Bioactive retention in 3D-printed strawberry snacks: Influence of fruit size and processing

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Abstract

Strawberries (*Fragaria* × *ananassa* Duch.) are rich in bioactive compounds and antioxidants, making them promising for functional food development. This study examined how fruit size (<15 g, 15–30 g, >30 g) affects the bioactive composition and antioxidant activity of fresh strawberries and three-dimensional (3D)-printed strawberry-based snacks using the "Albion" cultivar. Smaller fruits (<15 g) showed the highest total phenolic (134.02 ± 1.76 mg GAE/100 g) and anthocyanin contents (17.74 ± 0.26 mg Pg-3-Glc/100 g), with anthocyanins better retained in 3D-printed products (15.96 ± 0.30 mg Pg-3-Glc/100 g). Although 3D printing reduced anthocyanins by ~26%, antioxidant activity remained stable (DPPH–fresh: 295.73 ± 0.21 µmol TE/100 g *vs.* 3D-printed: 299.06 ± 0.21 µmol TE/100 g). A significant inverse correlation between pH and anthocyanins ($r = -0.999$, $p = 0.02$) emphasized acidity as a key factor in pigment stability. These results highlight the importance of fruit size and processing conditions for maximizing the nutritional value of 3D-printed functional foods.

Keywords: Strawberries; Fruit size; Bioactive compounds; 3D food printing; Functional foods

Introduction

Strawberry (*Fragaria* × *ananassa* Duch.) is a nutrient-rich fruit with a high content of bioactive compounds (BACs) that are beneficial for human health. It is an excellent source of vitamin C, folates, and dietary fiber, contributing to its potent antioxidant and anti-inflammatory properties (Newerli-Guz *et al.*, 2023). Strawberries contain more vitamin C than many other fruits, averaging 50–100 mg per 100 g of fresh weight, which enhances their antioxidant activity (Newerli-Guz *et al.*, 2023). High vitamin C content supports collagen synthesis, immunity, and protection against oxidative stress-related diseases (Alberts *et al.*, 2025). They also provide essential

minerals, such as potassium, magnesium, and manganese, which aid in metabolism (Vicente *et al.*, 2022). Rich in polyphenols, particularly flavonoids and ellagic acid, strawberries offer promising health benefits (Ezzat-Zadeh *et al.*, 2021). However, their 90% water content accelerates spoilage and shortens shelf life (Priyadarshi *et al.*, 2024). Thermal processing degrades heat-sensitive compounds like vitamin C and anthocyanins, making nonthermal techniques preferable for preserving nutrients and extending shelf life (Cano-Lamadrid & Artés-Hernández, 2022; Nhan & Quyen, 2023).

Nonthermal three-dimensional printing (3DP) technology has emerged as an innovative method for fabricating functional foods, enabling the preservation of heat-sensitive BACs and nutrients. It operates using an additive layer-by-layer approach, where food materials are deposited in precise layers to build a three-dimensional (3D) structure. The process typically involves extrusion, inkjet, or binder jetting techniques, allowing for controlled deposition of food pastes, purees, or gels based on digital designs. This method enables customization of texture, shape, and nutrient composition while maintaining the functionality and structural integrity of ingredients (Zhao *et al.*, 2025). Advantages include the ability to produce complex geometries, personalized nutrition, standardization of BACs content, and the retention of ingredient functionality without thermal degradation. However, challenges persist, such as ensuring the structural integrity of printed foods, achieving consistent texture and flavor, and maintaining microbial safety during storage (Guo *et al.*, 2023). Beyond technological limitations, the success of 3D food printing also relies on consumer acceptance and scalability. While customization is appealing, some consumers may view printed foods as less natural, making clear communication of nutritional and safety benefits essential. At the same time, scaling up from laboratory to industrial production remains challenging because of costs, standardization, and consistent quality. Overcoming these barriers will be key for 3D food printing to become a viable mainstream technology (Fisher, 2022).

Researchers have developed 3D-printed snacks using fruit bases to produce functional products with enhanced BACs and antioxidant properties (Tomašević *et al.*, 2021). In addition, 3D food printing has been explored for the valorization of fruit and vegetable industry by-products, transforming them into personalized foods with specific properties like shape, texture, and nutritional content (Tyupova & Harasym, 2024). For instance, the successful creation of 3D-printable formulations using orange peel waste (OPW), highlighting favorable rheological characteristics and effective preservation of bioflavonoids and antioxidant compounds was demonstrated (Tan *et al.*, 2023). In another study, enriching 3D-printed cookies with encapsulated polyphenols led to a significant enhancement of phenolic content—up to a 173% increase—as well as improved interactions between moisture and BAC stability (Oliveira *et al.*, 2021). These advancements demonstrate the potential of 3DP technology in developing innovative, nutritionally tailored fruit-based functional foods.

In the context of strawberry-based functional foods, extrusion-based 3DP has been utilized to create snacks enriched with BACs. A study investigated the incorporation of corn and wheat starches at different concentrations (10%, 15%, and 20%) into strawberry matrices. The findings indicated that a 15% starch addition, particularly with corn starch, optimized the preservation of BACs and antioxidant activity. In addition, the application of specific printing parameters (referred to as program 2 in the study) enhanced the textural properties and geometric stability of the printed snacks. Microbiological assessments revealed that the addition of natural antimicrobial agents, such as citral, a natural monoterpene, at a concentration of 75 mg/L, effectively inhibited pathogenic bacteria and ensured the microbial safety of the products during storage. These results underscore the potential of 3D printing technology in developing innovative, nutritionally tailored strawberry-based functional foods with desirable sensory properties and longer shelf life (Bebek Markovinović *et al.*, 2023).

Regarding the quality of strawberries and their derived products, studies indicate that fruit size is a key factor in quality assessment. The size of a strawberry has a significant impact on its overall quality, and in turn, on the quality of the processed products. However, research has also shown a negative correlation between fruit size and total soluble sugar content, suggesting that larger fruits may have a lower sugar concentration, which could impact the flavor profile of processed products (Cockerton *et al.*, 2021). In addition, the size of strawberry is influenced by factors such as cell size distribution and plant vigor, with larger plant crown diameters leading to larger fruit size and improved physicochemical properties (An *et al.*, 2024; Fagherazzi *et al.*, 2021). Other studies emphasize the importance of considering fruit mass and size in agricultural practices and food processing to optimize the content of BACs and, consequently, the nutritional and functional quality of fruit-based products (Sharma *et al.*, 2019; Trujillo-Mayol *et al.*, 2020; X. Zhou *et al.*, 2023). Therefore, optimizing strawberry size through cultivation practices is essential for enhancing both the quality of fresh fruit and the properties of strawberry-based products.

Understanding these interrelated factors is essential for optimizing ingredient selection and processing conditions in 3D food printing. This study innovatively

investigates the impact of strawberry fruit size on the BAC profile and antioxidant activity of both fresh and 3D-printed strawberry snacks, an aspect not previously explored in 3D food printing research. Furthermore, we examine how pH influences monomeric anthocyanin (ANT) stability during thermal processing in 3D printing, providing novel insights into preserving these sensitive compounds under printing conditions. The findings offer evidence-based strategies to maintain functional compounds in 3D-printed fruit products, ultimately enhancing their nutritional value and potential for personalized nutrition applications.

Materials and Methods

Schematic overview of the experimental program

A schematic flow diagram (Figure 1) is presented to provide a visual summary of the experimental design and workflow. The major stages include sample collection and classification, preparation of strawberry purées, 3D printing of fruit snacks, extraction of BACs, and analytical determinations. This diagram directly reflects the study's objective to compare bioactive profiles and antioxidant activity in relation to fruit size and processing method. All experimental procedures were performed in accordance with standard protocols approved by the Faculty of Food Technology and Biotechnology, University of Zagreb and the Division of Horticulture and Landscape Architecture, Faculty of Agriculture, University of Zagreb.

Strawberry fruit material

Strawberries (*Fragaria* × *ananassa* Duch., cv. "Albion") with three different fruit size classes (up to 15 g, 15–30 g, and over 30 g) were harvested on the same day in May 2023 from the plants grown in soilless cultivation at Jagodar-HB d.o.o. in Donja Lomnica, Zagreb County (Croatia). Immediately after harvesting, the strawberries were transported to the laboratory, washed with tap water, and destemmed. A total of 20 fruits from each size class were used to measure the total soluble solids content (SSC, °Brix) and the pH value after homogenization with a blender (SilverCrest, Kompernass GMBH, Bochum, Germany), which was used to obtain purees of consistent homogeneity. These purees were subsequently used to prepare extracts and for 3D printing. Spectrophotometric determination of BACs and antioxidant activity was conducted on all extracts. In total, six samples were analyzed: extracts from fresh strawberry samples (A, B, and C) and extracts from 3D-printed samples (3D–A, 3D–B, 3D–C) (Table 1).

The categorization into three distinct weight classes, small (<15 g), medium (15–30 g), and large (>30 g), was

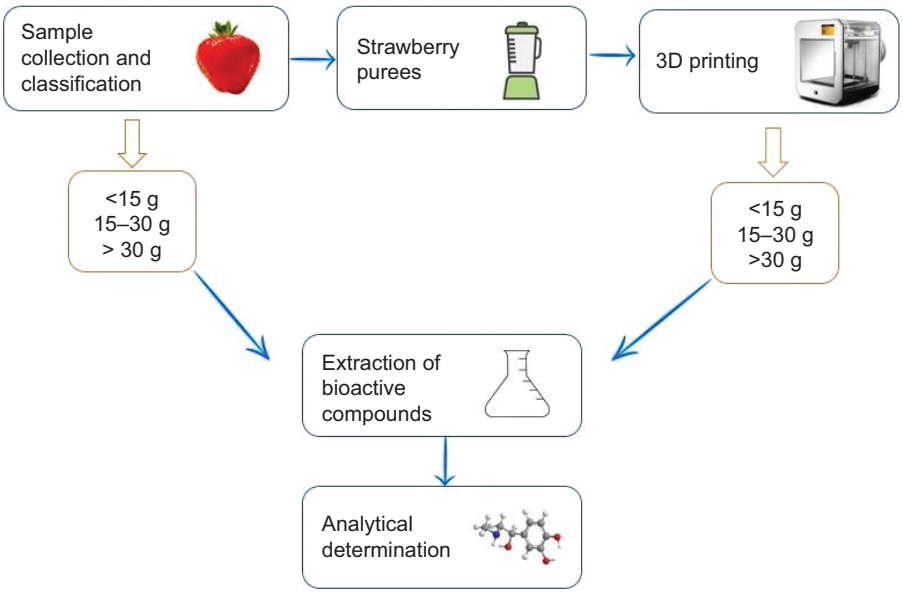

Figure 1. Workflow of the study examining the effect of strawberry fruit size on bioactive compounds (BACs) and 3D printing outcomes. Strawberry samples were collected and classified into three size categories (<15 g, 15–30 g, and >30 g). Purees were prepared and subjected to 3D printing. Both fresh and 3D-printed samples were then processed for the extraction of BACs, followed by analytical determination of their composition and antioxidant activity.

Table 1. Design of an experiment.

Sample ID	Sample	Fruit weight	Sample code
1	Puree	<15 g	A
2	Puree	15–30 g	B
3	Puree	>30 g	C
4	3D-printed snack	<15 g	3D–A
5	3D-printed snack	15–30 g	3D–B
6	3D-printed snack	>30 g	3D–C

based on common horticultural classification standards and reflects the natural variation in fruit size within the "Albion" cultivar. This stratification allowed for a controlled assessment of how fruit mass affects the chemical composition and functional properties of both raw and processed samples. Each weight class was represented in two forms: fresh purée and a corresponding 3D-printed snack. This pairing enabled direct comparison between the untreated and processed states of each fruit size group, isolating the effect of 3D printing on the retention of BACs. The use of a consistent sample size (20 fruits per weight category) ensured statistical validity and minimized biological variability. By maintaining uniformity in the processing conditions, such as puree preparation, starch concentration (15%), and 3D-printing parameters, the design ensured that any observed differences in the measured outcomes could be attributed primarily to the fruit size and not to inconsistencies in sample handling or printing performance. This experimental setup thus provides a robust framework for understanding the interactions between fruit morphology, food printing technology, and nutritional quality.

3D printing of strawberry fruit snacks

For the preparation of 3D-printed strawberry snacks, an extrusion-based Foodini 3D printer (Natural Machines, Barcelona, Spain) was used. Following the published results (Bebek Markovinović *et al.*, 2023), wheat starch (Denes Natura Kft., Pécs, Hungary) was used at a concentration of 15%. The strawberry and starch mixture were preheated to 65°C with constant stirring on an LLG – uniSTIRRER 7 magnetic stirrer (Lab Logistics Group GmbH, Meckenheim, Germany) to induce the gelatinization of the added hydrocolloids, thereby ensuring an adequate viscosity that is essential for successful 3D printing.

The printing process was performed at ambient temperature, as the Foodini printer itself does not apply heat during deposition. The printer was configured with the following parameters: a printing speed of 14,000 mm/min, a line thickness of 3.4 mm, a flow rate setting of 1.65 (a dimensionless value specific to the Foodini software), and a first-layer nozzle height of 4.5 mm. The printing material was loaded into 100 mL cartridges equipped with 4 mm nozzle diameters. The design selected for printing was a three-layered heart shape, with final product dimensions of 53 mm in length, 51 mm in width, and 12 mm in height (Figure 2) measured by Vernier caliper gange, digital (BOCHEM Instrumente GmbH, Weilburg, Germany). The printing design and process were controlled using Foodini Creator Software. No postprocessing steps were applied, and the snacks were either consumed immediately or stored at 4°C prior for analysis.

Figure 2. Strawberry snack produced by 3D printing (sample 3D-A).

Determination of SSC and pH

The determination of SSC is based on the direct reading of soluble dry matter using a digital refractometer (ATAGO PAL-3, Atago Co., LTD, Tokyo, Japan), while the pH of both samples was determined using a pH meter (FiveEasy, Mettler-Toledo, Greifensee, Switzerland).

Extraction of bioactive antioxidants

An extraction solvent of 40 mL (aqueous methanol solution, 80% v/v, with 1% formic acid, v/v) was added to 10 g of the sample. The prepared mixture was extracted using an ultrasonic processor (UP400St, Hielscher Ultrasound Technology, Germany) at amplitude 50%, pulse 100%, and extraction time 5 minutes. After extraction, the extract was filtered into a 50 mL volumetric flask, made up to the mark with the extraction solvent, and stored at 4°C until analysis (Bebek Markovinović *et al.*, 2024a).

Determination of BACs and in vitro antioxidant activity

All extracts were spectrophotometrically analyzed for their BAC content and antioxidant activity using a UV/Vis spectrophotometer (LLG-uniSPEC 2 spectrophotometer, Buch and Holm, Meckenheim, Germany).

Determination of Total Phenols (TP)

An extract of 400 µL, 400 µL of Folin–Ciocalteu reagent (previously diluted five times with distilled water), and 4 mL of 7.5% sodium carbonate solution (w/v) are pipetted into a test tube. The reaction mixture is left to stand for 20 minutes at room temperature, and the absorbance is measured at a wavelength of 725 nm. The determination is carried out in parallel, and the extraction solvent is used as a blank. The calibration curve was prepared using gallic acid standard solutions at concentrations ranging from 10 to 250 mg/L. The results were expressed as mg gallic acid equivalents (GAE) per 100 g of sample (Yuan *et al.*, 2018).

Determination of Total Flavonoids (TFs)

An extract of 0.5 mL, 1.5 mL of 96% ethanol, 0.1 mL of 10% aluminum chloride, 0.1 mL of 1 M potassium acetate, and 2.8 mL of distilled water were mixed in a glass tube. The reaction mixture is then left to stand for 30 minutes, after which the absorbance (optical density of the solution) is measured at a wavelength of 415 nm. A blank sample is prepared in the same way, but instead of extract, the extraction solvent is used, and instead of 10% aluminum chloride, the same volume of distilled water (0.1 mL) is added. The calibration curve was prepared using quercetin standard solutions at concentrations ranging from 10 to 200 mg/L. The results were expressed as mg quercetin equivalents (QE) per 100 g of sample (Chang *et al.*, 2020).

Determination of Total Hydroxycinnamic Acids (HCAs) and Total Flavonols (FLs)

An extract of 250 µL, 250 µL of 1 g L^{-1} HCl in 96% ethanol, and 4.55 mL of 2 g L^{-1} HCl were mixed in a glass tube. For the determination of total HCAs, the absorbance is measured at 320 nm, while for the determination of total flavanols, the absorbance is measured at 360 nm. A blank sample is prepared in the same way, but instead of extract, the extraction solvent is used. The HCA content was calculated from the calibration curve obtained using chlorogenic acid solutions (10-600 mg/L), and the results were expressed as mg chlorogenic acid equivalent (CAE) per 100 g sample. The FL content was calculated from the calibration curve obtained from quercetin solutions (10–600 mg/L), and the results were expressed as mg QE per 100 g of sample (Howard *et al.*, 2003).

Determination of Total ANTs

The pH differential method (Lee *et al.*, 2005) was used to determine anthocyanins. One milliliter of the extract was mixed with 4 mL of potassium chloride buffer pH 1.0 (0.025 M), and 1 mL of the extract was mixed with 4 mL of sodium acetate buffer pH 4.5 (0.4 M). After 20 minutes, the absorbance was measured at 520 nm and 700 nm. The anthocyanin content was expressed as mg pelargonidin-3-glucoside equivalent (Pg-3-Glc) per 100 g of sample.

Antioxidant activity

DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenging activity assay

An extract of 1.5 mL and 3 mL of a 0.5 mM DPPH solution was mixed in a test tube and kept at room temperature in the dark for 20 minutes. The absorbance was measured at 517 nm. A calibration curve was constructed from different concentrations of Trolox solutions (10–150 µM), and the results were expressed as µmol Trolox equivalent (TE) per 100 g of sample (Shortle *et al.*, 2014).

FRAP (Ferric Reducing Antioxidant Power) assay

An extract of 600 µL and 4500 µL of FRAP reagent (prepared from acetate buffer (0.3 M), 2.5 mL of TPTZ reagent (2,4,6-tris-2-pyridyl-s-triazine; 10 mM), and 2.5 mL of iron (III) chloride (20 mM) in a 10:1:1; v/v/v ratio) were pipetted into glass tubes, mixed and heated at 37°C for 10 minutes. The absorbance was measured at 593 nm. A calibration curve was constructed by different concentrations of Trolox solutions (10–150 µM), and the results were expressed as mmol TE per 100 g of sample (Benzie, 1996).

Statistical Analysis

To characterize the sample, descriptive statistics were utilized. MANOVA was applied to assess the discrete variables. A significance level of $\alpha \leq 0.05$ was used for all tests. The analyses were conducted using SPSS software (version 22) and Statgraphics Centurion XVII (StatPoint Technologies Inc., Warrenton, VA, USA).

Results and Discussion

Physicochemical properties of fresh strawberries and 3D-printed strawberry snacks

The influence of fruit size and 3DP technology on variations in SSC and pH values was analyzed, and the results are presented in Table 2. In fresh strawberries, no statistically significant difference in SSC was observed between fruits weighing <15 g and those weighing 15–30 g. The SSC differences between fruits weighing <15 g and those >30 g were not statistically significant (p=0.02). The only significant difference was found between strawberries in the 15–30 g range and those weighing more than 30 g, where fruits weighing 15–30 g had a higher SSC (9.40 ± 0.17%) compared to those >30 g (8.75 ± 0.17%). However, in 3D-printed products, no statistically significant differences in SSC were observed based on the weight of the strawberries used for printing (p=0.98). The addition of hydrocolloids in 3D-printed fruit-based products likely helps to maintain a consistent SSC by stabilizing water distribution. This ensures uniformity in texture and composition without significantly altering the SSC in 3D-printed products.

Analysis of pH values in fresh strawberries of different weights revealed no statistically significant difference between fruits weighing 15–30 g and those weighing >30 g, both of which had higher pH values than fruits weighing <15 g. These findings indicate that larger strawberries (>15 g) generally have a higher pH value than smaller fruits (<15 g) (p=0.03). In contrast, no statistically significant differences in pH values were observed in the 3D-printed products, regardless of the mass of the strawberries used for printing (p≤0.01). The measured pH values align with the results reported by other authors (Simkova *et al.*, 2023), who found pH values ranging from 3.30 and 3.82 in fresh strawberries. In addition, the same authors reported an SSC value in fresh strawberries ranging from 7.89% in smaller fruits to 9.08% in larger ones. These values were slightly lower than those obtained in this study, but these differences could be attributed to the fact that SSC is a cultivar-dependent parameter (Patel *et al.*, 2023; Šamec *et al.*, 2016).

Bioactive properties and antioxidant activity of fresh strawberries and 3D-printed strawberry-based snacks

Monitoring the bioactive and antioxidant activity of 3D-printed fruit snacks is crucial for positioning them as a potential ingredient for functional foods, ensuring that they offer health benefits beyond basic nutrition by preserving essential phytochemicals and enhancing consumer appeal (Tavares *et al.*, 2025). Therefore, this study focuses on determining the bioactive and antioxidant activities of 3D-printed strawberry snacks made from fruits of different masses to assess how these differences affect the parameters studied. The average total polyphenolic content in fresh and 3D-printed samples was 126.33 ± 1.01 mg/ 100 g. Among the subgroups, condensed tannins (CTs) were the most abundant (86.75 ± 0.55 mg/100 g), followed by HCAs (52.74 ± 0.53 mg/100 g), FLs (31.34 ± 0.39 mg/ 100 g), and TFs, which showed the lowest value (6.24 ± 0.07 mg/100 g) (Table 3). Differences were observed between fresh and 3D-printed products. Fresh fruits contained more total polyphenols (146.54 ± 1.43 mg/100 g) than 3D-printed snacks (106.12 ± 1.43 mg/100 g) (p≤0.01). They also had higher flavonoid (6.82 ± 0.09 mg/100 g) and CT content (103.14 ± 0.78 mg/100 g). In 3D-printed products, these values decreased to 5.66 ± 0.09 mg/100 g and 70.36 ± 0.78 mg/100 g, respectively. On the other hand, HCAs and FLs were more abundant in 3D-printed samples than in fresh fruits (p≤0.01). The HCA content in 3D-printed products was 64.05 ± 0.74 mg/100 g, compared to 41.44 ± 0.74 mg/100 g in fresh fruits. The lower phenolic content in 3D-printed products compared to fresh fruit is primarily attributed to the addition of starch to the fruit mass, as well as potential oxidative and thermal

Table 2. Soluble solids content and pH in fresh strawberries and 3D-printed strawberry-based snacks.

Variables	N	Soluble solids content (SSC) (%)	pH
Fresh strawberry fruit		p = 0.02†	p = 0.03†
<15 g	4	9.09 ± 0.17^{a,b}	3.41 ± 0.01^{b}
15–30 g	4	9.40 ± 0.17^{a}	3.47 ± 0.01^{a}
>30 g	4	8.75 ± 0.17^{b}	3.45 ± 0.01^{a}
3D-printed strawberry snacks	n	p = 0.98‡	p ≤ 0.01†
<15 g	4	31.39 ± 0.68^{a}	3.49 ± 0.00^{a}
15–30 g	4	31.22 ± 0.68^{a}	3.57 ± 0.00^{a}
>30 g	4	31.41 ± 0.68^{a}	3.60 ± 0.00^{a}

*Mean values marked with different letters are statistically different from each other at p ≤ 0.05.

Table 3. The content of phenolic compounds in fresh strawberries and 3D-printed strawberry snacks.

Variables	N	Total phenolic content (TPC)	Hydroxycinnamic acids (HCAs)	Flavonols (FLs)	Total Flavonoids (TFs)	Condensed tannins (CTs)
Product type		$p \leq 0.01^{\dagger}$	$p \leq 0.01^{\dagger}$	$p \leq 0.01^{\dagger}$	$p \leq 0.01^{\dagger}$	$p \leq 0.01^{\dagger}$
Fresh fruit	6	146.54 ± 1.43^{a}	41.44 ± 0.74^{b}	24.09 ± 0.55^{b}	6.82 ± 0.09^{a}	103.14 ± 0.78^{a}
3D-printed snack	6	106.12 ± 1.43^{b}	64.05 ± 0.74^{a}	38.59 ± 0.55^{a}	5.66 ± 0.09^{b}	70.36 ± 0.78^{b}
Weight		$p \leq 0.01^{\dagger}$	$p = 0.12^{\ddagger}$	$p = 0.98^{\ddagger}$	$p = 0.07^{\ddagger}$	$p \leq 0.01^{\dagger}$
<15 g	4	134.02 ± 1.76^{a}	53.91 ± 0.91^{a}	31.39 ± 0.68^{a}	6.52 ± 0.12^{a}	92.97 ± 0.96^{a}
15–30 g	4	122.23 ± 1.76^{b}	50.78 ± 0.91^{a}	31.22 ± 0.68^{a}	6.14 ± 0.12^{a}	81.52 ± 0.96^{c}
>30 g	4	122.75 ± 1.76^{b}	53.54 ± 0.91^{a}	31.41 ± 0.68^{a}	6.07 ± 0.12^{a}	85.77 ± 0.96^{b}
Average	12	126.33 ± 1.01	52.74 ± 0.53	31.34 ± 0.39	6.24 ± 0.07	86.75 ± 0.55

Results are expressed as mean ± standard error. Values represented with different letters are statistically different at $p \leq 0.01$; †indicates a significant factor in multifactor analysis; ‡indicates a nonsignificant factor in multifactor analysis. TPC – Total phenolic content (mg GAE/100 g); HCA – Hydroxycinnamic acid (mg CAE/100 g); FL – Flavonol (mg QE/100 g); TF – Total flavonoid (mg QE/100 g); and CT – Condensed tannin (mg CE/100 g).

degradation, which could cause structural alterations during the printing process (Zhou *et al.*, 2023). Another research produced functional printed snacks from *Arbutus unedo* fruit, assessing rheological, antioxidant, and bioactive properties. The printed products contained high polyphenol levels (approximately 632.6 mg/100 g), including predominant CTs. Results showed significant effects of printing parameters on bioactive retention and antioxidant capacity (Bebek Markovinović *et al.*, 2024b).

However, when comparing the influence of strawberry fruit weight on the bioactive composition, it was found that different fruit weights affect the proportions of specific BACs. Fruits weighing <15 g had the highest total phenolic compounds (134.02 ± 1.76 mg/100 g), compared to fruits weighing 15–30 g (122.23 ± 1.76 mg/100 g) and >30 g (122.75 ± 1.76 mg/100 g), with no statistically significant difference between the latter two groups ($p \leq 0.01$). Among the phenolic compounds, a statistically significant difference was observed only in the proportions of CTs, which were the highest in fruits weighing <15 g (92.97 ± 0.96 mg/100 g), followed by those >30 g (85.77 ± 0.96 mg/100 g). In comparison, the lowest CTs content was found in fruits weighing 15–30 g (81.52 ± 0.96 mg/100 g) ($p \leq 0.01$). No statistically significant differences were observed in the proportions of other polyphenolic compounds, including HCAs, FLs, and TFs, between the different weight classes of strawberry fruits.

The results for ANT content and antioxidant activity in fresh and 3D-printed strawberry snacks, based on product type and fruit weight, are presented in Table 4. The ANT content was higher in fresh fruits (18.81 ± 0.21 mg/100 g) compared to 3D-printed products (13.89 ± 0.21 mg/100 g) ($p \leq 0.01$). Regarding fruit weight, the highest ANTs were observed in strawberries weighing <15 g (17.74 ± 0.26 mg/100 g), while no statistically significant difference was found between fruits weighing 15–30 g and >30 g ($p \leq 0.01$).

Antioxidant activity varied depending on the analytical method used. The DPPH method indicated slightly higher antioxidant activity in 3D-printed products (299.06 ± 0.21 µmol TE/ 100 g) compared to fresh fruits (295.73 ± 0.21 µmol/TE 100 g) ($p \leq 0.01$). In contrast, the FRAP method showed an opposite trend, with fresh strawberries exhibiting higher antioxidant activity (1030.80 ± 17.26 µmol TE/100 g) than the 3D-printed products (862.00 ± 17.26 µmol TE/100 g). When comparing antioxidant activity with strawberry fruit mass, no statistically significant effect was observed using the DPPH method. However, the FRAP method revealed higher antioxidant activity in fruits weighing <15 g and >30 g, with no statistically significant difference between these two groups, while strawberries weighing 15–30 g exhibited a lower antioxidant activity ($p \leq 0.01$).

The average ANT content in fresh and 3D-printed strawberry-based products was 16.35 ± 0.15 mg/100 g. The average antioxidant activity measured by the DPPH method was 297.39 ± 0.15 µmol TE/100 g, while the FRAP method yielded an average antioxidant activity of 946.38 ± 12.21 µmol TE/100 g. In a previous study (Bebek Markovinović *et al.*, 2024a), the effects of varying wheat starch concentrations (6%, 8%, and 10% w/w) and different 3D printing programs on the antioxidant properties of snacks made from strawberry and strawberry tree (*Arbutus unedo* L.) fruits were investigated. The results indicated that increasing starch content led to a decrease in the levels of most BACs. However, the antioxidant activity remained unaffected by changes

Table 4. The content of monomeric anthocyanins and antioxidant activity in fresh strawberries and 3D-printed strawberry snacks.

Variables	N	Monomeric anthocyanins (ANTs)	DPPH	FRAP
Product type		$p \leq 0.01^{\dagger}$	$p \leq 0.01^{\dagger}$	$p \leq 0.01^{\dagger}$
Fresh fruit	6	18.81 ± 0.21^{a}	295.73 ± 0.21^{b}	1030.80 ± 17.26^{a}
3D-printed snack	6	13.89 ± 0.21^{b}	299.06 ± 0.21^{a}	862.00 ± 17.26^{b}
Weight		$p \leq 0.01^{\dagger}$	$p = 0.22^{\ddagger}$	$p = 0.02^{\dagger}$
<15 g	4	17.74 ± 0.26^{a}	297.40 ± 0.26^{a}	966.93 ± 21.14^{a}
15–30 g	4	15.93 ± 0.26^{b}	297.76 ± 0.26^{a}	875.17 ± 21.14^{b}
>30 g	4	15.38 ± 0.26^{b}	297.02 ± 0.26^{a}	997.03 ± 21.14^{a}
Average	12	16.35 ± 0.15	297.39 ± 0.15	946.38 ± 12.21

Results are expressed as mean ± standard error. Values represented with different letters are statistically different at $p \leq 0.01$; †indicates a significant factor in multifactor analysis; ‡indicates a nonsignificant factor in multifactor analysis. ANT – Monomeric anthocyanin (mg Pg-3-Glc/100 g); DPPH assay (μmol TE/100 g); and FRAP assay (mmol TE/100 g).

in starch concentration or printing parameters. This suggests that while starch dilution reduces certain bioactive components, the overall antioxidant activity is preserved, possibly because of the stability of specific antioxidant compounds during processing. The incorporation of fruit by-products into 3D-printed snacks has also been studied for its impact on antioxidant properties. For instance, OPW, which is rich in vitamins and antioxidants, was successfully transformed into edible snacks using 3D printing technology. The formulated ink, derived from OPW, maintained its antioxidant activity throughout the printing process, highlighting the potential of upcycling fruit waste into functional foods with health benefits (Tan *et al.*, 2023). In addition to the results obtained in this study, other research has also demonstrated that 3D printing technology can effectively produce fruit-based snacks with substantial antioxidant activity.

The effect of strawberry fruit weight on the bioactive properties of fresh strawberries and 3D-printed strawberry-based snacks

Based on the results presented in Table 5, variations in the bioactive properties of fresh fruits and 3D-printed products made from strawberries were observed, depending on the different fruit masses. In fresh strawberries, the TPC varied according to fruit weight. Fruits weighing more than 30 g (154.71 ± 2.62 mg/100 g) had a higher TPC compared to those weighing less than 15 g (147.13 ± 2.62 mg/100 g). Interestingly, TPC in fruits weighing 15–30 g did not differ statistically from that of fruits weighing either less than 15 g or more than 30 g (p=0.05). When examining specific subgroups of phenolic compounds, it was found that the proportions of HCAs, FLs, and CTs were not significantly affected by fruit mass. The only exception was TFs, which were present in higher amounts in fruits weighing less than 15 g (106.4 ± 1.36 mg/100 g) and more than 30 g (105.29 ± 1.36 mg/100 g), whereas fruits weighing 15–30 g had a lower TF content (97.74 ± 1.36 mg/100 g) (p=0.54).

Based on the results presented in Table 6, it is evident that the mass of strawberry fruits influences the proportions of ANTs in both fresh and 3D-printed strawberry-based products. In fresh fruits, the highest proportion of anthocyanins was found in the smallest fruits (<15 g), while no statistically significant differences were observed between fruits in the 15–30 g and >30 g categories (p=0.26). A similar trend was noted in 3D-printed products, where the highest ANT content was found in products made from strawberries weighing <15 g (15.96 ± 0.30 mg/100 g). In contrast, anthocyanin levels did not differ significantly between 3D-printed products made from strawberries in the 15–30 g (13.42 ± 0.30 mg/100 g) and >30 g (12.28 ± 0.30 mg/100 g) categories. A study on 3D-printed functional strawberry snacks explored the effects of food design and processing on the stability of antioxidant BACs, including anthocyanins. The research found that the 3D printing process can influence the retention of anthocyanins, with variations depending on the printing parameters and formulation (Huang *et al.*, 2024). However, the anthocyanin content in the 3D-printed snacks was approximately 26% lower than in fresh fruit. These results indicate that anthocyanin content decreases during processing, which aligns with findings in the literature that anthocyanins are volatile compounds prone to degradation because of temperature, light, pH, oxygen, and other environmental factors (Saini *et al.*, 2024). The significant loss of anthocyanins in food is primarily attributed to degradation reactions that occur during heat processing (Chen *et al.*, 2022).

Table 5. Influence of fruit mass on the polyphenolic compound content in fresh strawberries and 3D-printed strawberry snacks.

Variables	Mass	N	Total phenolic content (TPC)	Hydroxycinnamic acids (HCAs)	Flavonols (FLs)	Total Flavonoids (TFs)	Condensed tannins (CTs)
		p = 0.05†	p = 0.08‡	p = 0.66‡	p = 0.54‡	p = 0.03†	
Fresh fruit	<15g	2	147.13 ± 2.62[b]	41.89 ± 1.29[a]	23.91 ± 0.96[a]	106.40 ± 1.36[a]	6.93 ± 0.16[a]
	15–30g	2	137.79 ± 2.62[a.b]	37.52 ± 1.29[a]	23.67 ± 0.96[a]	97.74 ± 1.36[b]	6.60 ± 0.16[a]
	>30g	2	154.71 ± 2.62[a]	44.90 ± 1.29[a]	24.68 ± 0.96[a]	105.29 ± 1.36[a]	6.95 ± 0.16[a]
			p ≤ 0.01†	p = 0.19‡	p = 0.66‡	p = 0.54‡	p = 0.01†
3D-printed snack	<15g	2	120.90 ± 2.34[a]	65.93 ± 1.29[a]	38.87 ± 0.96[a]	79.54 ± 1.36[a]	6.11 ± 0.16[a]
	15–30g	2	106.67 ± 2.34[b]	64.03 ± 1.29[a]	38.77 ± 0.96[a]	65.29 ± 1.36[b]	5.68 ± 0.16[a]
	>30g	2	90.80 ± 2.34[c]	62.18 ± 1.29[a]	38.14 ± 0.96[a]	66.25 ± 1.36[b]	5.19 ± 0.16[a]
Average		12	126.33 ± 1.01	52.74 ± 0.53	31.34 ± 0.39	6.24 ± 0.07	86.75 ± 0.55

Results are expressed as mean ± standard error. Values represented with different letters are statistically different at p ≤ 0.01; †indicates a significant factor in multifactor analysis; ‡indicates a nonsignificant factor in multifactor analysis. TPC – Total phenolic content (mg GAE/100 g); HCA – Hydroxycinnamic acid (mg CAE/100 g); FL – Flavonol (mg QE/100 g); TF – Total flavonoid (mg QE/100 g); and CT – Condensed tannin (mg /100 g).

Table 6. Influence of fruit mass on monomeric anthocyanins and antioxidant activity in fresh strawberries and 3D-printed strawberry snacks.

Variables	Mass		Monomeric Anthocyanins (ANTs)	DPPH	FRAP
Fresh fruit		N	p = 0.26‡	p = 0.26‡	p = 0.08‡
	<15g	2	19.51 ± 0.41[a]	295.77 ± 0.37[a]	1004.00 ± 29.90[a]
	15–30g	2	18.43 ± 0.41[b]	296.40 ± 0.37[a]	963.53 ± 29.90[a]
	>30g	2	18.48 ± 0.41[b]	295.01 ± 0.37[a]	1124.70 ± 29.90[a]
			p ≤ 0.01†	p = 0.26‡	p = 0.08‡
3D-printed snack	<15g	2	15.96 ± 0.30[a]	299.02 ± 0.37[a]	929.84 ± 29.90[a]
	15–30g	2	13.42 ± 0.30[b]	299.12 ± 0.37[a]	786.81 ± 29.90[a]
	>30g	2	12.28 ± 0.30[b]	299.02 ± 0.37[a]	869.34 ± 29.90[a]
Average		12	16.35 ± 0.15	297.39 ± 0.15	946.38 ± 12.21

Results are expressed as mean ± standard error. Values represented with different letters are statistically different at p ≤ 0.01; †indicates a significant factor in multifactor analysis; ‡indicates a nonsignificant factor in multifactor analysis. ANT – Monomeric anthocyanin (mg Pg-3-Glc/100 g); DPPH assay (µmol TE/100 g); and FRAP assay (mmol TE/100 g).

A possible explanation for the difference in ANT content between fresh and 3D-printed samples is the sensitivity of anthocyanins to elevated temperatures. These temperatures are applied during the heating process required to achieve a homogeneous mixture for 3D printing. This assumption aligns with previous studies, which have reported anthocyanin reductions ranging from 5% to 44% in pasteurized strawberries, strawberry purées, and pulp (Salazar-Orbea *et al.*, 2021). Similarly, other authors (Salazar-Orbea *et al.*, 2023) observed reductions in anthocyanins approximately 5% to 44% in pasteurized strawberry, strawberry purees, and pulp. Although anthocyanins are susceptible to degradation under thermal conditions, their presence still highlights the promising potential of 3D printing technology in the development of functional foods. Through careful selection of fruit size and optimization of processing parameters, the bioactive properties of 3D-printed fruit snacks can be effectively preserved and even enhanced.

In addition, no statistically significant differences in antioxidant activities, as determined by the DPPH and FRAP methods, were observed in either fresh fruits (p=0.26) or 3D-printed products (p=0.08), regardless of fruit weight.

Based on all the obtained results, it can be observed that smaller fruits (<15 g) exhibited higher levels of several groups of BACs, including total phenolic compounds, anthocyanins, and flavonoids, compared to larger fruits. Consequently, 3D-printed snacks made from smaller strawberries retained a higher concentration of phenolic compounds and anthocyanins than those produced

from larger fruits. This suggests that smaller strawberries may be the most suitable for 3D printing in the context of functional food production.

Antioxidant activity values differ between DPPH and FRAP assays because they are based on distinct chemical principles—radical scavenging versus redox potential. Each method selectively detects different antioxidant compounds, leading to method-dependent variations in measured antioxidant capacity. Therefore, multiple assays are essential to comprehensively evaluate antioxidant potential in complex food matrices like fruit-based snacks.

Correlation effect outcomes

To further understand the interdependence between fruit acidity and anthocyanin stability, a correlation analysis was performed between the measured pH values and the ANT content across all strawberry samples, including both fresh and 3D-printed products. The results revealed a strong inverse correlation between pH and anthocyanin levels. In fresh strawberries, a negative trend was observed ($r = -0.87$; $p = 0.02$), though it did not reach statistical significance because of limited sample size. In contrast, 3D-printed products showed a statistically significant negative correlation ($r = -0.99$; $p = 0.02$), indicating that slight increases in pH during processing can markedly reduce anthocyanin retention. When both datasets were combined ($n = 6$), the correlation remained highly significant ($r = -0.99$; $p \leq 0.01$), suggesting that higher pH is consistently associated with lower anthocyanin content, regardless of processing state. These findings reinforce the notion that acidic environments favor anthocyanin stability, while elevated pH levels—potentially exacerbated by starch addition or heat exposure during 3D printing—promote anthocyanin degradation. Consequently, pH should be considered a critical control point when optimizing formulations for 3D-printed functional foods to maximize anthocyanin retention.

Conclusions

This study demonstrated that smaller strawberries contain higher levels of phenolics and anthocyanins. During 3D printing, anthocyanins were reduced by about 26%, while pH was identified as a key factor directly affecting their stability. The main advantage of 3D-printed functional foods lies in their optimization through the careful selection of fruit size and control of pH. These factors can maximize the retention of BACs and improve the nutritional value of printed snacks, supporting their potential for personalized nutrition and functional food development.

Disclosure

A.M. Khaneghah serves as Editor-in-Chief of QASCF; this role did not influence the peer-review or editorial decision of this manuscript.

Data Availability Statement

The original contributions presented in this study are included in the article. Further inquiries can be directed to the corresponding author.

Author Contributions

Conceptualization was done by B.D. and D.B.K.; methodology was the concern of B.D. and D.B.K.; software was looked into by P.P., D.P.H. and B.P.; validation was done by P.P., B.P., A.B.M., I.Š., I.M.B., L.M.B., and D.P.H.; formal analysis was the responsibility of A.B.M., I.Š., I.M.B., L.M.B., and D.P.H.; investigation was taken care of by A.B.M., I.Š., and D.P.H.; resources were managed by A.M.K., B.D. and D.B.K.; data curation was done by P.P.; writing—original draft preparation was looked into by B.D., D.B.K., and P.P.; writing—review and editing was done by A.B.M., I.Š., D.P.H., I.M.B., L.M.B., A.M.K. and B.P.; visualization was the purview of B.P. and P.P.; supervision was done by B.D. and D.B.K.; project administration was taken care of by D.B.K.; funding acquisition was done by D.B.K. All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflicts of interest.

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