



Application of High-Energy Laser coupled with Gamma – Ionizing Irradiations as New Sterilizing Technique

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ABSTRACT

The present investigations introduce for first time new trend of coupling of two sterilizing sources, high energy Nd-Laser and Gamma – irradiations which has oxidative nature by additional to its thermal effects. The investigated samples will be examined before and after radiations to confirm its internal structure (XRD, microstructure (SEM, AFM, Raman-Spectra) and killing ratio for polluted bacteria. Furthermore some selected biological tests will be checked to be sure from toxicity ratio after radiations. Also many of investigational parameters will be tested such as strength of irradiation dose and irradiation dosage time to achieve maximum healthy sterilizing ratio per minimum time to validate application and wide scale application of this promising sterilizing technique.

Keywords: Nd-Laser; Gamma-ray; Sterilizing; X-Ray; SEM; AFM

1. INTRODUCTION

Sterilization process can be defined as any process that effectively and efficiently kills or eliminates almost all micro-organisms like fungi, bacteria, viruses, spore forms [1-10].

There are huge numbers of researchers [2-7] who investigate different sterilization methods depending on the purpose of the sterilization and the material that will be sterilized. The choice of the sterilization method alters depending on materials and devices for giving no harm. These sterilization methods are mainly: dry heat sterilization, pressured vapor sterilization, ethylene oxide (EtO) sterilization, formaldehyde sterilization, gas plasma (H_2O_2) sterilization, peracetic acid sterilization, e-beam sterilization and gamma-sterilization.

Blood or tissue remaining on poorly cleaned instruments acts as a shield to microorganisms during the sterilization process, the number of cracks and crevices on an instrument that might harbor microorganisms [6-8]. Microorganisms collect in, and are protected by, scratches, cracks and crevices such as the serrated jaws of tissue forceps.

Finally, here is no single sterilization process for all the pharmaceuticals and medical devices. It is hard to assess a perfect sterilization method because every method has some advantages and disadvantages. For these reasons, in the present research project we will introduce application of high energy laser as secure healthy technique

Radiation processing refers to the use of radiation to change the properties of materials under irradiations on an industrial scale.

The term 'ionizing radiation' relates to all radiation capable of producing ionization cascades in matter. The energy range characteristic of ionizing radiation begins at about 1000 eV and reaches its upper limit at about 30 MeV [11-15].

Gamma rays are generally used for the sterilization of gaseous, liquid, solid materials, homogeneous and heterogeneous systems and medical devices, such as syringes, needles, cannulas, etc. Gamma irradiation is a physical means of decontamination, because it kills bacteria by breaking down bacterial DNA, inhibiting bacterial division [16-30]. Energy of gamma rays passes through hive equipment, disrupting the pathogens that cause contamination. These photon-induced changes at the molecular level cause the death of contaminating micro-organisms or render such organisms incapable of reproduction. The gamma irradiation process does not create residuals or impart radioactivity in the processed hive equipment that consider the most important advantage for sterilizing by gamma-irradiations [22-28].

Radiolytic products of water are mainly formed by indirect action on water molecules yielding radicals OH, e^-_{aq} and H. The action of the hydroxyl radical (OH.) must be responsible for an important part of the indirect effects. Drying or freezing of living organisms can reduce these indirect effects [31-33]. If we consider pure water, each 100 eV of energy absorbed will generate: 2.7 radicals OH., 2.6 e^-_{aq} , 0.6 radicals H., 0.45 H_2 molecules and 0.7 molecules H_2O_2 [34]. Greez et al [31] have investigated the action of radiation on bacteria and viruses and effective use of optimized, high dose (50 kGy) gamma irradiation for pathogen inactivation of human bone allografts. They confirmed that sterilizing by gamma irradiation is physical and experimental condition dependent.

Many researchers have investigated the different types of microorganism, mainly bacteria and, less frequently, moulds and yeasts, which found on many medical devices and pharmaceuticals [35-44].

Many hypotheses have been proposed and tested regarding the mechanism of cell damage by radiation. Some scientists proposed the mechanism thought 'radiotoxins' that are the toxic substances produced in the irradiated cells responsible for lethal effect. Others proposed that radiation was directly damaging the cellular membranes. In addition, radiation

effects on enzymes or on energy metabolism were postulated. The effect on the cytoplasmic membrane appears to play an additional role in some circumstances [45].

Kaplan et al. [45] investigated the effects of gamma irradiation of whole chicken carcasses on bacterial loads and fatty acids and contradict with some hypotheses mentioned above.

The aim of the present investigations are aiming to apply new advanced couple irradiation technique Nd-Laser plus Gamma-ray as new combined sterilizing technique can be yield to 100% ratio of bacteria killing.

2. EXPERIMENTAL

2. 1. Samples preparation

Two different samples of dry milk were selected to be the target of both Nd-laser and gamma-Irradiation. The same two samples were wet by physiological solution polluted by *Escherichia coli* symbolized as *E. coli* for *simplicity* as model of bacteria can be present in milk as general (*E. coli* $\sim 2,4 \times 10^5$ cell/cm³).

2. 2. Laser and Gamma-irradiation sources

The two wet samples of milk polluted with certain concentration of *Escherichia coli* (*E. coli* $\sim 2 \times 10^5$ and 4×10^5 cell/cm³). The applied laser Nd-pulsed laser has the following parameters: wavelength $\lambda = 1.06 \mu\text{m}$, pulsed rate $\eta = 10^{-3}$ s. The targets were irradiated by two different doses of Nd-laser beam irradiations the 1st 10 W/cm² for 5 min., 2nd 20 W/cm² for 10 min. The irradiation was carried out in air without any external heating. The energies of pulsed Nd-laser were sufficient to melt homogeneously the surface and near surface layers. SEM was used for monitoring the morphological changes. After laser-irradiation doses all sample were irradiated with gamma-ray 10 MR at distance = 20 cm using ¹³⁷Cs as source for Gamma-Ray.

2. 3. Phase Identification

The X-ray diffraction (XRD) measurements were carried out at room temperature on the fine ground samples using Cu-K α radiation source, Ni-filter and a computerized STOE diffractometer / Germany with two theta step scan technique.

Scanning Electron Microscopy (SEM) measurements were carried out at different sectors in the prepared samples by using a computerized SEM camera with elemental analyzer unit (PHILIPS-XL 30 ESEM /USA).

Atomic force microscopy (AFM): High-resolution Atomic Force microscopy (AFM) is used for testing morphological features and topological map (Veeco-di Innova Model-2009-AFM-USA). The applied mode was tapping non-contacting mode. For accurate mapping of the surface topology AFM-raw data were forwarded to the Origin-Lab version 6-USA program to visualize more accurate three dimension surface of the sample under investigation. This process is new trend to get high resolution 3D-mapped surface for very small area $\sim 0.1 \times 0.1 \mu\text{m}^2$.

2. 4. Electrical measurements

RT- DC-electrical conductance of the prepared materials were performed on the pellet surface by using two probe circuit and graphite paste was used as connective matter as shown in Fig. 1.

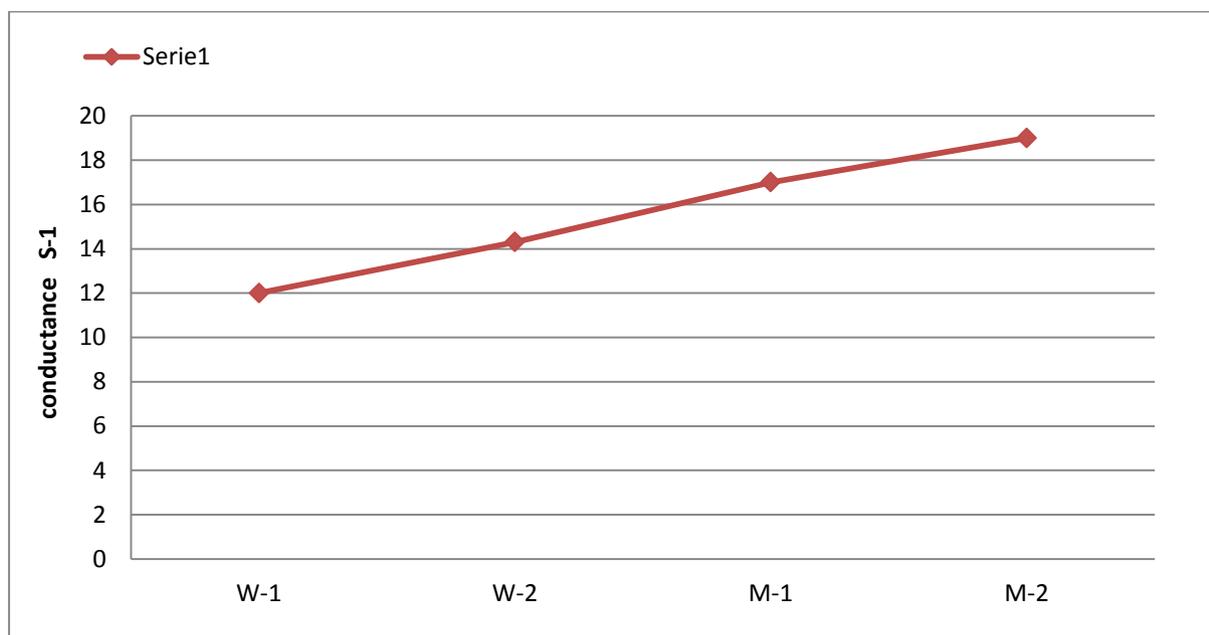


Fig. 1. Conductance Measurements.

The conductance measurements were performed for all solutions under investigations, all solutions are conductive as clear in Fig. 1.

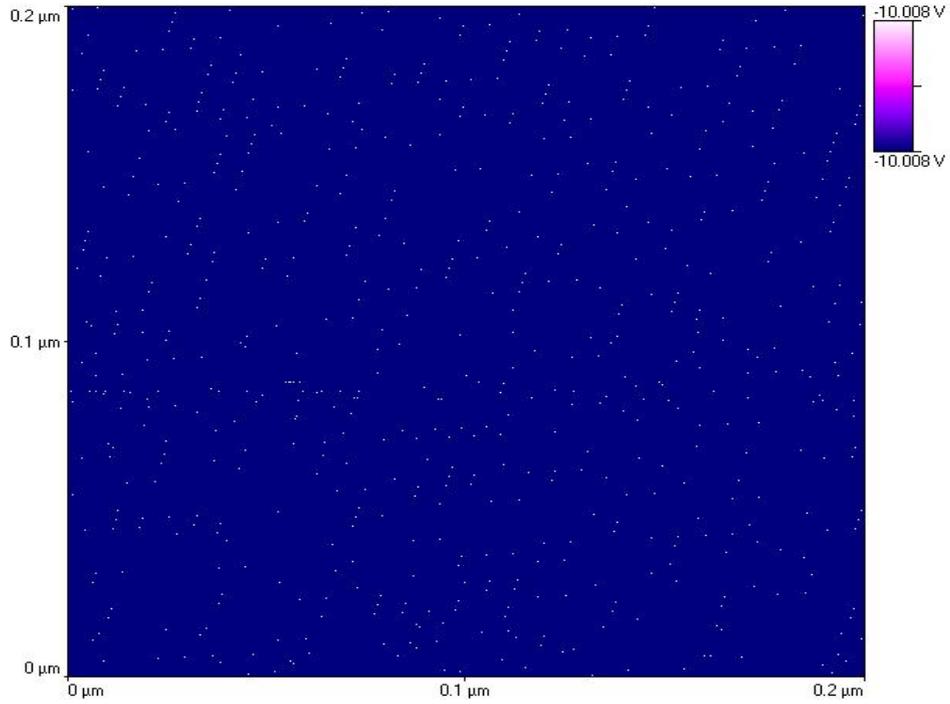
2. 5. FT-Infrared Spectroscop

The infrared spectra of the solid products obtained were recorded from KBr discs using a Shimadzu FT-IR Spectrophotometer in the range from 400 to 4000 cm^{-1} .

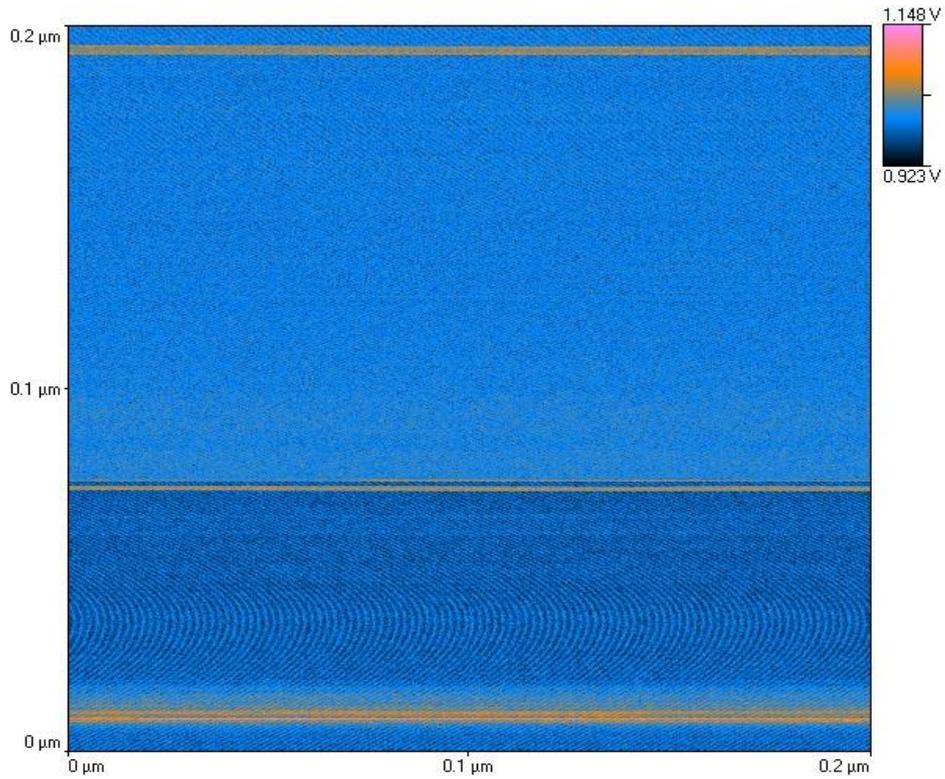
3. RESULTS & DISCUSSION

Two different samples of dry milk were selected to be the target of both Nd-laser and gamma-Irradiation .The two samples symbolized as M1 and M2 respectively. The M1 & M2 samples were wet by physiological solution polluted by *Escherichia coli* symbolized as *E. coli* for simplicity as model of bacteria can be present in milk as general (two conc. of *E. coli* $\sim 2,4 \times 10^5 \text{ cell/cm}^3$).

Many of spectral and structural investigations of the samples (M1, M2) under investigations were performed as function of irradiation dose whatever it Laser or Gamma-irradiations.



(a) M1-sample



(b) M2-Sample

Fig. 2(a,b). AFM-Investigations of Grain size of applied Milk-powder M1 and M2 samples.

Fig. 2(a) shows AFM-TM-deflection centers which describes the distribution dots represents pinning centers inside bulk of dry milk sample that be responsible for conduction inside bulk of material .The estimated grain sizes were found in between 1.3-3.2 μm for sample M1 while 2.1-4.1 μm is for M2-sample. Fig. 2(b) display surface topology of M2-sample with magnification factor = 0.2 μm , it was noticed that there are two different levels of array parallel to each other with average grain size in between 2.1-4.1 μm which is relatively high in contrast with grain size of sample-M1 due to difference in solvent applied in extraction process.

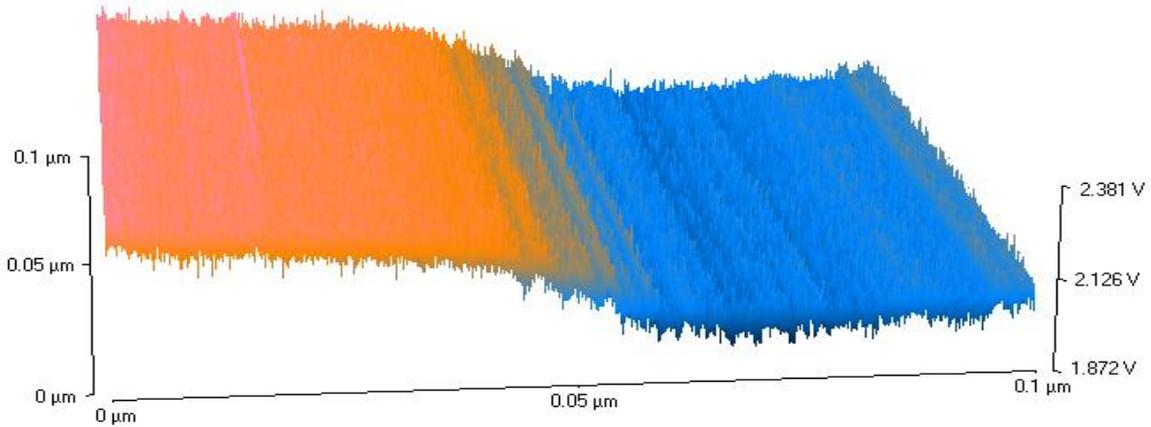


Fig. 3(a). 3D-AFM-Surface topology of M1-sample before laser-irradiation dose.

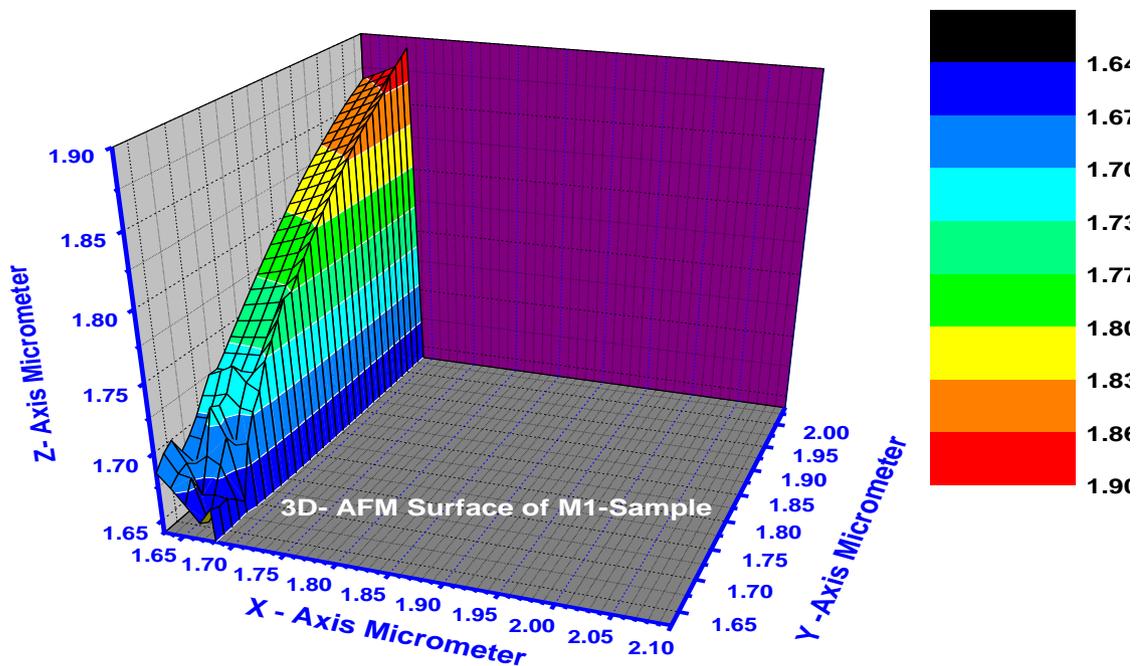


Fig. 3(b). Visualized 3D-AFM-Surface topology of M1-sample before laser-irradiation dose for area 0.05x0.05 μm^2

Figs. 3(a,b) describe real and visualized 3D-AFM-Surface topology of M1-sample before laser-irradiation dose. The visualized investigations confirmed the results obtained from AFM regarding averages of particle as well as grain size. From Fig. 3b it was notified that the maximum heights were ranged in between 1.86-1.90 μm (orange-red zones) while minimum heights recorded 1.64-1.67 μm (black-blue zones).

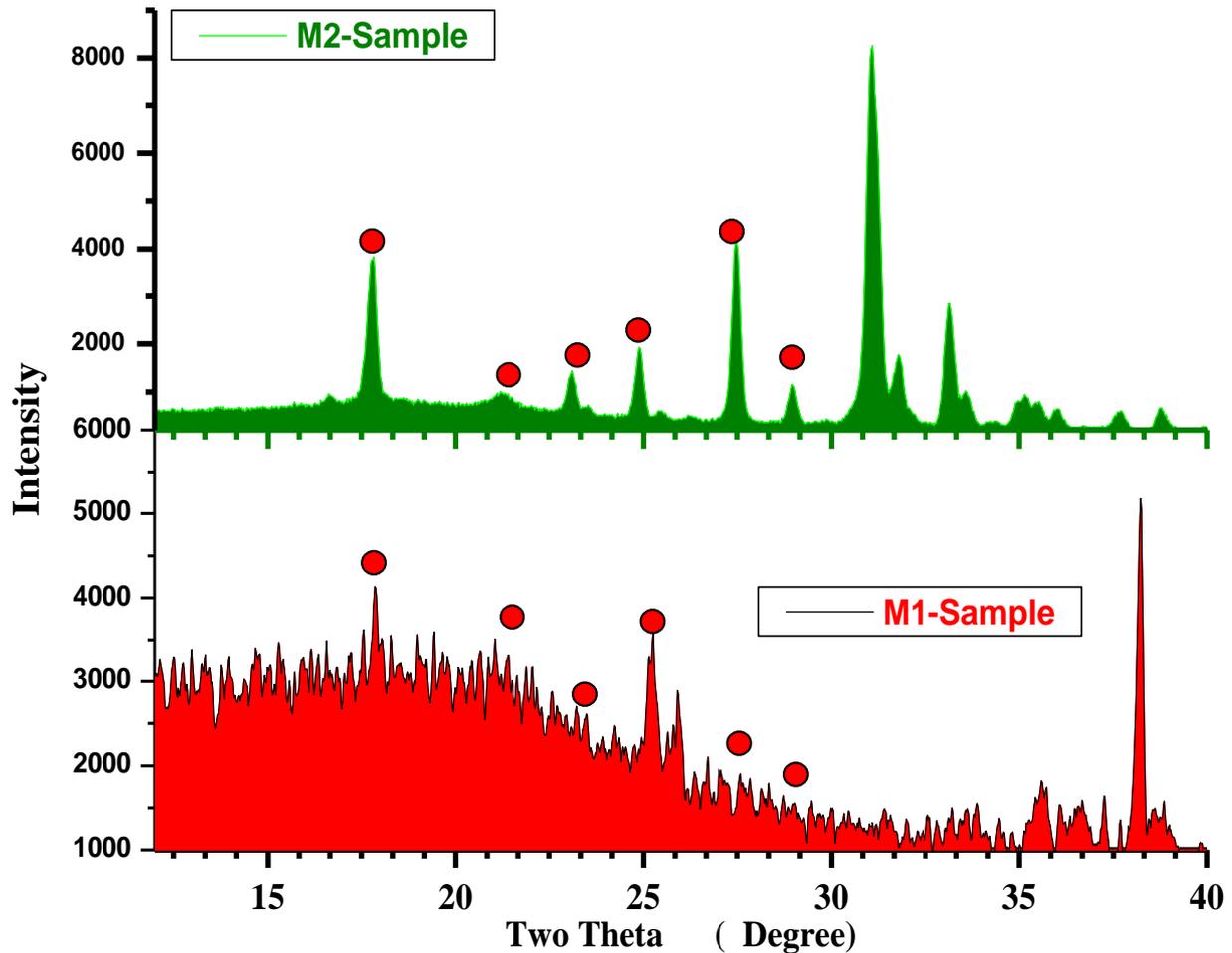


Fig. 4(a,b). RT-XRD-pattern recorded for samples (M1&M2) respectively.

Fig. 4(a,b) displays fingerprint pattern evaluated for dry milk samples M1, M2 respectively. Although the two dry milk sample from two different factory the internal lattice structure is fitted by ratio higher than 60 % percent as clear in Fig. 4(a,b) where red circle refer to fingerprint of internal lattice structure which is mainly lactose beside certain amount of lipids as impurity phases.

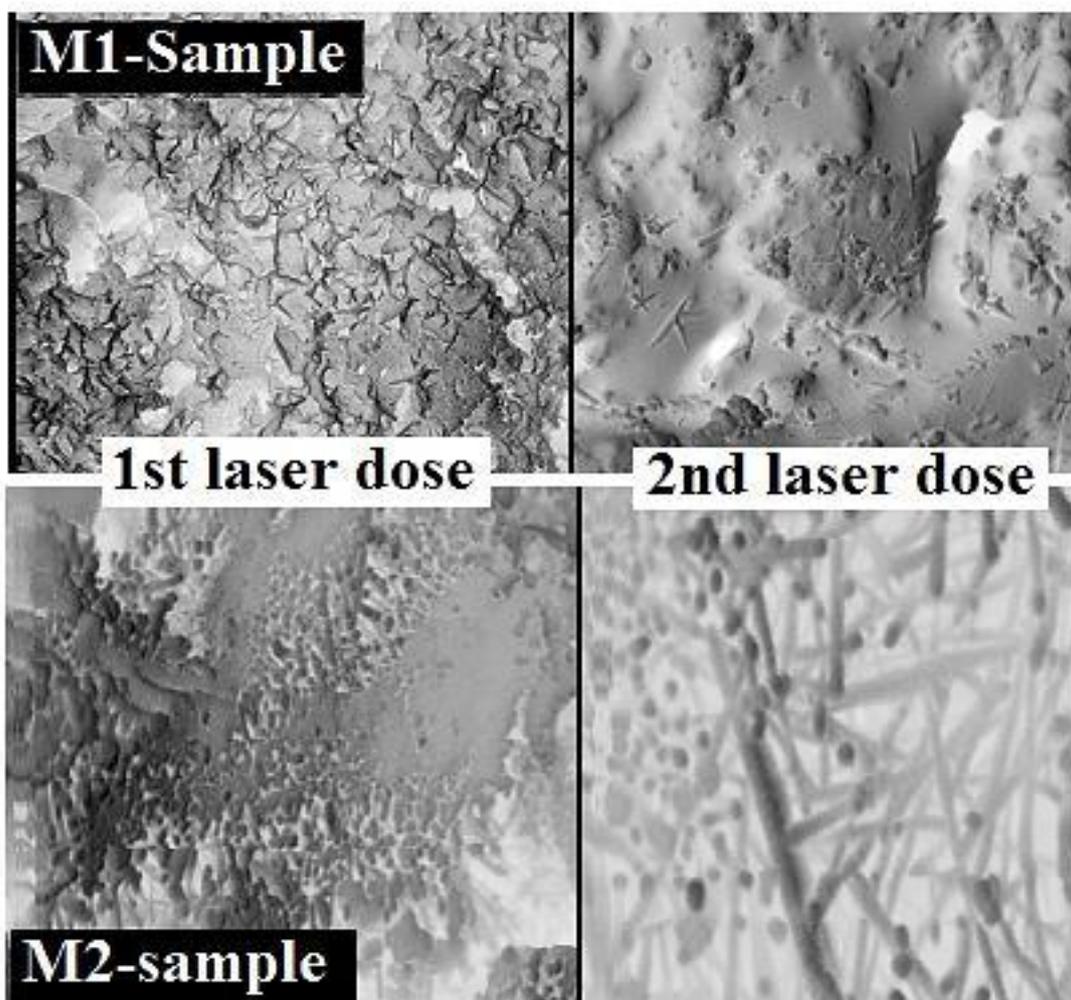
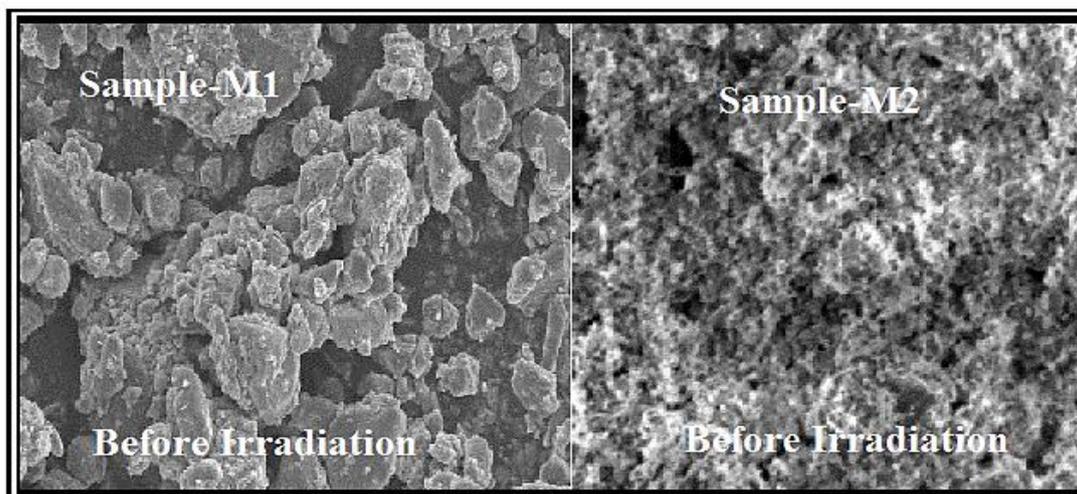


Fig. 5(a,b). SE-micrographs recorded for M1 and M2-samples Before and After 1st and 2nd laser irradiation dose.

From Fig. 5(a,b) one can notify that the average grain size of sample M1 & M2 before irradiation was found in between 1-4.5 μm which is consistent with data evaluated from AFM-investigation mentioned before. But the topological features of sample M1 & M2 after 1st and 2nd laser irradiation dose are completely different such the surface's layers and near surface layers reformed as small aggregated zones with different grains shapes as clear in Fig. 5(b).

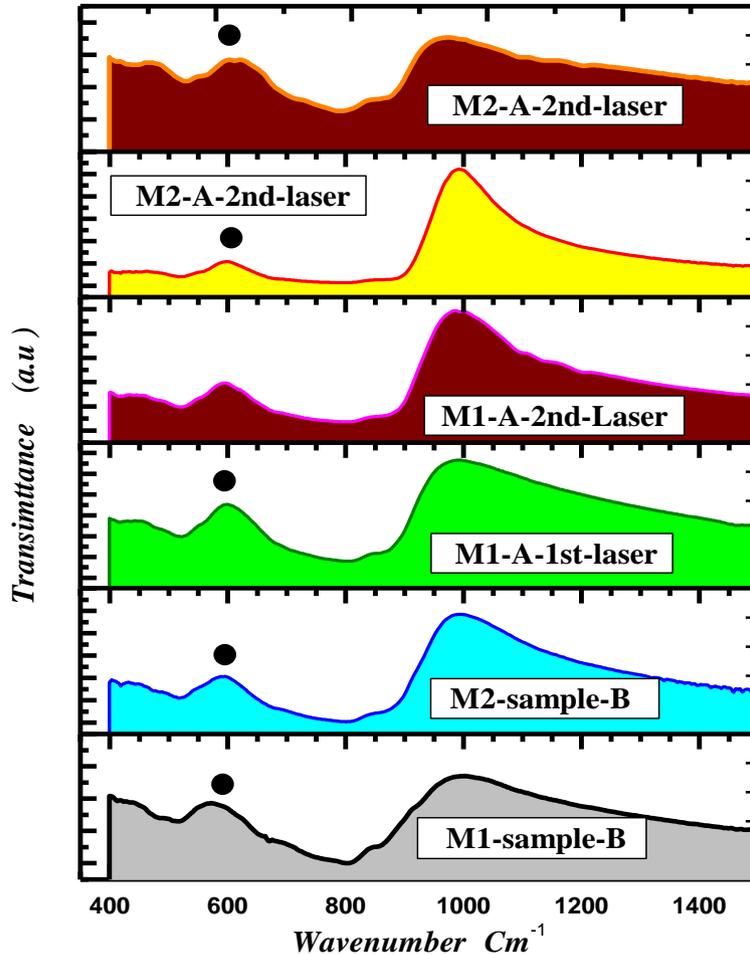


Fig. 6: IR-spectra recorded for dry milk samples (M1 & M2) After and Before laser dose irradiations.

Fig. 6 displays IR-spectra recorded for dry milk samples (M1 & M2) after and before laser dose irradiations. It was observed that dry milk samples (M1, M2) still having the function groups even after 2nd irradiation Nd-laser dose as clear in Fig. 6. The black circles refer to broad characteristic peak for applied milk sample and no remarkable differences as laser dose increase. These results support the opinion that declare that applied Nd-laser irradiation dose within the save limits in which crystal structure of milk does not changed as proved in our X-ray measurements

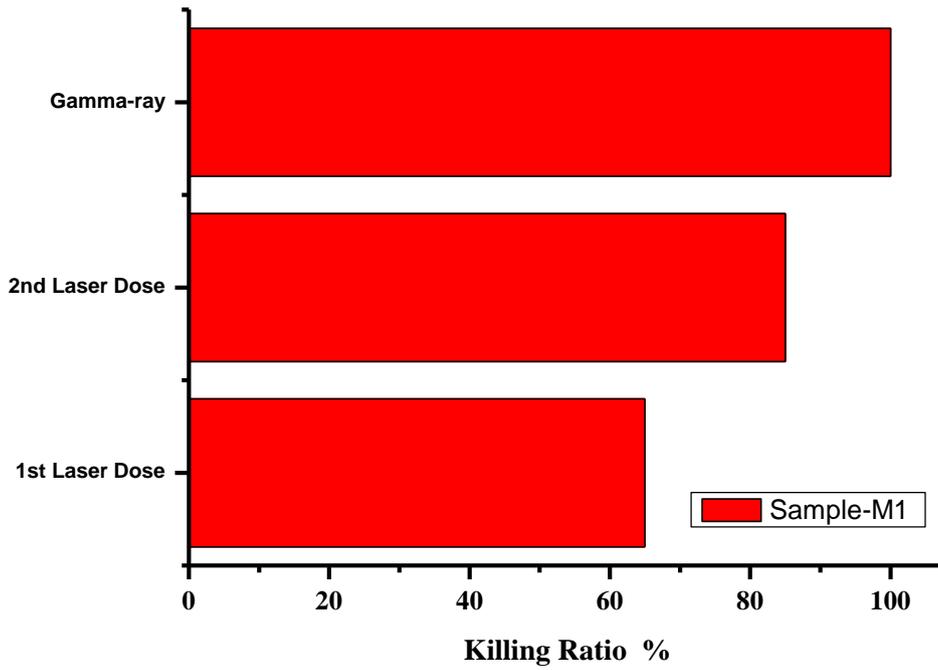


Fig. 7(a). Killing ratios after sterilizing process with Nd-Laser and Gamma – Ray for Sample M1.

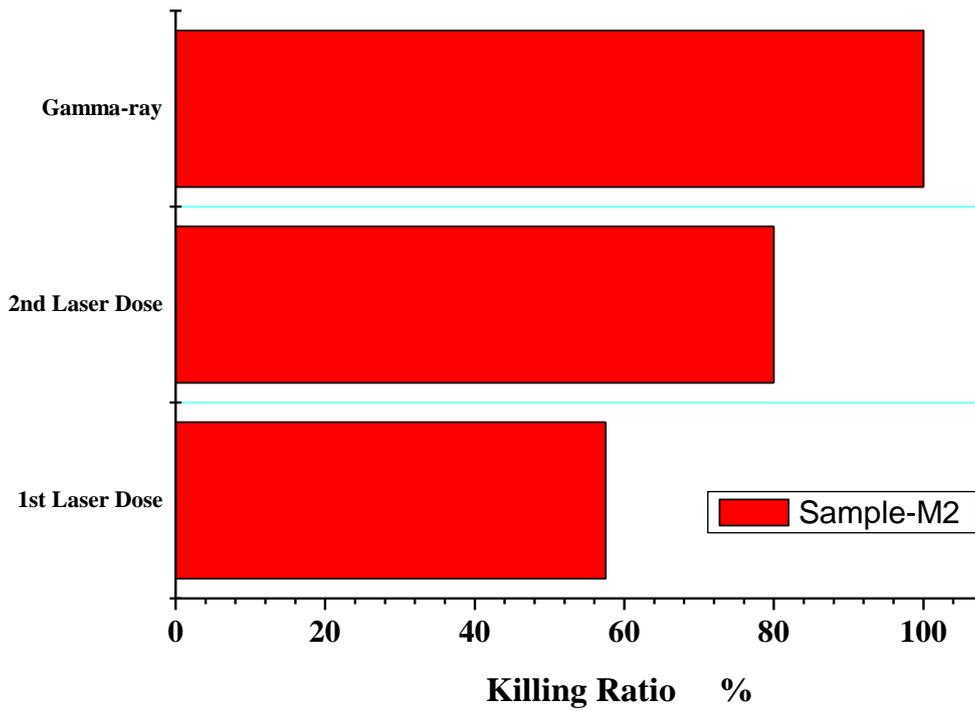


Fig. 7(b). Killing ratios after sterilizing process with Nd-Laser and Gamma – Ray for Sample M2.

Table 1. Killing ratios after dual sterilizing process with Nd-Laser and Gamma – Ray.

Sample No.	Sample M1 <i>E. coli</i> 2×10^5 cell/cm ³	Sample M2 <i>E. coli</i> 4×10^5 cell/cm ³	Killing Ratio %	
			M1	M2
1 st Laser Dose 10 W/cm ² for 10 min.	1.3×10^5	2.3×10^5	65	57.5
2 nd Laser Dose 20 W/cm ² for 20 min.	1.7×10^5	3.2×10^5	85	80
Gamma-Dose 10 MR at 20cm for 20 min.	$\sim 2 \times 10^5$	$\sim 4 \times 10^5$	100	100

Sterilizing process was tested applying only one source of sterilizing (Nd-Laser or γ -Ray), and all results confirmed that ratio of killing impossible to reach 100% even though the allowed time of sterilizing is double (Fig. 7(a,b)). From Table 1 one can observe that samples M1, M2 recorded 65,57.5% killing ratios after 1st laser respectively while they recorded 85, 80% killing ratio after 2nd irradiation Nd-Laser dose (20 W/cm² for 20 min.). These results confirmed that we need extra technique to terminate E-Coli bacteria (i.e. to reach 100% killing ratio) but after simultaneous irradiation with gamma-ray (10 MR at 20 cm for 20 min.) the examination of killing ratios reach ~ 100 % which is our major goal from this dual sterilizing technique.

4. CONCLUSIONS

The conclusive remarks inside this article can be summarized in the following points;

- 1- Applied sterilizing techniques are eco-friend.
- 2- No harmful bi-products produced after sterilizing.
- 3- Techniques applied are available and economically efficient.
- 4- Applied techniques are reproducible and fast.

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