إستخدام قشور البرتقال ومستخلصاتها الطبيعية المختلفة كمضافات غذائية ذات قيمة عالية في إنتاج بسكويت الوافل المقرمش

Utilization of orange peel and their various natural extracts as valuable food additives in production of crispy waffle

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المستخلص:

الهدف من الدراسة الحالية هو استخدام قشر البرتقال ومكوناته الطبيعية العديدة كمضافات غذائية مفيدة في صنع الفطائر المقرمشة (الوافل). تعتبر كل من قشور البرتقال ومستخلصاتها المذيبة من المضافات الغذائية الجيدة. يمكن استخدام مستخلصات من الفطريات التي نمت على هذه القشور لتعزيز الصفات المضافة للطعام. يمكن صنع فطائر الوافل وغيرها من المنتجات الغذائية ذات القيمة الغذائية المضافة من قشور البرتقال الطازجة ومستخلصها الإيثانولي. من ناحية أخرى، يمكن استخدام المستخلصات الإيثانولية للفطر المزروعة على قشور البرتقال لنفس الهدف. أجريت التحاليل الكيميائية لمستخلصات قشر البرتقال. بالإضافة إلى ذلك، تم تضمينها في فطائر الوافل واجراء التقييم الحسى للمنتجات. أظهرت النتائج أن قشور البرتقال احتوت على رطوبة 10.22٪، بروتين 8.35٪ ، دهون 2.53٪ ، رماد 6.45٪ ، ألياف خام 13.45٪ ، كربوهيدرات 58.98٪. كانت المكونات الفينولية الناتجة بواسطة استخدام الميثانول والإيثانول وخلات الإيثيل أفضل من الأسيتون والنيكلوروميثان وثاني كلورو ميثان في استخلاص المركبات الفينولية من قشور البرتقال. كان قشور البرتقال مصدرًا غنيًا لمضادات الأكسدة والأحماض الفينولية الطبيعية والفلافونويد. تحتوي المنتجات الثانوية لقشور البرتقال على منتجات فعالة مضادة للجراثيم وكمية عالية من مضادات الأكسدة، وفقًا للبحث حول القدرة المضادة للأكسدة ومكونات النشاط المضاد للميكروبات لمستخلصات قشر البرتقال. قبل وبعد التخمير، أظهرت التقييمات الحسية لفطائر الوافل مع قشور البرتقال ومستخلصاتها درجة عالية من القبول، وأوصت النتائج بإمكانية استخدام قشور البرتقال ومستخلصاتها لإنتاج منتجات المخابز المتمثلة في الوافل ذات القيمة الغذائية العالية.

الكلمات المفتاحية: قشور البرتقال، مستخلصات المذيبات والفطريات، التخمر الفطري، مضادات الميكروبات، مضادات الأكسدة، الفينول، الوافل.

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Abstract

The present study's goal was to employ orange peel and its numerous natural components as beneficial food additives in the creation of crispy waffles. Both orange peels and their solvent extracts are regarded as good food additives. Extracts from the fungus that grew on these peels may potentially be employed to boost food's additive qualities. Waffles and other value-added goods like them might be made from fresh orange peels and their ethanolic extract. On the other hand, ethanolic extracts of fungus developed on orange peels may be used for the same objective. Chemical analyses of orange peel extracts were performed. Additionally, they were included in waffle and taste tested. The results showed that the orange peels included 10.22% moisture, 8.35% protein, 2.53% fat, 6.45% ash, 13.45% crude fibre, and 58.98% carbs. Phenolic components from plant materials, methanol, ethanol, and ethyl acetate extracted phenolic compounds from orange peels better than acetone, n-hexane, and dichloromethane. Orange peels included a high concentration of natural phenolic acids and flavonoids. According to a study on the antioxidant capacity and antimicrobial activity components of orange peel extracts, Citrus sinensis fruit by-products exhibit effective antibacterial activity products and a large number of antioxidants. Orange peels included a high concentration of natural phenolic acids and flavonoids. According to a study on the antioxidant capacity and antimicrobial activity components of orange peel extracts, Citrus sinensis fruit by-products exhibit effective antibacterial activity products and a large number of antioxidants. Before and after fermentation, Sensory evaluations of waffles with orange peel and extract showed a high degree of acceptance, and the results recommended the possibility of using orange peel and extract to produce bakery products represented in waffles with high nutritional value.

Keywords:

Orange peels; solvent and fungal extracts; fungal fermentation; antimicrobial, total antioxidant, total phenolic, waffle

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Introduction

Fruits are rich in numerous phytochemicals, offering potential health benefits, including the prevention of diseases. The antioxidant properties of phenolic compounds, which enable them to neutralize free radicals, contribute to these health-promoting effects **(El-wardany, 2016). The food industry generates a large amount of solid and liquid waste as a result of food production, preparation, and consumption. Food processing waste can be recycled as raw materials. converted into higher-value byproducts, or used as food or feed (Nandini et al., 2013). According to FAO, 1.3 billion tons of various types of food lost annually across the supply chain could feed up to two billion people with no additional environmental effect (Williamson et al., 2016). Transforming waste materials into useful final products has become a popular area of research. This is particularly true in the food processing business, where wastes, effluents, residues, and byproducts may all be recovered and upgraded into higher-value products (Nandini et al., 2013). Citrus by-products are a promising economic source of bioactive compounds such as phenolic and flavonoid compounds, as well as having valuable technological and nutritional properties. Because of their low cost, these byproducts can be used as food additives in the food. industry (Al-Juhaimi, 2014; Galanakis, 2012). Citrus waste is high in flavonoids, carotenoids, dietary fiber, polyphenols, ascorbic acid, sugar, and other compounds. They are important in the evaluation of food quality because they contain bioactive compounds and dietary fiber (Sharma et al., 2017). On the international market, Oranges are very crucial fruits. In the case of juice production, over 70% of the oranges produced are employed in several industrial processes to generate juices, jams, and other goods, resulting in vast volumes of residues that account for approximately 50-60% of the processed fruit (Galanakis, 2012). Orange peels consist of 52 seeds, peels around 60-75%, and 23-33% membrane residues approximately, high in bioactive compounds like ascorbic acid, flavonoids, and phenolics, and pectin, all of which are beneficial to human nutrition. Citrus fruit contains Three flavonoids types. flavanones, flavones, and flavanols (Shawky et al., 2019). Orange peels contain flavonoids like hesperidin, narirutin, naringin, and eriocitrin. (Ghasemi et al., 2009). Orange peels offer valuable substances like natural products and bioactive phenolic compounds, making them ideal for intermediate food ingredients and health-promoting new products. (Ibrahim & Hamed 2018). Peels are believed to be natural products. that can function as an excellent low-cost antioxidant source (M'hiri et al., 2017). Fungi are high in nutritional value (Cerimi, 2019). Because they include both essential and non-essential amino acids, fungi are particularly nutritious as food and feed. Fungi have been utilized as food and in fermented beverages from the beginning**

of time (Iqbal et al., 2013). Fungi play various roles in food, including production, sources, and agents of spoilage. (Benedict et al., 2016). Many fungi are essential to the biotechnology field and Industrial applications include producing medications, foods, and beverages. Fungi are valuable sources of nutrition, used in cheeses, bread, rice, and soy sauce, and some of which are useful. In addition, yeasts and filamentous fungi secrete numerous enzymes and secondary metabolites in their growth medium. Many of these enzymes exhibit hydrolytic characteristics and find applications in various food processing industries, contributing to the improvement of odor quality. Conversely, secondary metabolites, encompassing antibiotics, alkaloids, enzymes, organic acids, carotenoids, toxins, and pigments, serve as valuable bioactive compounds. These secondary metabolites hold promising potential for applications in biotechnological and pharmaceutical fields (Praptiwi et al., 2018; Vaishnav & Demain, 2011; Zhang et al., 2002). Phenolic compounds are present in plant foods, with quality, type, and concentration varying significantly based on strain, genetics, and environmental conditions. (Kris-Etherton et al., 2002). Solid state fermentation of the plant materials was employed to enhance their phenolic to the increase in their antioxidant activity (Choung et al., 2001). The objective of this study is to make crispy waffle fortified by natural food additives extracts using orange peels and its fermented extracts orange peels of microorganisms grown on the peels.

Materials and Methods

Sample collection

Orange peels "balady orange" (Citrus sinensis) were purchased from the Makkah Juices Factory in Alexandria governorate, Egypt.

Chemicals

The solvents, reagents, and chemicals employed in the study were procured from El-Ghomhorya Company for Trading Drug, Chemicals, and Medical Instruments in Alexandria, Egypt. Additionally, all other chemicals utilized were of analytical grade.

Sample preparation

Orange peels were thoroughly washed under running tap water, after removing the tissues and fibers from it. The peels were chopped using a knife in small slices (about 1 cm2) to be ready for drying.

Drying method

During April 2020, five-kilograms of sliced orange peels were dried for a period of five days with an average of eight hours per day according to the method

of Abu-Arab et al., (2017). Until equilibrium moisture content was achieved to 10% moisture as (Manjarres-Pinzon et al., 2013; Adewole et al., 2014) used for orange peels. During the sun drying of sliced peels, air temperature and humidity measured using thermometer and hygrometer. The air temperature and relative humidity was recorded as 30 ± 2oC and 38 ± 6 %, respectively. Dried orange peels were ground to a fine powder and sifted through a 24-mesh size sieve. The powdered peels were then stored in dark glass airtight containers at room temperature until they were ready for use.

Proximate chemical composition of orange peels

Orange peels samples were analyzed for proximate chemical composition accordance with standard AOAC (2016). Protein (T.N. × 6.25, micro - kjeldahl apparatus, Velp company, Italy), fat (petroleum ether solvent) (soxhelt apparatus, Velp company. A muffle furnace maintained at 550°C for 5-6 hours used for ash content determination, Moisture, Crude fiber contents were determined in each analysis was carried out in triplicate. Total carbohydrates calculated by difference, Carbohydrates (%) = 100 - (% protein +% fiber + % moisture + % fat + % Ash).

Extraction of orange peels

Solvents extracted bioactive compounds from orange peel samples. including methanol, ethanol, ethyl acetate, acetone, dichloromethane, and n-hexane. For four hours at room temperature, 20 grams of peel powdered sample were mixed with 100 ml of solvents at 96 percent concentration. Extracts filtered, evaporated at 40°C under vacuum using Whatman No 42 -filter paper. After drying, the yield was calculated in grams of each extract then stored at 4°C in a closed vials (Ghareeb et al., 2016b).

Total Antioxidant Capacity (TAC)

Determination of total antioxidant capacity of each solvent extracts were evaluated using the phosphor-molybdenum method according to (Prieto et al., 1999; Ghareeb et al., 2016a; Ghareeb et al., 2016b).

Determination of total phenolic content (TPC)

By using Folin-Ciocalteu's assay the total phenolic content (TPC) was determined according to the procedure reported by (Ghareeb *et al*., 2014).

GC-MS analysis of the orange peels methanolic extracts

GC-MS analysis was performed according to the procedures reported by (Madkour et al., 2017). using a Thermo Scientific Trace GC Ultra/ISQ Single Quadrupole MS, TG-5MS fused silica capillary column

Fungal isolation

Soil samples were taken at a depth of 10 cm in the surrounding area of Mansoura Governorate, Egypt, in May 2020. The samples were sieved well, and air dried at 28°C for 5 days. Samples were kept at 10°C until they were used after drying. Soil samples were used to isolate fungal strains. The soils' microbes were counted using the serial dilution agar plating method. The soil suspension was serially diluted up to 10^6 dilutions. Then, at 28±2°C for 8 days, 100 µL of suspension from dilutions 10^3 to 10^6 was transferred to petri-dishes containing Czapex-Dox agar medium, the growth after two days was observed. The fungi were purified using the spore suspension and streak method after being isolated on culture medium from soil. The cultures were streaked onto fresh CD agar plates on a regular basis (every 6–8 days). The fungi were transferred three times on CD agar plates separately using the direct agar transfer method before being used for inoculation of liquid growth medium (Abdel-Aziz & Hezma 2013).

Fermentation and extraction

Czapek-Dox broth medium was mixed with orange peels in a 3:1 ratio, seeded with various fungi, then incubated for 15 days at 30°C before the extraction with ethyl alcohol (96 %). The antimicrobial properties of the extracts were tested (Abdel-Aziz & Hezma 2013).

Antimicrobial activity of the orange peel extracts

The antimicrobial activity of different extracts of orange peels were studied against different fungal strains according to (Ghareeb et al., 2015; Madkour et al., 2017).

Identification of fungal cultures

Fungal cultures were identified through a molecular biological approach involving DNA isolation, polymerase chain reaction (PCR) amplification, and sequencing of the Internal Transcribed Spacer (ITS) region. PCR amplification utilized ITS2 (GCTGCGTTCTTCATCGATGC) and ITS3 (GCATCGATGAAGAACGCAGC) primers, while sequencing employed ITS1 (TCCGTAGGTGAACCTGCGG) and ITS4 (TCCTCCGCTTATTGATATGC) primers. Purification of PCR products was performed using the Montage PCR Clean up kit (Millipore) to eliminate unincorporated PCR primers and dNTPs. Sequencing was carried out using the Big Dye Terminator cycle sequencing kit (Applied Bio Systems, USA), and the Applied Biosystems model 3730XL automated DNA sequencing system (Applied BioSystems, USA) was used to analyze the sequencing products. Candida sp. was utilized as a control, following the methodology described by Shawky et al. (2019).

Waffles making

Waffle control dough was made by mixing flour, salt, baking powder, and sugar in a bowl, then eggs, oil, milk, and vanilla were added to the flour mixture, then a portion of the mixture was distributed in a waffle machine at 60º C, for 10 minutes for baking until it became red and crispy (Huber & Schoenlechner, 2017). Experimental waffle was made by adding proportions of orange peels, which are 3%, 5% and 10% in succession. The waffle was also made by adding orange peels extracts in proportions of 0.05%, 0.1% and 0.2 according to the ratio reported by Huang et al., (2009). Tables (1) indicate the quantities used for making waffle.

Table 1: Ingredient of orange peels waffles and orange peels extract waffles before and after fermentation

Ingredients (g)	*C.W	*O.P.W 3%	*O.P.W 5 %	*O.P.W 10%	*O.P.E.W 0.05%	*O.P.E.W 0.1 %	*O.P.E.W 0.2%
Wheat flour (g)	100	97	95	90	99.95	99.9	99.8
Baking powder (g)	10	10	10	10	10	10	10
Eggs (g)	50	50	50	50	50	50	50
Oil (ml)	50	50	50	50	50	50	50
Milk (ml)	100	100	100	100	100	100	100
Vanilla extract (g)	1	1	1	1	1	1	1
Sugar powder (g)	15	15	15	15	15	15	15
Salt (g)	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Orange peels powder (g)	0	3	5	10	0.05	0.1	0.2

C.W= Control waffle O.P. W= Orange peels waffle O.P.E. W= orange peels extract waffle

Organoleptic evaluation of waffle

The sensory evaluation Nine-point test was conducted hedonic scale as reported by (Watts et al., 1989; Zaker et al., 2017), it was implemented by a well-trained 30-members comprising postgraduate and academic staff members at Alexandria University, Specific Education Faculty- Home Economics Department. They were requested to evaluate the characteristics of the produced crispy waffle. The assessment of items was conducted using a 9-point Hedonic scale, where participants rated their preferences with descriptive adjectives spanning from 9, representing "like highly," to 1, indicating "dislike extremely."

Statistical analysis

Data analysis was carried out using the IBM SPSS 23 statistical package application, following the approach outlined by Kirkpatrick and Feeney (2012). Triplicate analyses were conducted for all measurements and samples. Mean differences were examined at a 5% significance level using the Duncan test.

Additionally, the significance of the model was assessed using Analysis of Variance (ANOVA).

Results

Proximate chemical composition of orange peels

Proximate composition provides a general nutritional value of a food. Data in Table (2) shows the chemical composition of orange peels powder. From the table, noticed that carbohydrates were the highest content (58.98±0.51%) followed by crude fiber (13.45±0.01%) moisture (10.22±0.015%) total protein (8.35±0.020 %), ash (6.45±0.55%), and crude fat (2.53±0.021%). Table (2) revealed also These results are identical with that was obtained by Gotmare & Gade, (2018) since they made a preliminary analysis of orange peels residue, and the results were as follows: moisture (9.0%), fiber (15.3%), and ash (7.8%). Such data are in accordance with Abdelwahab & Abouelyazeed, (2018) which showed that carbohydrates are the highest amount of orange peels (69.47±0.87). Also, Adewole, et al., (2014) found that to be the orange peels contain moisture 10.00 %±0.01, ash content 5.51%±0.02, and crude fiber 12.47%±0.54. Also, orange peels were possessing a high fiber level these results agreed with Oikeh et al., (2013); Abd El-ghfar et al., (2016), they found that crude fiber was (13.43 ± 0.03, 12.46±0.01) respectively.

Table 2: Proximate chemical composition of orange peels (dry weight/100g)

Components (g/100g)	Content
Moisture	10.22±0.015
Total protein	8.35±0.020
Crude fat	2.53±0.021
Ash	6.45±0.55
Crude fiber	13.45±0.01
Carbohydrate	58.98±0.51

*Each value represents the mean of three replicates ±SD.

Total phenolic content (TPC) and total antioxidant capacity (TAC) of different solvent extracts of dried orange peels

Polyphenols are a group of compounds that act as primary antioxidants and free radical terminators (Ghareeb et al., 2016b). Various solvent extracts of orange peels.

were evaluated for their TP as presented in table (3). The results are in the order: MeOH (264.41) > EtOH (256.35) > EtOAc (237.29) > Me2CO (201.19) > CH2Cl2 (166.02) > C6H14 (127.37) mg GAE/ g dry extract. The results revealed that methanol, ethanol, ethyl acetate and acetone solvents were suitable for extraction of phenolic compounds ($P \leq 0.05$) than dichloromethane and n-hexane Due to their

higher polarity and enhanced solubility of phenolic components derived from plant sources (Clauditz et al., 2006; Kim et al., 2010; Marina & Noriham, 2014). Of the acids and essential compounds, phenols and flavonoids They are important phytochemicals of the fruit for their antioxidant properties. When used as a natural source of antioxidants in functional foods, the chelation of redox-active metal ions and the inhibition of hydroperoxide conversion to reactive oxyradicals. Phenolic content considers as an indicator of antioxidant capability and as a preliminary screen for peels (Abd El-ghfar et al., 2016). Consequently, there are strong positive correlation of TPC results and TAC values as presented in table (3). Also, the results are in the order: MeOH (679.77) > EtOH (642.61) > EtOAc (509.38) > Me2CO (454.63) > CH2Cl2 (280.87) > C6H14 (111.47) mg AAE/g dry extract. Because of their increased polarity and effective solubility of phenolic compounds, polar solvents such as methanol, ethanol, ethyl acetate and acetone are more suitable for extraction process of antioxidant compounds ($P \leq 0.05$) than dichloromethane and n-hexane (Cheng et al., 2015; El-Faham et al., 2016). Orange peels are rich source of natural flavonoids. Orange peels have the highest concentration of flavonoids, which accounts up almost half of orange content. The radical-scavenging activities of all the extracts were increased as with the rising concentration of the phenol and antioxidants content in orange peel (Ghasemi et al., 2009).

Table 3: Total phenolic content (TPC) and total antioxidant capacity (TAC) of different solvent extracts of dried orange peels

Extract solvents	(TAC) (mg AAE[2]/g dry extract)	(TPC) (mg GAE[3]/ g dry extract)
Methanol (MeOH)	679.77± 3.82	264.41 ± 2.05
Ethanol (EtOH)	642.61 ± 4.94	256.35 ± 4.38
Acetone (Me_2CO)	454.63 ± 3.78	201.19 ± 3.37
Ethyl acetate (EtOAc)	509.38 ± 2.87	237.29 ± 4.83
Dichloromethane (CH_2Cl_2)	280.87 ± 2.65	166.02 ± 3.37
n-hexane (C_6H_{12})	111.47 ± 3.02	127.37 ± 2.57

[1]Results are (means ± S.E.) (n = 3). [2]AAE: Ascorbic acid equivalent. [3]GAE: Gallic acid equivalent.

GC-MS investigation of the methanolic extract of orange peels before and after fermentation

GC-MS chromatogram of the orange peels ethanolic extract before fermentation
The GC-MS analysis of the ethanolic extract of orange peels disclosed the

presence of 38 compounds (Figure 1). The cumulative peak areas of the identified components make up 94.46%. The potential chemical structures of the identified compounds are detailed in Table 4. The main determined compounds are 1,2-Benzenedicarboxylic acid, butyl 2-methylpropyl ester (30.87%), 4-Benzyloxybenzoic acid (14.30%), Tetrahydro linalool (8.59%), Tripropylene glycol 2 (5.52%), Benzoic acid, 2-hydroxy, 2-methylbutyl ester (5.28%), Linalyl propionate (4.63%), 2 Hydroxytricyclo [5.2.1(1,4).0(5,9).]dec-7-ene (3.76%), Benzoic acid, 2-hydroxy, pentyl ester (2.62%), 2-Propanol, 1,1'-[(1-methyl-1,2-ethan ediyl)bis(oxy)]bis (2.44%), and Cyclopenta[g]-2-benzopyran, 1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethyl (1.77%), for which represented 79.78% of the overall peak areas. The identification was achieved via using computer search user-generated reference libraries, incorporating mass spectra (Madkour et al., 2017; Shawky et al., 2019).

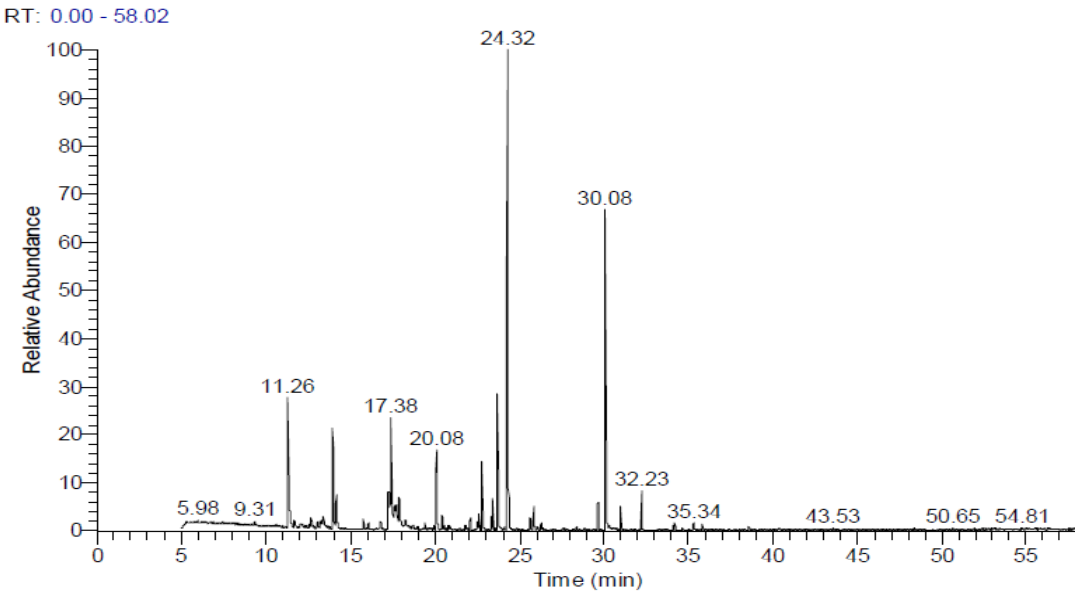


Fig. 1: GC-MS chromatogram of the orange peels methanolic extract before fermentation

Table 4: Chemical compositions of the orange peels before fermentation

No.	R_t	Area %[a]	M.W.	M.F.	Main Fragments	Identified compounds	Class/Category
1	9.32	0.22	126	$C_8H_{14}O$	39, 41, 55, 67, 79, 93, 108, 126	Cyclohexanemethanol, 4-methylene	Cycloalkane derivatives
2	11.26	8.59	158	$C_{10}H_{22}O$	43, 55, 69, 73, 111, 129	Tetrahydro linalool	Noncyclic monoterpenoid
3	11.67	0.41	84	C_6H_{12}	27, 29, 39, 41, 55, 69, 84	2-Pentene, 3-methyl	Alkene derivatives
4	12.66	0.61	154	$C_{10}H_{18}O$	43, 55, 69, 71, 93, 107, 121, 136	à-Terpineol	Monoterpenoids
5	13.06	0.38	236	$C_{16}H_{28}O$	41, 59, 67, 79, 93, 107, 135, 203, 218	2-Methyl-5-(1-adamantyl)pentan-2-ol	Cycloalkane derivatives
6	13.24	0.40	154	$C_{10}H_{18}O$	31, 43, 58, 69, 71, 93, 107, 121, 136	p-Menth-8-en-1-ol	Monoterpenoids
7	13.94	4.63	210	$C_{13}H_{22}O_2$	43, 59, 81, 93, 107, 121, 136	Linalyl propionate	Acyclic monoterpenoids
8	14.16	1.48	136	$C_{10}H_{16}$	39, 41, 77, 93, 107, 121, 136	à-Terpinene	Monoterpenoids
9	15.75	0.45	196	$C_{12}H_{20}O_2$	55, 69, 80, 93, 107, 121, 136, 154	Linalyl acetate	Monoterpene ester
10	16.72	0.29	174	$C_{10}H_{22}O_2$	27, 39, 41, 59, 82, 95, 123, 138	2,7-Dimethyl-2,7-octane diol	Alkane derivatives
11	16.79	0.24	210	$C_{15}H_{30}$	29, 41, 43, 55, 69, 71, 84, 112	2,4,6,8-Tetramethyl-1-undecene	Alkene derivatives
12	17.24	2.44	192	$C_9H_{20}O_4$	29, 31, 45, 59, 103, 117, 130, 161	2-Propanol,1,1'-[(1-methyl-1,2-ethan ediyl)bis(oxy)]bis	Alkane derivatives
13	17.38	5.52	192	$C_9H_{20}O_4$	45, 59, 103, 161	Tripropylene glycol 2	Alkane derivatives
14	17.59	0.61	134	$C_6H_{14}O_3$	31, 45, 59, 103	1-Propanol, 2-(2-hydroxypropoxy)	Alkane derivatives
15	17.75	0.71	318	$C_{14}H_{22}O_8$	43, 57, 73, 115, 159, 203	1,4-Diacetyl-3-acetoxymethyl-2,5 methylene-l-rhamnitol	Ketonic derivatives
16	17.88	1.20	190	$C_9H_{18}O_4$	41, 43, 57, 71, 85, 103, 117, 175	Butanoic acid, 4-(1,1-dimethylethoxy)-3-hydroxy, methyl ester,(R)	Aliphatic ester derivatives
17	19.37	0.29	170	$C_{12}H_{26}$	29, 41, 43, 57, 71, 85, 112, 155, 170	Undecane, 5-methyl	Alkane
18	20.08	3.76	150	$C_{10}H_{14}O$	27, 39, 66, 82, 91, 117, 132, 150	2-Hydroxytricyclo[5.2.1(1,4).0(5,9).]dec-7-ene	Alkene derivatives
19	20.87	0.22	150	$C_{10}H_{14}O$	27, 41, 79, 91, 107, 135, 150	3,5-Heptadienal, 2-ethylidene-6-methyl	Unsaturated aldehyde
20	22.50	0.26	158	$C_{10}H_{22}O$	27, 45, 55, 87, 115, 143	4-Nonanol, 4-methyl	Aliphatic alcohol

21	22.58	0.65	204	$C_{14}H_{20}O$	41, 57, 91, 117, 131, 147, 189	Lily aldehyde	Aldehyde derivatives
No.	R_t	Area %[a]	M.W.	M.F.	Main Fragments	Identified compounds	Class/Category
22	22.76	2.62	208	$C_{12}H_{16}O_3$	39, 43, 65, 92, 120, 138	Benzoic acid, 2-hydroxy, pentyl ester	Benzoic acid derivatives
23	23.30	0.50	154	$C_{10}H_{18}O$	27, 39, 41, 69, 81, 93, 109, 121, 154	Isogeraniol	Monoterpenoids
24	23.69	5.28	208	$C_{12}H_{16}O_3$	43, 55, 65, 92, 120, 138, 179	Benzoic acid, 2-hydroxy, 2-methylbutyl ester	Benzoic acid derivatives
25	24.32	30.87	278	$C_{16}H_{22}O_4$	41, 57, 76, 104, 149, 223	1,2-Benzenedicarboxylic acid, butyl 2-methylpropyl ester	Benzoic acid derivatives
26	25.62	0.46	222	$C_{15}H_{26}O$	41, 55, 83, 98, 109, 125, 138, 161, 207, 222	Patchouli alcohol	Sesquiterpenoid tertiary alcohol
27	25.84	1.02	220	$C_{13}H_{16}O_3$	27, 39, 55, 82, 93, 121, 138, 194, 220	cis-3-Hexenyl salicylate	Benzoate ester
28	26.29	0.23	128	C_9H_{20}	29, 41, 57, 85, 99, 113	Hexane, 3-ethyl-3-methyl	Alkane derivatives
29	29.65	1.77	258	$C_{18}H_{26}O$	141, 155, 171, 213, 243	Cyclopenta[g]-2-benzopyran, 1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethyl	Benzopyran derivatives
30	30.07	14.30	228	$C_{14}H_{12}O_3$	39, 65, 91, 109, 181, 208	4-Benzyloxybenzoic acid	Benzoic acid derivatives
31	30.96	0.92	270	$C_{17}H_{34}O_2$	43, 55, 74, 87, 97, 101, 143, 185, 227, 239	Pentadecanoic acid, 14-methyl, methyl ester	Fatty acid esters
32	32.23	1.53	284	$C_{18}H_{36}O_2$	43, 55, 88, 101, 115, 157, 199, 241,	Hexadecanoic acid, ethyl ester	Fatty acid esters
33	34.09	0.22	340	$C_{22}H_{44}O_2$	41, 43, 55, 67, 81, 96, 109, 123	(Z)-9-Docosene-1,22-diol	Aliphatic di-alcohol
34	34.19	0.36	296	$C_{19}H_{36}O_2$	41, 55, 69, 84, 97, 123, 180, 222, 264	9-Octadecenoic acid, methyl ester	Fatty acid esters
35	35.26	0.21	322	$C_{21}H_{38}O_2$	41, 67, 81, 95, 109, 123, 192, 291	11,14-Eicosadienoic acid, methyl ester	Fatty acid esters
36	35.35	0.25	282	$C_{18}H_{34}O_2$	55, 69, 88, 97, 101, 111, 123, 152, 194, 236	Ethyl 9-Hexadecenoate	Fatty acid esters
37	35.80	0.30	312	$C_{20}H_{40}O_2$	29, 43, 55, 73, 88, 101, 115, 157, 221	Octadecanoic acid, ethyl ester	Fatty acid esters
38	38.54	0.26	168	$C_{10}H_{16}O_2$	39, 41, 55, 81, 97, 108, 111, 132, 149, 150	Spiro[4.5]decan-1-one, 6-hydroxy	Cycloalkane derivatives
		T % 94.46					

[a] **Area%: Bold percentages refer to major identified compounds/ Rt: Retention time; M.W.: Molecular weight; M.F.: Molecular formula.**

GC-MS investigation of the orange peels after fermentation

The GC-MS analysis of the third sample revealed the presence of 14 compounds (Figure 2). The total peak areas of the identified ingredients constitute 62.59%. The potential chemical structures of the identified compounds are documented in Table 5. The primary detected compounds include: trans-13-Octadecenoic acid (14.36%), 1-decylaminomethyl-1,2,3,4-tetrahydroisoquinoline (10.86%), L-Isoleucine, methyl ester (9.82%), N, N-Diethyl-4-pyridylethylamine (7.58%), 9,12-Octadecadienoic acid, methyl ester (7.46%), and 5-cyclohexyl-5H-furan-2-one (5.33%), for which represented 55.41% of the overall peak areas.

The identification was made possible by computer searches that included mass spectra and user-generated reference libraries. (Madkour et al., 2017; Abdel-Wareth et al., 2019; Shawky et al., 2019; Khalaf et al., 2020). According to (Negro et al., 2016) and the results mentioned above, we could use the orange peel as a natural additive in bakery products and in the fields of nutrition, pharmaceutical, and cosmetics.

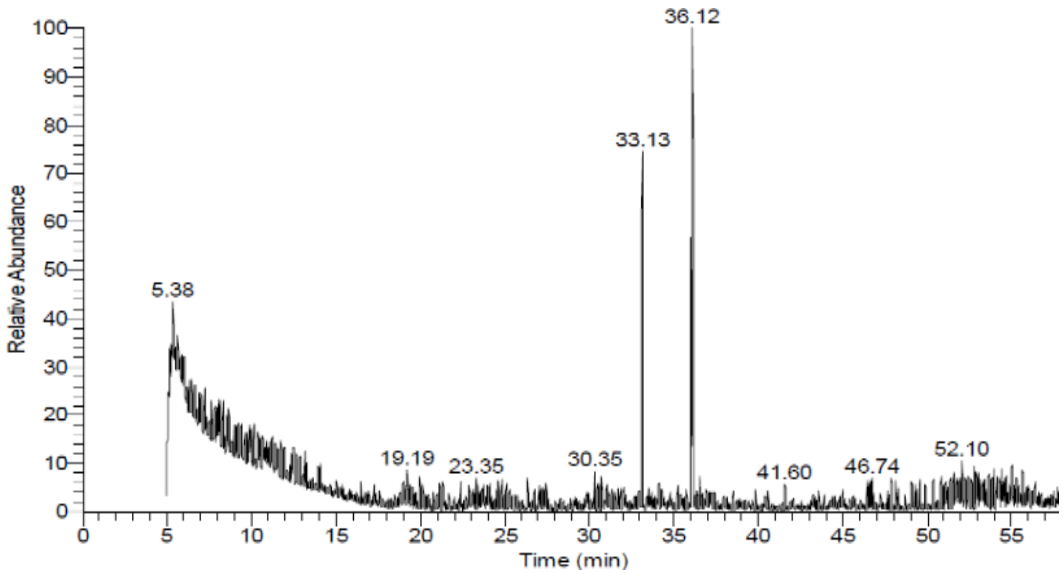


Fig. 2: GC-MS chromatogram of the orange peels after fermentation

Table 5: Chemical compositions of the orange peels after fermentation

No	R_t	Area %[a]	M.W.	M.F.	Main Fragments	Identified compounds	Class / Category
1	5.19	9.82	145	$C_7H_{15}NO_2$	41, 44, 57. 69. 86	L-Isoleucine, methyl ester	Amino acid ester
2	5.27	5.33	166	$C_{10}H_{14}O_2$	59, 84, 115, 166	5-cyclohexyl-5H-furan-2-one	Butenolide derivatives
3	5.37	7.58	178	$C_{11}H_{18}N_2$	58, 86, 105, 119	N,N-Diethyl-4-pyridylethylamine	Amine derivatives
4	9.34	0.79	740	$C_{50}H_{44}O_6$	181, 331, 481, 649	2,2',4,4',5-Pentabenzyloxy-6,6'-dimethyl Benzophenone	Benzophenone derivatives
5	19.19	0.77	276	$C_{15}H_{16}O_5$	49, 51, 84, 111, 139, 156	7-(2,3-Epoxy-3-methybutoxy)-6-methoxy Coumarin	Coumarin derivatives
6	29.85	0.98	686	$C_{41}H_{66}O_8$	93, 146, 253, 368, 460, 555, 671	(2R)8,13-epoxy-2,2-(8',13'-epoxy-2' bmethoxy-3'-oxolabdane-1'a,2'a-diyldioxy) 1a-hydroxylabdan-3-one	Triterpenoids
7	33.13	10.86	302	$C_{20}H_{34}N_2$	55, 73, 115, 131, 145, 229, 269, 296	1-decylaminomethyl-1,2,3,4-tetrahydroisoquinoline	Isoquinoline derivatives
8	36.04	7.46	294	$C_{19}H_{34}O_2$	41, 55, 67, 81, 95, 109, 149, 178, 263	9,12-Octadecadienoic acid, methyl ester	Fatty acid methyl ester
9	36.12	14.36	282	$C_{18}H_{34}O_2$	29, 41, 59, 69, 83, 97, 111, 151, 180	trans-13-Octadecenoic acid	A long-chain fatty acid
10	36.56	0.82	298	$C_{19}H_{38}O_2$	43, 55, 74, 87, 129, 143, 199, 255	Octadecanoic acid, methyl ester	Fatty acid esters
11	51.17	0.80	266	$C_{10}H_{18}O_6S$	73, 95, 120, 184, 207, 260	(+) Methyl [2,6-cis-4-(Methanesulfonyl) oxy-6-methyltetrahydropyran-2-yl)acetate	Pyran derivatives
12	52.97	1.28	611	$C_{38}H_{33}N_3O_5$	69, 133, 196, 207, 341, 365, 475, 538, 599	N-Cyclohexyl-1,7-dipyrrolidinylperylene-3,4: 9,10-tetracarboxylicacid3,4-anhydride-9,10-imide	Heterocyclic derivatives
13	53.17	0.87	692	$C_{44}H_{44}N_4O_{42}$	55, 72, 135, 224, 281, 401, 443, 510, 661	N, N'-Dicyclohexy -l1,7- dipyrrolidinyl perylene-3,4: 9,10- tetra-carboxylic acid bisimide	Heterocyclic derivatives
14	54.61	0.87	660	$C_{30}H_{28}O_{17}$	69, 135., 180, 207, 281, 402, 503, 628	Methyl pentaacetyl lactothamnolate	Carbohydrate derivatives
		T% 62.59					

[a] Area%: Bold percentages refer to major identified compounds/ Rt: Retention time; M.W.: Molecular weight; M.F.: Molecular formula.

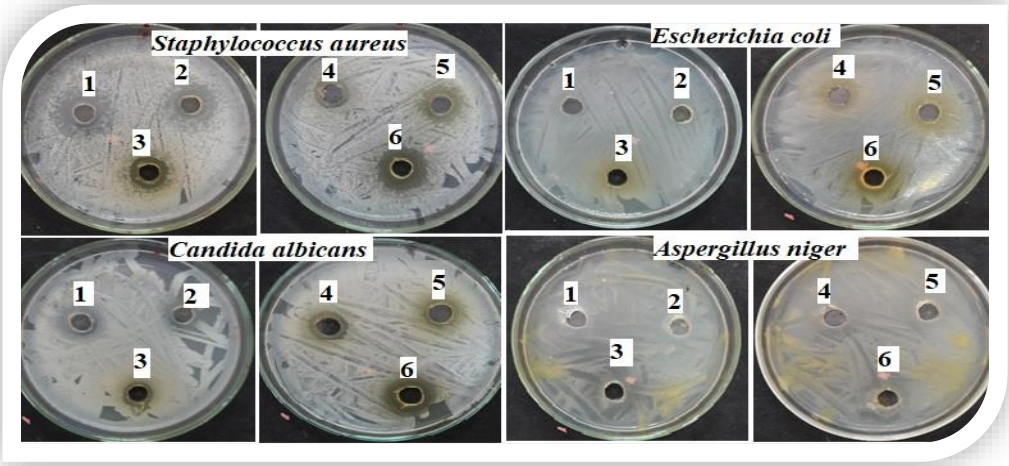
The antimicrobial activities of orange peels extracts

The increasing interest from consumers and the food industry in incorporating natural antibacterial agents into food products is notable. These compounds possess the capacity to break down cell walls and disrupt enzymes integrated into the cytoplasmic membrane, leading to cellular demise. The orange peel extract exhibited varied degrees of inhibition in the growth of tested microorganisms, as outlined in Table 6 and Figure 3. Except for Staphylococcus aureus treated with orange peel hexane extract, which displayed the lowest antimicrobial efficacy, the results indicated noteworthy differences ($p < 0.05$) in the inhibitory effects on the tested microorganisms, with inhibition zones ranging from 13 to 39 mm. The orange peel acetone extract demonstrated the highest inhibition against Aspergillus niger. Considering both economic and environmental perspectives, utilizing this byproduct as a source of cost-effective natural antimicrobials holds significant potential (Taveira et al., 2010; El-Faham et al., 2016).

Table 6: The antimicrobial activities of different orange peels extract against *Escherichia coli, staphylococcus aureus, Candida albicans* and *Aspergillus niger*

No	Solvent extract	Inhabitation Zone (ϕmm)			
		Staphylococcus aureus	*Escherichia coli*	*Candida albicans*	*Aspergillus niger*
1	Hexane	17	0	14	0
2	Dichloromethane	19	0	14	18
3	Methanol	18	0	31	25
4	Ethanol	16	15	29	12
5	Acetone	19	14	32	13
6	Ethyl acetate	25	18	17	12

1- Hexane 2- Dichloromethane 3- Methanol 4- Ethanol 5- Acetone 6- Ethyl acetate



Where 1- Hexane 2- Dichloromethane 3- Methanol 4- Ethanol 5- Acetone 6- Ethyl acetate

Fig. 3: The antimicrobial activities of different orange peels extracts against *Escherichia coli, staphylococcus aureus, Candida albicans* and *Aspergillus niger*

The antimicrobial activity of fermented orange peels

Orange peel extract contains antimicrobial compounds that can degrade cell walls, disrupt enzymes, and potentially cause cell death. The activity of different fungi which were grown on orange peels extract against different test microbes representing Various microorganism growth inhibition levels observed. as shown in table (7) and figure (4). the results of fermented orange peels to fight bacteria ($p<0.05$) inhibitory zones ranging from 0 to 35 mm, among inhibiting effects on the studied microorganisms. Consequently, the greatest inhibition was attained for Escherichia coli with ethanol, except for Aspergillus niger with fermented orange peels acetone extract, this showed the least amount of antibacterial activity (El-Faham et al., 2016). The results of the study agree with what reported by Yashaswini, (2018) which showed that orange peel could create antimicrobial chemicals, which would be necessary for microbial infection resistance. Antimicrobials and antibiotics derived from plants could be considered to be more effective, with fewer side effects.

Table 7: The antimicrobial activity of different fungi grown on orange peels

Extracts from Fungi grown on orange peels	*Staphylococcus aureus*	*Escherichia coli*	*Candida albicans*	*Aspergillus niger*
1	14	14	13	12
2	17	18	17	0
3	18	20	16	13
4	18	17	18	12
5	17	16	14	0

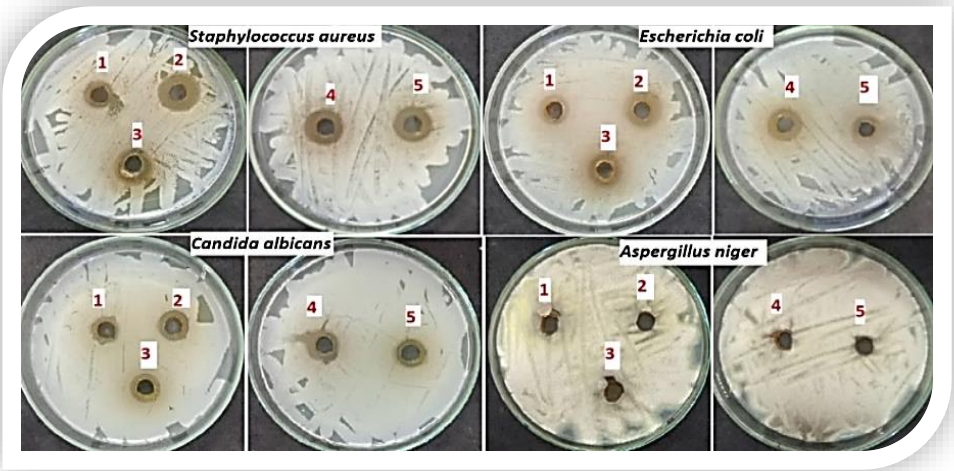


Fig .4: The antimicrobial activities of different fungal extracts against Escherichia coli, Staphylococcus aureus, Candida albicans and Aspergillus niger

Molecular Identification of the potent fungal isolate 4

The molecular approach (18S rRNA) as an accurate tool for identification was employed to distinguish fungal strains (4b and Asp) obtained from soil samples at Mansoura Governorate, a nucleotide sequence of 559 bp of the whole 18S rRNA gene of the fungal sp. In both strands, isolate 4 that was developed on orange peels was identified. BLAST search revealed 100% similarity to Talaromyces purpureogenus strain CBS 129768 (accession number MH865647.1). The phylogentic tree of this fungal was also constructed (Figure 5).

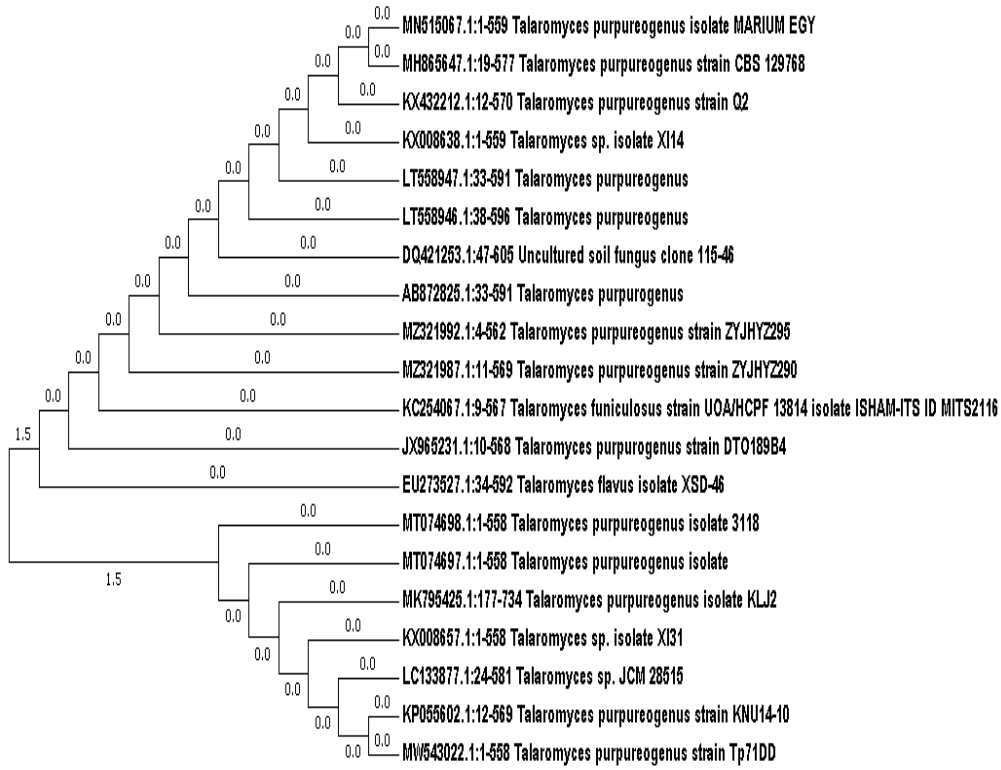


Fig.5: Phylogenetic trees showing relationship of strain *Talaromyces purpureogenus MARIUM* EGY with other related fungal species retrieved from Gen Bank based on their sequence homologies of 18S rRNA

Sensory evaluation of produced waffle

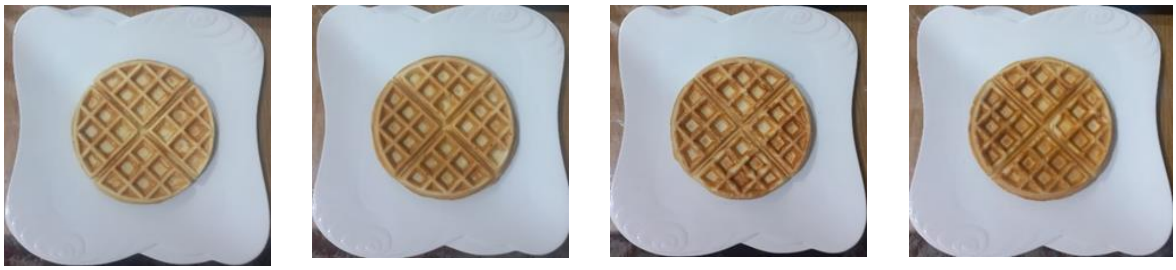
Sensory evaluation of orange peels powder waffle (n = 30)

The sensory assessment of the orange peel-derived natural additives used to make waffles (OP) is shown in Table (8). The color value steadily increased as the proportion of integrated orange peels increased, this is consistent with what was confirmed by several studies on bakery products fortified with orange peels compared to the controls (Sharoba et al., 2013; Zaker et al., 2017; Zoair et al., 2019). The statistically significant ($p < 0.05$) differences for taste, texture and color parameters were obtained regarding the sample containing 3% from orange peels addition. Concerning the study of the impact of orange peels powder on the sensory properties of waffle, data noted that the replacement up to 5% from orange peels powder had significant ($p < 0.05$) results and increased the scores for taste, texture, color and total acceptance as compared to control. It was observed that color, taste and texture had significant ($p < 0.05$) reduction, it was obtained only for 10 % (OPW). However, an improvement in color and taste of waffle refers to the yellowish color resulting from the natural pigments present in peels, this result agrees with El wardany (2016), where the bakery product that received the highest acceptability score was the formulation incorporating orange peels at (3%, 5%) level concentration, it recommends the importance of the balance between orange peels as additive to obtain a fortified product without harming the consumers' willingness to accept it. without harming the consumers' willingness to accept it.

Table 8: Sensory evaluation of orange peels powder waffle (n = 30)

Sample	Appearance	Taste	Texture	Color	odor	Acceptability
Control	$8.27^{ab}\pm0.98$	$8.23^{ab}\pm0.77$	$8.43^{a}\pm0.57$	$8.70^{a}\pm0.59$	$8.33^{a}\pm0.48$	$8.67^{a}\pm0.66$
OPW 3%	$8.47^{a}\pm0.63$	$8.50^{a}\pm0.51$	$8.23^{ab}\pm0.43$	$8.17^{b}\pm0.53$	$8.47^{a}\pm0/63$	$8.47^{a}\pm0.57$
OPW 5%	$8.30^{ab}\pm0.65$	$8.03^{bc}\pm0.67$	$7.90^{b}\pm0/76$	$8.10^{b}\pm0.71$	$8.30^{a}\pm0.65$	$8.33^{a}\pm0.48$
OPW 10%	$8.01^{b}\pm0.83$	$7.70^{c}\pm0.98$	$7.37^{c}\pm0.85$	$7.97^{b}\pm0.89$	$8.13^{a}\pm0.90$	$7.20^{b}\pm0.89$
(F)	1.81	5/98	14.43	6.43	1.21	9.27
(P)	0.140	0.001	.001	0.001	0.308	0.001

***Orange peels waffle =OPW, *Values are expressed as means ± SE Mean values within a column not sharing common superscript letters (a, b, c, d, e, f) were significantly different ($p < 0.05$)**



1-Control orange peels waffle 2- orange peels waffle 3% 3- orange peels waffle 5% 4-orange peels waffle 10%

Fig. 6: Sensory evaluation of orange peels powder waffle.

Sensory evaluation of orange peels extract waffle before and after fermentation (n = 30)

The sensory evaluation of orange peel extract waffle (OPEW) at different concentrations is summarized in Table 9, comparing them to the control sample. The data revealed significant differences between the control and waffle samples in certain characteristics both before and after fermentation. Notably, the orange peel extract waffle (OPEW) at a concentration of 0.05% received high acceptance and achieved the maximum score for sensory properties. A similar positive trend was observed for the 0.1% concentration sample compared to the control. However, at the 0.2% concentration level, the mean scores were lower, indicating that the sample was deemed unacceptable compared to the control, Table 9 also illustrates that the waffle containing 0.05% orange peel extract (OPEW) exhibited the highest total scores (8.40 ± 0.49, 8.43 ± 0.57) for orange peels before and after fermentation, respectively, followed by the 0.1% concentration level (8.17 ± 0.69, 8.10 ± 0.76), respectively.

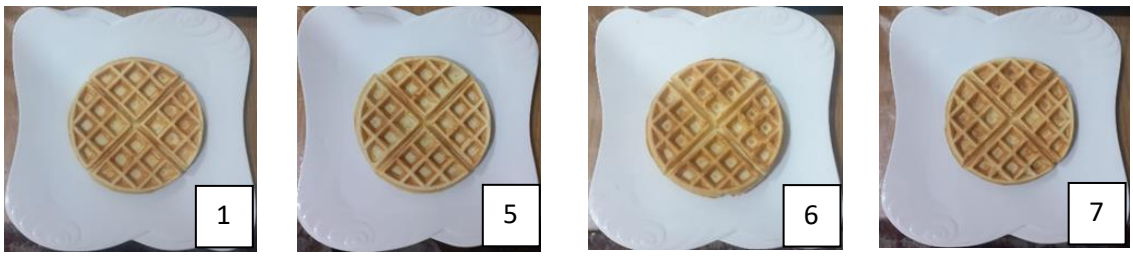
These results show that the orange peel extract could be added in amounts up to 0.1% in the formula of waffle without adversely affecting sensory characteristics of waffle. Appearance, taste and odor differences between the examined samples were not statistically significant (p 0.05). In the study by Elhassaneen et al., (2016). The appearance, odor, and taste of the waffle fortified with orange peel extract were not affected, indicating that each recipe may be well-received by customers. In contrast, a study by Pereira et al. (2020) found that waffle formulations containing 0.02% of orange peel extract showed statistically lower sensory grades for overall acceptance compared to the control sample. However, in the present investigation, the addition of orange peel extract

enhanced the antibacterial activity of the enriched waffle. In another study by Huang et al. (2009), the use of orange peel extract at levels of 0.5% and 0.1% was considered the best choice due to increased antioxidant and antibacterial activity, which are key acceptability criteria for certain types of waffle, Differences between the waffle samples made of orange peel extract compared to their structural properties are shown in Table No. (9). The changes in appearance, taste and odor did not negatively affect the overall impression. The study of Sagar et al., (2018) highlighted the significance of the balance when using orange peels to obtain a fortified product which will not negatively affect the acceptability of the trained panelist and consumers. On the other side, according to Sagar et al., (2018) using waste to produce diverse essential bioactive elements represents a crucial stride toward sustainable growth, especially waste from fruits such as oranges.

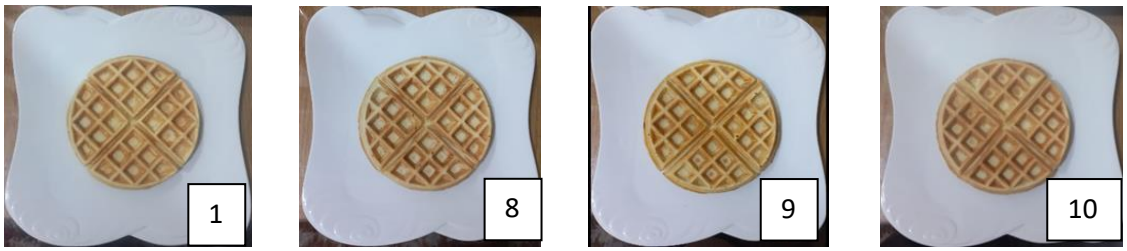
Table 9: Sensory evaluation of orange peels extract waffle before and after fermentation (n = 30)

Sample	Appearance		Taste		Texture		Color		odor		Acceptability	
	Before	after	before	After	before	after	before	After	before	after	before	after
Control	8.27^{ab} ±0.98	8.27^{a}± 0.98	8.23^{a}± 0.77	8.23^{a}± 0.77	8.43^{a}± 0.57	8.43^{a}± 0.57	8.70^{a}± 0.59	8.70^{a}± 0.59	8.33^{a}± 0.48	8.33^{a}± 0.48	8.67^{a}± 0.66	8.67^{a}± 0.66
OPEW 0.05%	8.23^{ab} ± 0.63	8.40^{a}± 0.68	8.30^{a}± 0.54	8.37^{a}± 0.56	7.83^{b}± 0.59	8.07^{b}± 0.52	8.40^{a}± 0.49	8.47^{a}± 0.51	8.17^{a}± 0.75	8.23^{a}± 0.62	8.40^{ab} ± 0.49	8.43^{ab} ± 0.57
OPEW 0.1%	8.50^{a}± 0.57	8.33^{a}± 0.66	8.23^{a}± 0.62	8.20^{a}± 0.48	8.01^{b}± 0.83	8.03^{b}± 0.81	8.37^{a}± 0.72	8.13^{b}± 0.43	8.10^{a}± 0.76	8.17^{b}± 0.74	8.17^{b}± 0.69	8.10^{bc} ± 0.76
OPEW 0.2%	7.97^{b}± 0.89	8.07^{a}± 0.83	8.13^{a}± 0.78	8.17^{a}± 0.75	7.50^{c}± 0.51	7.77^{b}± 0.50	7.97^{b}± 0.96	7.90^{b}± 0.71	7.53^{b}± 0.84	7.97^{b}± 0.56	7.57^{c}± 0.73	8.03^{b}± 0.77
(F)	2.31	0.981	0.30	0.542	1.15	5.99	5.30	1.48	5.45	1.93	5.49	5.47
(P)	0.080	0.404	0.82	0.654	0.001	0.001	0.002	0.001	0.002	0.128	0.001	0.001

*Orange peels extract waffle =OPEW، *Values are expressed as means ± SE. Mean values within a column not sharing common superscript letters (a, b, c, d, e, f) were significantly different ($p < 0.05$)



Before 1-Control orange peels waffle, 5- orange peels extract waffle 0.05%, 6- orange peels extract waffle 0. 1%, 7-orange peels extract waffle 0.2%



After 1-Control orange peels waffle, 8- orange peels extract waffle 0.05%, 9- orange peels extract waffle 0.1%, 10-orange peels extract waffle 0.2%

Fig. 7: Sensory evaluations of orange peels extract waffle before and after fermentation.

Conclusion

The quality of crispy waffle products was affected by addition orange peels and there extracts as natural food additives. The antioxidant capacity and antimicrobial activity constituents of orange peels extracts showed that *Citrus sinensis* fruit wastes contain useful antimicrobial activity products and a high content of antioxidants. Orange peels were rich source of natural phenolic acids and flavonoids. The orange peel extraction with methanol, ethanol, ethyl acetate and acetone were efficient in the extraction of phytochemical compounds. This study was focused on minimizing the waste of fruit juice industry. The study recommended that orange peel can be inserted into several bakery recipes to enhance the chemical composition of ingredients.

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