



Studying the impact of 3d printing technology on safety indicators of plant-based burger

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ABSTRACT

This study investigate the impacts of 3D printing technology on safety indicators in plant-based burgers. Experiments were performed in the refrigerator on days 0, 5, 7, 12, and 14 and at room temperature at 0, 24, and 96 h after printing. On days 7 and 12 in the refrigerator, PTC of printed samples was significantly higher than conventional. On day 1, the printed sample had higher pH, but on the other days, the pH decreased and no significant difference was reported. TVBN showed a significant ($p < 0.05$) increase in the refrigerator and room. Peroxide value and TBARS increased ($p < 0.05$) in all groups and the printed sample showed a higher value, which could be due to porosity during printing. The results of the sensory showed that the appearance and overall acceptance of the 3D printed sample were significantly ($p < 0.05$) higher than the conventional sample.

1. Introduction

3D printing of food is one of the most important technologies in the food industry in 2023. According to the statistics of scientific databases, researchers around the world are studying more in this field. Also, startups and food companies are increasing research and production in the field of food printing (Derossi, Corradini, Caporizzi, Oral, & Severini, 2023a). This technology has provided many benefits for the food industry and consumers. On a large scale, 3D printers can save and create economic benefits due to the avoidance of micro-circulation (Yoha & Moses, 2023). In addition, this technology has made it possible to prepare food according to the customer's taste. Also, this technology can produce food for various age groups and specific diseases (Kong, Zhang, Mujumdar, & Li, 2023). One of the important studies in this field is food production for dysphagia patients. Also, with this technology, personalized foods can be produced for cancer patients, which precisely adjusts their food plan. Other uses in the food industry include military use, schools, disabled people, restaurants, and space travel (Burke-Shyne, Gallegos, & Williams, 2020). Such as all new technologies, this

technology also has problems that have been tried to be solved. One of the existing challenges is food neophobia. Food neophobia expresses the fear of eating foods that have been produced with new technologies. Informing consumers about all the safety and health features of the product can be one of the ways to reduce food neophobia (Lee, Hwang, Kim, & Cho, 2021). Also, another way is to label this product correctly or launch information campaigns (Brunner, Delley, & Denkel, 2018). Another challenge that has been proposed in most studies as future research is the study of the safety features of products produced with 3D printers. The complete identification of the safety of these products will have a great impact on the attitude of consumers (Nei & Sasaki, 2023). Also, manufacturers of 3D printers can use safety information and improve the production of their machines based on it. In addition, with the identification of safety indicators such as microbial characteristics, peroxide value, thiobarbituric acid, volatile nitrogenous bases, pH, and sensory, consumption of three-dimensional foods in certain diseases will be done more easily and reliably. Studies conducted on 3D-printed polymers show that biofilms produced by microorganisms increase during the printing process. Also, in the study conducted on printed

Abbreviations: PBB, plant based burger; TSP, Textured Soy Protein; XG, Xanthan Gum; PCA, Plate Count Agar; TVC, Total viable count; PTC, Psychrophilic total count; PV, Peroxide value; TBARS, Thiobarbituric Acid; TVB-N, Total Volatile Nitrogen.

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fruits and vegetables, it was reported that the microbial growth is high and there is a need to disinfect the device (Burkard, Shah, Harms, & Denkel, 2023; Morya, Sandhu, Thakur, Neumann, & Awuchi, 2023). Plant-based products have become very trendy in the world. Due to ethical problems and the existence of economic and environmental challenges, the consumption of this group of foods is increasing. It has also been reported that the use of a plant-based diet improves cardiovascular, intestinal, liver, and brain health and plays a role in increasing life (Abedini et al., 2022; Derossi, Corradini, Caporizzi, Oral, & Severini, 2023b; Yu, Zhang, Bhandari, & Li, 2023). Therefore, in this article, Plant-based burger (PBB) were used to evaluate safety during the 3D printing process. According to the standards of the Food and Drug Organization, the formulation including soy protein, onion, bread crumbs, gluten, salt, spices, and sunflower oil was used for PBB. The review of many studies showed that xanthan gum had an excellent performance in improving the texture and printability of samples (Liu et al., 2023).

As mentioned, one of the most important challenges of 3D-printed food is safety, which has not been comprehensively investigated so far. Animal protein substitute products are another trend in the world's food industry. This study aims to investigate the effect of 3D printing technology on the safety indicators (microbial characteristics, peroxide value, thiobarbituric acid, volatile nitrogen bases, and pH) of plant-based burgers in the shelf life of refrigerator and room temperature. Due to the presence of food neophobia in foods produced with novel technologies such as 3D printers, sensory evaluation was another goal of this study. To better understand the result of this study, two groups including 3D-printed plant-based burgers and conventional burgers were evaluated.

2. Material and methods

2.1. Materials

Ingredients used to produce 80 g of plant-based burger samples include Textured Soy Protein (TSP) (purchased from the Tarkhineh company), onion, bread crumbs powder, Xanthan Gum (XG) (purchased from the Tarkhineh company), gluten powder, salt, spices, and sunflower oil (were purchased from the local market) (Table 1).

2.2. Sample preparation and storage condition

First, the TSP was soaked in water for 4 h. Then it was drained and ground with a meat grinder. Gum, onion, gluten, bread flour, oil, spices, and salt were added to the ground soy protein. At this stage, the ingredients were completely mixed in the blender. In this study, two samples (printed and conventional) were used to check in all situations. The storage conditions for evaluating the safety indicators were in two situations, including the refrigerator and room temperature. Experiments were performed in the refrigerator on days 0, 5, 7, 12, and 14. Also, the conditions outside the refrigerator were stored at room temperature (20 to 25 °C) and tests were performed at 0, 24, and 96 h after printing.

2.3. Experimental design

The PBB formulation was first optimized to achieve suitable rheological properties suitable for 3D printing. First, we used a formulation with 12% oil and the print machine had a 2 mm nozzle. Due to its high

viscosity, this formulation did not flow properly and could not be pumped from the nozzle diameter of 2 mm. For optimization, 15% oil and a new printer with a nozzle diameter of 4 mm were used, and the result was acceptable. We used Abtin-II 3D printer (Abtin company, Iran) (Fig. 1).

2.4. 3D printing parameters

FreeCAD software was used to prepare the required files and create the STL format (the acronym that stands for stereolithography). In addition, Slic3r software was used to convert the STL format to G-code (Fig. 2). The samples were printed in the conditions of 1 cm thickness and 9 cm diameter. The information on print factors is written in Table 2.

2.5. Total count viable

To determine the total number of aerobic mesophilic and psychrophilic bacteria, first, 5 g of the sample was mixed with 45 cc of 0.85% sterile physiological serum and homogenized for 60 s, and then the required dilutions were prepared with physiological serum. 0.1 cc of each dilution was spread on the Plate Count Agar (PCA) culture medium. Cultured plates were incubated at 35 °C for 48 h to count TVC (Total viable count) and at 7 °C for 10 days to count PTC (Psychrophilic total count) and after a period, colonies were counted. The colonies were reported as log CFU/g PBB (Farokhzad, Dastgerdi, & Nimavard, 2023).

2.6. Determination of lipid oxidation

Peroxide value (PV) and Thiobarbituric Acid (TBARS) were determined according to AOCS methods Cd 8–53 and Cd19–90, respectively (Farokhzad et al., 2023).

2.7. Chemical analysis

In brief, five g of the sample (Fig. 6) was homogenized with 45 ml of deionized distilled water for 1 min at one hundred rpm at room

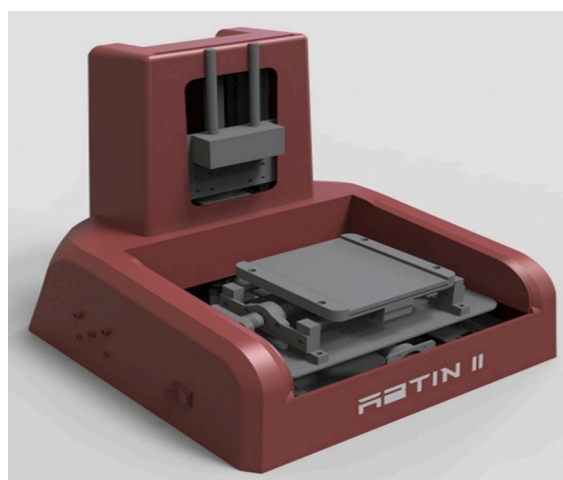


Fig. 1. 3d printer.

Table 1
Ingredient used to produce samples.

	TSP	Onion	Bread crumbs	XG	Gluten	Salt	Spices	Sunflower oil
100%	53%	15%	12%	2%	1%	1%	1%	15%
80 g	42.4	12	9.6	1.6	0.8	0.8	0.8	12

Textured Soy Protein (TSP) and Xanthan Gum (XG).

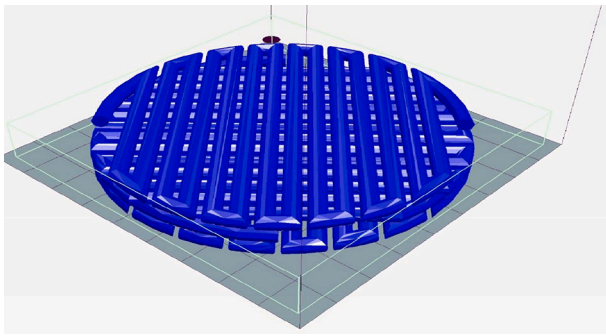


Fig. 2. 3D model.

Table 2

The printing settings are expressed as Slic3r term.

Printing adjusting	Sign	Value	Units	Definition
Nozzle diameter	D	4	mm	Nozzle diameter
Layer height	Z	4	mm	Layer height
Extrusion flow speed	Q	5–10	ml s ⁻¹	Continuous extrusion flow rate provided by the syringe pump
Flow rate	S	70–120	%	The volume of ink that passes through the extruder
Perimeter	P	3	–	Number of outline layers
Infill density	infill	100	%	Quantity of material filling the object

temperature, and pH turned into measured with a pH meter (Metrohm, Switzerland). Total Volatile Nitrogen (TVB-N) was determined in keeping with the strategies previously characterized (Farokhzad et al., 2023).

2.8. Sensory evaluation

A trained panel of 25 people evaluated the sensory properties. The 3D-printed burger samples and the conventionally produced burger samples were marked and a random coding was pre-imposed on the glass containers to be presented to the panel members. The sample pieces were placed at room temperature for 30 min and then distributed among the panel members. Sensory properties such as appearance, odor, texture, color, and overall acceptability were measured. Samples were judged using a 9-step hedonic test. 9 indicated that the sample was very good and a score of 1 indicated that it was very poor (Ghoneim et al., 2023). Because the consumer's final product is fried, first we fried them for 3–5 min at 180 °C with sunflower oil in a frying pan, and similar to the raw sample test, the sensory parameter was evaluated (Di Monaco et al., 2009).

2.9. Statistical analysis

The collected raw data were analyzed by SPSS software. First, the mean and standard deviation were calculated for each parameter. Then, to check the comparison between the groups, the necessary tests for being parametric or non-parametric were used, and the special tests of each were used according to the results. The necessary diagrams were drawn with Prism software. At first, for parametric and non-parametric tests Kolmogorov-Smirnov was used. All data were non-parametric except the TBARS test. Therefore, Kruskal-Wallis was used to check the significant difference between the parameters except for TBARS, and ANOVA was used for TBARS.

3. Results and discussion

3.1. Microbiological test

Tables 3 and 4 show the results of microbial analysis. Examination of the refrigerated samples showed a significant increase in microbial count during the 14 days of the study. Also, the examination of days 7, 12, and 14 showed that the 3D printed and conventional samples were higher than the standard limit of PBB in Iran (5 log CFU/g) for TVC. During the investigation, the samples that were produced by the conventional method had more microorganisms, which indicated cross-contamination during the investigation. One of the reasons for the increase in the growth of microorganisms in the conventional method is the use of hands more than in the 3D printing method. The 3D printer and the conventional sanitation device are excellent, but due to the difference in machine material, it can have a significant effect on the transfer of pollution. PTC analysis showed that 3D printed and conventional samples had no significant difference on day 1 and was not detected. But the examination on the 7th day showed that PTC increased significantly in 3D printed samples. Also, days 7 and 12 showed that PTC in 3D printed samples was significantly higher than conventional samples and it seems that the dominant microorganisms in the 3D printer are psychophilic bacteria. Examination of room samples showed that TVC increased significantly in all samples during 96 h of storage. It was also higher than Iran's standard limit for PBB in 96 h. Both samples were not significantly different at room temperature and at 0 h, but at 96 h, the conventional sample was significantly higher than the printed sample.

Farokhzad et al. (2023) investigated safety indicators in chicken burgers. The condition of their investigation was the refrigerator. They reported that PTC and PTC significantly increased during 20 days. Also, the samples on days 10 and 20 were higher than Iran's standard limit for burgers (Farokhzad et al., 2023). In another study, Bebek Markovinović et al. (2023) investigated the physicochemical characteristics of 3D printed Functional Strawberry Snacks. They examined the samples for 10 days and observed that the microbial growth increased significantly during the 10 days. No significant difference between the samples was reported on days 0 and 2. But days 4, 7, and 10 showed that the 3D printed sample had higher aerobic mesophilic bacteria (Bebek Markovinović et al., 2023). For a detailed discussion on the microbial properties of 3D printed foods, one should check Various stages of preservation of 3D printed food products. These steps include printing, cooking, storing, packaging, and dispensing (Singh, 2022). Examining pathogenic bacteria in 3D printers is very important and has not been investigated in meat and analog products to date. Also, different food items should be checked in terms of mold and yeast growth according to 3D printer machines and production and formulation conditions. Markovinovi et al. (2023) investigated the microbial characteristics of 3D

Table 3

Microbial characteristics of 3d printed burgers during 14 days in the refrigerator.

Microorganism type	Sample	Storage (Day)				
		0	5	7	12	14
Total viable count	Conventional	4 × 10 ^{3a}	6.6 × 10 ^{5b}	12.2 × 10 ^{5a}	18.4 × 10 ^{6b}	29.2 × 10 ^{6b}
	3D printed	2.4 × 10 ^{3a}	4.2 × 10 ^{3a}	9.8 × 10 ^{5a}	13.8 × 10 ^{6a}	24.4 × 10 ^{6a}
Psychophilic total count	Conventional	n.d	n.d	n.d.a	14.3 × 10 ^{5a}	23.5 × 10 ^{6a}
	3D printed	n.d	n.d	6.4 × 10 ^{3b}	18.4 × 10 ^{5b}	29.5 × 10 ^{6a}

Table 4
Microbial characteristics of 3d printed burgers during 96 h in the room.

Microorganism type	Sample	Storage (Hour)		
		0	24	96
Total viable count	Conventional	4.6×10^2 ^a	12.7×10^4 ^a	24.5×10^6 ^a
	3D printed	3.5×10^2 ^a	8.4×10^4 ^b	15.2×10^6 ^b

printed strawberry snacks. The storage conditions of their samples were 10 days at 4 °C. They reported that pathogenic bacteria, mold, and yeast were not found in all the printed samples. But on days 7 and 10, samples with vanillin (1 g L⁻¹) have high microbial content. Samples with more vanillin showed higher microbial content on the fourth day. The presence of aerobic mesophilic bacteria observed before storage could be due to handling during the preparation of the product and is limited to the surface. They suggested that more microbial properties should be investigated in printed foods (Bebek Markovinović et al., 2023).

Severini, Derossi, Ricci, Caporizzi, and Fiore (2018) printed a fruit-and-vegetable-based totally smoothie and monitored its microbiological profile over eight days while the product was saved at 5 °C in the air (20% O₂ and 80% N₂) or under a modified environment (5% O₂ and 95% N₂). Microbial concentrations (mesophilic flora, psychrophilic microorganisms, and yeasts) inside the samples started high, at between 4 and five log CFU/g, on Day 0, remained between Day 0 and Day 6 regardless of the food storage, then confirmed a decrease at Day 8. The authors explained this, to begin with high microbial contamination as delivered by way of the printer itself, via its pistons, its tubes, or the extruder, as they'd carefully washed the ingredients in advance (Severini et al., 2018). Liu, Bhandari, and Zhang (2020) investigated the growth and viability of probiotic bacteria exposed to a 3D printer. They reported that no significant difference in the growth of probiotic bacteria was observed during storage and 3D printing is a useful approach to produce functional foods. They also observed that it stayed at a much higher level (above 9.773 log CFU/g) than the recommended least dose in probiotic foods (6 log CFU/g) (Liu et al., 2020). In 3D printers, pressure can be a confusing factor to investigate the survival and death of organisms for future studies. In 3D printers, different pressures are usually created

depending on the physical characteristics of the food. Severini et al. (2018) used a pressure of 1.3 bar to print fruit and vegetable products. They reported that the microbial load of the final product was very high overall. Also, the first 3D-printed sample showed a very high microbial load (Severini et al., 2018).

3.2. Determination of lipid oxidation

According to Fig. 3 and Fig. 4, on the first day of investigation, no significant changes ($p > 0.05$) were observed in PV and TBARS between the 3D printed and conventional samples. But during storage, both parameters increased significantly ($p < 0.05$). Primary oxidation products in food are known as PV and secondary products are known as TBARS.

Comparing the beginning and the end of the chart, all the samples showed an increase in two parameters, and the highest PV and TBARS in the refrigerator and room conditions were related to the 3D printed sample, which was not significant compared to the conventional group ($p > 0.05$). However, the conventional sample had more TBARS (10.1 ± 0.13) in 96 h and in-room conditions than the 3D printed sample (9.98 ± 0.4) (Fig. 3). In addition, on the 7 days and in the refrigerator, TBARS of the conventional sample was significantly ($p < 0.05$) higher than the printed sample. The data shows that 3D printing technology can affect the oxidation factors in 14 days of storage in the refrigerator and 96 h of storage in the room, but it is not significant. Our assumption was that considering that porosity increases in 3D printing products, it can increase oxidation factors significantly. However the use of the same source for the production of conventional burgers and 3D-printed burgers did not show a significant difference in oxidation parameters. Also, some 3D printers have heat from the bottom, which can be investigated in future studies in PV and TBARS parameters. Fan, Zhang, Zhang, Ma, and Feng (2023) investigated TBARS in meat. They observed that the difference between the treatments was not significant on the first day, but it increased significantly until the 14th day. They reported that on the 14th day, a significant difference was observed between the samples (Fan et al., 2023). Farokhzad et al. (2023) investigated PV in chicken burgers. Comparing their results with our study shows that PV increases faster because chicken burgers can have more fat than veggie burgers

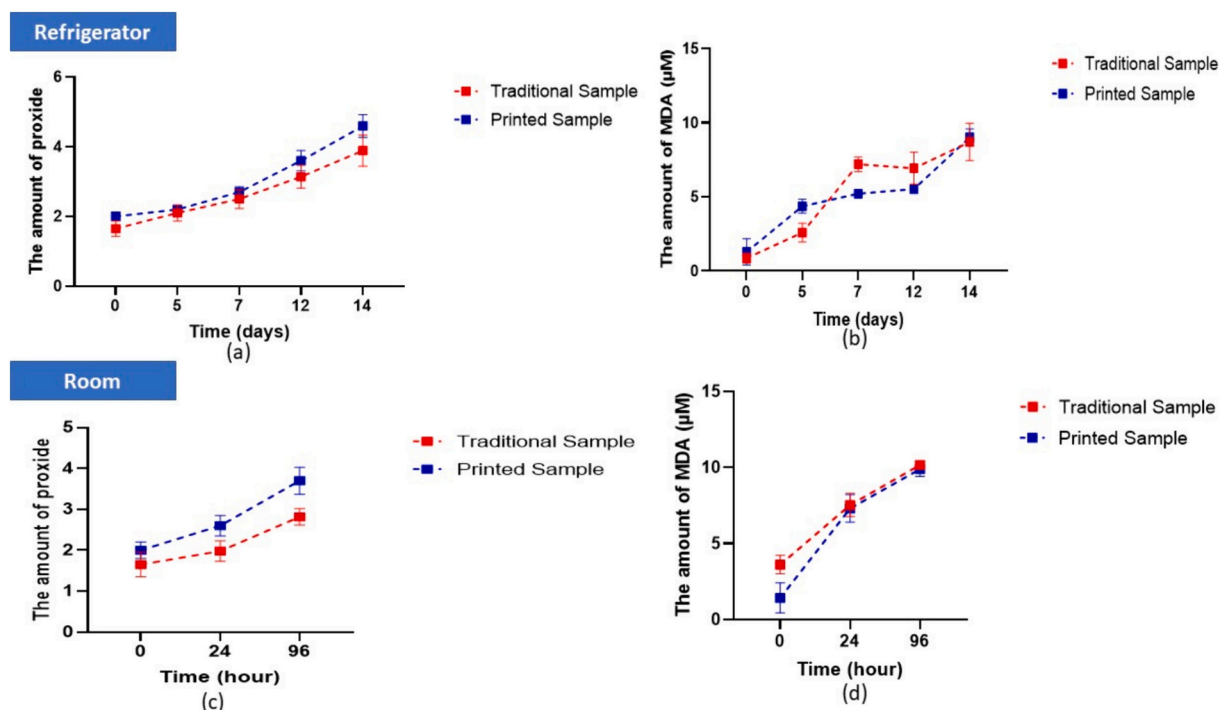


Fig. 3. Oxidation stability of 3d printed burgers during 14 days in the refrigerator and 96 h at room temperature.

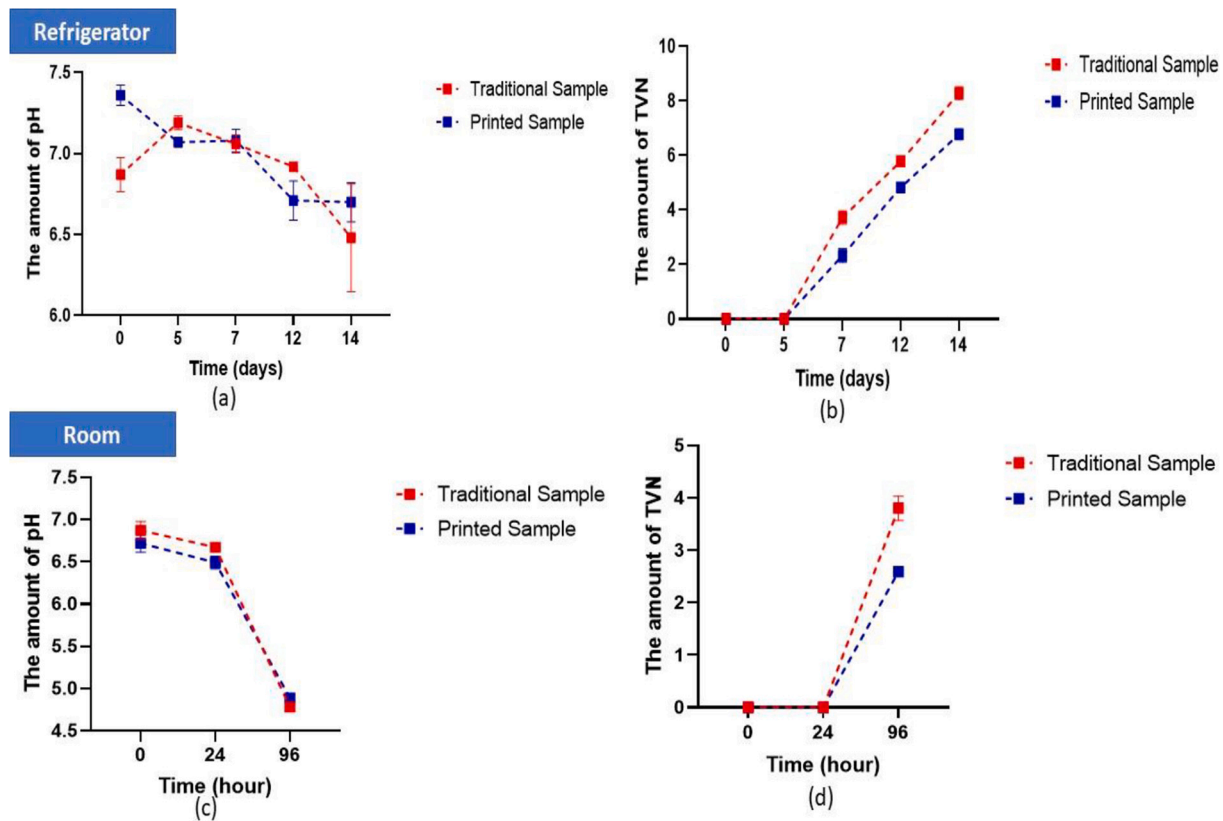


Fig. 4. Chemical characteristics of 3d printed burgers during 14 days in refrigerator and 96 h in room temperature.

and are more prone to oxidation. They also reported that PV increased significantly within 20 days of the examination (Farokhzad et al., 2023). Natural antioxidants in food such as spices can be damaged under the influence of product preparation processes for printing, including blending, mixing, and homogenization, and can accelerate the oxidation of samples (Severini et al., 2018).

3.3. Chemical analysis

Examining the pH value during 14 days in the refrigerator and 96 h in the room showed that all the samples decreased significantly. The printed and conventional samples were significantly different on day 1 in the refrigerator, and the printed and conventional samples showed 7.4 and 6.8, respectively. However, during the experiment, there was no significant difference between the samples, but all the samples decreased significantly. Examination of room samples showed no significant difference between printed and conventional samples at 0 and 24 h. But between 24 h and 96 h, the pH value decreased significantly. Because the production of TVBN was not high, the effect on pH increase was not significant and all the samples were sour.

Marrone et al. (2021) investigated the physicochemical characteristics of Black Angus beef burgers. They observed that the pH value of the samples decreased during the investigation period (Marrone et al., 2021). Panza, Conte, and Del Nobile (2022) investigated the shelf life of a prolonged fish burger for 26 days. They observed that there was no significant difference in the control samples. But the samples with antioxidants had a significantly lower pH value (Panza et al., 2022). The increase in the acidity of the samples can have multiple reasons, for example, it is related to the formation of dominant organic acids, oxalic acid, and malic acid. It can also be related to the activity of microorganisms and natural antimicrobial compounds in spices (Abedi, Lakzadeh, & Amouheydari, 2021; Amirhossein Abedini, Sadighara, Sani, & McClements, 2023). The investigation of TVB-N during 14 days in the

refrigerator and 96 h in the room showed that all the samples increased significantly. Examination of printed and conventional samples in the refrigerator showed that TVB-N was not found on days 0 and 5. But it increased significantly on days 7, 12, and 14. However no significant difference was observed between printed and conventional samples. However, conventional samples had more TVB-N, which could be due to a higher microbial load. Examination of room samples showed that TVB-N did not produce at 0 and 24 h, but increased significantly at 96 h. Also, the highest amount of TVB-N was observed in the conventional sample at 96 h, which can be due to the presence of proteolytic enzymes in microorganisms that can convert proteins into volatile nitrogen compounds.

Farokhzad et al. (2023) investigated the physicochemical characteristics of Chicken Burgers. They observed that TVB-N significantly increased during the 20 days of testing. They also reported that the highest formation increase was in the control samples, which could be due to the microbial load and proteolytic enzymes (Farokhzad et al., 2023). Mahmoud and Abu-Salem (2014) investigated the effect of adding Soybean Additives to address the burger. TVB-N in all samples increased significantly in 12 days. They observed that the highest amount of TVB-N formation was related to the samples with Protein Hydrolyzate produced from Soybean fermentation (Mahmoud & Abu-Salem, 2014).

3.4. Sensory evaluation

Fig. 5 shows the results of the sensory test. For refrigerator samples, it was done on days 0, 7, and 14. On days 5 and 12, participants did not report significant changes. Examination of the refrigerator samples showed that the appearance of the printed samples showed a significantly higher score. Also, the participants reported significantly higher texture and overall acceptance of the printed sample than the conventional sample. But there were no significant changes in color and odor

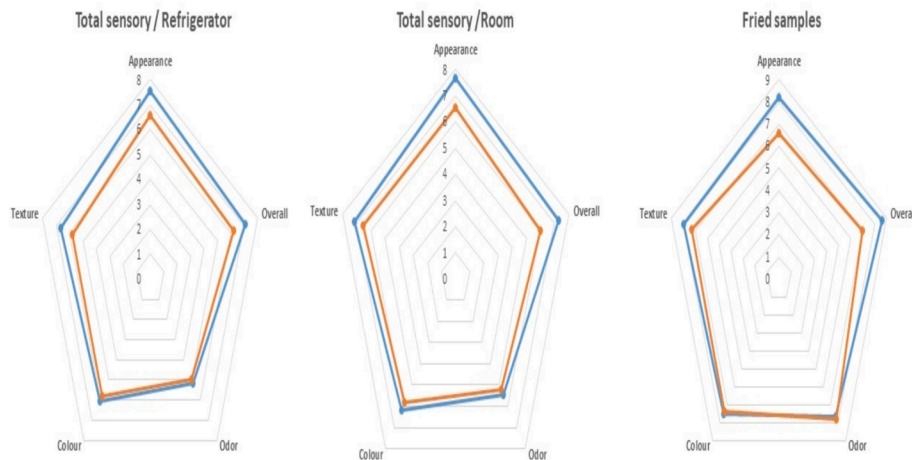


Fig. 5. Sensory characteristics of plant based burgers in refrigerator, room and fried samples. Blue: 3d printed sample, Red: conventional sample. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

and both samples did not get high scores. The sensory test conditions were in the room at 0 and 96 h, and 24 h did not show significant changes. Examination of room samples confirmed the above results, but there were no significant changes in the texture. Examination of the fried samples showed that appearance and general acceptance were significantly higher in the printed samples than in the conventional samples. In addition, texture analysis showed that the changes between the samples were not significant. Color and odor received significantly higher scores in the fried sample than in the refrigerator and room, which indicates the effect of frying on organoleptic and visual characteristics. The printed sample in appearance and overall acceptance were significantly higher than the conventional sample in 3 conditions, which confirms the impact of 3D printing technology on visual appeal.

Severini et al. (2018) investigated the physicochemical characteristics of 3D-printed vegetables. They reported that the sensory results of the 3D printed sample (4.60 ± 0.74) were significantly higher than the other food sample (3.37 ± 1.1). They also reported that other sensory parameters did not show significant differences. In addition, they observed that 3D printing technology had no negative effect on color changes and off-odor or off-flavor production (Severini et al., 2018). Agarwal et al. (2022) investigated the physicochemical characteristics

of 3D-printed gluten-free snacks. They used two conditions of a 3D printer and a conventional method to produce samples. They observed that there were no significant changes in color and odor between the two samples. Texture analysis showed that the conventional samples had a significantly higher score than the printed sample. But in light, moister, and soft parameters, the printed sample was significantly better than the conventional sample. They stated that cooking and printing time can affect sensory characteristics (Agarwal et al., 2022).

Bulut and Candogan (2022) produced functional chicken meat using a 3D printer to optimize the level of gelatin. In their sensory evaluation, they examined appearance, color, odor, flavor, texture, and overall acceptability. The 1.79% gelatin-added sample received significantly higher scores than the control in terms of appearance, color, odor, flavor, texture, and overall acceptability attributes ($P < 0.05$) Because gelatin improved the properties of viscosity, printability, and gelatinization (Bulut & Candogan, 2022). Jin et al. (2023) studied the sensory properties of 3D printed Mackerel Mince (*Scorpaenopsis scrofa*) using purple potato powder (PP) and Citric Acid (CA). The results of their investigation showed that the addition of PP and CA could improve the sensor characteristics in terms of color and make it pink. Furthermore, an increase in purple PP concentration and added CA reduced the fluidity and loss of water in mackerel mince increase in PP and CA concentrations enhanced the umami and sweet taste of mackerel mince but reduced the fishy and sour taste, and the degree of preference was within the acceptable range, except for PP1%-CA0%. Sensory evaluation showed that the best percentage of PP and CA samples in terms of color L^* , a^* , and b^* were 1.00–3.00% and 0.09–0.32%, respectively (Jin et al., 2023).

4. Conclusion

According to the results, safety parameters including microbial, pH, peroxide, TBARS, TVBN, and sensory were investigated. At first, it tried to present a more attractive structure than the conventional burger to have better visual features. 3D printing of the samples significantly improved the sensory characteristics related to appearance, texture, and overall acceptance, but had no significant effect on pH and TVBN. Also, the 3D-printed samples had higher peroxide values and PTC than the conventional burger. This study provides useful information for consumers and producers, especially people with food neophobia. But this study provides preliminary information, and future studies can study more detailed issues such as the effect of pressure on the microbial and nutritional properties of the 3D printed product. Another factor is the use of UV for printing. One of the suggestions we came up with during the study process was to use a camera to send the production process

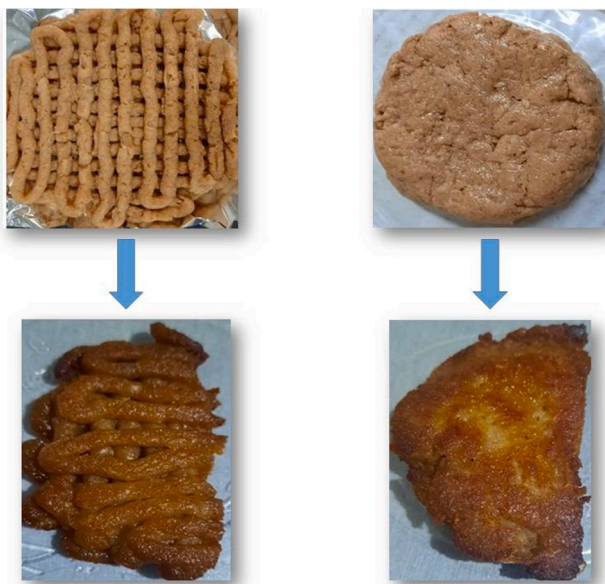


Fig. 6. The final product.

online to consumers with food neophobia. Certainly, the correct notification of the results of scientific tests and online observation of the production process can be among the useful approaches to reducing food neophobia. The challenge related to the sanitization of all 3D printer equipment still exists, it seems that different studies can focus on aspects of the migration of heavy metals from printer equipment to food or the migration of microplastics. Sterilization must cover all parts exposed to food, including cartridges, nozzles, and build platforms, and it can comply with regular microbial standards and tests. You should also pay attention to the remaining detergent and the material of the 3D printer. The amount of volume that a 3D printer can print during a complete process is a fundamental challenge for the safety debate. Because every time you change the nozzle and fill it and communicate with your hands and equipment, it contaminates the food. In addition, leftover food from the previous print can increase contamination. It seems that checking the printing process can be the solution to the issue of which printed sample the safety index exceeds the standards or the use of a tank connected to the nozzle can help in improving the safety index. Another suggestion we reached in this study was the use of bio-compatible nozzles and tanks. Likely, the use of materials such as polysaccharides and nanoparticles will greatly improve the safety of printed products, although you should try so that they are not a source of contamination or affect the sensory characteristics of the final product. Another important material in safety is the presence of an automatic and sterile tool for separating the printed sample. This issue is related to the design of 3D printers, but it can reduce hand contact and improve safety indicators if implemented correctly. Because we saw in the study that it is a challenge to separate samples using conventional tools. Using nozzles with the right diameter for production in a short time creates a great advantage. In this study, we selected a diameter of 4 mm for the nozzle as optimum through various tests, and a very fast printing time was observed. It seems that in industrial and high-scale production, nozzle diameter will be an important feature in the efficiency of production products. Multi-nozzle printers can be used for high-efficiency production on an industrial scale. Also, printers with intelligent control can report safety issues to manufacturers, which are a new generation of 3D printers. Large-scale production of new technologies always has many challenges. Due to the close relationship between the food industry and people's health, the industrialization of new technologies in this issue sometimes has more challenges such as safety and fear of consuming new products (food neophobia). Examining the safety of 3D printers in the application of food production allows food companies to target and plan more precisely for its use and expansion. Another important and influential factor in production is the comparison of the characteristics of the product produced with the new method and the conventional method. This comparison provides useful information for the industrialization of 3D-printed foods. For example, how is the growth of microorganisms in the 3D printed product compared to the conventional method or other factors such as pH, TVBN, peroxide, TBARS, and sensory properties. The safety evaluation of 3D printers makes it possible for the food industry to have a regular plan for device sanitization or to use interventions before and after product production. It also makes it possible to inform consumers and reduce food neophobia.

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CRediT authorship contribution statement

Amirhossein Abedini: Writing – original draft. **Hedayat Hosseini:** Data curation. **Nabi Shariatifar:** Funding acquisition. **Ebrahim Molaee-aghae:** Writing – original draft. **Parisa Sadighara:** Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The data that has been used is confidential.

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