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Original Article

Bio-activities and Application of Avocado Seed Powder in Soft Biscuits

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Abstract: Avocado is a very popular plant, widely used in Vietnam and around the world. Its fruit contains a lot of nutrients beneficial for health. During the processing avocado, the seed (accounting for 13 to 24% of the total avocado fruit weight) is often discarded as agricultural waste that requires further processing. However, avocado seeds are also known to contain bioactive compounds such as procyanidins, phenolics, fatty acids, amino acids, and weak acids which exhibit antioxidant, antibacterial, hypoglycemic, anti-diabetic, and anti-hypertensive properties. These compounds were applied in various pharmaceutical, biotechnological, chemical, and food industries. This study evaluated the antibacterial ability of avocado seed extracts on *Helicobacter pylori* HP09 and *Bacillus* spp., showing the high antibacterial activity of ethyl acetate extract. Avocado seed powder has been studied as a way to partially replace wheat flour in making soft biscuits. The supplement ratio of avocado seed powder was 10% of the total wheat flour weight. The other ingredients also include eggs, sugar, oil, vanilla, wheat flour, cocoa powder. The cookies were baked at 160 °C for 15 minutes. The results showed that incorporating avocado seed powder into soft cookies maintained their nutritional value, flavor, and appeal, while also providing health benefits.

Keywords: Avocado seed powder, *Bacillus* spp., bioactive, *Helicobacter pylori*, soft cookie.

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1. Introduction

Avocados (*Persea americana* Mill.) are widely cultivated in various regions of Vietnam, particularly in the Central Highlands (including Dak Lak, Gia Lai, Kon Tum, Lam Dong), and Southeast (Dong Nai). These areas are known for favorable ecological conditions supporting the growth of avocado trees. Avocado fruits are rich in nutrients, including antioxidants, vitamins (E, C, A), high level of potassium, and dietary fibre which are beneficial for health. In addition, the avocado has various uses as a natural cosmetic, having advantages in rapid skin penetration and acting as a superior natural sunscreen. The economic efficiency of avocado trees is very high. As a result, the area of avocado cultivation is continuously expanding, and the avocado production and processing industry is increasingly developing [1, 2].

Avocado seeds can constitute up to 13% to 24% of the fruit's weight. Therefore this byproduct is generated in significant quantities during processing. Solutions for utilizing avocado seeds, and managing waste from avocado seeds are extremely important to reduce negative environmental impacts [2]. Avocado seeds are known to contain numerous compounds with the ability to treat digestive disorders such as the anti-inflammatory, antioxidant, and antibacterial properties. Avocado seeds are rich in various nutrients and bioactive compounds, including protein, starch, lipids, dietary fiber, vitamins, minerals, and many phytochemicals. Carbohydrates are considered a significant component, comprising about 64.9%, while starch accounting for 91.2% of the total carbohydrate content in avocado seeds [3]. According to Liu et al., [4], the carbohydrate composition of avocado seeds includes 246.1 mg/g (starch), 18.5 mg/g (sucrose), 1.9 mg/g (hexose), 63.8 mg/g (d-mannoheptulose), and 88.3 mg/g (perseitol) dry weight [4].

Avocado seeds contain a significant phenolic content, including five groups: procyanidins, catechin, flavonol, hydroxycinnamic, and hydroxybenzoic acids [3]. In a study evaluating phenolic compounds in Hass and Fuerte avocado, four phenolic compounds were reported: trans-5-O-caffeoyl-d-quinic acid, procyanidin B1, catechin, and epicatechin. Within the terpenoid and phenylpropanoid groups, seven compounds were identified: estragole, isoestragole, cubebene, α-cubebene, α-germacrene, α-farnesene, and caryophyllene. Another significant lipid-soluble component in avocado seeds is polyhydroxylated fatty alcohol derivatives (PHFAs). Acetogenin (a type of PHFA) originates from unsaturated fatty alcohols. The total acetogenin content ranges from 1090 to 8330 μg/g DW in avocado seed [5]. Alkaloids, phytosterols, and tocopherols are also present in avocado seeds but in low concentrations.

Avocado seeds and their bioactive compounds show potential in cancer prevention for humans and animals, including prostate, breast, lung, colon, and liver cancer cells. Polyphenols from avocado seeds can inhibit prostate cancer cells (LNCaP), breast cancer cells (MCF7), lung cancer cells (H1299), and colon cancer cells (HT29) [6]. Supplementing the diet of diabetic rats with avocado seeds (2%–8%) has shown anti-diabetic and lipid-lowering effects by reducing blood glucose and cholesterol levels [7]. The water extract of avocado seeds has antioxidant properties and can prevent oxidative damage caused by free radicals [8]. In avocado seeds, phenolic compounds and procyanidin (catechin and epicatechin) contribute 38% of the antioxidant activity of the whole avocado. The ethanol extract of Hass and Fuerte avocado seeds contains a high content of phenolic compounds [9]. Water extracts of avocado seeds exhibit antioxidant properties and may prevent oxidative damage caused by free radicals [8]. Phenolic components and procyanidins (catechin and epicatechin) contribute 38% to the antioxidant activity of whole avocados [10]. Ethanol extracts of Hass and Fuerte avocado seeds contain high levels of phenolic components [9].

Organic extracts from seed avocado inhibited the growth in vitro of strains of *Candida* spp., *Cryptococcus neoformans* and *Malassezia pachydermatis* [11], *Staphylococcus aureus, Staphylococcus pyogenes, Corynebacterium ulcerans, Candida albicans, Escherichia coli, Salmonella typhi* [12] *Helicobacter pylori* [13].

According to Mahawan et al., [14] avocado seed powder is a source of nutrients, with high levels of minerals, carbohydrates, and fiber, but it is not suitable for bread processing due to its lack of gluten. However, when supplemented with avocado seed powder (25% and 50% supplementation levels to flour) cookies were acceptable in terms of sensory qualities. They were moderately favored for color, while aroma, taste, and overall impression were rated low due to the presence of tannins in avocado seed powder, causing a slightly bitter taste. To further investigate, the authors recommended further research focusing on removing tannins from avocado seed powder to improve its quality for both powder and finished products.

In 2020, Terati et al., [15] partially replaced avocado seed powder for wheat flour in the processing of barley cakes with four formulas: F0 (control-0% avocado seed powder), F1 (25% avocado seed powder), F2 (50% avocado seed powder), and F3 (75% avocado seed powder). The results showed that substituting avocado seed powder significantly affected the sensory quality of the cakes, with formula F3 being the most highly accepted compared to the others. The cakes were characterized by a bitter taste, aroma, coarse texture, and brown color. Cakes containing this avocado seed supplement contained 25.57% carbohydrates, 6.05% fats, 3.45% protein, 63.08% moisture, and 1.85% ash.

According to Nguyen et al., [16] the protein content in avocado seeds was 2.66%, nearly 1.7 times higher than that in the pulp, and vitamin C content in avocado seeds was also the highest among the vitamins found, at 4.22 mg/100 g. Starch was a major component in these avocado seeds, accounting for 17.9 g/100 g. The authors suggested that further research could focus on applying avocado seeds in food products such as avocado seed powder, avocado seed tea, and animal feed, rather than disposing of them into the environment, thereby causing pollution.

The objective of this study is to evaluate certain biological activities of Booth avocado seed powder collected in Vietnam, and apply it as a partial starch in soft cookies, in order to develop a cookie product enriched with beneficial bioactive compounds.

2. Materials and Methods

2.1. Materials

Avocado seeds: Booth avocado seeds were collected from beverage stores in Hanoi and classified under expert guidance (Botanist) to ensure that only Booth avocados were selected for the study.

Microorganisms: *Helicobacter pylori* and *Bacillus* spp. were isolated and preserved at the National Microbial Genetic Resources Center.

Cookie ingredients: flour, baking powder, cocoa powder, sugar, cooking oil, eggs, salt were purchased from the Abby baking supplies store.

Avocado seeds were washed, chopped and dried at 40-55 °C, before being ground into powder.

2.2. Methods

2.2.1. Extraction of Avocado Seed

Avocado seed powder was extracted using five solvents to get five extractions (methanol, ethyl acetate, chloroform, ethanol, hot water) at a ratio of 1:5 (g/ml). The extractions were conducted at ambient temperature for 24 hours with intermittent shaking. The mixtures were filtered through paper filters and the extraction solvents were then removed using the R-205-Büchi (Switzerland) rotary evaporator at 40 °C to obtain a known weight of extracts. The residues were collected and stored at 4 °C for further studies [17].

2.2.2. *Bacillus* spp. Resistant Test

The medium MPA (meat peptone agar) was autoclaved at 121 °C in 15 mins. Then, the medium was cooled to 45-50 °C, poured into petri dishes.

The methanol, ethyl acetate, chloroform, ethanol and hot water extractions, along with the control ampicillin were measured and diluted in DMSO to the concentrations of 0.2, 0.4, 2, 20, and 100 mg/ml.

Medium petri disks were seeded with 24-hour-old cultures of *Bacillus* spp. inocula (a standardized 10^6 CFU/ml ~ 0.5 McFarland Standard). Seven wells (6 mm in diameter) were cut into the agar media with a sterilized cork borer and then extracts with the concentrations of 0.2, 0.4, 2, 20, 100 mg/ml were poured into the wells. An antibiotic (ampicillin 100 ppm) and DMSO (100 ppm) were also used as a positive and negative control, respectively. Inoculated plates were then incubated at 37 ºC for 24 hours and zones of inhibition were measured in millimeters. Three replicates were prepared for each microorganism.

HTKK = the diameter of the clear zone (D) - the diameter of the well (d = 6 mm).

2.2.3. *H. pylori* Resistant Test

H. pylori HP09 was cultured on BA agar medium supplemented with 7% of sheep or horse blood and cultivated in micro-aerobic conditions at 37 °C. The BA agar medium was prepared using 23 g of a special nutrient substrate, 1 g of starch, 5 g of sodium chloride, 13 g of agar, and 1000 ml of sterilized water. The medium was then autoclaved at 121 °C in 15 mins. Then, the medium was cooled to 45-50 °C, provided with 7% sheep or horse blood and poured into petri dishes.

The methanol, ethyl acetate, chloroform, ethanol, hot water extractions were measured and diluted in DMSO to the concentration of 200 mg/ml. Pour 2 µl of each diluted extraction (equal 200 µg) onto the distilled paper, then waited until the paper dry and placed the paper on BA agar plates which were incubated with *H. pylori*. The plates were cultured under micro-aerobic conditions at 37 °C. A positive control was the paper supplemented with 20 mg/ml of amoxicillin. The diameter of the paper was 6 mm [18].

After 7-day cultivation, measured the clean zone of each paper on BA agar medium plates. HTKK = D-d (mm); D: the clear zone diameter of each paper; d: the diameter of the paper; HTKK – *H. pylori* resistant activity [19]. The extraction that had the highest HTKK was selected for the next experiments.

2.2.4. Process of Making Soft Cookies with Avocado Seed Powder Supplement

Experimental steps for the soft cookie making process:

Step 1: Mix and knead the wet and dry ingredients to form a dough.

Step 2: Shape into small round balls weighing 25 g.

Step 3: Bake at 160 °C for 15 mins.

Step 4: Cool the cookies at room temperature.

Step 5: Pack cookies tightly in plastic bags or glass jars to preserve freshness.

2.2.5. Evaluation of the Soft Cookie Product Quality

Evaluation of the effect of supplemented avocado seed powder ratio on the quality of the soft cookie: The cookie mixing formula was chosen with the ratio of avocado seed powder to total flour being 0, 10, 15, and 20%.

Evaluation of the effect of baking time on the quality of the soft cookie: The cookies were baked at 160 °C for 10, 12, 15 and 20 mins to evaluate the quality.

Determining moisture: Moisture content was determined according to TCVN 4069:2009.

Determining ash content: Ash content was determined according to TCVN 9939:2013.

Determining total nitrogen content: Total nitrogen content was determined according to TCVN 8125:2015.

Determining lipid content: Lipid content was determined according to TCVN 8555:2011.

Determining total carbohydrate: Total carbohydrate content was determined according to FAO/WHO (1998).

Sensory evaluation of the soft cookie: The 9-point scale method involves pleasant and unpleasant states. In this method, “like” and "dislike” states are measured on a hedonic scale. The evaluation subjects included families, friends, lecturers, and students from the VNU University of Science, aged 18 to 50 years. The information was then collected and analyzed to provide accurate results regarding the sensory evaluation of the soft cookie.

3. Results and Discussion

3.1. Evaluation of Antibacterial Activity of Avocado Seed Extracts

Avocado seeds were extracted with five different solvents: methanol, ethyl acetate, chloroform, ethanol, and hot water, obtaining five extracts of N1, N2, N3, N4, N5 respectively (Figure 1). Methanol, ethyl acetate, chloroform, and ethanol were effective solvents in the extraction of polar substances such as phenolic compounds.

3.1.1. Evaluation of Antibacterial Activity of Avocado Seed Extracts agaist *Bacillus* spp.

The antibacterial activity of avocado seed extracts was studied at different concentrations (0.2, 0.4, 2, 20, 200 mg/ml) against the pathogenic bacterium *Bacillus* spp. All avocado seed extracts showed antibacterial activity against *Bacillus* spp. (shown in Figure 2). The antibacterial activity of the avocado seed extract and ampicillin is represented by HTKK. HTKK of extracts of 200 mg/ml are presented in Table 2. The methanol extract (N1) was the highest, the water extract was the lowest.

Table 1. Ingredients in soft cookies

Formula / Ingredients	CT1	CT2	CT3	CT4
Flour	200 g	180 g	170 g	160 g
Avocado seed powder	0	20 g	30 g	40 g
Cocoa powder	10 g	10 g	10 g	10 g
Baking powder	5 g	5 g	5 g	5 g
Sugar	80 g	80 g	80 g	80 g
Oil	40 ml	40 ml	40 ml	40 ml
Egg	80 g	80 g	80 g	80 g
Salt	1/2 tsp	1/2 tsp	1/2 tsp	1/2 tsp
Vanilla	1 tsp	1 tsp	1 tsp	1 tsp

N1 N2 N3 N4 N5

Figure 1. Extraction from avocado seeds
(N1: methanol extract, N2: ethyl acetate extract, N3: chloroform extract, N4: ethanol extract, N5: hot water extract).

Table 2. *Bacillus* spp. resistant by extracts
(N1: methanol extract, N2: ethyl acetate extract, N3: chloroform extract,
N4: ethanol extract, N5: hot water extract)

Label (200 mg/ml)	Clean zone diameter D (mm)	HTKK
N1	31±1.2	25±1.2
N2	28±1.4	22±1.4
N3	12±1.6	6±1.6
N4	20±1.5	14±1.5
N5	6±1.6	-
Positive control	30±1.7	24±1.7
Negative control	6	0

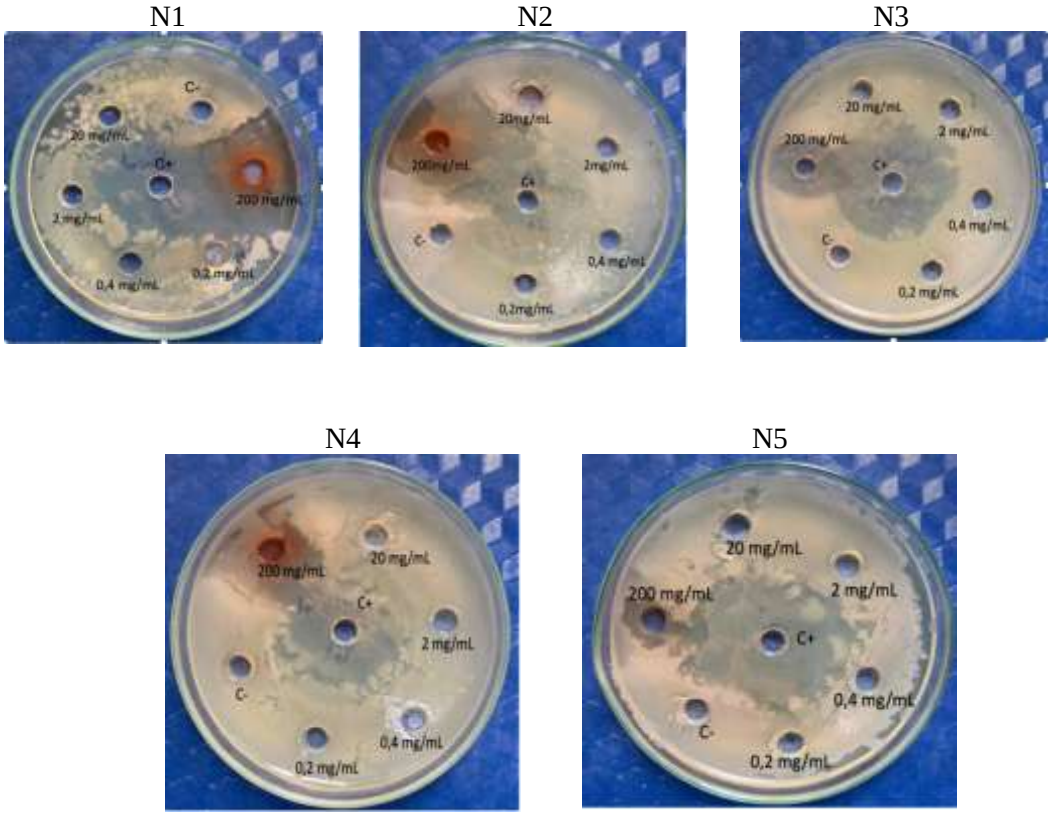

Figure 2. Antibacterial activity of extracts against *Bacillus* spp.
(N1: methanol extract, N2: ethyl acetate extract, N3: chloroform extract,
N4: ethanol extract, N5: hot water extract).

There have been studies reporting the activity of avocado seed extracts against pathogenic bacterial strains, including *Bacillus* spp. Kupnik et al., [20] reported the ability of Hass avocado seed extract to inhibit the pathogenic strain *B. cereus*. The ethanol extract showed activity with an inhibition zone 15-17 mm, which was half as effective as the methanol

extract of the avocado seed. Thus, the result in Table 2 shown that the ethanol extract of Booth 7 avocado seed has equivalent activity compared to that from other avocado varieties worldwide.

3.1.2. Evaluation of Antibacterial Activity of Avocado Seed Extracts against *Helicobacter pylori*

The extracts were tested with the *H. pylori* HP09 and the results are shown in Figure 3. As a negative control DMSO (C-) no-antibacterial zone was detected, so DMSO dissolves extracts and can be used as a negative control in antibacterial experiments without affecting the results. The positive control (20 mg/ml of amoxicillin, C+) exhibited anti-*H. pylori* activity, regarding clean antibacterial zone (D-d) of approximately 20 mm. Compared to the positive control, it can be seen that samples N2 and N3 exhibit weak antibacterial activity, with bacterial density appearing sparser compared to the other samples. Athaydes et al., [21] indicated that the avocado seed hexane extracts could resist two strains of *H. pylori* ATCC 43504 and 43629 which caused stomach ulcer [21]. Torres et al., [22] evaluated the ability of avocado peel extract (confirmed to be high in polyphenols) to resist *H. pylori*, with the HTKK of the antibacterial zone being small (11-12 mm) compared to amoxicillin (62 mm). The Booth 7 avocado seed extracts in this study may have the potential to inhibit *H. pylori*.

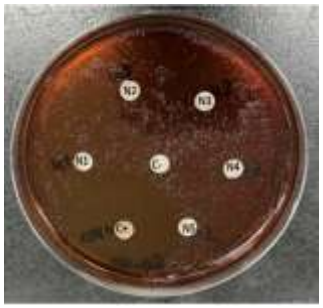

Figure 3. Ability of the extracts to against *H. pylori* HP09 (N1: methanol extract, N2: ethyl acetate extract, N3: chloroform extract, N4: ethanol extract, N5: hot water extract).

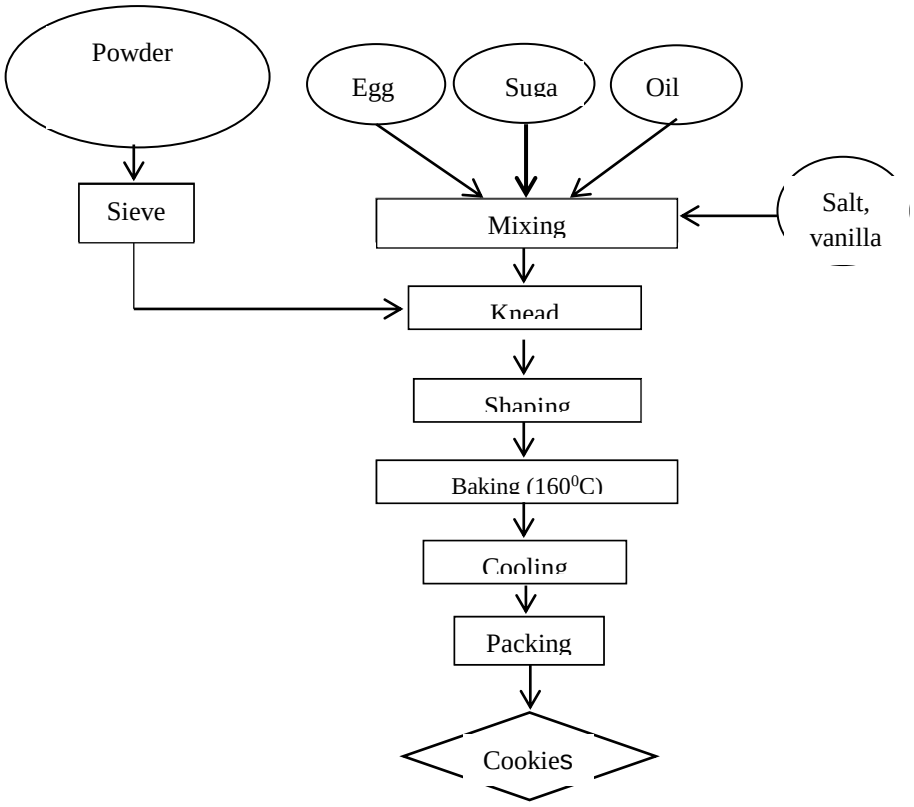

Figure 4. Process of making soft cookie.

3.2. Process of Making Soft Cookies with Avocado Seed Powder Supplement

The process of making soft biscuits was carried out as shown in Figure 4. The proportions of avocado seed powder and baking time were evaluated to choose the most suitable formulation.

3.2.1. Evaluation of the Effect of Supplemented Avocado Seed Powder Ratio on the Quality of the Soft Cookies

The analyses of the avocado seed powder for moisture, ash, total nitrogen, lipid, and total carbohydrates were 13.46, 1.94, 6.75, 11.64, and 58.21%, respectively. The moisture content of avocado seed was the same as that of baking flours, which is around 13 percent [23]. The ash content of the avocado seed powder was 1.94 and is higher than normal maize and wheat flour. The total nitrogen content, lipid content of the avocado seed powder were lower than that as reported by Ẹiofor et al., [24]. This difference may be attributed to the avocado variety and different ecological zones and soil. The seed powder has a high carbohydrate content, which is 58.21%. Thus, it can replace flour in some baking formulations.

Table 3. Sensory characteristics of soft cookies

Criteria	0% avocado seed powder	10% avocado seed powder	15% avocado seed powder	20% avocado seed powder
Color	Dark brown	Light brown	Light brown	Light brown
Texture	Soft, moist	Soft, moist	Soft, light dry	Dry, fragment
Taste	Sweet	Light bitter	Bitter	Unplease bitter
Overall preference	Like	Like	Acceptable	Unlike

As shown in Table 3, the sensory properties of the product decrease as the proportion of avocado seed powder increases. This is likely due to the tannin content in the avocado seed powder, which imparts a slightly bitter taste.

In terms of color, when there is no avocado seed powder (0%), the cookies have a darker brown color than when there is 10, 15 or 20% avocado seed powder. The color becomes more and more lighter because avocado seeds contain anthocyanin colored particles, which change color to bright orange when exposed to oxygen, overwhelming the brown color of cocoa powder in the cake.

In terms of texture, cookies supplemented with avocado seed powder mostly have a drier and more fragmented structure than proof cookies, the reason being that gluten - the substance that builds structure for cakes - tends to decrease.

The more avocado seed powder replaced wheat flour, the more bitter after taste that comes from the tannin content in avocado seed powder. In overview, cookies without avocado seed powder (0%) and those with 10% avocado seed powder were rated highly in terms of flavor, texture, and overall appeal. In contrast, cookies with 15% avocado seed powder were considered acceptable, while those with 20% were deemed unpleasant due to their bitter aftertaste.

From the sensory evaluation results, the 10% avocado seed powder ratio had the most popular flavor and achieved the highest value for color, texture, scent and taste (Figure 5). Furthermore, if compared with the control formula (0% avocado seed), the criteria values are almost equivalent and satisfactory with high acceptability. This can be a suitable formula to process soft biscuits supplemented with avocado seed powder.

3.2.2. Evaluation of the Effect of Baking Time on the Quality of the Soft Cookie:

The cookies were baked at 160 °C for 10, 12, 15, and 20 mins to evaluate the quality. The baking process plays an important role in the

cookie process. When the temperature acts on the dough the physical-thermal, chemical-biochemical and gelation processes take place simultaneously. Baking temperature causes starch to gelatinize and denature proteins. The coagulation protein makes the starch become gelatinized, forming a porous structure and a configuration of cookies.

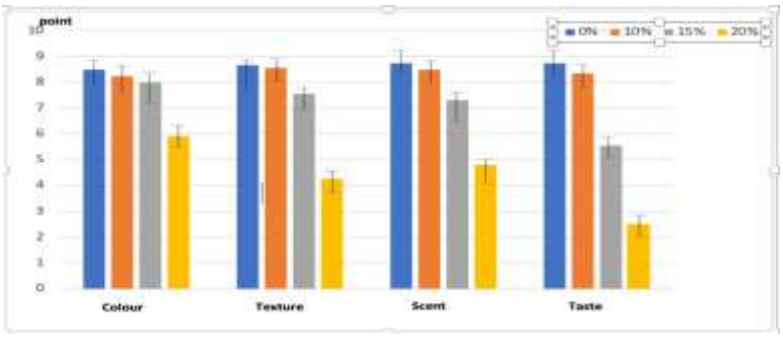

Figure 5. Acceptability of cookies.

Table 4. Effect of baking time on the cookie quality

	10 mins	12 mins	15 mins	20 mins
160 ^{0}C	Light colour, loose texture, too soft	Dry surface, wet inside, doneness	Moderate moisture, the dry surface uniform, soft and high sensory value	Low moisture, dry cookie, easy to crumble

The results show that the color, moisture, and structure values reached suitable levels when the cookies were baked at 160 °C for 15 mins. The cookies had a harmonious brown color, not too light and no scorched spots appeared in comparison to the other three formulas (Table 4 and Figure 6).

3.3. Evaluation of the Soft Cookie Quality

Compared to the control cookies (not containing avocado seed powder), soft cookies supplemented with 10% avocado seed powder had negligible changes in nutritional composition but higher lipid, glucid and ash content (Table 5). This difference may be due to the difference in ingredients between avocado seed powder and commercial wheat flour. However, the product still ensures good quality for consumers.

According to Atwater's 4-9-4 system, 1 gram of protein contains 4 calories, 1 gram of carbohydrates contains 4 calories, and 1 gram of fat contains 9 calories. Therefore, we have calculated the calories in 100 g of finished cake as follows:

Calories in 100 g cookies = 66.69 x 4 + 8.01 x 4 + 12.94 x 9 = 415.26 (kcal).

Table 5. Nutritional composition of cookies

Criteria	Unit	100 g cookies	
		Cotrol cookies (0% avocado seed powder)	Cookies with 10% avocado seed powder
Moisture	%	12.68±1.1	10.53
Protein (TN)	%	9.32±1.4	8.01
Lipid	%	12.74±0.8	12.94
Carbonhydrate	%	63.72±2.1	66.69
Ash	%	1.54±0.2	1.83

4. Conclusions

Five avocado seed extracts (methanol, ethyl acetate, chloroform, ethanol and hot water) demonstrated the ability to inhibit the *Bacillus* spp. strain to varying levels. Among the extracts, the water extract showed the weakest antibacterial activity, while the other four extracts exhibited stronger effects, with the methanol extract being the most potent.

The Booth 7 avocado seed extracts in this study may have the potential to inhibit *H. pylori*. Ethyl acetate and chloroform extracts could resist *H. pylori*.

Soft cookies supplemented with avocado seed powder achieved high quality, sensory value, and good texture when replaced with 10% avocado seed powder, baked at 160 °C for 15 mins, resulting in a soft, moist structure and appropriate color. The nutritional composition was almost equivalent to the control cookies (0% avocado seed powder), with higher levels of carbohydrates, lipids, and ash.

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