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Research Article

Reused N95 Face Masks Performance: Effect of Gamma Irradiation Method on Residual Bioburden and Functional Material Resistance

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Abstract: Due to the pandemic of Covid-19, the use of face masks has been very intense. There is a need for an innovative solution to enable reuse of face masks that have been exposed to viruses or bacteria. Face mask sterilization by gamma irradiation could be one of the solutions. However, to ensure that the quality of the sterilized mask is still at a satisfactory level, chemical, thermal, and mechanical tests need to be conducted. Firstly, microbial analysis or bioburden is carried out. The effective dose for reducing microbes is found 23.2 kGy. These results are reinforced by several functional tests. FTIR measurement found no crucial functional group changes from polyester and polypropylene to masks. At 15 kGy and 25 kGy absorbed gamma irradiation, the elongation tensile strength of the mask slightly increased by 1.3 and 0.8 points higher than the initial. The tensile strength value decreased from 3.02 MPa to 2.27 MPa and 2.53. MPa for 15 kGy and 25 kGy. After several sterilization techniques, DSC curves showed melting point of polyester and polypropylene do not significant changes. SEM-EDS analysis show no presence of bacteria of viruses 0% mass of nitrogen at each of several methods of sterilization. After gamma irradiation, the morphology of polypropylene surfaces became slightly flawed, spaced, and fractured.

Keywords: Bioburden; COVID-19; Face mask; Functional test; Gamma irradiation

1. Introduction

The SARS-CoV-2 virus caused the COVID-19 pandemic, which affected people worldwide. This virus results in a massive economic impact and increased medical waste. In Indonesia, the amount of medical waste generated until July 2021 had reached 18,460 tons (Amanah, 2022). Additionally, safety protocols need to be implemented in various settings, which has resulted in a high demand for face masks. This has led to the need for new solutions, such as the reuse of face masks, which

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require collaboration among companies, governments, and scientists to address these challenges (Pérez et al., 2022).

Several methods can be used to sterilize face masks, such as chemical and physical sterilizations. Chemical sterilization uses disinfection methods to expose the viruses. Mostly they measured the effect of some disinfectant variants, reduction of viral infectivity and viral half-life. Physical sterilization used UV-C irradiation and ozone exposure over time (Nicolau et al., 2021). Coating and filming surfaces by several substances, as shown, reduced the activity and titer of the viruses after 1 hour (Viana Martins et al., 2022). SARS-CoV-2 is inactivated by 20 kGy of gamma irradiation, with potential deformation of the FFP respirator occurring at 24 kGy (Rubio-Romero et al., 2020). However, the high doses of irradiation cause damage to the polymer face mask system (Naikwadi et al., 2022), and UV sterilization might not reach the inner layer due to shadows (Bentancor et al., 2021). Dry or steam heating can be used for sterilization, but a lot of materials are sensitive to high temperatures and heat (Yuen et al., 2022; Anderegg et al., 2021).

A sterilization process was carried out on used face masks to eliminate bioburden, which is the number of microorganisms on the surface. This research will focus on physical sterilization by gamma irradiation and compare the procedures to the other methods. At the final stage, this research intend to determine sterilization inactivated dose of bacteria, fungi and also SARS-CoV-2 by gamma irradiation (Yap et al., 2020). Additionally, this study aims to verify that the inactivated dose of gamma radiation will not significantly affect the chemical and mechanical properties of the face mask. So that the contaminated mask can be reused after sterilization. This research has the recent invention i.e., N95 face masks SARS-CoV-2- contaminated can be reused after sterilization by Co-60 gamma irradiation without lack the quality of the material.

2. Methods

2.1. Analysis of Bioburden

This research was divided into three stages; first, analyzing the bioburden of used masks. Second, sterilization of used masks through the γ-irradiation method and comparison with other methods like UV light, heating, and autoclaving. Third, functional analysis of sterilized used masks.

The 3M™ N95 type 8210, St. Paul, USA. Face masks were obtained from the nuclear area health clinics used by medical doctors, nurses, and analysts. The workers used double layers of masks, where the first was a 3-ply head loop, and the second layer was N95 masks. The average time of wearing a mask was 4 hours, and the bioburden was counted using the dilution method of the total bacterial and fungal. Nutrient agar (NA) andpotato dextrose agar (PDA) was used as media for total bacteria and fungi. The verification dose could be determined from the Sterility Assurance Level (SAL) table in ISO 11137 based on the bioburden data. (Marsit et al., 2014). The radiation dose estimated to produce a guaranteed level of sterility, with a value of SAL 10-2, was used to determine the sterilization. A total of 100 used mask samples were placed in sterile PE plastic bags and irradiated at the calculated dose.

Furthermore, 100 samples were irradiated and tested for sterility to determine the number of non-sterile samples. After receiving the verification dose, the sterilization dose is determined based on ISO 11137 with SAL 10-6. The sterility assurance level (SAL) is the probability that a microbe is still alive in a product unit after the sterilization process. For an accepted verification dose, the sterilization is determined based on the table of sterility assurance level (SAL) = 10-6 in ISO 11137 (Trandafir et al., 2012).

2.2. Sterilization

2.2.1. Gamma irradiation

Irradiation was performed using a Co-60 source Gamma Cell-220, Nordion, Canada, Upgraded irradiator by Izotop, Hungary (dose rate 3,867.1 Gy/hour). The temperature in the chamber of the Gamma Cell is about 35-37 °C. Furthermore, a Red Perspex batch PT 4034 dosimeter, Harwell

Company UK, was used to determine the distribution of absorbed doses. Several dosimeters are placed on different parts of the sample. The absorbed dose was determined by converting the measured absorption value at 640 nm using a UV-Vis spectrophotometer (Agilent, Malaysia) and the thickness of the dosimeter obtained using a screw micrometer.

2.2.2. Non-γ-irradiation

There were three non-γ-irradiation methods used for sterilizing masks. First, the masks were irradiated using a UV source (Cleaver Scientific Ltd.) at room temperature for one hour. Subsequently, the autoclave method was carried out for approximately two hours at a pressure of 1 ATM and a temperature of 121°C using Hirayama Manufacturing equipment. The masks were also subjected to heat using a Fisher Isotemp Incubator 200 Series Oven (Heraeus Thermo Scientific) at a temperature of 70°C for 1.5 hours.Functional Tests

Several methods were observed to analyze the functional properties of face masksbefore and after irradiation treatment. Meanwhile, conventional surgical masks were usedfor the experiments and manufactured according to the United States NIOSH-42CFR84 for clinical device use. These three-layered masks consist of thermoplastic elastomer, aluminum, polyurethane, polypropylene, and polyester.

The membrane surface morphology test was performed using a scanning electron micrograph (SEM, JSM-6510LA, JEOL Ltd. Japan.) with a magnification of 2000 times and avoltage of 30kV.

FTIR spectrometer analysis (Shimadzu, IR Prestidge-21) is applied for allmeasurements using the reflectance method. For each measurement, IR data were characterized in the range of 4000 cm^{-1} to 400 cm^{-1}, with 64 spectra per second at 4 cm^{-1} resolution (Qiu et al., 2016). At the end of FTIR measurements, all data were 3-point baseline corrected. This method can predict subject matter modification in the polymer structure and identify functional group changes before and after Co-60 irradiation. Furthermore, the thermal properties of the 3M face mask were analyzed usingdifferential scanning calorimetry (DSC-60, Shimadzu) in the range of 30 °C up to 250 °C. DSC is an analytical tool for this method to observe the thermal study of the differences in melting point, glass transition/crosslinking/decomposition, and corresponding enthalpyof face mask materials (Sirin et al., 2022).

The tensile strength of the mask analyzed by the Universal Testing Machine (UTM,Z005, Zwick Roell) can provide information in the stress-strain graph. The measurement test parameters follow the ASTM D 638 Standard Test Method for Tensile Properties of Plastics with a load cell of 2.5 kN. The mask is cut as shown in Figure 1. using a dumbbell cutter ASTM 1822-L or the same ASTM D 638 type V. The polymer surface morphology test was performed using a scanning electron micrograph with a magnification of 500 times and a voltage of 20 kV.

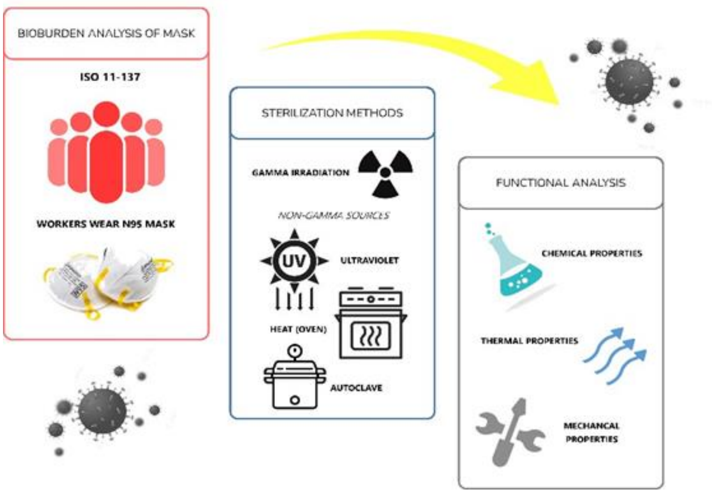

Figure 1 Three stages of the research

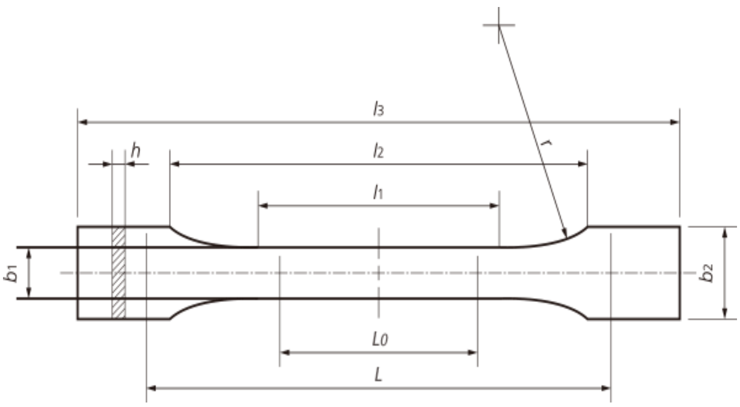

Figure 2 The specimen test of tensile strength

Meanwhile, SEM was used to image the surface shape inside the face mask at variation magnification power before and after gamma irradiation.

3. Results and Discussion

3.1. Microbial Analysis and Bioburden

The total microbial contamination in used N95 masks presented different results for each method. Figure 3 shows differences between blank in UV, Oven, and irradiation methods for the number of microbial detected. The UV and oven methods still detected bacteria, but those in the oven method were fewer. In addition, no fungi were detected in the oven method, unlike in UV. The microbial analysis was not conducted in the autoclave method because the samples were broken. According to the results, among three methods of non-γ-irradiation sterilization, the autoclave is the best for reducing the number of microbes since the process are sufficient for 21 minutes, and the samples are not damaged (Scaglione et al., 2022). Bioburden analysis was carried out based on ISO 11137. The average value of the entire batch is used to determine the verification dose when the average bioburden per batch is less than twice the total. The mean bioburden of batches 1,2, and 3 was less than twice the average of the entire batch. Therefore, the verified dosesof SAL 10^{-2} and sterilized SAL 10^{-6} were 9.6 and 23.2 kGy, respectively. Tests with 100 samples using a verification dose showed that only 1 showed bacterial growth, while the test results with a sterilization dose indicated the absence of bacteria and fungi.

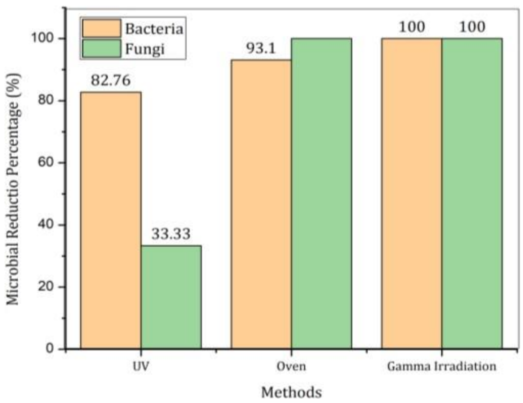

Figure 3 Comparison data of microbial analysis for the sterilization method

The oven and autoclave are heating methods, while UV irradiation is a process that utilizes non-ionizing radiation where this non-ionizing source has low energy. When this energy encounters a material, it does not have enough quantum to inoculate the atoms (Boschini et al., 2011). However, it produces heat that can cause an inactivation reaction and denatured protein in certain viruses by observing a certain time and temperature, which can cause the virus to be reduced (Kampf et al., 2020). This reaction damages DNA, but the impact is only for small microbes and viruses (Tomita, 2010).

In contrast, ionizing radiation, such as high-energy X-rays/gamma radiation, will damage DNA by direct deposition or secondary interactions with surrounding atoms or molecules. Secondary interactions occur with the surrounding water molecules, forming free radicals, OH- responsible for 90% of the resulting DNA damage. The direct and indirect interactions can lead to significant double-strand breaks, often resulting in cell death (Iliakis et al., 2019). Therefore, ionizing radiation effectively extinguishes microbes or viruses in a sterilization dose.

3.2. Functional Analysis

3.2.1. Fourier Transform Infra-Red (FTIR)

FTIR spectra of the new N95 mask are shown in Figure 4. Shell of N95 consists of polyester (PEs) material with an identical absorption peak of unsaturated polyester (Yanget al., 2014) and polyester-fibers (Deka et al., 2015). Figure 4 shows the valence band of –OH groups at 3421 cm^{-1}, for –COO groups at 1629 cm^{-1} and 1580 cm^{-1}. There are 2 types of carbonyl groups, namely -COO is associated with long-chain carbon from polyester, and - COO binds to an aromatic ring. Bands of the –CH group are specific at 2962, 2951, and 2882 cm^{-1} for –CH, -CH2, and -CH3, respectively. After irradiation, the absorbances of –OH bands (Fig. 4b, Fig. 5b) increased due to the oxidation process from gamma irradiation. The intensity became stronger because the energy of gamma irradiation generated new hydroxyl groups. Radicals of •H and •OH, which is generated by gamma irradiation, merged and produced hydroxyl groups (Navarro et al., 2018). The new (Fig. 4a) and used N95 (Fig. 5a) mask shifts at each FTIR spectra. The effect of gamma irradiation doses breaks the polyester molecular chain to decrease the molecular weight.

Gamma rays are powerful ionizing radiation, well- penetrated to the materials. Therefore, affect polymer structure by generating free radicals, ions, and peroxides. These types can bond to each molecule to form a longer polymer chain.As a consequence, the absorption intensity of some peaks has changed, showing the relative change of carbonyl and hydroxyl groups (Motaleb et al., 2021). Methine group absorption can substantially change after gamma irradiation treatments because it can break the chemical bond of –CH in polyester. The high energy of gamma irradiation generates many free radicals •H from the –CH bond and could react with any other •RCOOand •O to change the adsorption intensity of –CH, -C=O, and –OH bands (Svoboda et al., 2021). In addition to gamma irradiation, the effect of autoclave, oven, and UV irradiation methods (Fig. 5c) was observed on the N95 mask (Figure 5). FTIR spectra show no crucial changes in each peak. Furthermore, slight changes at 3417, 2824, and 1630 cm^{-1} were analyzed with different sterilization methods. From three different sterilization methods, the mask with higher temperatures affected molecule's rearrangement (Jung and Lee, 2021).

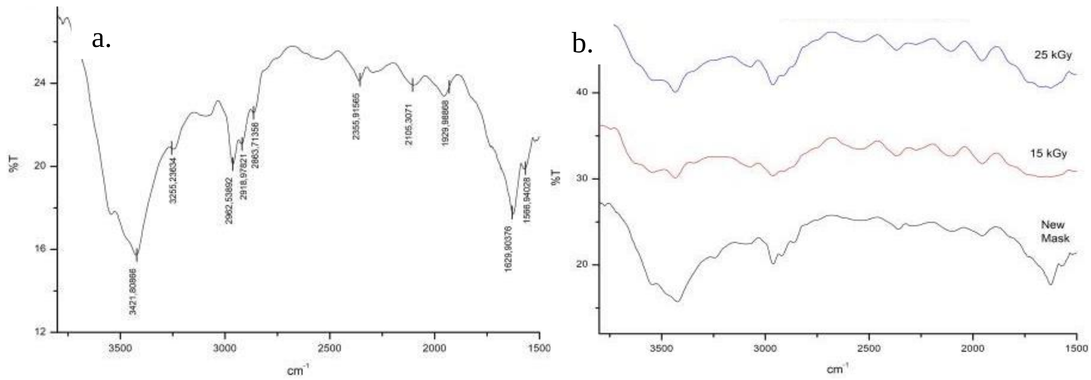

Figure 4 FTIR spectra of new shell part N95 mask and irradiation N95

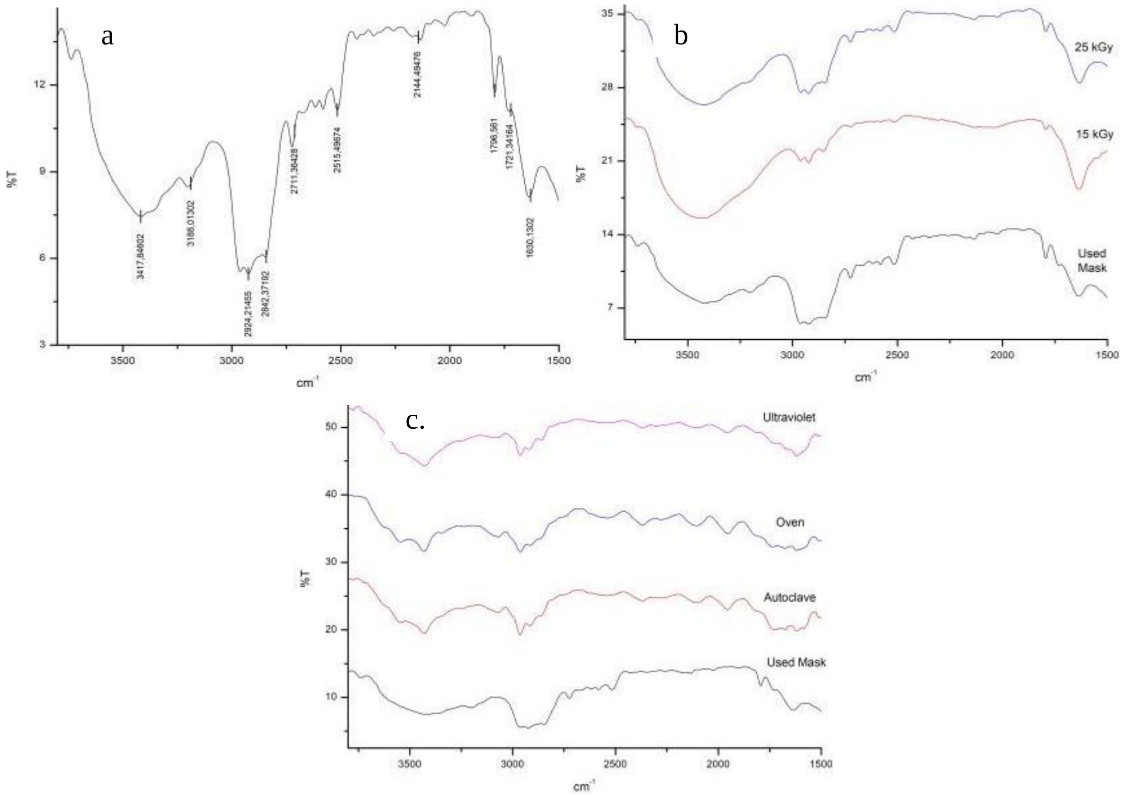

Figure 5 FTIR spectra of Used N95 mask, irradiation Used N95 mask and Used mask with Autoclave, Oven and UV treatment

3.2.2. Differential Scanning Calorimetry (DSC)

The shell part of the N95 mask consists of polyester (PEs), and when separated from the whole part, a small amount of polypropylene (PP) was obtained from the filter part. PEs melting point is around 130-170°C, depending on the processing methods. Furthermore, it has a melting point below 165 °C, but with present techniques, PP has around 170°C (Olejnik et al., 2020; Nayak et al., 2013). Thermal conductivity of PP is affected by its pore and shape of PP also (Zulkarnain et al., 2017). Pore size and shape determine the density of PP, more of density the melting point of the polymer becomes higherbut has low reactivity. In Figure 6a and 6b, the new N95 mask consists of two curves at a melting point of 156.05 ^{0}C, which belongs to PP. The DSC curve of the new N95 mask shows an intense exothermic phenomenon after reaching the temperature of 125°C, which is related to the melting of PP. Thermoxidative degradation of PP occurred at a temperature of 200-225°C, and the mass loss has to be confirmed by thermogravimetry analysis (Parthasarathy et al., 2013).

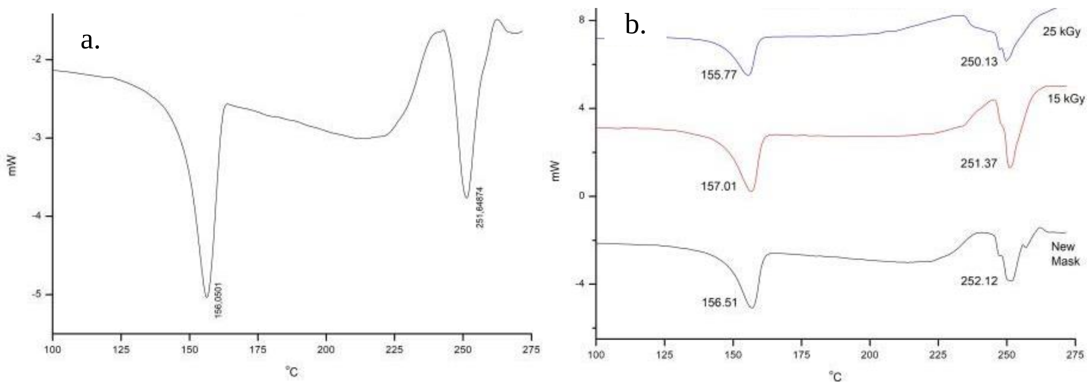

Figure 6 DSC spectra of new shell part N95 mask and irradiation N95 mask

PP experiences surface degradation and produces volatile PP oligomers, called fragmentation of polymers through irradiation (Noguchi and Yamasaki, 2020; Romano et al., 2018). DSC curves in Figure 7a. show each melting point from the endothermic peak 156- 158 °C for PP and 250-253 °C for PEs. The melting point of control, γ-irradiated up to 25 kGy (Fig 7b), and several sterilization methods (Fig. 7c) do not change when only each sample has the same concentration of PP polymers. PEs shift at 250-253 °C due to different particle sizes and shapes (Bouza et al., 2011).

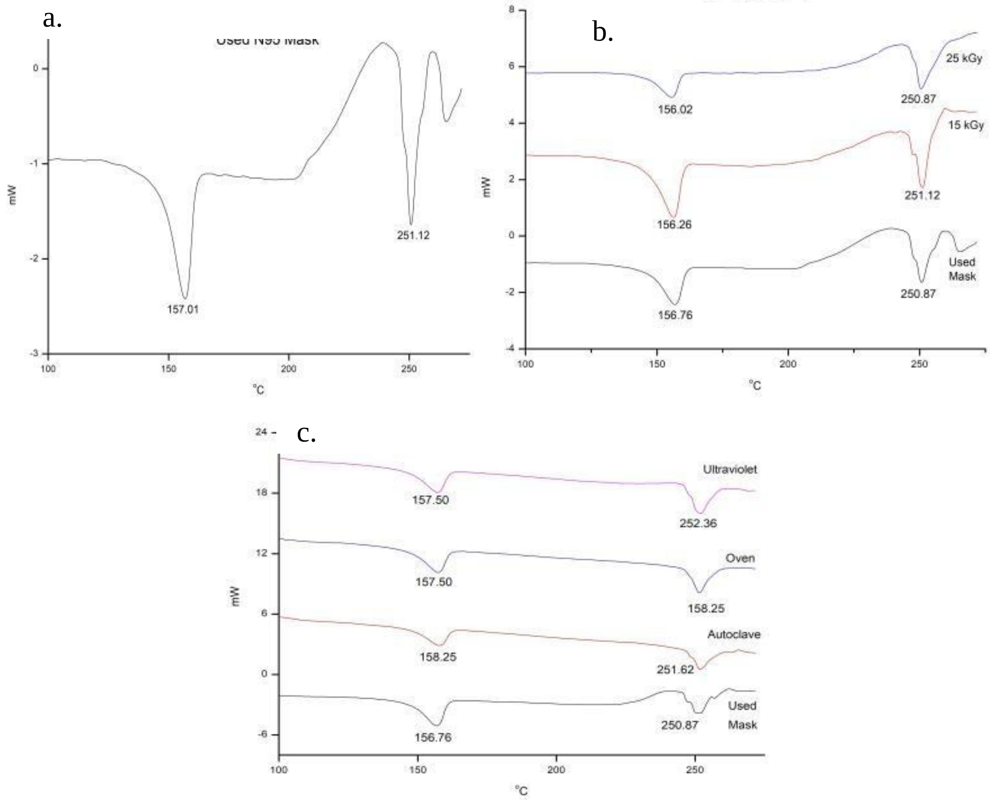

Figure 7 DSC spectra of shell part used N95 mask, irradiation used N95 mask and used N95 mask with several methods of sterilization

3.2.3. Universal Testing Machine Analysis

The elongation at tensile strength (ETS) and tensile strength (TS) of the new N95 mask was analyzed to be 7.23 % (avg.) and 3.02 MPa (avg.), respectively. Furthermore, theN95 mask was treated by gamma irradiation at 15 kGy and 25 kGy. The ETS value of the mask slightly increased by 1.3 points to 8.5 % at a 15 kGy absorbed dose. Figure 8 shows the effect of gamma irradiation on the ETS and TS values of the new N95 mask. The TS values of the 15 kGy irradiated mask have a shrimpy lowering to 2.27 MPa. Kilmartin-Lynch et al. found another study about new N95 mechanical properties. It has 3.27 MPa of tensile strength, with the best result at 3.67 MPa. The tensile strength of the mask was analyzed by 3.02 MPa (avg.) with a similar method ASTM D638-2014. After gamma irradiation, TS was measurably reduced to 2.27 MPa (15 kGy) and 2.53 (kGy). N95 masks, which contain PP and are exposed to the dose rate of gamma irradiation, may cause molecular structural changes. Remarkably, the changes in crystallinity are due to PP chain scission degradation (Romano et al., 2018: Gahleitner et al., 2011), (Otaguro et al., 2010). In this experiment, the mechanical properties were also investigated. Used masks with various gamma irradiation dose rates were compared and heated by autoclave (121°C), oven (70 °C, 2h), and UV (2h) treatment.

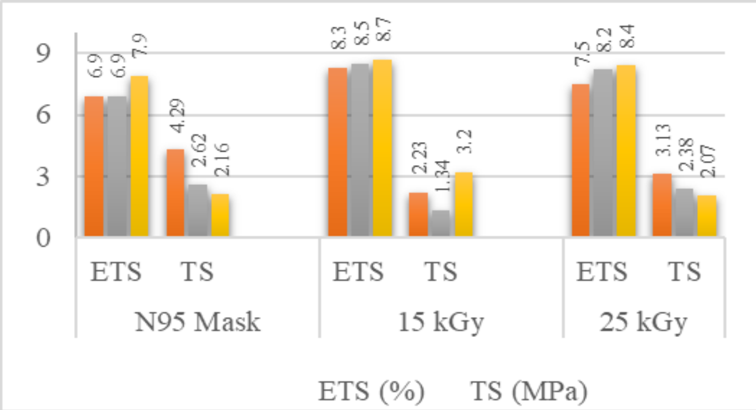

Figure 8 Elongation at tensile strength and tensile strength analysis of new shell part N95 maskand irradiation N95 mask

According to the result in Figure 9., the mechanical properties have improved the elongation to tensile strength values. The ETS increased by 6.1 points. Gamma irradiation of the used mask also increased by 2.6 points of ETS, even though the mechanical properties of PP at the exactdose rate should be decreased (Svoboda et al., 2021). However, it may be caused by the accumulation of heat from the mask at the medical laboratory before being treated by gamma irradiation. Theoretically, molecule reinforcement by heat (irradiation, oven, autoclave, and UV in this research) produced rougher fiber of PP. This condition increased the bond between fibres and matrices and then improved the mechanical properties of PP (Shieddieque et al., 2021). The N95 mask had a 27% elongation improvement after being used at the medical laboratory. This improvement may be induced by the overheated PP particles, as macro chains self-crosslink and transform into homopolymers (Serbetci et al.,2007). The degree of deterioration had not occurred because of the increase in the levels of ETS following numerous heating treatments. The heat may affect the PP molecule structure and cause a crosslink between the PP chain to generate small chains (Ostergard, 2011).

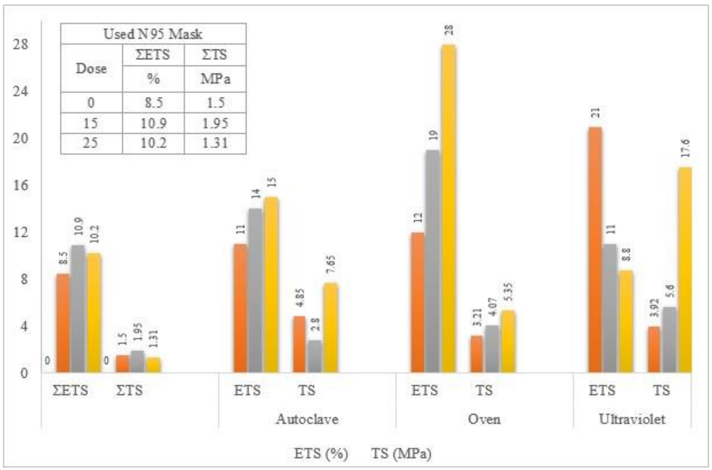

Figure 9 Elongation at tensile strength and tensile strength of Used N95 mask with Autoclave, Oven and UV treatment

3.2.4. Surface Morphology Analysis

The SEM examined the morphology of the N95 mask with several treatments at room temperature and exams up to 1000 times magnification. SEM images from N95 and before irradiation indicated that the shell layer of PP from the N95 mask was made up of non-woven polypropylene fibers tangled to form a three-dimensional porous structure (Nam et al., 2016).

Used masks and masks treated by gamma irradiation have cracks and defects. Small particles are formed at fiber surfaces. Figure 10 shows the meltdown microstructure shell layer at higher magnification. Initial N95 masks contain PP with smooth surfaces. After gamma irradiation, the morphology generated more rift, and the polymer interacting with ionizing energy contributed to physical, chemical, and psycho-chemical changing (Abiona and Osinkolu, 2010). The escalation of the gamma irradiation dose rate conducted flaws, hollowed spaces, fractures, spheroids, and scalds on the PP surface (Keene et al., 2014; Mathakaria et al., 2014; Martínez-Barrera et al., 2013). The elementas analysis of N95 masks was observed by EDS. Mask surfaces indicate no nitrogen (protein) presence after several sterilization methods. Kampf et al. (2020) analyzed gamma irradiation and heat at several methods to lead inactivation and protein denaturationof certain viruses.

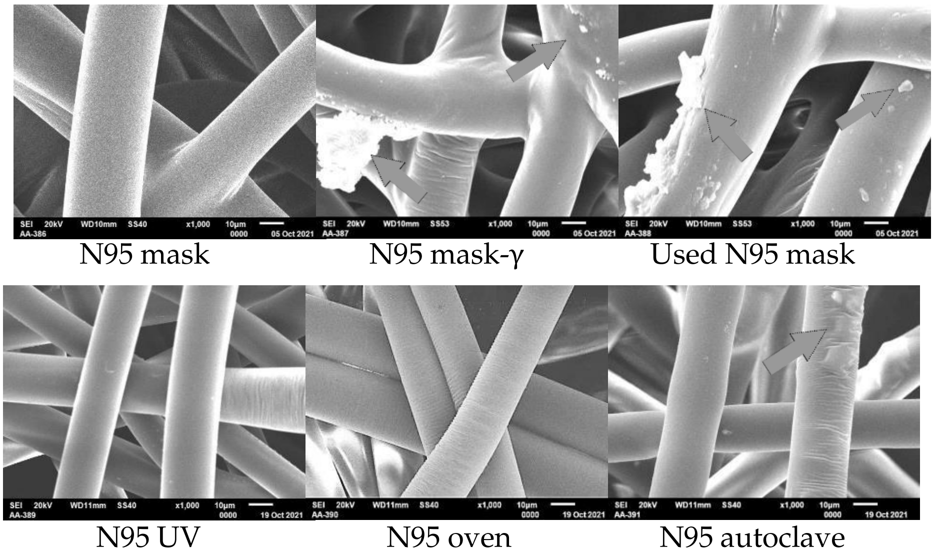

Figure 10 SEM images of N95 mask with several methods of sterilization

4. Conclusions

The bioburden results showed that a sterilization dose of 23.2 kGy is more effective than the non-γ-irradiated method. Referring to its mechanical, psycho-chemical, thermal, and morphological properties, the N95 mask is still in a reasonable and non-damaging stage toreused. Zero bacteria, fungi, and viruses were found after the mask was treated with a gamma irradiation dose of up to 25 kGy. However, further EDS analysis to amount of nitrogen for the control N95 mask is needed to verify the microbial analysis method. The weathering Test of Polymer is important to examine how often the N95 mask can be reused after sterilization.

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Author Contributions

The authors indicated made substantial contributions to the following task of research: Initial conception, drafting text/intellectual input (ARS, ALY, IS), design of experiment (ARS. ALY, IS, DT, DD, BS), provision of resources (DT, D, HY, MYY, RF), collection data (ARS, ALY, IS, MYY, RF, BS), perform for sample irradiation, analysis and interpretation data for FTIR spectroscopy, DSC analysis, UTM analysis, bioburden standard methods (ARS, ALY, IS, DD, MYY, BS, RF), writing and revision of paper (ARS, ALY, IS, DD). All authors approved the content of manuscript and agree to be held accountable for the work.

Conflict of Interest

The authors declare no conflict of interest. The funding support had no role in the design of the study; collection, analyses, interpretation of the data; in the writing of manuscript; or In the decision to publish the results.

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