

# Effectiveness of Animal-Waste Scatterers in Room Temperature Vulcanizing Silicone on the Ultrasound Properties of Breast Tissue-Mimicking Phantoms

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## Abstract

This study aimed to determine the effectiveness of animal-waste-derived scatterers incorporated into room temperature vulcanizing (RTV) silicone on the ultrasound properties of breast tissue-mimicking phantoms. Specifically, the study evaluated the effects of shrimp shell powder, eggshell powder, and fishbone powder on echogenicity and speckle characteristics under standardized ultrasound imaging conditions. A quantitative true experimental post-test-only comparative research design was employed. Four phantom formulations were fabricated: RTV silicone with shrimp shell powder and eggshell powder (1:1), RTV silicone with shrimp shell powder, eggshell powder, and fishbone powder (1:1:1), RTV silicone without scatterers, and an agar-based reference phantom. Ultrasound images were acquired using a 10 MHz linear transducer and analyzed through region-of-interest (ROI) analysis using ImageJ software. Results demonstrated that the incorporation of animal-waste-derived scatterers significantly increased echogenicity and speckle compared to RTV silicone without scatterers. Among the fabricated formulations, the RTV silicone phantom containing shrimp shell powder and eggshell powder (1:1) produced ultrasound properties most closely resembling the agar-based reference phantom. Statistical analysis further revealed significant differences among the fabricated phantoms in terms of echogenicity and speckle. The findings suggest that calcium-rich animal-waste-derived materials possess potential as sustainable and cost-effective acoustic scatterers for ultrasound breast phantom fabrication and other tissue-mimicking phantom applications.

**Keywords:** *breast tissue-mimicking phantom, RTV silicone, animal-waste-derived scatterers, echogenicity, speckle, ultrasound imaging*

## 1. Introduction

Cancer remains one of the leading causes of mortality worldwide, accounting for approximately 9.7 million deaths globally in 2022 (National Cancer Institute, 2024). Among all cancer types, breast cancer is recognized as the most commonly diagnosed malignancy among women across most countries worldwide, contributing significantly to global morbidity and mortality (World Health Organization, 2025). In the Philippines, breast cancer continues to represent a major public health concern, with thousands of newly diagnosed cases and deaths annually, highlighting the importance of improving early detection strategies and accessible diagnostic imaging modalities.

Ultrasound imaging has become an important complementary tool in breast cancer evaluation because it is non-ionizing, relatively affordable, widely accessible, and capable of detecting abnormalities in dense breast tissue where mammography sensitivity may be reduced (Kalakattawi et al., 2025). In addition to lesion detection, ultrasound imaging is extensively utilized in biopsy guidance, disease monitoring, and treatment evaluation. The increasing reliance on ultrasound imaging has intensified the demand for realistic breast tissue-mimicking phantoms for training, equipment calibration, quality assurance, and imaging research.

Breast tissue-mimicking phantoms are artificial models developed to replicate the acoustic and imaging behavior of biological tissues under controlled conditions (Ng et al., 2021). These phantoms are widely utilized in medical imaging research and education because they provide a reproducible and safe environment for evaluating ultrasound performance without exposing patients to unnecessary risks. Phantoms also contribute significantly to the improvement of ultrasound scanning skills among radiologic technologists, sonographers, and trainees.

Several materials have been explored for phantom fabrication, including gelatin, agar, silicone, and commercially manufactured synthetic polymers. Agar- and gelatin-based phantoms can reproduce acceptable acoustic properties similar to soft tissue; however, they remain susceptible to microbial degradation and structural instability over time (Aldehani et al., 2025). Commercially manufactured phantoms provide standardized performance and high reproducibility but are often expensive and inaccessible to resource-limited institutions (Bliznakova et al., 2024). RTV silicone-based phantoms, on the other hand, are highly durable, flexible, and resistant to environmental degradation, making them promising materials for long-term phantom applications (Kumar et al., 2024).

In ultrasound imaging, echogenicity and speckle are important parameters in determining tissue realism and acoustic behavior. Echogenicity refers to the ability of tissues or phantom materials to reflect ultrasound waves, producing image brightness, while speckle refers to the granular image texture resulting from the interference of scattered ultrasound waves within heterogeneous structures (Christensen et al., 2024). The incorporation of acoustic scatterers within phantom matrices has been demonstrated to significantly influence these ultrasound image properties.

Recent studies have explored the use of additives such as graphite, aluminum oxide, and calcium carbonate to improve ultrasound scattering behavior and tissue mimicry (Chintada et al., 2022). However, many synthetic scatterers remain relatively expensive and environmentally unsustainable. Consequently, there is growing interest in

the exploration of biodegradable and waste-derived materials as alternative acoustic scatterers for phantom fabrication.

Shrimp shell powder, eggshell powder, and fishbone powder possess calcium-rich mineral components capable of producing acoustic impedance mismatches that influence ultrasound scattering behavior (Tarafdar et al., 2021; Opriş et al., 2023; Muñoz et al., 2024). Shrimp shells and eggshells are composed primarily of calcium carbonate, while fishbone powder contains hydroxyapatite, a calcium phosphate mineral structurally similar to biological tissues. These mineral compositions suggest their potential utility as ultrasound scatterers capable of improving echogenicity and speckle formation in phantom materials.

Despite the known mineral composition and biomedical applications of these materials, limited studies have investigated their effectiveness as acoustic scatterers in RTV silicone-based breast tissue-mimicking phantoms. Therefore, this study aimed to determine the effectiveness of animal-waste-derived scatterers incorporated into RTV silicone on the ultrasound properties of breast tissue-mimicking phantoms using quantitative ImageJ-based analysis of echogenicity and speckle.

## **2. Objectives**

This study aimed to determine the effectiveness of animal-waste-derived scatterers incorporated into room temperature vulcanizing (RTV) silicone on the ultrasound properties of breast tissue-mimicking phantoms.

Specifically, the study aimed to:

1. Determine the echogenicity and speckle characteristics of fabricated RTV silicone-based phantoms;
2. Determine whether significant differences exist among the fabricated phantom formulations; and
3. Identify the formulation that most closely resembles the ultrasound properties of the agar-based reference phantom.

## **3. Materials and Methods**

This study utilized a quantitative true experimental post-test-only comparative research design. The study was conducted at the Radiologic Technology Department of Lorma Colleges using a Mindray diagnostic ultrasound machine equipped with a 10 MHz linear transducer under standardized imaging conditions.

Shrimp shells, eggshells, and fishbones were collected and thoroughly cleaned to remove residual organic matter. The materials were air-dried and oven-dried before being ground into fine powders using a blender. The powdered materials were subsequently sieved through an 80 µm mesh to obtain uniform particle sizes.

Room temperature vulcanizing (RTV) silicone served as the base material for the fabricated phantoms. Silicone oil was added at 40% w/w relative to the RTV silicone base mass to improve flexibility and acoustic transmissibility. Animal-waste-derived scatterers were incorporated at a fixed concentration of 2% w/w relative to the RTV silicone base mass.

Four phantom formulations were fabricated:

- Sample 1: RTV silicone + shrimp shell powder (SSP) + eggshell powder (ESP) (1:1)

- Sample 2: RTV silicone + shrimp shell powder (SSP) + eggshell powder (ESP) + fishbone powder (FBP) (1:1:1)
- Sample 3: RTV silicone without scatterers
- Sample 4: Agar-based reference phantom

All mixtures were thoroughly stirred to ensure homogeneous distribution of scatterers before being poured into molds. RTV-based phantoms were allowed to cure completely at room temperature before ultrasound scanning.

Each phantom was scanned individually using a Mindray diagnostic ultrasound machine equipped with a 10 MHz linear-array transducer. Imaging parameters including gain, depth, dynamic range, and time gain compensation were standardized and maintained across all scans to ensure consistency and comparability.

Ultrasound images were analyzed using ImageJ software through region-of-interest (ROI) analysis. Three non-overlapping ROIs of equal size were selected within homogeneous regions of each phantom image. Mean gray-level intensity was operationally defined as echogenicity, while the standard deviation of gray-level intensity represented speckle texture.

Descriptive statistics including mean and standard deviation were computed for echogenicity and speckle measurements. One-way analysis of variance (ANOVA) and Tukey Honestly Significant Difference (HSD) post hoc analysis were performed using the Statistical Package for the Social Sciences (SPSS) to determine statistically significant differences among phantom formulations at a 0.05 level of significance.

#### 4. Results

This section presents the quantitative ultrasound properties of the fabricated breast tissue-mimicking phantoms in terms of echogenicity and speckle obtained through region-of-interest (ROI) analysis using ImageJ software. The results also present the statistical analysis performed to determine significant differences among the fabricated phantom formulations and identify the formulation that most closely resembled the agar-based control phantom.

**Table 1**  
**Ultrasound Properties of Fabricated Phantoms**

Phantom Sample Formulation	Echogenicity (Mean $\pm$ SD)	Speckle (Mean $\pm$ SD)
Sample 1 RTV + SSP + ESP (1:1)	32.17 $\pm$ 2.46	13.39 $\pm$ 0.11
Sample 2 RTV + SSP + ESP + FBP (1:1:1)	27.19 $\pm$ 1.07	11.95 $\pm$ 1.58
Sample 3 RTV Silicone (No Scatterers)	10.99 $\pm$ 0.06	2.64 $\pm$ 0.08
Sample 4 Agar-Based Control	37.05 $\pm$ 1.30	11.47 $\pm$ 0.73

*Note.* SSP = shrimp shell powder; ESP = eggshell powder; FBP = fishbone powder. Echogenicity values represent mean gray-level intensity (image brightness/acoustic intensity), while speckle values represent the standard deviation of gray-level intensity (ultrasound texture/backscatter variation) obtained through region-of-interest (ROI) analysis using ImageJ software.

Table 1 presents the quantitative ultrasound properties of the fabricated breast tissue-mimicking phantoms in terms of echogenicity and speckle obtained through ROI

analysis using ImageJ software. Among the fabricated phantom samples, the agar-based control phantom demonstrated the highest echogenicity with a mean value of  $37.05 \pm 1.30$ , followed by Sample 1 consisting of RTV silicone with shrimp shell powder and eggshell powder (1:1), which obtained a mean echogenicity of  $32.17 \pm 2.46$ . Sample 2 consisting of RTV silicone with shrimp shell powder, eggshell powder, and fishbone powder (1:1:1) demonstrated a lower echogenicity value of  $27.19 \pm 1.07$ , while the RTV silicone phantom without scatterers exhibited the lowest echogenicity value of  $10.99 \pm 0.06$ .

In terms of speckle, Sample 1 demonstrated the highest mean speckle value at  $13.39 \pm 0.11$ , followed by Sample 2 with a mean speckle value of  $11.94 \pm 1.58$ . The agar-based control phantom demonstrated a mean speckle value of  $11.47 \pm 0.73$ , while the RTV silicone phantom without scatterers produced the lowest speckle value of  $2.64 \pm 0.08$ .

The findings indicate that the incorporation of animal-waste-derived scatterers substantially increased both echogenicity and speckle within the RTV silicone matrix. RTV silicone without scatterers demonstrated weak acoustic scattering and minimal ultrasound texture formation, suggesting that RTV silicone alone possesses relatively homogeneous acoustic characteristics with limited internal heterogeneity capable of producing realistic acoustic backscatter behavior.

The increased echogenicity and speckle observed in Sample 1 suggest that the incorporation of shrimp shell powder and eggshell powder enhanced ultrasound wave scattering and acoustic backscatter behavior within the phantom matrix. The elevated echogenicity indicates increased ultrasound reflectivity and image brightness, while the higher speckle value suggests the formation of more heterogeneous internal acoustic structures capable of generating more realistic ultrasound texture patterns.

Meanwhile, the incorporation of fishbone powder in Sample 2 resulted in lower echogenicity and speckle values compared to Sample 1. Although Sample 2 still demonstrated substantially higher ultrasound properties relative to RTV silicone without scatterers, the addition of fishbone powder may have influenced scatterer distribution, particle uniformity, or internal dispersion within the RTV silicone matrix. Variations in particle composition, density, and distribution may therefore have moderated the overall scattering efficiency and acoustic backscatter behavior within the phantom material.

One-way analysis of variance (ANOVA) revealed statistically significant differences among the fabricated phantom formulations in terms of echogenicity and speckle. For echogenicity, the ANOVA produced an F-value of 172.57 with a p-value of less than 0.001, indicating statistically significant differences among the fabricated phantom groups. Similarly, speckle analysis yielded an F-value of 94.14 with a p-value of less than 0.001, confirming statistically significant differences in ultrasound texture characteristics among the fabricated phantoms.

The statistically significant findings indicate that variations in scatterer composition and formulation substantially influenced ultrasound image brightness, texture formation, and acoustic backscatter behavior within the fabricated phantoms. The large F-values further suggest that the observed variations were primarily associated with phantom composition rather than random variation alone.

Tukey's Honestly Significant Difference (HSD) post hoc analysis further demonstrated that all pairwise comparisons for echogenicity yielded statistically significant differences ( $p < 0.05$ ), indicating that each phantom formulation produced

distinct acoustic intensity and image brightness characteristics. For speckle, statistically significant differences were observed between Sample 1 and Sample 3, Sample 2 and Sample 3, and Sample 3 and Sample 4. However, no statistically significant differences were observed between Sample 1 and Sample 2, Sample 1 and Sample 4, and Sample 2 and Sample 4, suggesting similarity in ultrasound texture characteristics and acoustic backscatter behavior among these phantom formulations.

The comparison between Sample 1 and the agar-based control phantom yielded a non-significant p-value of 0.102 for speckle, indicating statistically comparable ultrasound texture characteristics between the two phantom formulations. Although Sample 2 demonstrated numerically closer speckle values relative to the agar-based control phantom, Sample 1 demonstrated higher speckle intensity and more pronounced ultrasound texture formation, suggesting greater internal heterogeneity and acoustic backscatter variation within the RTV silicone matrix.

Overall, the findings indicate that the incorporation of animal-waste-derived scatterers improved the acoustic behavior and ultrasound texture characteristics of RTV silicone breast tissue-mimicking phantoms. Among the fabricated RTV silicone formulations, Sample 1 demonstrated the most favorable combination of echogenicity, speckle formation, and tissue-mimicking capability relative to the agar-based control phantom.

## **5. Discussion**

The findings of the study demonstrated that the incorporation of animal-waste-derived scatterers significantly influenced the ultrasound properties of RTV silicone breast tissue-mimicking phantoms. The increased echogenicity observed in the fabricated phantoms containing shrimp shell powder, eggshell powder, and fishbone powder suggests enhanced ultrasound wave scattering and acoustic backscatter behavior caused by increased acoustic impedance mismatch within the RTV silicone matrix.

RTV silicone without scatterers demonstrated the lowest echogenicity and speckle values, indicating weak ultrasound scattering behavior and minimal ultrasound texture formation. This finding suggests that RTV silicone alone possesses relatively homogeneous acoustic characteristics with limited internal heterogeneity capable of generating realistic ultrasound texture patterns and acoustic backscatter variation. The low standard deviation values observed in Sample 3 further indicate consistent ROI measurements associated with the uniform internal structure of RTV silicone without embedded scatterers.

The incorporation of shrimp shell powder and eggshell powder in Sample 1 substantially increased image brightness and ultrasound texture formation. The higher echogenicity and speckle values observed in this formulation indicate stronger acoustic backscatter and more heterogeneous internal acoustic structures capable of generating realistic ultrasound texture patterns. These findings suggest that the incorporated scatterers improved the tissue-mimicking capability of RTV silicone by increasing internal acoustic heterogeneity.

The observed improvements in ultrasound properties may be attributed to the mineral composition of the incorporated animal-waste-derived materials. Shrimp shell powder and eggshell powder are rich in calcium carbonate, which increases acoustic impedance mismatch within the RTV silicone matrix and enhances ultrasound wave

reflection and scattering behavior (Tarafdar et al., 2021; Jin et al., 2024). These calcium-rich microstructures likely contributed to the stronger acoustic scattering behavior and more defined speckle patterns observed in Sample 1.

Although the incorporation of fishbone powder in Sample 2 also improved ultrasound properties relative to RTV silicone without scatterers, the formulation demonstrated lower echogenicity and speckle values compared to Sample 1. Fishbone powder contains hydroxyapatite, a calcium phosphate mineral associated with acoustic scattering behavior because of its density and stiffness relative to soft phantom matrices (Muñoz et al., 2024; Hussin et al., 2023). However, differences in particle morphology, dispersion, and interaction within the RTV silicone matrix may have influenced the scattering efficiency and acoustic backscatter behavior observed in Sample 2.

The statistically significant differences observed among the fabricated phantom formulations indicate that scatterer composition, concentration, and internal distribution substantially influenced ultrasound image brightness and texture formation within the fabricated phantoms. The large ANOVA F-values further suggest that the observed differences in echogenicity and speckle were primarily associated with phantom composition rather than random variation alone.

The findings are supported by Filippou et al. (2022), who reported that the incorporation of scattering structures within tissue-mimicking phantoms increases acoustic backscatter and alters ultrasound attenuation properties because of enhanced internal heterogeneity. Similarly, Armstrong et al. (2023) explained that tissue-mimicking materials containing embedded scattering agents generate more realistic ultrasound texture patterns and image brightness due to increased acoustic impedance variation and speckle formation. Kamalinia et al. (2023) further demonstrated that variations in material composition and internal structural properties significantly influence echogenicity and ultrasound image appearance in tissue-mimicking materials.

The Tukey HSD post hoc analysis further demonstrated that Sample 1 and Sample 4 exhibited statistically comparable speckle characteristics, suggesting similarity in ultrasound texture formation and acoustic backscatter behavior between the RTV silicone phantom containing shrimp shell powder and eggshell powder and the agar-based control phantom. Although Sample 2 demonstrated numerically closer speckle values relative to the agar-based phantom, Sample 1 exhibited higher speckle intensity and more pronounced ultrasound texture formation, indicating greater internal heterogeneity and acoustic scattering complexity.

Overall, the findings demonstrate the potential application of sustainable and biodegradable animal-waste-derived materials as low-cost acoustic scatterers for ultrasound breast phantom fabrication. The study contributes to the growing interest in environmentally sustainable biomedical imaging materials by transforming discarded biological materials into functional components for tissue-mimicking phantom development.

## **6. Conclusion**

The findings of the study demonstrated that the incorporation of animal-waste-derived scatterers significantly improved the ultrasound properties of RTV silicone breast tissue-mimicking phantoms in terms of echogenicity and speckle characteristics. The fabricated phantoms containing shrimp shell powder, eggshell powder, and fishbone

powder exhibited higher echogenicity and more defined speckle formation compared to RTV silicone without scatterers, indicating enhanced acoustic backscatter behavior and improved ultrasound texture characteristics. These findings suggest that the incorporation of calcium-rich animal-waste-derived materials increased internal acoustic heterogeneity within the RTV silicone matrix, thereby improving the tissue-mimicking capability of the fabricated phantoms.

The study further demonstrated statistically significant differences among the fabricated phantom formulations in terms of echogenicity and speckle, indicating that scatterer composition substantially influenced ultrasound image brightness, acoustic behavior, and texture formation within the fabricated phantoms. Variations in the type and combination of incorporated scatterers affected the overall scattering efficiency and internal acoustic properties of the RTV silicone matrix. The statistically significant findings confirmed that the observed differences in ultrasound properties were primarily associated with phantom composition rather than random variation, highlighting the importance of scatterer selection and formulation in ultrasound phantom fabrication.

Among the fabricated RTV silicone formulations, the phantom containing shrimp shell powder and eggshell powder (1:1) demonstrated the most favorable ultrasound properties relative to the agar-based control phantom. The formulation exhibited statistically comparable speckle characteristics and improved ultrasound texture formation compared to the other RTV silicone-based phantoms evaluated in the study, suggesting enhanced acoustic similarity and more realistic tissue-mimicking behavior. These findings further suggest that calcium-rich animal-waste-derived materials possess potential as sustainable, low-cost, and effective acoustic scatterers for ultrasound breast phantom fabrication and other tissue-mimicking phantom applications. Future investigations may further optimize scatterer concentration, particle size, and internal distribution to improve the acoustic performance and tissue-mimicking capability of RTV silicone-based phantoms.

Furthermore, future studies may evaluate additional acoustic parameters such as attenuation coefficient, speed of sound, and acoustic impedance to provide more comprehensive assessment of phantom performance and establish closer approximation to actual breast tissue properties. The incorporation of vacuum degassing techniques, varying RTV silicone hardness levels, and additional biodegradable or waste-derived biomaterials may likewise be explored to improve phantom uniformity, acoustic realism, and reproducibility. Additional phantom samples, repeated trials, and comparative investigations are also recommended to further strengthen the reliability and potential applicability of fabricated tissue-mimicking phantoms in ultrasound training, quality assurance, and imaging research.

## **7. Acknowledgements**

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## 9. Appendices

### APPENDIX A Certification of Exemption from Review

**LORMA**  
COLLEGES

REC Form 007  
CERTIFICATE OF EXEMPTION FROM REVIEW

**CERTIFICATION OF EXEMPTION FROM REVIEW**

REC Reference # 2025-242

To: Aishwarya L. Barroren, Claudia Ail A. Cerros, Audrey C. Gonzalez, Karlie Mae S. Jaldoc, and Cliv Webber A. Salas

From: LORMA Colleges - Research Ethics Committee

Date: January 13, 2025

This is to certify that the Research Proposal entitled, "EFFECTIVENESS OF ANIMAL WASTE SCATTERED IN BIODEGRADABLE MICROENCAPSULES WITH BIOPOLYMER ON THE ULTRASOUND PROPERTIES OF BREAST TISSUE-MIMICKING PHANTOMS" submitted by Aishwarya L. Barroren, Claudia Ail A. Cerros, Audrey C. Gonzalez, Karlie Mae S. Jaldoc, and Cliv Webber A. Salas, of College of Bachelor Technology, has been reviewed by the Research Ethics Committee of LORMA Colleges and found that all ethical considerations are in place to conduct the research in the stated basis of the study. Hence, this research proposal is exempted from review.

*P. VERA, LPT*  
Chairwoman, REC

## **10. Author(s) Biodata**

Ashley Rose I. Barrocan, Charles Azil A. Corpuz, Audrey C. Gonzales, Karylle Mae S. Jadsac, and Clyd Webber A. Solano are fourth-year Bachelor of Science in Radiologic Technology students from Lorma Colleges who collaboratively conducted a study focused on the utilization of animal-waste-derived scatterers in room temperature vulcanizing (RTV) silicone for breast tissue-mimicking phantom fabrication. Guided by their research adviser, Mr. Mark Burgonio, and research instructor, Mrs. Marites C. Pagdilao, the researchers pursued this study to contribute to the advancement of ultrasound imaging, tissue-mimicking phantom development, and sustainable biomedical materials for educational, research, and quality assurance applications.

Driven by their interest in ultrasonography, medical imaging, and innovative phantom fabrication, the researchers explored the potential application of shrimp shell powder, eggshell powder, and fishbone powder as acoustic scatterers capable of improving echogenicity and speckle characteristics in RTV silicone-based breast phantoms. Through this study, the researchers aimed to support the development of realistic, sustainable, accessible, and affordable ultrasound breast tissue-mimicking phantoms.

The researchers aspire to further contribute to the advancement of radiologic technology and medical imaging through research, innovation, and professional practice in the field of diagnostic imaging and ultrasonography.