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Functionality of Muffins Fortified with Apple Pomace: Nutritional, Textural, and Sensory Aspects

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Abstract: Apple pomace resulting from juice processing and thermal processing (50 °C/12 h and 80 °C/5 h) were optimized in order to obtain apple pomace powders with an organoleptic profile suitable as ingredients in food products. Proximate composition (moisture, titratable acidity, ash, soluble substances, and fat content) was analyzed using ISO standard methods. The Folin–Ciocâlteu method, a DPPH (2,2-Diphenyl-1-picrylhydrazyl) test, and flavonoid determination were used to assess antioxidant potential and bioactive compound contents. Antimicrobial potential and cytotoxic and anti-proliferative potential were also assessed. Aroma was characterized by a volatile compound analysis performed using ITEX/GC-MS for the by-products, the powder, and some muffins developed with the proposed functional ingredient. For the sensory analysis of muffins, the acceptability was evaluated using the hedonic test and texture analysis was done using the CT3 Texture Analyzer. The cytotoxic potential of the methanol extracts of the by-products ranged between 70.42 and 78.80%. The hedonic test revealed that the evaluators highly appreciated the aroma, which was attributed to the volatile compounds responsible for the pleasant aroma. The addition of 20% apple pomace powder led to similar texture and sensory scores to the reference samples.

Keywords: apple pomace; muffins; volatile compounds; antioxidants; flavonoid; nutritional

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1. Introduction

Recent trends in global food markets have shown that there are more and more consumers who demand value-added foods—functional foods. Apple (*Malus domestica* Borkh) is an ancient fruit, widely cultivated in temperate regions, with continuous increases in production rates [1].

Despite the fruit's widespread availability, its consumption share remains relatively consistent, while the products which are produced from it show less innovation (including juice, wine, jams, and dried products). A total of 70–75% of apples are consumed fresh, while the rest of the proportion is processed, resulting in significant waste (seeds and peels) [2].

Residual agricultural products and food processing by-products are often considered a problem, but in the case of apples the peel is further valorized in pectin production. However, the conversion of such materials into more valuable resources would represent a good contribution to the valorization of apple pomace [3].

In the fruit juice industry, apple pomace can account for up to 60% of the fruit mass and represents a challenge for the industry in determining its value [4]. Apple pomace is a valuable source of phytochemicals, primarily composed of epidermis and pulp, with a minor amount of seeds and stems [5–7].

The amount of both saturated and unsaturated free fatty acids and minerals (especially Ca, K, Mg, Na, and P) in fruit apple pomace has been shown in research studies. These compounds have the potential to be significant components of dietary food supplements [2].

Consumers are demanding changes in the food market, demonstrating willingness to pay higher prices for good-quality foods, with benefits for their health. There is a hypothesis suggesting that consuming foods with higher levels of dietary antioxidants might decrease oxidative stress and help avoid chronic diseases [8]. Oxidative stress can result in cancer and cardiovascular diseases apparition, which are the primary causes of early death in Europe and the USA [9].

Antioxidant compounds are a large group of metabolites possessing, besides antioxidant capacity, antimicrobial activity, and they also contribute to the color, astringency, and bitterness of fruits. Apples are abundant in phenolic compounds, with higher concentrations in the peel compared to the flesh. The composition differs between the flesh and the peel. The flesh includes catechins, procyanidins, phloridzin, phloretin glycosides, caffeic acid, and chlorogenic acid. In addition to these compounds, the peel also contains flavonoids such as quercetin and cyanidin glycosides [10,11]. In other fruits and vegetables, it has also been reported that the peels have higher phytochemical concentrations or higher antioxidant potential [12,13].

Food products obtained from fruits and vegetables contain a lot of soluble fibers, especially pectins, which lower the level of glucose in the blood and influence lipid metabolism [14]. The general state of health is enhanced by dietary fibers, which have a positive impact on particular physiological functions in the human body. The consumption of high-fiber products increased as a result of the health benefits, which motivated food scientists to explore novel fiber sources as food constituents [14]. The viscosity, texture, sensory characteristics, and shelf-life of products can be improved by fiber's properties. Fiber-rich by-products can be added to foods to increase emulsion or oxidative stabilities, enhance water and oil retention, and serve as inexpensive and non-caloric bulking agents [15].

Cookies are widely eaten by consumers of all age groups around the world due to their nutritional value, affordability and extended shelf-life. Because of these mentioned features, cookies may represent an appropriate matrix for incorporating various bioactive compounds [16].

Currently, some research investigations are centered on using apple pomace in cakes and other sweet bakery products to improve their flavors and nutritional compositions [6,17]. A sensory analysis conducted on cakes with pomace showed high scores and overall acceptability for all samples except for the sample containing 30% pomace [6]. Due to its pleasant fruity aroma, apple pomace has the potential to be used as a flavoring agent in cakes. However, additional research is required to determine its viability for commercial-scale implementation.

In this context, apple pomace powders were developed at different time–temperature protocols and were further incorporated in muffins. The objectives of this study were as follows: (i) to evaluate the functional properties of the apple by-products and powders developed from it/flour, (ii) to examine the antimicrobial potential and the cytotoxic and anti-proliferative potential of the apple pomace and its volatile compounds, and (iii) to assess the consumer acceptability and textural analysis of the muffins.

2. Materials and Methods

2.1. Materials

All reagents were of analytical grade. Analytical reagents and chemicals were purchased from Sigma Aldrich (St. Louis, MO, USA). Bacterial reference strains were pro-

cured from Oxoid Ltd. (Hampshire, UK) and culture mediums, such as Muller Hinton Agar, from Merck (Darmstadt, Germany), while the substances for the culture medium were procured from Gibco Life Technologies, Paisley, UK. The DLD-1 line (Colorectal Adenocarcinoma) was from the European Cell Culture Collection. The MTT compound [3-(4,5-dimethylthiazolyl)-2,5-diphenyl-tetrazolium bromide] was purchased from Sigma Aldrich (St. Louis, MO, USA). The raw materials for the muffin production were bought from a local market, from specialized stores in Romania.

2.2. The Production of Apple Pomace Powder

By-products resulting from apple juice production from a local factory were procured and frozen at −18 °C. To produce the powder, the frozen by-products were dried in the oven at 80 °C for 5 h or at 50 °C for 12 h (Memmert UF55, Germany). After that, the by-product was grinded with a mortar and pestle, resulting in a fine powder, with no agglomerations (Figure 1).

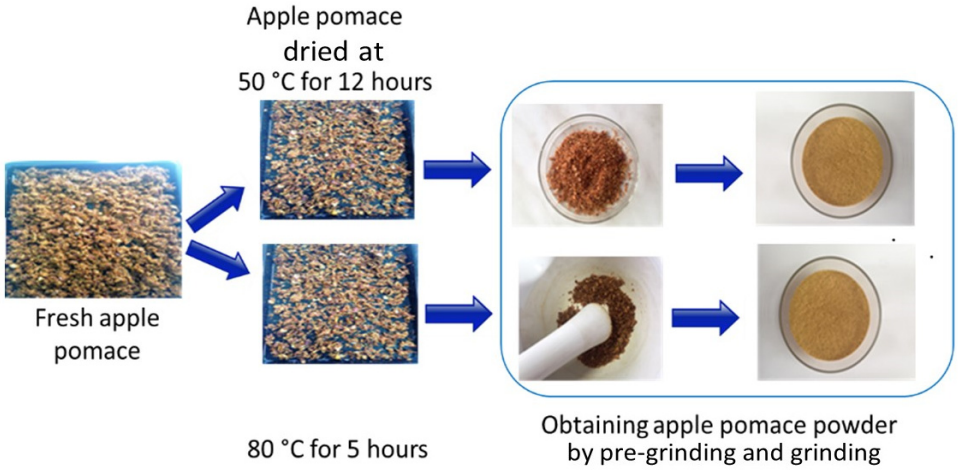


Figure 1. The process of obtaining apple pomace powders.

2.3. Optimization of the Muffin Formulations with Apple Pomace Powder

The powder obtained by grinding the apple pomace dried at 80 °C for 5 h was selected in order to obtain the muffins. The wheat flour from the composition of the muffins was substituted with 10%, 20%, 30%, or 40% apple pomace powder and the resulting prototypes are illustrated in Figure 2. Further, the muffins were subjected to a sensory and textural analysis in order to be characterized.

2.4. Physicochemical Characterization of the Apple Pomace Powders

The water content was determined by drying in an oven at 103 ± 2°C for 3 h, the determination being repeated until the weight was constant. The weight difference between the samples before and after the drying procedure represents the calculated moisture content. The total content of ash was assessed by calcination at 550–600 °C. The titrable acidity was assessed by neutralization with sodium hydroxide solution (0.1 N) in the presence of methylene blue as an indicator. The total soluble substance [°Brix] determination was conducted with a refractometer following ISO2173:2003. The fat content was determined in a Soxhlet apparatus.

2.5. Determination of Total Polyphenol Content by Folin–Ciocâlteu Method

The total polyphenol content was assessed using the Folin–Ciocâlteu method, slightly modified. The 25 µL sample was mixed with 1.8 mL of distilled water and 120 µL of Folin–Ciocâlteu reagent. Five minutes later, a Na_2CO_3 7.5% solution (340 µL) was added to ensure that the redox reaction between the phenolic compounds and the Folin reagent occurred under basic conditions (pH 10). The samples were incubated for 90 min at room temperature. The spectrophotometer Shimadzu UV-VIS 1700 was used to measure the

absorbance at 750 nm. Methanol was used as the control sample. The total polyphenol content was quantified based on the fresh weight (FW) in Gallic acid equivalents (GAE)—mg GAE 100 g $^{-1}$ [18].

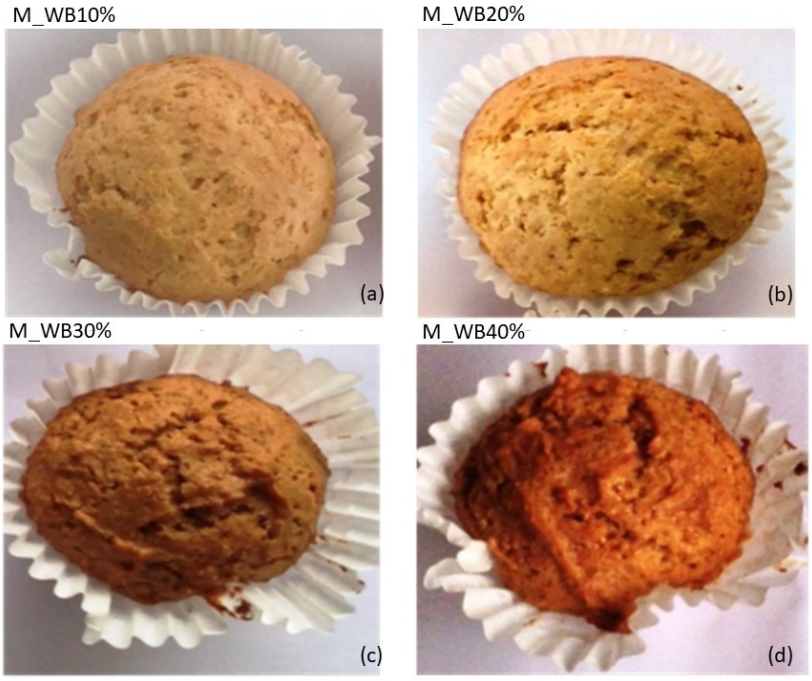


Figure 2. Prototypes of muffins with different concentrations of apple pomace powder substituting the wheat flour: (**a**) with 10% replacement of wheat flour, (**b**) with 20% replacement of wheat flour, (**c**) with 30% replacement of wheat flour, and (**d**) with 40% replacement of wheat flour.

2.6. Determination of the Total Flavonoid Content

The apple pomace extracts were evaluated for their total flavonoid level using the colorimeter technique, as reported by Vlaic et al. [18]. The alcoholic extracts were diluted with distilled water to a final volume of 5 mL, by adding 300 µL of a 5% $NaNO_2$ solution. After 5 min, the mixture was subjected to the addition of 300 µL of a 10% solution of $AlCl_3$ and after another 6 min, with 2 mL of a NaOH 1N solution. The UV-VIS 1700 Shimadzu spectrophotometer was used to measure the absorbance at 500 nm. The total flavonoid content was quantified in mg quercetin equivalent per 100 g of fresh sample.

2.7. DPPH-Scavenging Activity

The antioxidant capacity was measured by assessing the Free Radical Scavenging effect over 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) [19]. The antioxidant potential (%) was calculated based on the ratio of the difference between the absorbance of the sample and the blank, and the absorbance of the blank:

$$\text{DPPH scavenging effect (\%)} = [(A_0 - As) \times 100]/A_0$$

where A_0 is absorbance of the blank and As is absorbance of the samples.

2.8. The Antimicrobial Potential and the Microbial Contamination of the Samples

The in vitro antimicrobial potential of the apple juice by-products was evaluated using the following reference strains: *Staphylococcus aureus* ATCC® 6538P, *Bacillus cereus* ATCC® 14579, *Escherichia coli* ATCC® 25922, *Salmonella enteritidis* ATCC® 13076, and *Salmonella typhimurium* ATCC® 14028.

The bacterial strains were prepared as an inoculum. For preparing the inoculum, a suspension of 4–6 identical bacterial colonies and sterile saline solution was prepared

as a representative inoculum. The size of the inoculum was standardized on a nephelometric system, through the McFarland scale. For the antibacterial screening tests carried out on the vegetal by-products, different bibliographical sources [20,21] indicate different potential inoculum sizes. In the present study, the bacterial inoculum was Inocul (1)—number 0.5 on scale McFarland (for which the turbidity corresponds to a concentration of 1.5×10^8 CFU/mL); the value 0.5 is indicated for in vitro testing of the susceptibility towards synthesized antimicrobial agents. The bacterial inoculum was uniformly distributed on Muller Hinton Agar. The plaques were kept in the proximity of a gas bulb for 10–15 min in order to be aired, and then the tested products were spread on the marked plaques, each probe in duplicate. The results were read after 24 h of incubation at 37 °C and the size of the inhibited induced zones was assessed, zones where there is no presence of microbial colonies. The results were expressed as means of the values of the diameters of the two duplicated samples.

2.9. The Cytotoxic and Anti-Proliferative Potential of the Methanolic Extracts of the by-Products

For testing the selected extracts, DLD-1 cellular lines were used. The DLD-1 line (Colorectal Adenocarcinoma) was used at passage 31. The cells were grown in RPMI 1640 (Sigma-Aldrich) medium, which was supplemented with 10% fetal serum (Sigma-Aldrich) and 1% Antibiotics Antimycotics (Gibco).

The tests were conducted on 96-well plates, the cell concentration being 1×10^5. After 24 h of incubation, the cell cultures were treated with the selected products and after 24 h of exposure the MTT test was conducted in order to evaluate the viability. The MTT test is based on detecting the reduction of the MTT compound [3-(4,5-dimethylthiazolyl)-2,5-diphenyl-tetrazolium bromide] by the mitochondrial dehydrogenase and the formation of formazan, a blue-colored compound. The process reflects the normal functioning of the mitochondrial cells and also the viability of the cells. After the removal of the growth medium, the cells were treated with 100 µL solution of MTT (0.5 mg MTT/mL/tampon/HBSS) and incubated for 3 h at 37 °C. The following steps were the removal of MTT and the distribution of 100 µL dimetilsulfoxid (DMSO) (Sigma Aldrich (St. Louis, MO, USA)) in each well plate for the solubilization of the formazan particles. Then, the optical densities (ODa) of the compounds were measured at 450 nm using a spectrophotometer. The results of the MTT test (the optical densities for each sample and the correspondent diluent) were expressed as mean value ± the standard deviation and further expressed as viability percent, resulting from the division of the optical density of the sample by the optical density of the reference (the cells grown in the same standard conditions but without treatments).

$$\% \text{ viability} = (\text{mean OD of the sample}/\text{mean OD of reference}) \times 100$$

The intensity of the cytotoxic potential was parsed by statistical comparison of the results of each sample to 100% (which is the viability of the reference).

2.10. Extraction and Analysis of Volatile Compounds Using ITEX/GC-MS Technique

For the extraction of volatile compounds, a sealed headspace vial containing 1 mL of sample was incubated at 60 °C for 20 min using a CombiPAL AOC-5000 autosampler (CTC Analytics, Zwingen, Switzerland). Using a headspace syringe equipped with a Tenax trap (ITEX-2TRAPTXTA, (G23)-Siliconert 2000, Tenax ta 80/100 mesh, CTC Analytics, Zwingen, Switzerland), the volatiles from the headspace were repeatedly adsorbed (30 strokes) onto the trap and then directly desorbed into the GC-MS injector. The separation of the volatile compounds was performed on a Shimadzu GC-MS QP-2010 (Shimadzu Scientific Instruments, Kyoto, Japan) model gas chromatograph–mass spectrometer equipped with a CombiPAL AOC-5000 autosampler (CTC Analytics, Zwingen, Switzerland) and with a ZB-5 ms capillary column of 30 m × 0.25 mm i.d. and 0.25 µm film thickness (Phenomenex, Torrance, CA, USA). The program for the column oven temperature was as follows: 40 °C (maintained for 5 min) raised to 180 °C with 5 °C/min and then to 240 °C with 15 °C/min (hold for 5 min). The carrier gas was helium, at a constant flow of 1 mL/min;

the injector, ion source, and interface temperatures were set at 250 °C. The MS detector was used in electron impact ionization (EI) mode in a scan range of 40–650 m/z. The split ratio was 1:2. The separated volatile compounds were tentatively identified by comparing the obtained mass spectra with those from NIST27 and NIST147 mass spectra libraries and verified by comparison with retention indices drawn from www.pherobase.com and www.flavornet.org (for columns with a similar stationary phase to ZB-5 ms). The results are expressed as percentage of total peak area (100%).

2.11. Characterization of the Muffins Containing Apple Pomace Powder

2.11.1. Textural Analysis

The textural profile of the muffins was obtained by using a CT3 Texture Analyzer (Brookfield Engineering Labs, Middleboro, MA, USA) according to the method described by Chis, 2020, with modifications [22]. The muffins were subjected to a double compression test under the following conditions: 50% target deformation, 1 mm s^{-1} test and post-test speed, 5 g trigger load, and 5 s recovery time. Hardness, total work, resilience, springiness, cohesiveness, and gumminess were used as indicators for the texture profile analysis. Each muffin sample was cut to obtain samples of 25 mm × 25 mm × 25 mm (l × w × h). In order to avoid drying, the samples were tested immediately after preparation. The specific texture parameters were analyzed using Texture Pro CT V1.6 software (Brookfield Engineering Labs, Middleboro, MA, USA).

2.11.2. Sensory Evaluation of Different Muffin Prototypes

The sensory analysis was performed using 55 evaluators, students from the Faculty of Food Science and Technology, aged between 18 and 55 years old. The evaluators were selected based on their availability, and their interest regarding consumption of an assortment of new muffins. Four samples of freshly made (within 6 h) muffins were evaluated in each session under normal daylight conditions. All samples were anonymously coded, and two replications of each sample were presented to panelists. They were asked to evaluate the color, taste, aroma, texture, and overall acceptability using a 9-point hedonic scale ranging from 9 (like extremely) to 1 (dislike extremely), as described by Chis et al. [22]. Water was also provided for rinsing the palate between samples.

2.12. Statistical Analysis

The analysis of variance was performed using Minitab 16.1.0 and Tukey comparison tests were used at a significance level $\alpha = 0.05$. Classical and relative eta-squared was used to compare the effects of different factors in the same design.

3. Results and Discussion

3.1. Physicochemical Characterization of the Apple Pomace and the Obtained Powders

The apple juice by-products were used in the analysis frozen, without thawing or any special refrigeration treatment. The by-products contained 21.18% dry matter and a considerable content of minerals (2.4%), low acidity (0.95%), and a total fat content of 2.07%, as can be seen in Table 1.

Table 1. Changes in the physicochemical properties of the by-products as a result of thermal treatments.

Apple By-Product	Dry Matter [%]	Acidity [%]	Minerals [%]	Fat [%]
Frozen	21.18 ± 0.53	0.95 ± 0.07	2.40 ± 0.51	2.07 ± 0.13
F	92.21 ± 0.57	3.76 ± 0.33	4.10 ± 0.81	3.62 ± 0.01
Dried 80 °C/5 h	91.90 ± 0.42	2.15 ± 0.05	3.88 ± 0.10	3.52 ± 0.06

The biologically active compound quantification revealed a total of 578.1 mg/100 g of flavonoids and 37.59 mg/100 g of phenolic compounds, the antioxidant potential being

75.07%. Due to the fact that the water-soluble carbohydrates are extracted during the juice pressing, the by-products are not obtained in soluble substances, presenting a concentration of 0.5 °Bx.

The influence of the processing conditions on the biologically active compounds present in the by-products, specifically heat treatments at two different time–temperature protocols, followed by grinding, was also analyzed.

Regarding the thermal treatment involved in their processing, the dry matter of the by-products increased from 21.18% (the value corresponding to the frozen sample) up to ~92% for the dried samples.

The acidity of the apple pomace powders increased in comparison to that of the frozen sample as follows: 2.15% for the sample subjected to 50 °C for 12 h and 3.76% for the sample subjected to 80 °C for 5 h, it being concluded that the drying of the samples at higher temperatures could induce fermentative alterations (Table 1). The ash content determined in the frozen sample was similar with what has been reported (0.5–6.10%) in other studies [23].

When the juice is pressed out from the fruits, the majority of the water-soluble carbohydrates are also extracted and the by-products remain with low amounts of soluble substances, 0.5 °Bx in the frozen sample and 2 °Bx for the dried samples, due to an increase in the concentration as a result of water evaporation. Other authors report that apple pomace contains both soluble and insoluble fibers, the first in lower amounts due to the previously mentioned reasons [23]. During drying, the concentration also increases the DPPH antioxidant potential from 75.93% corresponding to the frozen sample to 92.29% corresponding to the sample dried at 80 °C for 5 h, highlighting that shorter thermal treatments, even at higher temperatures, could enhance the activity of some biologically active compounds. The powder obtained after drying the sample at 50 °C for 5 h contained 54.78 ± 1.92 mg GAE/100 g of total polyphenols and a total flavonoid content of 1837.64 ± 2.32 mg QE/100 g (Table 2), which is more than what was determined for the other apple pomace powder, where extractions were assisted by ultrasound [24]. Among the main polyphenols determined in apple pomace (air-dried at 70 °C) resulting after the extraction of juice from mixed red apple cultivars and the Antonówka cultivar Flavan-3-ols, quercetin glycosides and phloridzin were determined, accounting for a total polyphenol content of 516 ± 32 mg/g [25].

Table 2. Changes in the biological active compounds of the by-product as a result of thermal treatments.

Apple By-Product	Soluble Substances [°Brix]	DPPH Antioxidant Activity [%]	Total Flavonoid Content [mg QE/100 g]	Total Polyphenolic Content [mg GAE/100 g]
Frozen	0.50 ± 0.00	75.07 ± 0.20	578.1 ± 2.30	37.59 ± 0.64
Dried 50 °C/12 h	2.00 ± 0.00	89.59 ± 0.82	1837.64 ± 2.32	54.78 ± 1.92
Dried 80 °C/5 h	2.00 ± 0.00	92.29 ± 0.27	1661.25 ± 1.99	51.61 ± 2.56

Due to the fact that the products displayed high radical scavenging activity of the 1-dipheny l-2-picrylhydrazyl (DPPH) compound, it was decided to test the effect of the by-products on the DLD-1 (Colorectal Adenocarcinoma) cellular line, as seen in Figure 3. The cytotoxic potential of the methanolic extracts of the by-products was evaluated by statistical comparison of the viability obtained for each product reported to 100% (% the viability of the reference, the methanol). The methanolic extracts of the apple pomace samples registered values ranging between 70.42 and 78.80%, it being concluded that the sample subjected for the longest period to thermal treatment had the lowest viability, displaying the highest cytotoxic effect, in comparison to the rest of the samples.

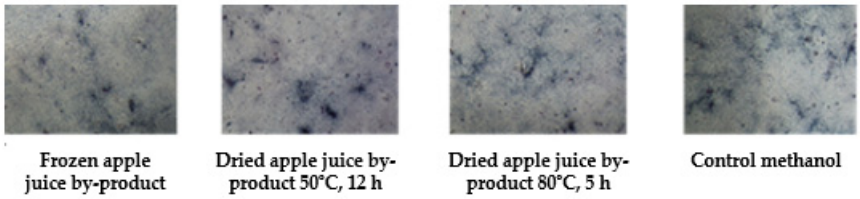


Figure 3. Microscopic analysis of the cellular viability of the line DLD-1 (Colorectal Adenocarcinoma) after treatments with the methanolic extract of apple juice by-products.

The behavior of the tested samples towards the bacterial strains was relatively similar, the antimicrobial effect depending on the type of extract. The samples do not present antimicrobial behavior towards Gram-negative bacteria (*Salmonella enteritidis* ATCC® 13076, *Salmonella typhimurium* ATCC® 14028), but they exhibit antimicrobial effect against Gram-positive bacteria (Table 3).

Table 3. The viability of DLD-1 (Colorectal Adenocarcinoma) cell line after treatments with methanolic extracts of the apple juice by-product samples.

Sample Parameter	Control Methanol	Frozen Apple By-Product	Dried Apple By-Product (50 °C/12 h)	Dried Apple By-Product (80 °C/5 h)
DO repetition 1	0.394	0.301	0.246	0.302
DO repetition 2	0.370	0.290	0.292	0.300
Mean of the DO	0.382	0.296	0.269	0.301
% Viability	100%	77.36	70.42	78.80

The frozen by-product and the dried by-product (80 °C/5 h and 50 °C/12 h) were inoculated on three types of media (agar with glucose, agar MacConkey, and agar Sabouraud with chloramphenicol) and they were incubated at 37 °C for 24 h. There were no characteristic colonies for microbial development (bacteria or yeasts), and no NTG was established. However, the pomace did have an inhibition effect over the *Staphylococcus aureus* (frozen sample or pomace dried at 50 °C/12 h), or over the *Bacillus cereus*, as can be seen in Table 4. The antimicrobial potential of apple pomace is attributed to the ursolic acid from its composition [23,26]. The acidity of the samples can also be strongly correlated with the antimicrobial potential, the sample dried at 50 °C/12 h presenting the highest content of organic acids.

Table 4. Inhibition zone diameter (mm) measured in comparison to the reference strains.

Code	Sample	*Salmonella enteritidis*	*Salmonella typhimurium*	*Staphylococcus aureus*	*Bacillus cereus*
3	Frozen by-product	-	-	9.5 ± 0.41	-
4	Dried by-product 80 °C/5 h	-	-	-	10.5 ± 0.41
1	Dried 50 °C/12 h	9 ± 0	-	10 ± 0.82	9.5 ± 0.41

3.2. Volatile Compounds Determined from Apple Pomace before and after Its Processing into Powder

Based on a comparison with custom libraries derived from NIST, a total of 29 compounds were putatively identified in the aroma profile in the raw by-product resulting from the processing of apple fruits, and a total of 25 compounds were identified in the powder of the dried apple pomace subjected to the thermal treatment at 80 °C. Their concentrations and names are presented in Table 5.

Table 5. The volatile fingerprints of raw apple pomace and powder.

Volatile Compounds	Odor Perception	Raw By-Product	Apple Pomace Powder
Alcohols and aldehydes			
2-metil-1-butanol	Wine, Onion	6.48	N.D.
1-pentanol	Balsamine, Oil, Sweet, Chemical Mint	0.64	0.13
1-Hexanol	Ethereal, Oil, Alcohol, Green, Fruity, Sweet, Woody, Floral	18.52	20.98
1-Dodecanol	Coconut, Earthy, Honey, Soapy, Wax	0.66	N.D.
2-Methyl-1-butanol	Pleasant, Roasted, Wine, Onion, Fruity Fusel Alcoholic Whiskey	N.D.	3.93
1-Octen-3-ol	Mushroom, Green, Vegetative	N.D.	0.25
Aldehydes and Ketones			
Hexanal	Fresh, Green/Sharp, Earthy Overall Intensity Good, Green Apple, Fruity, Grass-Like	51.37	24.43
2-Hexenal	Apple, Green	1.44	N.D.
Heptanal	Soap, Orange Peel, Tallow	0.38	0.58
2-Heptenal	Floral, Green, Fatty	0.17	0.72
Benzaldehyde	Almond, String, Sharp, Sweet, Bitter, Cherry	0.53	30.46
Nonanal	Aldehydic, Rose, Waxy, Citrus, Orange, Floral	0.19	0.74
Octanal	Aldehydic, Waxy, Citrus, Orange Peel, Green, Fatty	N.D.	0.22
2-Octenal, (E)-	Fresh Cucumber, Fatty, Herbal, Banana, Waxy, Green Leaf	N.D.	0.27
5-Hepten-2-one	Citrus, Green, Musty, Lemongrass, Apple	4.97	1.29
Acetophenone	Floral, Amond	0.12	N.D.
ethanone, 1-[4-(1-methylethyl)phenyl]	Musk-related odors	N.D.	0.46
Terpenes and terpenoids			
beta-Myrcene	Balsamic, Must, Spice	0.14	N.D.
Limonene	Citrus, Mint	0.57	N.D.
alpha-Farnesene	Mild, Green-Floral, Herbaceous, Sweet, Warm, Woody	0.67	N.D.
alpha-Pinene	Pine, Turpentine	0.21	
beta-Pinene	Fresh Minty, Eucalyptus, Camphoraceous note with a spicy, peppery nutmeg nuance	N.D.	0.2
p-Cymene	Fruity, Sweet	N.D.	0.11
D-Limonene	Lemon, Orange	N.D.	5.36
Eucalyptol	Liquor, Mint, Pine	N.D.	0.14
beta-Linalool	Nf	N.D.	0.29
alpha-Terpineol	Oily, Anise, Minty, Peach-Like	N.D.	0.31
Acids			
Butanoic acid, ethyl ester	Green Grass, Fruit	0.42	N.D.
Butanoic acid, propyl ester	Nf	0.28	N.D.
Butanoic acid, butyl ester	Nf	0.91	N.D.
Butyl 2-methyl butanoate	Nf	1.43	N.D.
n-Butyric acid 2-ethylhexyl ester	Nf	0.55	N.D.
Butanoic acid	Rancid, Cheese	1.4	N.D.
Benzoic Acid	Faint Balsam, Urine, Wine-Like, Very Weak	0.11	N.D.
Butanoic acid, hexyl ester	Sweet, Slightly Waxy, Fruity, Apple, Apple Peel Aroma	4.44	N.D.
Hexanoic acid	Sour, Fat, Sweat, Cheesy	2.61	0.22
Octanoic acid, hexyl ester	Nf	0.11	N.D.
Propanoic acid	Pungent, Rancid, Soy	0.16	0.17
Acetic acid, hexyl ester	Sharp, Acrid, Vinegar, Sour	N.D.	0.2

Table 5. *Cont.*

Volatile Compounds	Odor Perception	Raw By-Product	Apple Pomace Powder
Miscellaneous compound			
Toluene	Nf	N.D.	0.13
Furfural	Sweet Woody, Almond, Fragrant, Baked Bread	N.D.	5.19
2-Pentylfuran	Sweet	N.D.	1.6
Styrene	Sweet, Balsamic, Floral, Plastic	0.44	N.D.
Isopentyl hexanoate	Sweet, Fruity	0.08	N.D.

Nf—not found; N.D.—not detected.

These belong to several classes of organic compounds, such as 4 types of alcohols, 8 types of aldehydes, 4 types of terpenes and terpenoids, and 11 types of acids, but also isopentyl hexanoate and styrene.

Regarding the types of compounds identified in the apple pomace powder, four were alcohols, eight were aldehydes, six were types of terpenes and terpenoids, three were types of acids, but there was also toluene, furfural, 2-Pentylfuran, and ethanone. Alcohols are formed by the reduction of corresponding aldehydes, by the action of the enzyme alcohol dehydrogenase. Alcohols are the second most important compounds that contribute to the aroma of ripe apples, after esters [27]. The main alcohols identified in the raw by-product resulting from the processing of apple fruits are 1-hexanol with a concentration of 18.64%, imprinting an odor of green, fruity, sweet, woody, and floral; 2-metil-1-butanol in a concentration of 6.48%, which imprints a smell of wine and onion; 1-pentanol in a concentration of less than 0.64, impregnating an odor of balsamine, oil, sweet, and mint; in smaller quantities are also found phenol 0.12% and 1-dodecanol 0.66%.

Following the heat treatment, changes occurred regarding the concentration of compounds in the alcohol class as follows: the 1-hexanol content increased, reaching the value of 20.98%; new compounds of this class appeared, namely 2-Methyl-1-butanol, which gives an odor of pleasant and 1-Octen-3-ol, which imprints an aroma of mushroom, green, and vegetative. Aldehydes and ketones derive mainly from the catabolism of fatty acids but can derive from branched-chain amino acids such as isoleucine, valine, and leucine. There are reports of more than 25 aldehydes in apple fruits, but in our study the following aldehydes were determined in the by-products and the powders of apple pomace: hexanal, 51.37% in the by-product and 24.43% in the powder, developing aroma such as fresh, green/sharp, earthy overall intensity good, green apple, fruity, and grass-like [28]; 2-Hexenal was 1.44% in the by-product and undetectable in the powder, this being associated with a smell of green apple. 5-Hepten-2-one accounted for 4.97% in the by-product and a lower amount in the powder, while low amounts of nonanal 0.19% were detected in the by-product, the odor perception being that of aldehydic, rose, waxy, citrus, orange, and floral, a higher amount being detected for the powder. Heptanal was determined in lower amounts in the by-products in comparison to the powder, giving an aroma of soap, orange peel, and tallow. Benzaldehyde was determined in low amounts in the by-product, 0.53%, but increased a lot in the thermally treated sample, the powder presenting a very high benzaldehyde content of 30.46%. Benzaldehyde is the volatile compound responsible for a typical cherry aroma and can be synthesized from amino acids (aromatic amino acid and phenylalanine) [29]. The rest of the identified compounds were lower in content, such asacetophenone (0.12% in the by-product and ND in the powder) could lead to a perception of floral and almond odors. Terpenes and terpenoids are compounds identified both in the by-product obtained from the processing of apple fruits and the powder. beta-Myrcene 0.14% is associated with balsamic, must, and spice odors; limonene was also detected at 0.57% level in the by-product, conferring an aroma of citrus and mint; another therpene, alpha-Pinene, was also detected but only in the by-product at a level of 0.21%, offering pine, turpentine green-floral, herbaceous, sweet, warm, and woody aromas.

The most abundant acid found in the by-product of apple processing is butanoic acid, hexyl ester, or hexyl butyrate at 4.44%, which belongs to the class of organic compounds

known as fatty acid esters. Hexyl butyrate has a sweet, slightly waxy, fruity, apple, and apple peel aroma, with a similar green/soapy taste. Another important acid is hexanoic acid at 2.61%, having as its perception sour, fat, sweat, and cheesy odors. The rest of the acids are in lower concentrations, according to Table 5.

Following the drying operation at 80 °C, new compounds appeared, such as the following: D-Limonene at 5.36%, associated with a lemon and orange aroma; beta-Pinene was also at a low content of 0.2%, leading to a fresh minty, eucalyptus, and camphoraceous note with a spicy, peppery, and nutmeg nuance; alpha-Terpineol was found at 0.31% with an oily, anise, minty, and peach-like odor, but also other compounds were present, such as beta-Linalool at 0.29% and p-Cymene at 0.11%.

In the powder obtained from the thermally treated sample, the compound furfural was also detected at a level of 5.19 %. Furfural has been reported as an index of storage temperature abuse in commercially processed citrus juices. In citrus fruit juices, furfural is reported to stem from the decomposition of ascorbic acid. In the literature, both aerobic and anaerobic degradation of ascorbic acid have been linked with furfural formation. The oxidative reaction involves the degradation of ascorbic acid to dehydroascorbic acid, followed by the hydration of dehydroascorbic acid to ketogluconic acid, and finally decarboxylation and dehydration to furfural non-oxidative degradation occurs under acidic conditions, in which ascorbic acid degrades into furfural and 3-deoxy-L-pentose [30]. Another co-eluting compound, 2-pentylfuran 1.6%, may have a positive contribution to aroma, as its odor description is known as sweet [31].

3.3. Volatile Compounds Determined in the Muffin Prototypes

Volatile compounds are a measure of the aroma of a product, which is one of the most important factors that determine the consumer's preference regarding baked products. The profiles are mainly produced during the action of enzymes, or during the production process, which involves fermentations or thermal treatments and some reactions during baking (Maillard and caramelization). The ingredients and elaborating techniques could greatly contribute to the final aroma [32,33]. The GC/MS analysis of the volatile fraction of the muffin allowed the identification of 18 volatile compounds, accounting for around 90% of total peak area in the GC (Table 6). Quantitatively, the most important chemical groups are the aldehydes, terpenes and terpenoids, acids, and toluene. Aldehydes are well known to be odor-active compounds responsible for malty/chocolate notes [34]. Hexanal could have been formed during the baking process and imparts herbal characteristics to the muffins' flavor [35]. Octanal is derived from oleic acid oxidation. Low quantities of maltol (0.05–0.06) were generated; thus, the Maillard or caramelization reactions [36] which occurred during backing were not of high intensity. Concerning the aldehydes, 2-methyl butanal, 2-methyl propanal, and 3-methyl butanal are Strecker's aldehydes coming from valine, isoleucine, and leucine, respectively; these aldehydes are well known to be odor-active compounds responsible for chocolate/malty notes [34]. Concerning acids, they are originated from a lipid oxidation reaction. The acids are associated with cheese and waxy notes [35]. In muffin products, from the terpenes and terpenoids group, the most representative were limonene and beta-Myrcene, which are responsible for the lemon, orange, fresh sweet, balsamic, must, and spice aroma notes, meanwhile eucalyptol and gamma-Terpinene are responsible for liquor, turpentine, herbaceous, fruity, sweet mint, and pine flavors. In the muffin elaboration, lemon essence was used as an ingredient, which explains the high content of D-limonene (87.52–83.67) and some compounds such as beta-Pinene, p-Cymene, and limonene.

Table 6. The volatile fingerprints of muffins prepared with different concentrations of apple pomace powder.

Volatile Compounds	Odor Perception	M_W0	M_WB10%	M_WB20%	M_WB30%	M_WB40%
Alcohols and aldehydes						
Hexanal	Fresh, Green, Fatty, Fruity, Sweaty, Aldehydic, Grass, Leafy	1.29	1.38	1.4	1.18	1.18
Heptanal	Soap, Orange Peel, Tallow, Aldehydic, Waxy Citrus, Green Orange Peel	0.29	N.D.	N.D.	N.D.	N.D.
Octanal		0.2	N.D.	N.D.	N.D.	N.D.
2-Methyl propanal	Fresh Sweet, Mint, Floral	0.11	0.12	0.13	0.12	0.13
2-Methyl butanal	Musty, Cocoa, Coffee, Nutty	0.32	0.33	0.33	0.32	0.33
3-Methyl butanal	Ethereal, Aldehydic, Chocolate, Peach, Fatty	0.79	0.80	0.79	0.80	0.80
Maltol	Sweet, Caramel, Cotton Candy, Jam, Fruity, Baked Bread	0.05	0.06	0.06	0.05	0.06
Terpenes and terpenoids						
beta-Myrcene	Balsamic, Must, Spice	3.07	2.68	3.12	2.66	2.64
alpha-Pinene	Pine, Turpentine	1.18	1.27	0.83	0.89	0.70
beta-Pinene	Fresh Minty, Eucalyptus, Camphoraceous note with a spicy, peppery, nutmeg nuance	2.00	1.74	1.43	1.58	1.23
p-Cymene	Citrus, Sweet, Herbal, Spicy	1.95	2.36	1.47	2.36	2.11
D-Limonene	Lemon, Orange, Fresh Sweet	84.64	83.67	86.97	87.04	87.52
Eucalyptol	Liquor, Mint, Pine	0.07	N.D.	N.D.	N.D.	N.D.
alpha-Thujene		0.49	0.55	0.35	0.35	0.32
gamma-Terpinene	Turpentine, Herbaceous, Fruity, Sweet	1.35	1.46	1.32	0.6	1.64
Acids						
Propanoic acid	Pungent, Rancid, Soy	1.33	2.83	1.07	1.12	0.59
Miscellaneous compound						
Toluene	Nf	0.09	N.D.	N.D.	N.D.	N.D.

3.4. Textural Characterisation of the Muffin Prototypes

Regarding the textural analysis, the most similar prototype to the reference was the sample with 20% substitution of wheat flour with apple pomace powder. As can be seen in Table 7, the classification of the experimental prototypes of the muffins, in terms of textural results, highlights that the firmness, adhesiveness, cohesiveness, gumminess, and chewiness of the sample increase with the increase in the replacement levels. On the other hand, it is remarkable that the prototype containing 20% processed apple juice by-product displayed smaller results for elasticity and cohesiveness, suggesting that the evaluators might prefer this prototype due to the decreased cohesiveness. Taking into consideration the comments of the evaluators, we consider relevant their opinion on the sweet–sour aroma of the sample containing 20% processed apple juice by-product.

Table 7. Results regarding textural characterization of the muffin prototypes.

Parameters	Muffin Samples				
	M_W0	**M_WB10%**	**M_WB20%**	**M_WB30%**	**M_WB40%**
Firmness [g]	1055	754	1497	1757	1980
Total work [mJ]	162.0	111.7	238.5	254.6	283.7
Fracturability [g]	1055	750	1459	1757	1994
Deformation [%]—first fracture	17.9	21.5	19.0	22.1	23.2
Peak load [dyn/cm^2]	23,418.6	16,737.1	33,230.0	39,001.4	42,300.2
Consistency [g]	721	573	629	907	1124
Work done in cycle 2 [mJ]	52.6	41.0	46.5	67.6	89.39
Elasticity	0.75	0.82	0.72	0.73	0.75
Cohesiveness	0.29	0.33	0.17	0.23	0.30
Gumminess [g]	309	246	250	409	598
Chewiness [mJ]	40.0	35.0	35.9	50.8	80.23
Mean consistency load [g]	888	664	1063	1332	1690

M_WB0 is 100% wheat flour + 0% apple juice by-products, M_WB10% is 90% wheat flour + 10% apple juice by-products, M_WB20% is 80% wheat flour + 20% apple juice by-products, M_WB30% is 70% wheat flour + 30% apple juice by-products, and M_WB40% is 60% wheat flour + 40% apple juice by-products.

3.5. Sensory Evaluation of Different Muffin Prototypes

The substitution of the wheat flour with apple pomace powder was detectable during the sensory analysis. There was a decreasing trend for aroma and taste acceptability, probably due to the inclusion of the by-product powder, but the statistical analysis revealed no significant differences between these values, as can be seen in Table 7. The mean values of sensory attributes ranged between 7.71 and 8.19 for the tested samples. The muffin prepared with 100% wheat flour obtained the maximum hedonic scores. The mean scores decreased as the proportion of apple pomace powder increased. The highest value of appreciation was recorded for the muffin obtained with 20% apple juice by-products while the lowest value was for the muffin with 40% powder, which showed a significantly different overall acceptability score ($p > 0.05$; Table 8). Overall, there was no significant difference ($p > 0.05$) among the control sample and the muffins containing 10%, 20%, 30%, and 40% apple pomace powder. These results strengthen the hypothesis that muffin supplementation up to 20% apple juice by-products is optimal in terms of sensory and texture parameters while improving the antioxidant content of the samples.

Table 8. Sensory evaluation of muffin prototypes.

Muffin Samples	Color	Taste	Aroma	Texture	Overall Acceptability
M_W0	8.04 ± 0.8 [a]	7.66 ± 1.46 [a]	8.04 ± 1.16 [a]	8.57 ± 0.59 [a]	8.19 ± 0.81 [a]
M_WB10%	7.85 ± 1.06 [a]	7.90 ± 0.70 [a]	8.00 ± 0.83 [a]	8.42 ± 0.59 [a]	7.90 ± 0.62 [a]
M_WB 20%	7.71 ± 1.00 [a]	7.76 ± 0.76 [a]	7.90 ± 0.83 [a]	8.38 ± 0.66 [a]	8.09 ± 0.76 [a]
M_WB 30%	7.71 ± 1.18 [a]	7.71 ± 1.05 [a]	7.71 ± 1.14 [a]	8.33 ± 0.65 [a]	8.00 ± 0.88 [a]
M_WB 40%	7.61 ± 1.20 [a]	7.66 ± 1.31 [a]	7.42 ± 1.28 [a]	8.28 ± 0.64 [a]	7.71 ± 1.18 [a]

[a] no significant ($p < 0.05$), M_WB0 is 100% wheat flour + 0% apple juice by-products, M_WB10% is 90% wheat flour + 10% apple juice by-products, M_WB20% is 80% wheat flour + 20% apple juice by-products, M_WB30% is 70% wheat flour + 30% apple juice by-products, and M_WB40% is 60% wheat flour + 40% apple juice by-products.

4. Conclusions

This study presents an alternative to capitalize on apple pomace powder to obtain a handcrafted muffin product. The by-product resulting from apple juice production and some powders obtained after its thermal processing were also evaluated in terms of physicochemical properties. Muffins were obtained after optimizing the process of drying and grinding apple pomace to obtain a powder with a high antioxidant potential, rich in biologically active substances. Following the analyses performed, it was demonstrated that the samples were characterized by a high content of phenolic compounds responsible for

numerous physiological, biological, and biochemical functions due to their strong antioxidant activity. The cytotoxic potential of the methanol extracts of the apple pomace ranged between 70.42 and 78.80%, it being concluded that the sample subjected for the longest period to thermal treatment was the lowest in viability, displaying the highest cytotoxic effect. The behavior of the tested samples towards the bacterial strains was relatively similar and an antimicrobial effect towards the Gram-positive bacteria was revealed. In regard to the detected volatile compounds, different compounds were registered for the apple by-product in comparison to the powder obtained after the thermal process. Hexanal was in lower amounts in the thermally processed pomace, for which some acids but also acetic acids were not detected; instead, the presence of some miscellaneous compounds was observed, such as Furfural and 2-Pentylfuran, which might be able of conferring a sweet aroma. Apple pomace volatile compounds positively influenced the final muffin aromatic profile, leading to pleasant odor and taste perceptions. Even if no statistically significant difference was recorded during the sensory analysis regarding the scored parameters, the overall acceptability was the highest for the reference muffin 8.19 $\pm$ 0.81, followed by the muffin with 20% apple pomace powder and that with 30% apple pomace powder. In the textural analysis, the muffin with 30% apple pomace powder registered the highest firmness in comparison to the reference or the muffin with 20% apple pomace powder, and also a higher value for gumminess and chewiness. In what concerns the volatile compound profile, higher values of hexanal were detected for the muffin with 20% apple pomace powder in comparison to the reference, which might impart to the product some chocolate notes, but also higher release of beta-Myrcene and D-Limonene.

Apple pomace is a valuable by-product which, if used as an ingredient, will diversify muffins and improve their nutritional properties. The strength of the current work is that muffins with special, positive sensory attributes were obtained, while sensory evaluation indicates overall acceptability, even when 40% of apple pomace powder was used. The opportunities related to the fortifications of muffins with apple pomaces are that this ingredient is widely available, both industrially and in households, easy to process prior to usage (cleaning, drying, and milling), and that consumers are interested in reducing waste and improving the nutritional status of food products. In what concerns the upscaling of the process, some threats might occur because supplementary processing, storage, and transportation of a novel ingredient include costs, but also collaborations between industries (e.g., juice factories and bakeries), which would be possible only if they were more supported by governments.

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