broken by the ultrasonic waves is higher.

In addition to the time factor, power can also affect the extraction results. Higher ultrasonic power produces stronger shear forces, which can increase the extraction yield and efficiency of the process. In addition, a very high intensity or prolonged UAE can result in significant protein denaturation. Therefore, it is imperative to optimize the power parameters during the extraction experiment, and one must be careful to choose the power level that is adjusted to the characteristics of the sample [13]. In the DNA extraction process, especially using the ultrasound-assisted extraction method, the two main parameters that significantly affect the extraction results are power and duration. These two factors play important roles in cell lysis efficiency, DNA release, and the quality of the resulting DNA. High power (above 400 W) significantly increases DNA

yield from processed meat products but needs to be controlled to avoid DNA denaturation.

High-intensity ultrasonic waves can break down bacterial cells through cavitation. If cavitation occurs, a very strong motion is generated near the bubble. The bacteria are subjected to great mechanical stress, and the walls are greatly stretched; if the elasticity is exceeded, the wall tear, and the bacteria die. In addition, near cavitation, there is turbulence that rotates violently. The cell wall can be damaged by the shear stress generated by turbulence. Cavitation derived from ultrasonic waves can also cause the porosity of the cell membrane to widen, allowing cavitation bubbles to enter. Cavitation bubbles affect the performance of capcase-3 enzymes, leading to the apoptosis of bacterial cells.

Another mechanism is nuclear degradation, which results in cell lysis [13]. These changes can be observed in the cell nucleus that undergoes phynosis or lysis, as well as in the cytoplasm that undergoes coagulation and appears pink. Interference from foreign chemicals or the accumulation of toxic substances can inhibit mitochondrial ATP production, which plays an important role in cell transport, especially at the Na+ pump. ATP deficiency results in Na+ ions being unable to be removed from the cell, thus drawing water in and causing the cell to swell and the cytoplasm to appear cloudier.

The duration of extraction also has a significant effect on DNA results. Extraction over a short period can cause the cells to not be wholly destroyed, so the DNA may not be completely extracted. The optimal extraction time using ultrasound was 10–15 min. Longer durations can reduce DNA quality due to excessive heat and pressure [17]. The effectiveness of DNA extraction is greatly influenced by the power and time. High power is needed to lyse the cells, and the time must be controlled to avoid degradation. The results of this test are in line with previous work, who stated that the success of DNA extraction is influenced by several factors, such as tissue type, cell destruction method, and the condition of the enzyme used [19]. However, in certain situations, variations in time or energy intensity (power) do not always cause significant differences in the amount or quality of DNA produced, especially if the extraction process has reached an optimal level of efficiency.

The drug sample showed excellent DNA extraction results, with the highest extraction percentage value reaching 1,477.42 % at 450 watts of power for 40 min. Although the crude DNA pellets were lower than the sausage's (6.87 ± 1.77 ng), the percentage value was very high because the total extracted DNA matrix was lower and more selective to the target DNA. This shows that the ultrasound method is very effective for samples with a more defined matrix, such as drugs, because the active ingredients are not as complex as processed food ingredients. This statement is supported by Lokhande et al., (2024) who stated that ultrasonic-assisted extraction allows better recovery of nucleic acids from pharmaceutical samples through complex matrix disruption caused by cavitation [20].

Meanwhile, DNA extraction from cosmetic samples showed a moderate percentage, with a value of 138.49 %, and the crude DNA pellets reached 0.98 ± 0.15 ng. Although the value is lower than that of drugs, these results indicate that the ultrasound method remains effective in the extraction process of cosmetics containing chemicals, such as preservatives, emollients, and surfactants, which can inhibit the extraction process. As

stated by [21], the nature of the sample matrix and the presence of PCR inhibitors can significantly affect the recovery and purity of the extracted DNA.

In this study, analysis of variance (ANOVA) was conducted to determine the extent to which sample type, extraction tool power, and extraction time affected the results of the crude DNA pellets that were successfully extracted. F and p values are important indicators in statistical analysis for assessing the significance of variables in a model and its effectiveness. The F value indicates how well the model fits the data compared to the variation caused by random errors, and the p-value indicates the importance of each variable in the model. Overall, these values indicate whether the observed relationships in the model are statistically significant or coincidental. The ANOVA results are shown in Table 5. The ultrasound method significantly influenced the crude DNA pellets, as indicated by the p-value of the linear model <0.05.

Table 5. Two-way ANOVA

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	4	4,387,966	1,096,991	11.92	0.000
Linear	4	4,387,966	1,096,991	11.92	0.000
Sample type	2	4,288,090	2,144,045	23.29	0.000
Power (Watt)	1	43,780	43,780	0.48	0.499
Extraction time (min)	1	56,096	56,096	0.61	0.445
Error	19	1,749,006	92,053		
Lack-of-Fit	7	206,444	29,492	0.23	0.970
Pure Error	12	1,542,562	128,547		
Total	23	6,136,971			

4 Conclusions

The maximum crude DNA pellets can be obtained by ultrasound method with a power of 450 watts and a time of 40 min for pork sausage, detusitrol drugs, and cosmetics, reaching 25.13 %, 1, 444.42 %, and 138.49 % compared to extraction results with commercial kits, respectively. It can be concluded that a higher cavitation intensity can optimally lyse cells. However, it should also be noted that high power over a long time can cause DNA degradation if not adequately controlled or adjusted to the sample used. Thus, ultrasound can be an efficient, fast, and environmentally friendly alternative for DNA isolation, especially for detecting non-halal materials.

Acknowledgments. We would like to express our deepest gratitude to all those who contributed to the success of this research. This research was funded by Institut Teknologi Sepuluh Nopember (ITS) and managed under the Strategic Research Grant (SRG) Type D Scheme (Contract No. 1685/PKS/ITS/2026)

Disclosure of Interests. The authors declare no conflict of interest.

References

1. Pramila, C., Ahda, Y., Putri, D. H., Achyar, A.: Optimization of Multiplex Real Time Polymerase Chain Reaction (qPCR) Based Pork Contamination Detection in Beef Processed Food Products. Jurnal Serambi Biologi **8**(3), 466-472 (2023).
2. Mukhlis, Wardiman, D., Yetri, A.: Indonesian Muslim Societies in the Digital Age, Opportunities, Challenges, and Future Prospects. Digital Muslim Review **2**(2), 194-216 (2024). https://doi.org/10.32678/dmr.v2i2.46.
3. SNI 99004: General requirements for halal food. National Standardization Agency of Indonesia (BSN), Jakarta (2021).
4. Salsabila, N., Suciati, Y., Astiwara, E. M.: Analisis Kandungan DNA Babi pada Produk Daging Olahan di Pasar-Pasar Kelurahan Cempaka Putih dan Tinjauannya Menurut Pandangan Islam. Junior Medical Juornal **2**(1), 124–132 (2023). https://doi.org/10.33476/jmj.v2i1.3789.
5. Law of the Republic of Indonesia Number 8, 1999, Consumer Protection, Jakarta, Indonesia
6. Law of the Republic of Indonesia Number 33, 2014, Halal Product Guarantee, Jakarta, Indonesia
7. Artika, I. M., Dewi, Y. P., Nainggolan, I. M., Siregar, J. E., Antonjaya, U.: Real-Time Polymerase Chain Reaction: Current Techniques, Applications, and Role in COVID-19 Diagnosis. Genes **13**(12), 2387 (2022). https://doi.org/10.3390/genes13122387.
8. Gunawan, S., Nurkhamidah, S., Rachmaniah, O., Rahmawati, Y., Meka, W., Fahmi: Manajemen industri produk pangan halal di Indonesia. Andi Press, Yogyakarta (2025).
9. Hutami, R., Bisyri, H., Sukarno, S., Nuraini, H., Ranasasmita, R.: Ekstraksi DNA dari Daging Segar untuk Analisis dengan Metode Loop-Mediated Isothermal Amplification (LAMP). Jurnal Agroindustri Halal **4**(2), 209-216 (2018). https://doi.org/10.30997/jah.v4i2.1409.
10. Prasetyo, M. A. A., Sanjaya, G. E.: DNA Identification of Pork and Beef using Polymerase Chain Reaction Method in Food Industry. Journal of Healtcare Technology and Medicine **9**(2), 1024-1030 (2023). https://doi.org/10.33143/jhtm.v9i2.3344.
11. Mortas, M., Awad, N., Ayvaz, H.: Adulteration detection technologies used for halal/ kosher food products: an overview. Discover Food **2**(15), 1-23 (2022), https://doi.org/10.1007/s44187-022-00015-7.
12. Zhang, J., Yu, P., Fan, L., Sun, Y.: Effects of ultrasound treatment on the starch properties and oil absorption of potato chips. Ultrason. Sonochem (70), 105347 (2021). https://doi.org/10.1016/j.ultsonch.2020.105347.
13. Shen, L., Pang, S., Zhong, M., Sun, Y., Qayum, A., Liu, Y., Rashid, A., Xu, B., Liang, Q., Ma, H., Ren, X.: A comprehensive review of ultrasonic assisted extraction (UAE) for bioactive components: Principles, advantages, equipment, and combined technologies. Ultrason Sonochem (101), 106646 (2023). https://doi.org/10.1016/j.ultsonch.2023.106646.
14. Nath, O., Fletcher, S.J., Hayward, A., Shaw, L.M., Agarwal, R., Furtado, A., Henry, R.J., Mitter, N.: A Comprehensive High-Quality DNA and RNA Extraction Protocol for a Range of Cultivars and Tissue Types of the Woody Crop Avocado. Plants (Basel) 11(3), 242 (2022). https://doi.org/10.3390/plants11030242.
15. Khoiriah, U. L., Baydillah, P., Darmawan, R., Nasori, N., Taufany, F., Gunawan, S.: Lysis Buffers for Detecting Pork Content in Food Products. Indonesian Journal of Chemistry **25**(4), 1051-1063 (2025). https://doi.org/10.22146/ijc.101620.
16. Zabidi, A., Fauzi, F., Abd Razak, F., Rosli , D., Jamil , M., Wan Ibrahim, W., Yahaya , N.: Screening Porcine DNA in Collagen Cream Cosmetic Products. Food Research **4**(1), 151-156 (2020). https://doi.org/10.26656/fr.2017.4(S1).S05.

17. Jun, Y. W., Kant, M., Coskun, E., Kato, T.A., Jaruga, P., Palafox, E., Dizdaroglu, M., Kool, E.T.: Possible Genetic Risks from Heat-Damaged DNA in Food. ACS Central Science **9**(6), 1170–1179 (2023). https://doi.org/10.1021/acscentsci.2c01247.
18. Kanifah, U., Lutfi, M., Susilo, B.: Characterization of Red Betel Leaf (Piper crocatum) Using Ultrasonic Assisted Extraction (UAE) (Study Of Solvent And Extraction Time). Jurnal Bioproses Komoditas Tropis **3**(1), 73–79 (2015). https://jbkt.ub.ac.id/index.php/jbkt/article/view/164.
19. Sajali, N., Wong, S. C., Hanapi, U. K., Jamaluddin, S.A.B., Tasrip, N. A., Mohd Desa, M. N.: The Challenges of DNA Extraction in Different Assorted Food Matrices: A Review. Journal of Food Science **83**(10), 2409–2414 (2018). https://doi.org/10.1111/1750-3841.14338
20. Lokhande, S., Salvi, B.V., Shende, P.: Potential of ultrasonic processing in biomedical applications. RSC Pharmaceutics **1**, 204-217 (2024). https://doi.org/10.1039/D4PM00010B.
21. Qiu, L., Zhang, M., Chitrakar, B., Bhandari, B.: Application of power ultrasound in freezing and thawing Processes: Effect on process efficiency and product quality. Ultrason. Sonochem (68), 105230 (2020). https://doi.org/10.1016/j.ultsonch.2020.105230.

Open Access This chapter is licensed under the terms of the Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License (http://creativecommons.org/licenses/by-nc-nd/4.0/), which permits any noncommercial use, sharing, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license and indicate if you modified the licensed material. You do not have permission under this license to share adapted material derived from this chapter or parts of it.

The images or other third party material in this chapter are included in the chapter's Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the chapter's Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder.