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FOOD SAFETY-BASED EVALUATION OF 3D PRINTED OBJECTS

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ABSTRACT

The 3D printing technology involves a digital manufacturing machine that produces three-dimensional objects according to designs created by the user via 3D modelling or computer-aided design/manufacturing (CAD/CAM) software. This work analyzes the processing timeline of the filament (material for 3D printing) from unboxing to the extrusion trough the nozzle. It is an important task to analyze the growth of bacteria on 3D printed surface and in gaps between the layers. By default, 3D printed object is not food safe after longer usage and direct contact with food (even though the food safe filaments were used), but there are solutions for this problem. We tested several methods to prevent or reduce the emerging microbial contamination. These methods are coating with epoxy resin, using antimicrobial and high temperature-resistant filaments. The best results were obtained by epoxy resin coating, where the object was cleanable like any other injection molded plastic object with smooth surface. Very good results have also been obtained by boiling the objects, and it is good to see that nowadays more and more special filaments have food safe certificate and can withstand boiling temperatures too. Using antibacterial filaments reduced bacterial colonies by 80%, which is not a perfect solution.

Keywords: 3D printing, epoxy coating, antimicrobial filaments, heat-resistant filaments

1. INTRODUCTION

Additive manufacturing (AM) also known as 3D printing – recently has undergone rapid growth, replacing the use of the technology from prototyping to producing end-user parts and products [1].This technology uses a variety of materials, including polymers, metals, ceramics, and biomaterials. This type of AM has been known as a promising manufacturing process in various industries, including the food industry. This is a relatively advanced technology that started with growth in the mid-80s due to the latest developments made in computing and control systems. The 3D Printing technology involves a digital manufacturing machine that produces 3D objects according to designs created by a user via computer-aided design/manufacturing (CAD/CAM) software [2].3D printing systems can be classified as fused deposition modeling (FDM) or fused filament fabrication (FFF), stereolithography (SLA) and selective laser sintering (SLS). In this work, only FDM technology is investigated as the most popular 3D printing method. In the 3D printing world, a significant change takes place when patents expired. The patent on FDM expired in 2009 and as a result, prices of FDM printers decreased from over $10,000 to less than $1,000, which caused expansion of consumer-friendly 3D printers. Advances in 3D printing technology are giving companies the opportunity to explore 3D printing applications that were not previously possible. As the cost of 3D printers falls and the speed at which they can help companies scale to mass production increases, it will become easier to transform the entire manufacturing industry [3]. Using 3D printed objects in the food industry does not mean that they always have direct contact with food, for example they can be used as elements which support food processing parts like pipe holders, gears *etc*. 3D printing is a digital process of building the object layer by layer, connecting the layers through phase transitions or chemical reactions. A 3D printed object is considered as food safe if it can have direct contact with the food without any toxic effect even after cleaning, storing, and reusing of the object. An important part of this work is analyzing the processing timeline of the filament (material for 3D printing) from unboxing to the extrusion trough the nozzle. Another important task is analyzing the microbial growth on 3D printed surface, and checking the methods how can we prevent or reduce this process. The act of microorganisms attaching to inert, solid surfaces to form a biofilm is known as biofouling. Biofouling is a problem in many branches of science and business, including medicine, and is estimated to cost billions of dollars annually [4]. 3D printed materials are among the newest sectors to experience biofouling. In the medical industry, 3D printed materials are also used for manufacturing prosthetic

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limbs. Both the overall antibacterial properties and the ability of such 3D printed prostheses to lessen biofilm adhesions have not yet been tested in contrast to conventional prostheses, which have been studied and developed to have anti-biofilm and antimicrobial properties [5]The first step in the formation of a biofilm is bacterial attachment [6]. A growing body of research shows that surface topography has a significant influence on bacterial attachment and subsequent biofilm formation. Cells frequently arrange themselves to maximize their contact area with the surface with topographic features at the micrometric scale, which is comparable to the size of prokaryotic cells. This is advantageous for attachment [7] .Some of the methods to reduce or prevent microbial contamination/biofilm formation may be surface smoothing and using a proper design. Proper design of 3D printed objects should be important to avoid sharp corners and use smooth edges. If it is possible to print 3D printed object without supports, the cleanability of the objects is improved [8]. Previous research has proven that, by default, 3D printing object is not food safe after longer usage and direct contact with food (even the food safe filaments were used), but there are solutions for this problem, for example by using food grade epoxy resin [9]. Because 3D-printed items can be used for various purposes, they are also subjected to a variety of environmental factors. This increases the need for surface treatment with coatings that would offer both protection and the desired aesthetic appearance of 3D-printed products when used in harsher environments, such as those with elevated relative humidity and ultraviolet light irradiation [10]. Since surface chemistry and wettability are directly related to coatings' ability to adhere to substrates, it is crucial to have a thorough understanding of these properties in order to create an effective surface system made up of polymers and coating assets. The physical and chemical characteristics of coatings, however, must also be understood in order to design an ideal surface system. The surface treatment of polymers is only possible with suitable coatings because of specific surface properties. In fact, these coatings typically contain a variety of resins (acrylics, alkyd, polyurethane, polyvinyl acetate, melamine, polyester) and solvents (water, butyl alcohol, isopropyl alcohol, propylene glycol methyl ether) [11]. In addition, coatings can be applied to 3D-printed objects using a variety of technologies, depending on the coating type and properties, object shape, and object size [12].Additionally, a new method for prevention of bacterial contamination was tested and that is usage of temperature-resistant filaments to obtain 3D printed objects, which can be cleaned using boiling water. NonOilen^{TM} is a PLA (polylactic acid)-like filament, which has food safe certificate, and it can withstand temperatures up to 110 °C, meaning that the 3D printed parts can be cleaned in boiling water. The aim of this study was to evaluate the 3D printed object in different perspectives of food safety. To improve the surface of 3D printed objects, coating with epoxy resin was investigated in terms of strength, thermal resistance and cleanability (by smoothing the surface of 3D printed objects). Another aim of this study was to test the effect of high temperature on the 3D printing objects, in order to answer the question whether the used filaments were resistant to processing temperature or not. The study was also focused on the reduction of bacterial contamination with heat treatment or boiling to minimize bacterial attachment and growth on smooth surface of 3D printed object. Hence this study has mainly examined 3D printed objects in direct contact with food from the food safety aspect, in order to minimize microbial load and bacterial growth that affect the quality of the food products.

2. MATERIALS AND METHODS

2.1. 3D printer and filaments

Prusa i3 MK3 FDM 3D printer (Stratasys Ltd, USA) with 0.4 mm stainless steel nozzle and 0.05 - 0.2 mm of layer height was used. The maximum speed was 200 mm s^{-1} and maximum nozzle temperature, and heat bed temperature were 300 and 120 °C, respectively.The filaments used for food safe 3D printed object were PLA3d850 Polylactic Acid (Jaen, Spain) with print temperature: 215 °C, heat deflection temperature (0.45 MPa) 55 °C. GRIPHEN^{R} polyethylene terephthalate glycol (PETG) (Arla plast AB Vastanavagen, Sweden) with printing temperature: 250°C and heat deflection temperature (0.45 MPa) 68 °C. PLACTIVE by Copper3D (a Chilean/US company) which was non-toxic, mostly for medical applications in the form of copper ions. PUREMENT BNK-PMTBK175 Antimicrobial PLA filament (Green Catridge Technology, USA) with filament of 1.75 mm diameter and 0.6 kg. It commonly used for food industry applications in silver ions form, Prusament PLA Regularly used for reference which was not antimicrobial. and Philament (Filaticum) exist in silver ions variation which was antimicrobial since essentially food safety compliance, were filaments used for antimicrobial 3D printing filaments. It has been certified by the System for Identifying motivated Abilities (SIMA), and antimicrobial registered by Food and Drug Administration (FDA) and Restriction of Hazardous Substances (RoHS)Test. Nonoilen by Filamentum (Czech.s.r.o, Czech Republic) filament based PLA , with its working temperature of 175-195 °C, heated bed: 0-50 °C, temperature resistance up to 110 °C without annealing, tough material was used for filament of 3D printed object that printed at high temperature.

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Epoxy resin (resin and hardener) XTC-3D (INCHEM Ltd, Hungary), with mixing ratio by volume 2A:1B, pot life 10 minutes, thin working time 20 minutes, thin film react time 90 minutes, tack free time 120 minutes, cure time 2100 minutes and mixed viscosity 350 mPa·s A and B refers to resin and hardener respectively. This epoxy was used for improvement of the 3D printed object surface. Notice this type do not have food safe certificate, only the cleanability was investigated.

2.2. 3D printing test specimens for temperature testing

Nonoilen by Filamentum filament-based PLA used at its working temperature of 175-195 °C, heated bed: 0-50 °C, temperature resistance up to 110 °C without annealing, tough material. The filament NonOilenTM, which resembles PLA has a food safety certificate, and can withstand temperatures of up to 110 °C, allowing 3D printed parts to be cleaned in boiling water. The temperature test was carried out in an oven recording the temperature of the first deformation.

2.3. Microbial contamination analysis

Three-dimensional printed cups made of the aforementioned filaments were subjected to simulated application experiment, consisting of 10 day-long storage of UHT-treated commercial milk (ultra-high temperature pasteurization, at least 140 °C), with everyday cup exposure to milk matrix for 15 min, followed by a specific cleaning procedure. Different cleaning procedures were investigated, including standard cleaning using commercial dishwasher and tap water, as well as boiling in hot water (100 °C, 5 min) for heat-resistant filaments. The. investigation of microbial contamination was performed using the swab of the cups' inner surface followed by suspending of the collected microbial cells in sterile saline.

The 10, 100- and 1000-fold diluted suspensions (1 mL) were mixed with melted and tempered PCA medium (plate count agar, Himedia Laboratories, India, 50±1 °C) for the plate count assay. The incubation was carried out at 30 °C for 48 h, followed by enumeration of the emerged colonies. The cell number was given as CFU/cm^2, taking into account the sample dilution and the inner surface area of the tested 3D-printed cups.

2.4. Mechanical testing

Test specimens for the mechanical testing are 3D printed with 0.2 mm layer height on Prusa MK3S 3D printer (Stratasys Ltd, American). Tensile (pulling) test objects are printed in horizontal position, where the smallest cross section area is 4×4 mm. Experimental objects for the layer adhesion tests are 3D printed in vertical position with the same smallest cross section area as presented in Fig. 1.

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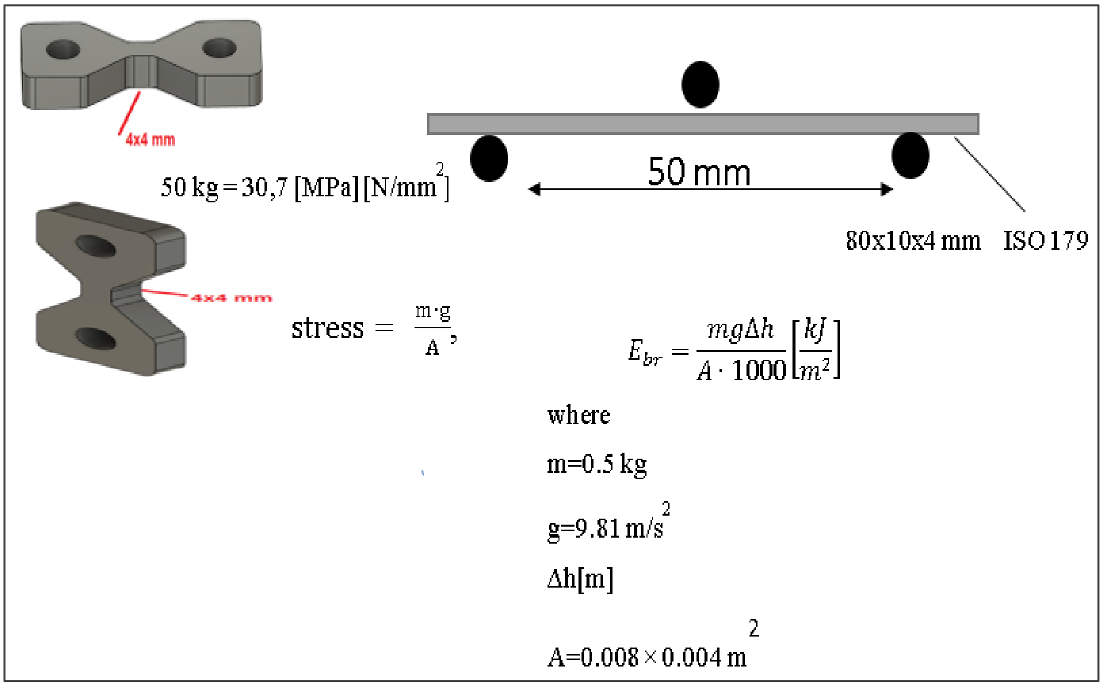

Figure. 1 Mechanical Test specimens

$$\text{Stress} = \frac{gm}{A} \qquad (1)$$

$$E_{\text{br}} = \frac{gm\Delta h}{1000A} \qquad (2)$$

where E_{br} / kJ m^{-2} is ….; m = 0.5 kg is ….; g = 9.81 m s-2; Δh is…… and $A = 8 \times 4$ mm^2.

The load was generated by a chain hoist and the attached crane scale recorded the break load. With this equipment the maximal break load only could be measured, without information about the deformation, prolongation, or the yield point. The IZOD impact test is based on ISO 180 using 0.5 kg hammer. Izod impact is the kinetic energy needed to initiate fracture and continue the fracture until the specimen is broken. The three (3) point bending test is based on ISO 179 with distance between supports of 50 mm. During the heat-deformation test, the objects were placed into the kitchen oven with M10 nut on the top as a small load. The reference thermometer in the oven was used to record the temperature of the first deformation.

3. RESULTS AND DISCUSSION

3D printed cups were produced from different filaments considering the food safety aspect through FDM 3D printing system in order to test their mechanical properties, heat deformation, processing temperature resistance (cleanability via boiling) and food safety from the point of view of bacterial growth. The cups were also tested for tensile (pulling) and layer adhesion, where break load expressed in terms of kilogram. Nonoilen and PLA filaments printed cups were analyzed break load of tensile and layer adhesion test as well as bending and 2mm deformation load test. The IZOD impact in kJ m^{-2} of Nonoilen and PLA printed cups also evaluated. Acrylonitrile styrene acrylate (ASA), PLA and Nonoilen based 3Dprinted cups were conducted for heat resistance at higher boiling temperature. Commercial milk was added in to 3D printed cups for 30 minutes. After that different commercial cleaning were investigated, using dish soap and tap water, as well as heat treatment like boiling in hot water at 100 °C for 5 minutes which was applicable for heat resistant filaments. Bacteria colony was performed commercially cleaned boiled in hot water cups. Food grade two components epoxy resin was used to 3D printed cups in order to create stronger and smooth surface for easier cleaning. 3D printed cups were used for testing bacterial growth on it. These cups were made from Polyethylene

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terephthalate glycerol (PETG) with addition of epoxy, PETG 0.05 mm, PETG 0.2 mm, PLA 0.2 mm, PLA 0.05 mm layer height and Poly smooth.

3.1. Effect of temperature on 3D printing filaments deformation

Temperature resistance of 3D printing filaments was evaluated, and the results are given in Fig.2. It was verified that, between the tested materials, the Nonoilen filament was the most heat resistant and it can withstand boiling temperatures (100 °C) without deformation. The temperature test results in oven showed that the Nonoilen filament's maximum working temperature without the deformation is 120 °C, for ASA 95 °C and for PLA 50 °C. According to these numbers, in most cases, the regular PLA cannot be cleaned in the dishwasher either.

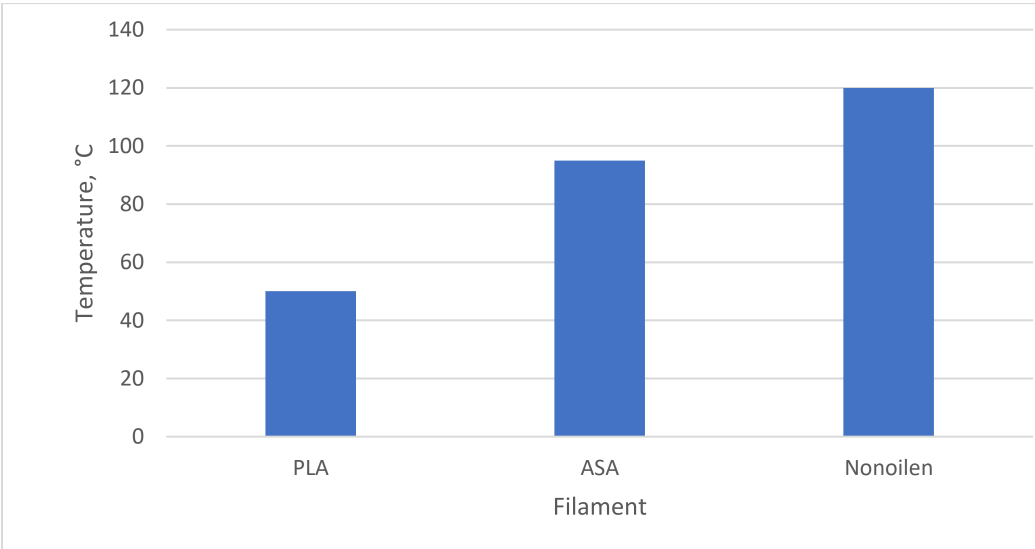

Figure 2 Deformation temperature of PLA, ASA and Nonoilen

3.2. Mechanical properties of 3D printed objects

From the food safety aspect, the microbial contamination experiments are of the utmost importance, but for statical design it is always good to know the mechanical properties of the tested material. The mechanical properties of the 3D-printed specimens produced using PLA and Nonoilen filaments are demonstrated in Figure 9 in terms of tensile and layer adhesion test (a), 2 mm deformation and maximum load test (b), and IZOD impact test (c). The results for the 3D printed specimens obtained using antimicrobial filaments in terms of the tensile and layer adhesion tests are presented in Fig.3.

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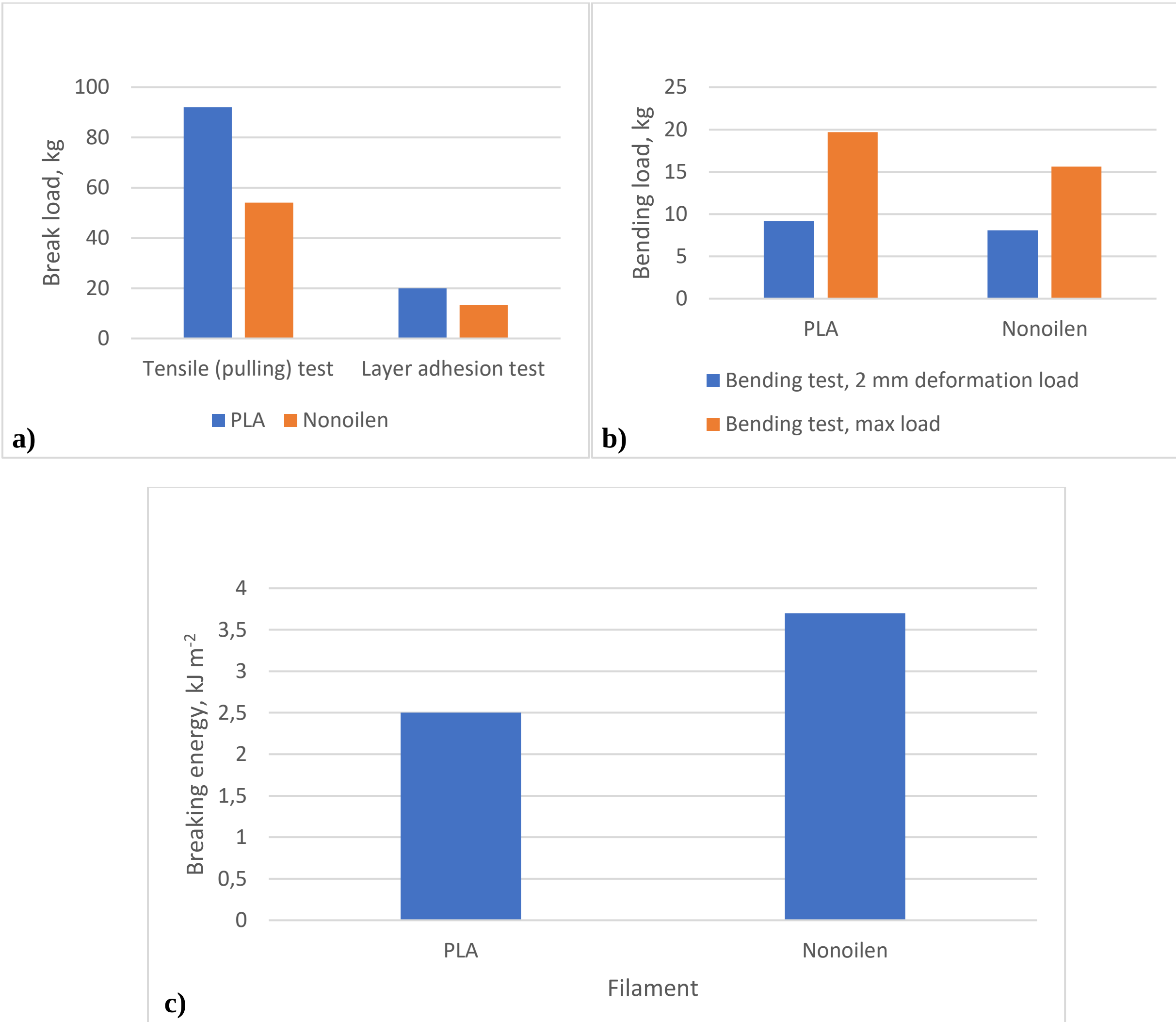

Figure 3 Mechanical properties test of 3D printed cups made of PLA and Nonoilen filament: a) Tensile and Layer adhesion test in break load test, where cross section area was 4 × 4 mm, (b) 2 mm deformation load and maximum load in bending test, bending test based on ISO 179 within supports distance of 50 mm, (c) IZOD impact test based on ISO 180 and 0.5 kg hammer used.

In tensile and layer adhesion in break load test with smallest cross section area of 4 mm x 4 mm, PLA has showed good mechanical behavior. PLA also represented better mechanical performance than Nonoilen filament in deformation and maximum bending test based on ISO179. On the other hand, Nonoilen filament indicated higher resistance to the applied force over PLA in IZOD impact test, which was based on ISO 180.

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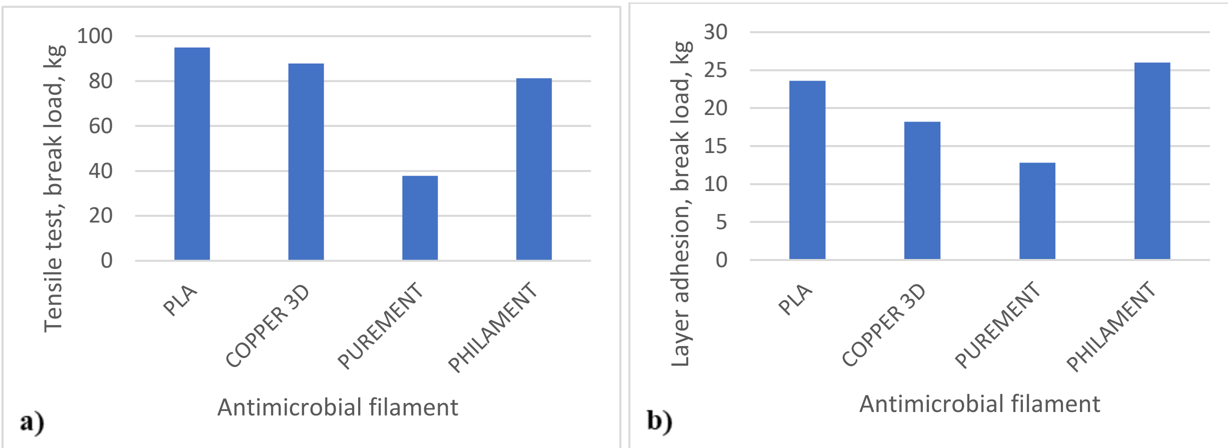

Figure 4: Mechanical properties tests of the 3D printed from antimicrobial filaments via break load (kg), (a) tensile (pulling) test, (b) layer adhesion test.

From the above Fig 4, the two most important experiments are tensile test (with test object printed in horizontal position) and the layer adhesion test (same object but printed in vertical position). Print object vertically, take substantially longer time, since it's standing tall and requires more attention layers stacked sequentially one on top of another. Only the break load was measured, where the smallest cross section area was 4x4 mm. The objects were printed using PLA, Copper3D, Purement and Philament antimicrobial filaments. In the pulling test shown in Figure 10, break load reaches maximum of 95 kg for PLA antimicrobial filament and the lowest break value of 37.8 kg is observed for objects made of Purement antimicrobial filament. In the layer adhesion test, Philament-based cup had the highest break load of 26 kg, and the lowest break load of 12.8 kg is identified in case of the Purement antimicrobial filament. In both tensile and layer adhesion test, the Purement antimicrobial filament was the weakest in terms of mechanical properties among the tested materials.

3.3. Results of the microbial contamination tests

3D printed cups made of different filaments and exposed to surface smoothing were examined for the material coating and layer height effects to microbial contamination reduction (Tab. 1). Furthermore, 3D printed cups made of antimicrobial filaments were investigated for their antimicrobial properties in terms of overall bacterial load reduction compared to the regular PLA-based cups (Fig.5b). Nonoilen filament-based cups were also examined in terms of microbial contamination to investigate the effect of the applied cleaning procedure on the overall bacterial load (Fig. 5a).

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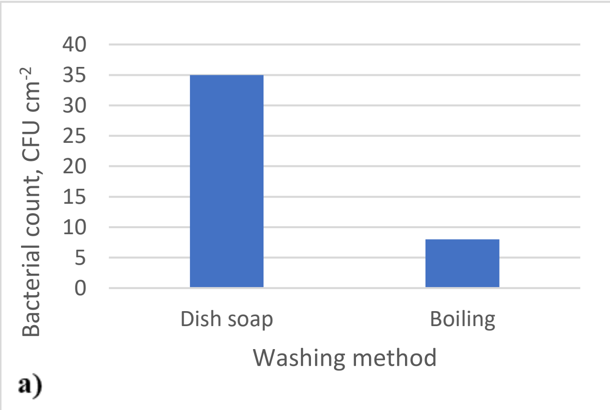

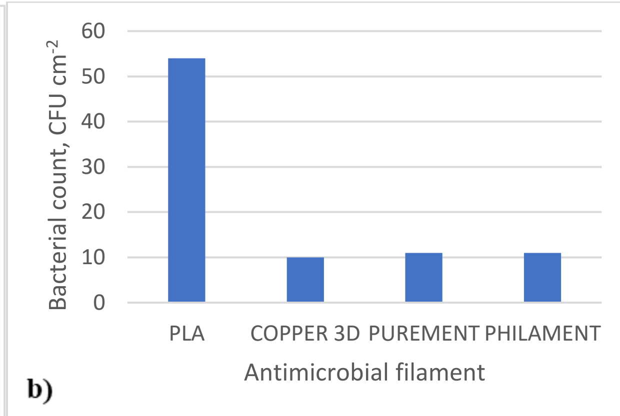

Figure 5. (a) Effect of cleaning the Nonoilen filament-based 3D printed cups using a dish soap vs. boiling) on bacterial growth; (b) Bacterial contamination reduction at the 3D printed cups using antimicrobial filaments vs. regular PLA.

Considering the results given in Figure, cleaning of the 3D printed objects via boiling is a more effective method compared to dish soap cleaning, emphasizing the benefit of physical (heat-based methods) over chemical cleaning. The lowest bacterial contamination was observed in objects made of Copper3D filament. The antimicrobial filament of copper 3D, purment and philament based presented the better results over PLA filament against the tested microbial. These antimicrobial filaments act as antimicrobial agent against the microbial growth on the surface of the material. Philament^{TM} antibacterial material incorporates metal ions which are known to have antibacterial properties. These inhibit the growth of bacteria by blocking enzymatic reaction. The antibacterial properties of copper3d filaments were confirmed by two microbiology laboratories in Chile and USA stated that, the nono-additives contains all mechanical properties of the material like hardens, flexibility, odor neutral, easy to print and hydrophobic. The antimicrobial filament material in PUREMENT registered by FDA and RoHS Test, who verified that it contains no harmful organic substances and is eco-friendly product that has consistent antimicrobial effect.

Table 1. The effects of material coating and layer height on microbial contamination

Object	**Material**	**Layer height, mm**	**CFU/cm^2**
Control		-	0
Injection molded	PET	-	0
1	PETG + Epoxy	Smoothed	0
2	PETG	0.05 mm	35
3	PETG	0.2 mm	194
4	PLA	0.2 mm	106
5	PLA	0.05 mm	71
6	Polysmooth	Partly IPA smoothed	512

3D printed objects coated with epoxy resin are easily cleanable thanks to the smooth surface of the objects. Based on the results given in Table 1 injection molded, and epoxy coated test objects have shown complete absence of bacterial contamination, *i.e.*, without recorded formation of bacterial colonies at the end of experiment. According to these results, the layer height does not show significant effect on bacterial colony formation.

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4. CONCLUSIONS

This work proved that all of the investigated methods (coating with epoxy resin, using antimicrobial and high temperature-resistant filaments) can improve food safety of the 3D printed object, but the extent of this effect varies. From the aspect of food safety, the best results were obtained by coating the 3D printed object with epoxy resin, where the object has shown good cleanability like any other injection molded plastic object with a smooth surface. Using antibacterial filaments reduced bacterial colonies by 80 % (compared to the reference - regular PLA), which is not a perfect solution, but the biggest advantage of this method is that it does not require any post processing and the object is ready out of the 3D printer. Very good results were obtained in terms of heat stability of the 3D printed objects. PLA filament-based 3D printed object have respectable resistance to tensile load.3D printing is a versatile technology that can create objects with different sizes, shapes, and materials. In addition to mechanical property, chemical, electrical, optical and surface properties will be emphasizing the need for future research in this field in order to the printed materials fit for the proposed objectives.

REFERENCES

[1] A. Harris The Effects of in-home 3D printing on product liability law, J Sci Policy Gov. 2015; 6(1) http://www.sciencepolicyjournal.org/uploads/5/4/3/4/5434385/harris_new_ta1_1.2.2015_lb_mg.pdf.

[2] K. Ramachandraiah Potential development of sustainable 3D-printed meat analogues. Sustainability. 2021; 13(2): 938 https://doi.org/10.3390/su13020938.

[3] N. Nachal, J. A. Moses, P. Karthik, and C. Anandharamakrishnan, 'Applications of 3D Printing in Food Processing', no. May 2018, pp. 123–141, 2019.http//dx.doi.org/10.1007/s12393-019-09199-8

[4] D. C. H. Jr, P. Palmer, H. Ji, G. D. Ehrlich, and J. E. Król, 'Bacterial Biofilm Growth on 3D-Printed Materials', vol. 12, no. May, pp. 1–13, 2021.https://dx.doi.org/ 10.3389/fmicb.2021.646303

[5] L. Pagani, Q. Qi, X. Jiang, and P. J. Scott, 'Towards a new definition of areal surface texture parameters on freeform surface', Measurement, vol. 109, pp. 281–291, 2017. (http://creativecommons.org/licenses/by/4.0/).

[6] G. Feng, Y. Cheng, S. Wang, D. A. Borca-tasciuc, R. W. Worobo, and C. I. Moraru, 'Bacterial attachment and bio fi lm formation on surfaces are reduced by small-diameter nanoscale pores : how small is small enough ?', Nat. Publ. Gr., no. May, 2015. https://dx.doi.org/10.1038/npjbiofilms.2015.22;

[7] N. M. Æ. James et al., 'Escherichia coli , Pseudomonas aeruginosa , and Staphylococcus aureus Attachment Patterns on Glass Surfaces with Nanoscale Roughness', pp. 268–273, 2009. https://dx.doi.org/10.1007/s00284-008-9320-8

[8] M. Hasan, B. Tasneem, G. T. Amer, C. C. Hull, C. Deckard, and L. Hornbeck, 'Design Fabrication and Testing of a 3D Printer', pp. 2334–2344, 2019. http://ieomsociety.org/pilsen2019/papers/563.pdf

[9] J. Sun, Z. Peng, W. Zhou, J. Y. H. Fuh, G. S. Hong, and A. Chiu, 'A Review on 3D Printing for Customized Food Fabrication', Procedia Manuf., vol. 1, pp. 308–319, 2015. (http://creativecommons.org/licenses/by-nc-nd/4.0/

[10] M. Reza, A. Zolfagharian, M. Jennings, and T. Reinicke, 'Structural performance of 3D-printed composites under various loads and environmental conditions', Polym. Test., vol. 91, no. April, p. 106770, 2020. https://www.researchgate.net/publication/335080694W

[11] J. Wu, E. Bouwman, and J. Reedijk, 'Chelating ligands as powerful additives to manganese driers for solvent-borne and water-borne alkyd paints', vol. 49, pp. 103–108, 2004. https://dx.doi.org/0.1016/j.porgcoat.2003.08.019

[12] M. Lanzetta, E. Sachs, M. Lanzetta, and E. Sachs, 'Improved surface finish in 3D printing using bimodal powder distribution', 2013. https://dx.doi.org/10.1108/13552540310477463

Others:

ISO 179: https://standards.iteh.ai/catalog/standards/sist/b316c2ec-7c1f-48e1-abd6- f15e6db53bbe/iso-179-2- 2020

ISO 180: https://standards.iteh.ai/catalog/standards/sist/b89edb91-b564-4c95-b614-f3fcc5cfcc39/iso-180-2019