DOI: 10.21608/pssrj.2022.115574.1159

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

نيفين الورداني[١] ، محمد عبد العزيز[٢]، مريم عبد القادر[١]، مسعد غريب [٣]

[١]قسم الاقتصاد المنزلي– كلية التربية النوعية – جامعة الاسكندرية، مصر.

[٢]قسم كيمياء الكائنات الدقيقة – المركز القومى للبحوث، القاهرة، مصر.

[٣]قسم الكيمياء العلاجية – معهد تيودور بلهارس للابحاث، القاهرة، مصر.

nevenmr@gmail.com, mohabomerna@yahoo.com, amrym4025@gmail.com, m.ghareeb@tbri.gov.eg.

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

Neveen A. Elwardany[1], Mohamed S. Abdel-Aziz[2], Mariam A. Abdelkader[1] Mosad A. Ghareeb[3].

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

نيفين الورداني[1] ، محمد عبد العزيز[٢] ، مريم عبد القادر [١] ، مسعد غريب [٣]

*[١]قسم الاقتصاد المنزلي– كلية التربية النوعية – جامعة الاسكندرية ، مصر.

*[٢]قسم كيمياء الكائنات الدقيقة – المركز القومى للبحوث ، القاهرة ، مصر.

*[٣]قسم الكيمياء العلاجية – معهد تيودور بلهارس للابحاث ، القاهرة ، مصر.

المستخلص: اجريت هذه الدراسة بهدف الاستفادة من قشور البرتقال ومستخلصاتها الطبيعية المختلفة كمضافات غذائية ذات قيمة عالية لانتاج الوافل المقرمش. تعتبر قشور البرتقال من المضافات الغذائية الجيدة وكذلك مستخلصاتها بالمذيبات المختلفة. كما يمكن استخدام مستخلصات الفطريات المزروعة على هذه القشور لتحسين القيم المضافة للغذاء. يمكن استخدام قشور البرتقال الطازجة ومستخلصها الإيثانولي لإنتاج منتجات غذائية ذات قيمة عالية مثل الوفل. وفي الوقت ذاته يمكن استخدام المستخلصات الإيثانولية للفطريات المزروعة على قشور البرتقال لنفس الغرض. تم اجراء تحليل لمستخلصات قشور البرتقال كيميائيا. من جانب اخر تم إضافتها إلى الوافل وتم اجراء للتقييم الحسي للمنتجات. أظهرت النتائج أن قشور البرتقال احتوت على نسبة رطوبة ١٠.٢٢٪، بروتين ٨.٣٥٪، دهون ٢.٥٣٪، رماد ٦.٤٥٪، ألياف خام ١٣.٤٥٪، كربوهيدرات ٥٨.٩٨٪. اوضحت النتائج ان الميثانول ، والإيثانول ، وخلات الإيثيل أفضل من الأسيتون، والهكسان، وثاني كلورو ميثان في استخلاص المركبات الفينولية من قشور البرتقال. كما اظهرت النتائج ان قشور البرتقال تحتوى على نسبة عالية من المركبات الفينولية والفلافونويد ومضادات الأكسدة. أظهرت النتائج وجود فروق معنوية ($p < 0.05$) بين تأثيرات التثبيط على الكائنات الحية الدقيقة المختبرة ، حيث تراوحت مناطق التثبيط من ١٣ إلى ٣٩ مم. ومن ثم تم الحصول على أعلى نسبة تثبيط للفطر اسباجلس نيجر (**Aspergillus niger**) مع مستخلص الأسيتون مع قشور البرتقال. نستنتج من ذلك ان هذا المنتج الثانوي ذا فائدة كبيرة من الجوانب الاقتصادية والبيئية كمصادر لمضادات الميكروبات الطبيعية منخفضة التكلفة. اوضحت نتائج التقييم الحسى للوافل المقرمش مع قشور البرتقال ومستخلصاتها (قبل وبعد التخمر) تحسنا كبيرا فى التقبل العام. حيث تبين ان الوافل المقرمش المحتوى على قشور البرتقال بنسبة ٣%، ٥% هو الاعلى معنويا من حيث التقبل، كما اظهرت النتائج ان الوافل المدعم بمستخلصات قشور البرتقال (قبل وبعد التخمر) كانت معاملة الوافل المقرمش المحتوية على نسبة ٠.٠٠٥% من مستخلصات قشور البرتقال هى الافضل معنويا من حيث التقبل العام مقارنة بنسبة ٠.٠١%، ٠.٠٢% وكانت الاقرب للعينة الضابطة (الضابطة) قبل وبعد التخمر. تبين نتائج هذه الدراسة ان قشور البرتقال ومستخلصاتها تحتوى على نسبة عالية من الفينولات ومضادات الاكسدة ، كما انها تعد مصادر غنية بمضادات الميكروبات، ومن ثم فانه يوصى باستخدام هذه القشور ومستخلصاتها في انتاج مخبوزات كالوافل ذات قيمة غذائية عالية.

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

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

Neveen A. Elwardany[1], Mohamed S. Abdel-Aziz[2], Mariam A. Abdelkader[1] Mosad A. Ghareeb[3]

[1]Home Economics Dept, Fac, of Specific Education, Alexandria Univ, Egypt
[2]Microbial Chemistry Department, National Research Centre, Dokki, Cairo 12622, Egypt.
[3]Medicinal Chemistry Department, Theodor Bilharz Research Institute, Warrak El-Hadar, Imbaba (P.O. 30), Giza 12411, Egypt

Abstract

The present study aimed to utilize orange peel and their various natural extracts as valuable food additives in production of crispy waffle. Orange peels are considered as a good food additive as well its solvents extracts. Extracts of fungi grown on these peels could also be used to improve the additive values of food. Fresh orange peels and their ethanolic extract could be used to produce value-added products like waffle. Meanwhile, ethanolic extracts of fungi grown on orange peels could be used in the same purpose. Orange peels extracts were analyzed chemically. They were also added to waffle and subjected to sensory evaluation. The results showed that the orange peels contained moisture 10.22%, protein 8.35%, fat 2.53%, ash 6.45%, crude fiber 13.45%, and carbohydrates 58.98%. Phenolic components from plant materials, methanol, ethanol, and ethyl acetate were better than acetone, *n*-hexane, and dichloromethane in extracting phenolic compounds from orange peels. Orange peels were rich source of natural phenolic acids and flavonoids. The antioxidant capacity and antimicrobial activity constituents of orange peels extracts showed that *Citrus sinensis* fruit by-products contain useful antimicrobial activity products and a high content of antioxidants. Before and after fermentation, sensory evaluations of the waffle with orange peels and extract revealed a high level of acceptability. Therefore, the result recommended that orange peels and their extract could be used in the production of bakery products such as waffle with high nutritional value.

Keywords:

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

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

Neveen A. Elwardany[1], Mohamed S. Abdel-Aziz[2], Mariam A. Abdelkader[1] Mosad A. Ghareeb[3].

Introduction

Fruits contain thousands of phytochemicals, providing them with potential health benefits that help in protecting the body from diseases. The antioxidant activity of phenolic compounds is due to their ability to scavenge free radicals (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 wastes can be recycled as raw materials, converted into higher-value byproducts, or used as food or feed (Nandini *et al.*, 2013). Annually, 1.3 billion tons of different types of food wasted throughout the supply chain could feed as many as two billion people without any additional impact on the environment as identified by FAO (Williamson *et al.*, 2016). Transforming waste materials into useful final products has become a popular area of research. This is especially true in the food and food processing industry, where wastes, effluents, residues, and by-products can all be recovered and upgraded into more high-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 one of the most important fruits. About 70% of oranges produced are used in many industrial processes to make juices, jams, and other products, resulting in massive amounts of residues that account for about 50-60% of the processed fruit in the case of juice production (Galanakis, 2012). Orange peels are a type of residue that consists of seeds (0-9%), peels (60-75%), and membrane residues (23-33 %). Orange peels are high in bioactive compounds such as ascorbic acid, flavonoids, phenolic compounds, and pectin, all of which are beneficial to human nutrition. Citrus fruit contains three types of flavonoids: flavanones, flavones, and flavanols (Shawky *et al.*, 2019). Hesperidin, narirutin, naringin, and eriocitrin are the main flavonoids found in orange peels (Ghasemi *et al.*, 2009). Since orange peels contains a variety of valuable substances (natural products and bioactive phenolic compounds), it can be used as a raw material for intermediate food ingredients or as an ingredient in high-value new products with health benefits (Ibrahim & Hamed 2018). Peels are also thought to be natural by 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 many roles in relation to food. Some fungi used in food production, some are food sources themselves and some are agents of food spoilage (Benedict *et al*., 2016). Many fungi are essential to the biotechnology field and have industrial applications in the production of certain medications, foods, and beverages. Fungi are considered as an excellent and valuable source in nutrition, and they are used in cheeses, bread, rice and soy sauce (Praptiwi *et al.*, 2018). Many fungi synthesize secondary metabolites and some of which are useful. In addition, yeasts and filamentous fungi secrete a plethora of important enzymes in the growth medium together with other secondary metabolites. Most of these enzymes are hydrolytic in nature and could be employed in different food processing industries as well as in refinement off odder quality. On the other hand, secondary metabolites are being considered as useful bioactive substances containing antibiotics, alkaloids, enzymes, organic acids, carotenoids, toxins and pigments which have potential application in biotechnological and pharmaceutical fields as well (Praptiwi *et al.*, 2018; Vaishnav & Demain, 2011; Zhang *et al*., 2002). Phenolic compounds are present almost in all plant foods, but their quality, type and concentration vary strongly according to the plant strain and genetic factors as well as the environmental conditions (Kris-Etherton *et al*., 2002). Solid state fermentation of the plant materials was employed to enhance their phenolic contents leading 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

All chemicals' solvents and reagents were purchased from El-Ghomhorya Company for Trading Drug, Chemicals and Medical Instruments, Alexandria Egypt. All other chemicals used were of analytical grade.

Sample preparation

Orange peels were thoroughly washed under running tap water, after removing the tissues and fibers from it. Peels were chopped with a knife in small slices (about 1 cm^2) 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, the air temperature and relative humidity were determined using thermometer and hygrometer. The air temperature and relative humidity was recorded as 30 ± 2°C and 38 ± 6 %, respectively. Dried peels were ground to a fine powder and passed through a 24-mesh size sieve; the orange peels powder was stored at room temperature in dark glass airtight containers until needed.

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 method using semiautomatic apparatus, Velp company, Italy), fat (petroleum ether solvent) (soxhelt semiautomatic apparatus, same company. A muffle furnace maintained at 550°C for five hours used for ash content determination, Moisture, Crude fiber contents were determined in each analysis was carried out in triplicate. Total carbohydrates were calculated by difference, Carbohydrates (%) = 100 - (% moisture + % protein + % fat + % Ash + % fiber).

Extraction of orange peels

Different solvents were used to extract bioactive compounds from orange peels samples, including methanol, ethanol, ethyl acetate, acetone, dichloromethane, and *n*-hexane. For four hours at room temperature, twenty grams of each peel powdered sample were stirred with 100 ml of each solvent at a concentration of 96 percent. To remove peel particles, the extracts were filtered through Whatman No 42-filter paper and evaporated at 40°C under vacuum. After drying, the yield was calculated in grams of each extract then stored in a closed vial at 4°C (Ghareeb *et al.*, 2016b).

Determination of Total Antioxidant Capacity (TAC)

Total antioxidant capacity (TAC) of each solvent extract was evaluated using the phosphomolybdenum method according to the reported procedures (Ghareeb *et al.*, 2016a; Ghareeb *et al.*, 2016b; Prieto *et al.*, 1999).

Determination of total phenolic content (TPC)

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

GC-MS analysis of the orange peels methanolic extract

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

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, and air dried at 28°C for 3–5 days. Samples were kept at 10°C until they were used after drying. Soil samples were used to isolate fungal strains. The microbes in the soil were counted using the serial dilution agar plating method. The soil suspension was serially diluted up to 106 dilutions. Then, at 28 2°C for 6–8 days, 0.1 mL of suspension from dilutions 103 to 106 was transferred to petri dishes containing Czapex-Dox agar medium, and growth was observed after two days. 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 fungus was transferred three times on CD agar plates 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, and incubated at 30°C for 15 days before being extracted with ethyl alcohol (96 %). The antimicrobial properties of the extracts were tested (Abdel-Aziz & Hezma 2013).

Antimicrobial activity

The antimicrobial activity of different extracts of orange peels was evaluated against different fungal strains according to the reported procedures (Ghareeb *et al.*, 2015; Madkour *et al.*, 2017).

Identification of fungal cultures

Fungal cultures were identified according to a molecular biological protocol by DNA isolation, amplification (PCR) and sequencing of the ITS region. The primers ITS2 (GCTGCGTTCTTCATCGATGC) and ITS3 (GCATCGATGAAGAACGCAGC) were used at PCR while ITS1 (TCCGTAGGTGAACCTGCGG) and ITS4 (TCCTCCGCTTATTGATATGC) were used at sequencing. The purification of the PCR products was carried to remove unincorporated PCR primers and dNTPs from PCR products by using Montage PCR Clean up kit (Millipore). Sequencing was performed by using Big Dye terminator cycle sequencing kit (Applied Bio Systems, USA). Sequencing products were resolved on an Applied Biosystems model 3730XL automated

DNA sequencing system (Applied BioSystems, USA). *Candida* sp. was used as control according to 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
Milk (ml)	100	100	100	100	100	100	100
Eggs (g)	50	50	50	50	50	50	50
Oil (ml)	50	50	50	50	50	50	50
Baking powder (g)	10	10	10	10	10	10	10
Salt (g)	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Powder sugar (g)	15	15	15	15	15	15	15
Vanilla extract (g)	1	1	1	1	1	1	1
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 was performed using a nine-point hedonic scale as reported by (Watts *et al*., 1989; Zaker *et al*., 2017), it was carried out by a well-trained 30-members comprising postgraduate and academic staff members of Home Economics Department-Specific Education Faculty-Alexandria University. They were requested to evaluate the characteristics of the produced crispy waffle and rating the products on a 9-point Hedonic scale with corresponding descriptive terms ranging from 9 'like extremely' to 1 'dislike extremely'.

Statistical analysis

Statistical analysis of data was carried out using the IBM SPSS 23 statistics package program (Kirkpatrick & Feeney, 2012). Triplicate analyses were performed for all measurements for all samples. Differences between the means were compared by Duncan test at 5% of significance. The significance of the model was evaluated by ANOVA.

Results

Proximate chemical composition of orange peels

Proximate composition provides a general nutritional value of a food. Data in Table (2) shows the proximate chemical composition of orange peels powder. From such data it could be noticed that carbohydrates were the largest compound (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 consistent with that was mentioned by Gotmare & Gade, (2018) since they made a preliminary analysis of orange peels residue, and the obtained results were as follows: moisture (9.0%), fiber (15.3%), and ash (7.8%). Such data are in accordance with Abdelwahab & Abouelyazeed, (2018) which mentioned that the greater portion of orange peels is carbohydrates (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, ١٢.46±0.01) respectively.

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

Component (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). Different 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) > Me_2CO (201.19) > CH_2Cl_2 (166.02) > C_6H_{14} (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 owing to their higher polarity and good solubility for phenolic components from plant materials (Clauditz *et al.*, 2006; Kim *et al.*, 2010; Marina & Noriham, 2014). Phenolic acids and flavonoids are significant fruit phytochemicals for their antioxidant properties, chelation of redox-active metal ions, inhibition of hydro peroxide conversion to reactive oxyradicals when utilized as a natural source of antioxidants in functional foods and inactivation of lipid free radical chains. Phenolic content consider 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) > Me_2CO (454.63) > CH_2Cl_2 (280.87) > C_6H_{14} (111.47) mg AAE/g dry extract. Due to higher polarity and good solubility of phenolic compounds, the polar solvents like 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 increasing 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	(TPC) (mg GAE[3]/ g dry extract)	(TAC) (mg AAE[2]/g dry extract)
Methanol (MeOH)	264.41 ± 2.05	679.77± 3.82
Ethanol (EtOH)	256.35 ± 4.38	642.61 ± 4.94
Acetone (Me_2CO)	201.19 ± 3.37	454.63 ± 3.78
Ethyl acetate (EtOAc)	237.29 ± 4.83	509.38 ± 2.87
Dichloromethane (CH_2Cl_2)	166.02 ± 3.37	280.87 ± 2.65
n-hexane (C_6H_{12})	127.37 ± 2.57	111.47 ± 3.02

[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 investigation of the methanolic extract of orange peels

GC-MS examination of the methanolic extract of orange peels revealed that it comprises 38 compounds (Figure 1). The total peak areas of the identified ingredients constitutes 94.46%, the prospects of the chemical structures of the identified compounds are recorded in table (4): The main detected 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).

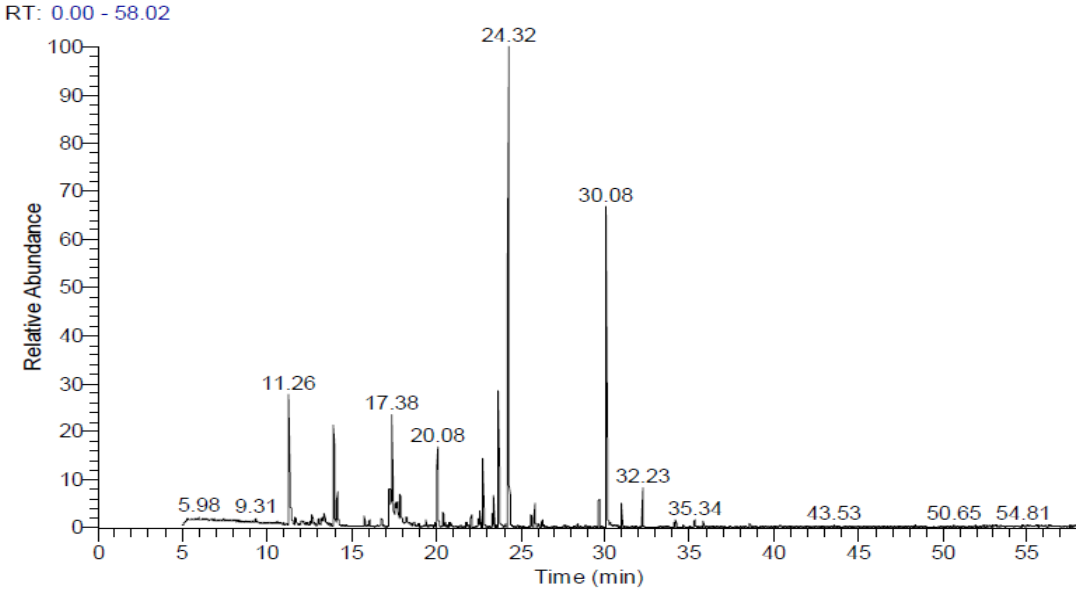


Fig. 1: GC-MS chromatogram of the orange peels methanolic extract before fermentation

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

GC-MS examination of the 3 comprises 14 compounds (Figure 2). The total peak areas of the identified ingredients constitutes 62.59%, the prospects of the chemical structures of the identified compounds are recorded in table (5): The main detected compounds are 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 achieved via using computer search user-generated reference libraries, incorporating mass spectra (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 an natural additive in bakery products and in the fields of nutrition, pharmaceutical, and cosmetics.

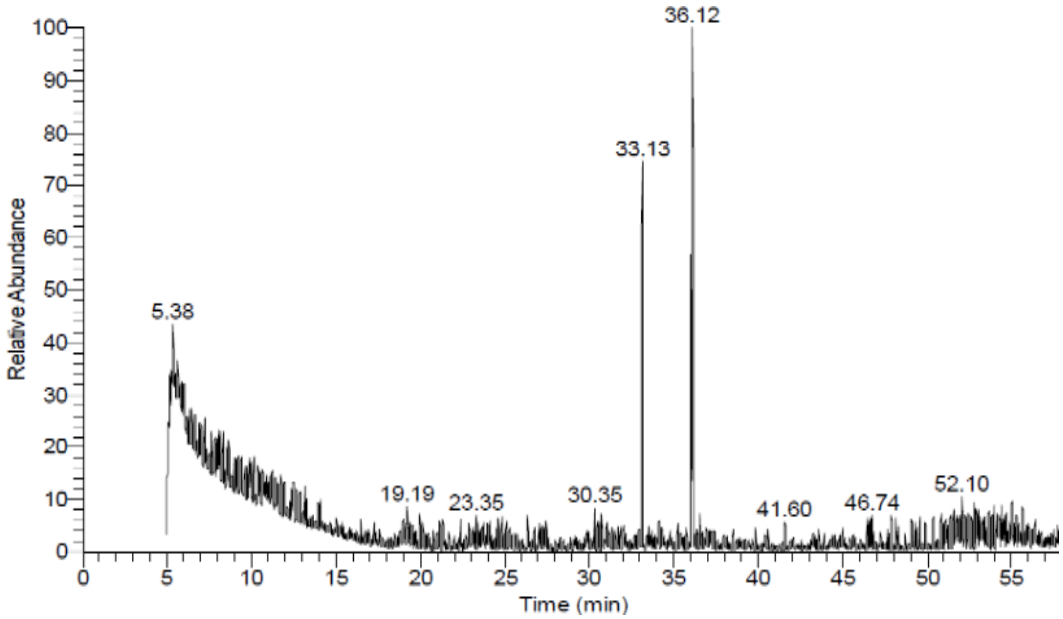


Fig. 2: GC-MS chromatogram of the orange peels after fermentation

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-methybuto xy)-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-tetracarboxylic acid 3,4-anhydride-9,10-imide	Heterocyclic derivatives
13	53.17	0.87	692	$C_{44}H_{44}N_4O_4$ 2	55, 72, 135, 224, 281, 401, 443, 510, 661	N,N'-Dicyclohexy-l1,7-dipyrrolidinyl perylene-3,4: 9,10-tetracarboxylic acid bisimide	Heterocyclic derivatives
14	54.61	0.87	660	$C_{30}H_{28}O_{17}$	69, 135., 180, 207, 281, 402, 503, 628	Methylpentaacetyllactothamnolate	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.

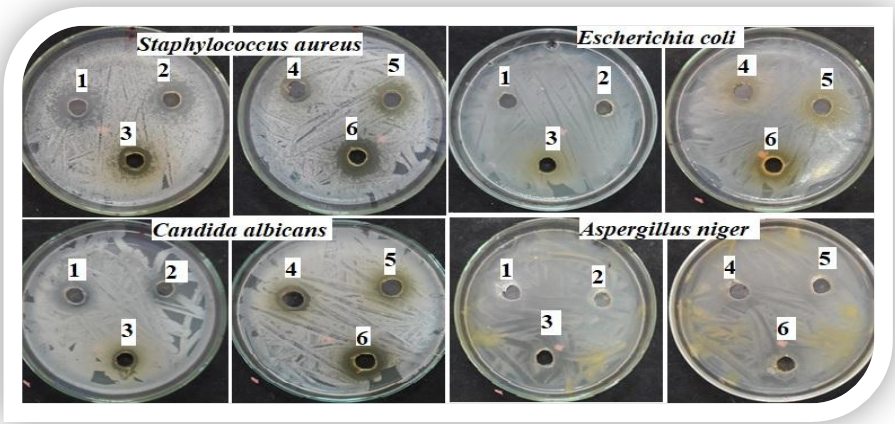
The antimicrobial activities of orange peels extracts

The use of natural antimicrobial compounds in foods has gained much attention by the consumers and the food industry. These compounds can degrade the cell wall and disrupt cytoplasmic membrane-integrated enzymes, which may eventually lead to cell death. The orange peels extract showed various degrees of inhibition against the growth of investigated microorganisms as shown in table (6), figure (3) Thus, with the exception and *Staphylococcus aureus* with orange peels hexane extract, which exhibited the lowest antimicrobial value, the results showed that there were significant differences ($p < 0.05$) among inhibition effects on the tested microorganisms, with inhibition zones ranging from 13 to 39 mm. Hence, the highest inhibition was obtained for *Aspergillus niger* with orange peels acetone extract. This by-product could be of great benefit from economic and environmental perspectives as sources of low-cost natural antimicrobials (El-Faham *et al.*, 2016; Taveira *et al.*, 2010).

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



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

The antimicrobial compounds which were grown on orange peels extract can degrade the cell wall and disrupt cytoplasmic membrane-integrated enzymes, which may eventually lead to cell death. The activity of different fungi which were grown on orange peels extract against different test microbes representing showed various degrees of inhibition against the growth of investigated microorganisms as shown in table (7) and figure (4). the results showed that there were significant differences ($p<0.05$) among inhibition effects on the tested microorganisms, with inhibition zones ranging from 0 to 35 mm. Hence, the highest inhibition was obtained for *Escherichia coli* with ethanol, except for *Aspergillus niger* with fermented orange peels acetone extract, which exhibited the lowest antimicrobial value (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

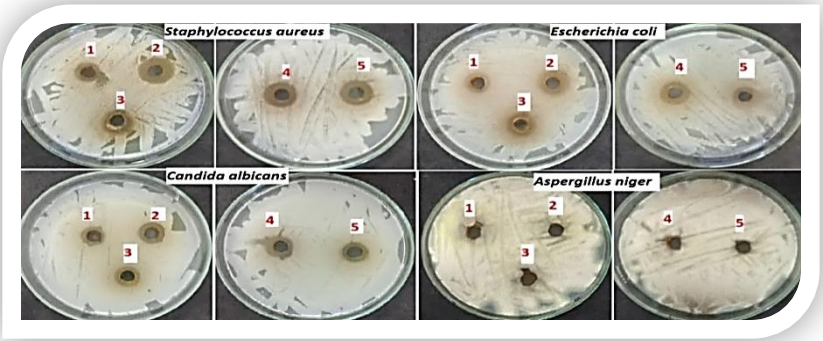
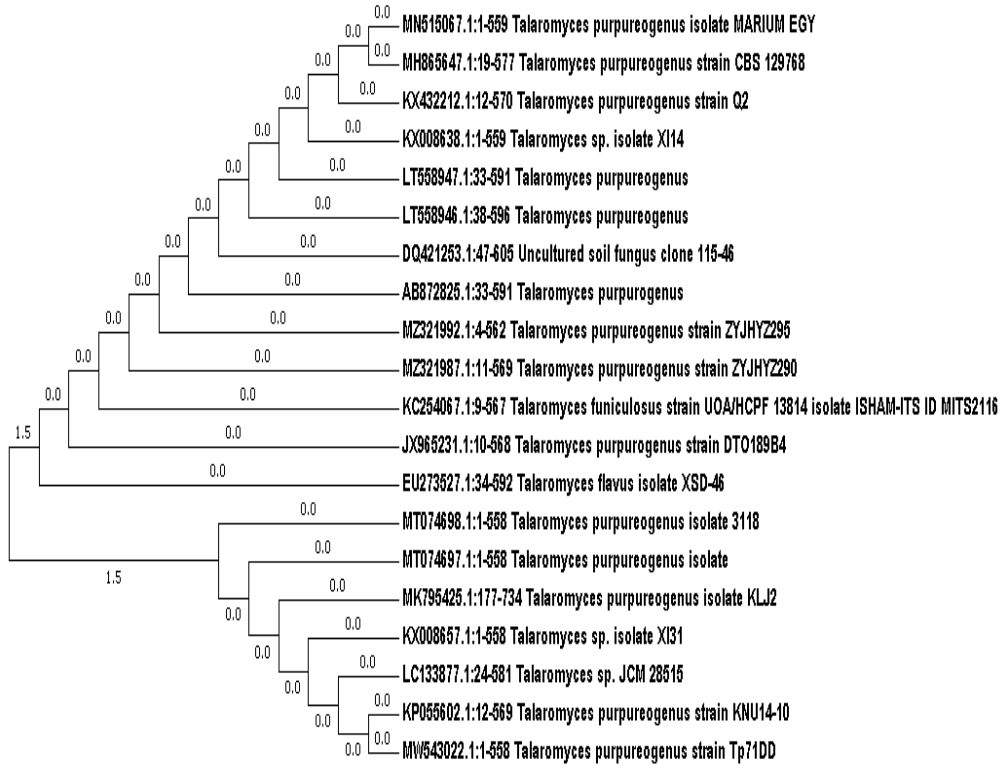


Fig .4: The antimicrobial activities of different fungal extracts against Escherichia coli, Staphylococcus aureus, Candida albicans and Aspergillus niger

Molecular Identification of the potent fungal isolate 4

The molecular approach (18S rRNA) as an accurate tool for identification was used to identify 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. isolate 4 grown on orange peels was determined in both



strands. 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 evaluation of waffle product made by using natural additives extracted from orange peels (OP) shown in table (8). The color value increased gradually with the higher percentage of incorporated orange peels, this is in line 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 parameter were obtained regarding the sample containing 3% form orange peels addition. Concerning with the study of the impact of orange peels powder on the sensory properties of waffle, data noted that the replacement up to 5% form orange peels powder had significant ($p < 0.05$) result 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 highest acceptability score of bakery product was the forfeited product with orange peels at (3%, 5%) level concentration, it recommend the importance of the balance between orange peels as additive to obtain a fortified product with no negative effect on the acceptability of the consumers.

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%	$8.4^{٧a}\pm0.63$	$8.50^{a}\pm0.51$	$8.23^{ab}\pm0.43$	$8.17^{b}\pm0.53$	$8.47^{a}\pm0/63$	$8.47^{a}\pm0.57$
OPW 5%	$8.٣٠^{ab}\pm0.٦٥$	$8.03^{bc}\pm0.67$	$7.90^{b}\pm0/76$	$8.10^{b}\pm0.71$	$8.30^{a}\pm0.65$	$8.33^{a}\pm0.48$
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)

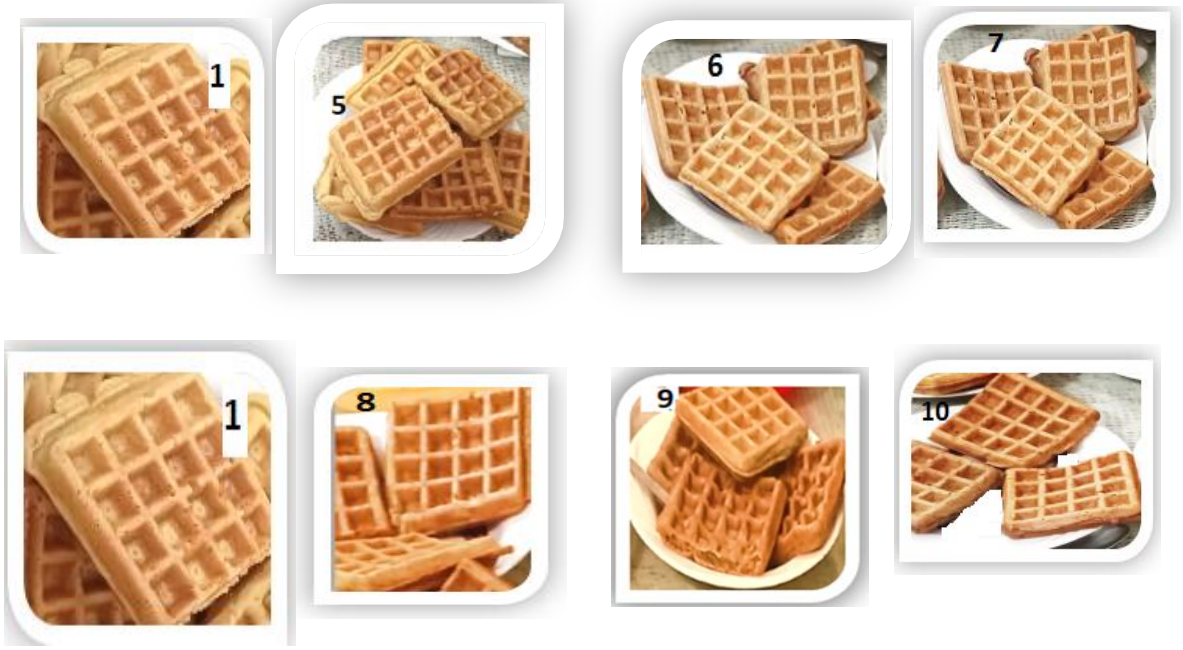
The sensory evaluation of orange peels" extract waffle (OPEW) with different concentrations compared with control sample is summarized in table (9). Data indicated that there were significant differences between the control and the waffle samples in some characteristics before and after fermentation. Orange peels extract waffle (OPEW) at level of concentration at 0.05%, were highly accepted and exhibited the maximum score of sensory properties. Similar trend was also seen for 0.1% sample level compared to control sample. Meanwhile, at 0.2% level, the mean scores were lower which indicate that the sample were unacceptable as compared to control sample. Also, as shown in table (9) the acceptability of waffle containing 0.05% level orange peels extract waffle (OPEW) had higher total scores (8.40± 0.49, 8.43± 0.57) before and after fermentation for orange peels, respectively followed by 0.1% at level (8.17± 0.69, 8.10± 0.76) respectively. These results indicate that the orange peels extract could be added in amount up to 0.1% in the formula of waffle without adversely affecting sensory characteristics of waffle. Appearance, taste and odor did not yield any statistically significant ($p < 0.05$) differences between the studied samples. In the study by Elhassaneen *et al.*, (2016) appearance, taste and odor of waffle fortified orange peels extract was also not affected this means that each formulation could have positive acceptance among consumers. On the other hand, a study conducted by Pereira *et al.*, (2020), showed the waffle formulations containing 0.02% of orange peels extract showed statistically different lower sensory grades for overall acceptance in comparison to the control sample. Considering the obtained results of this study, the added orange peels extract had a beneficial effect on antimicrobial activity of the fortified waffle. While, according to Huang *et al.*, (2009), using orange peels extract at level 0.5%, 0.1%, can be declared as the best choice because of increased antioxidant activity and antimicrobial activity as an important acceptance parameter in those kinds of waffle.

Differences between orange peels extract waffle samples according to their textural parameters are presented in table (9). The changes in appearance, taste and odor did not negatively affect the overall impression. The study of Sagar *et al.*, (2018) highlighted the importance 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 hand, according to Sagar *et al.*, (2018) using waste to produce various crucial bioactive components is an important step toward sustainable development, especially the waste that originates from fruits as orange.

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 **2- orange peels extract waffle 0.05%** **3- orange peels extract waffle 0. 1%**
4-orange peels extract waffle 0.2%, **After 5-Control orange peels waffle** **6- orange peels extract waffle 0.05%**
7- orange peels extract waffle 0.1% **8-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.

Referances

Abd El-ghfar, M. A., Ibrahim, H. M., Hassan, I. M., Fattah, A. A., & Mahmoud, M. H. (2016). Peels of lemon and orange as value-added ingredients: chemical and antioxidant properties. *Int. J. Curr. Microbiol. App. Sci, 5*(12), 777-794 .

Abdel-Aziz, M. S., & Hezma, A. (2013). Spectroscopic and antibacterial evaluation of nano-hydroxapatite polyvinyl alcohol biocomposite doped with microbial-synthesized nanogold for biomedical applications. *Polymer-Plastics Technology and Engineering, 52*(14), 1503-1509 .

Abdelwahab, A. S., & Abouelyazeed, A. (2018). Bioactive Compounds in Some Citrus Peels as Affected by Drying Processes and Quality Evaluation of Cakes Supplemented with Citrus Peels Powder. *Journal of the Advances in Agricultural Researches, 23*(1), 44-67 .

Abdel-Wareth, M. T., El-Hagrassi, A. M., Abdel-Aziz, M. S., Nasr, S. M., & Ghareeb, M. A. (2019). Biological activities of endozoic fungi isolated from Biomphalaria alexandrina snails maintained in different environmental conditions. *International Journal of Environmental Studies*-٧٨٠ ،(٥)٧٦ ، ٧٩٩.

Abou-Arab, E. A., Mahmoud, M. H., & Abu-Salem, F. M. (2017). Functional properties of citrus peel as affected by drying methods. *American Journal of Food Technology, 12*(3), 193-200 .

Adewole, E., Adewumi, D., Jonathan, J., & Fadaka, A. (2014). Phytochemical constituents and proximate analysis of orange peel (citrus Fruit). *Journal of advanced Botany and Zoology, 1*(3), 1-2 .

Al-Juhaimi, F. Y. (2014). Citrus fruits by-products as sources of bioactive compounds with antioxidant potential. *Pak. J. Bot, 46*(4), 1459-1462 .

AOAC. (2016). Official methods of analysis of AOAC INTERNATIONAL 20th edition, Appendix D, Guidelines for collaborative study procedures to validate characteristics of a method of analysis. *Gaithersburg, MD, USA* .

Benedict, K., Chiller, T. M., & Mody, R. K. (2016). Invasive fungal infections acquired from contaminated food or nutritional supplements: a review of the literature. *Foodborne pathogens and disease, 13*(7), 343-349 .

Cerimi, K., Akkaya, K. C., Pohl, C., Schmidt, B., & Neubauer, P. (2019). Fungi as source for new bio-based materials: a patent review. *Fungal biology and biotechnology, 6*(1), 1-10 .

Cheng, S.-Y., Wang, B.-J., & Weng, Y.-M .(٢٠١٥) . Antioxidant and antimicrobial edible zein/chitosan composite films fabricated by incorporation of phenolic compounds and dicarboxylic acids. *LWT-Food Science and Technology, 63*(1), 115-121 .

Choung, M.-G., Baek, I.-Y., Kang, S.-T., Han, W.-Y., Shin ،D.-C., Moon, H.-P., & Kang, K.-H. (2001). Isolation and determination of anthocyanins in seed coats of black soybean (Glycine max (L.) Merr.). *Journal of agricultural and food chemistry, 49*(12), 5848-5851 .

Clauditz, A., Resch, A., Wieland, K.-P., Peschel, A., & Götz, F. (2006). Staphyloxanthin plays a role in the fitness of Staphylococcus aureus and its ability to cope with oxidative stress. *Infection and immunity, 74*(8), 4950-4953 .

El-Faham, S., Mohsen, M., Sharaf, A., & Zaky, A. (2016). Utilization of mango peels as a source of polyphenolic antioxidants. *Current Science International, 5*(04), 5 .٥٤٢-٢٩

Elhassaneen, Y., Goarge, S., Sayed, R., & Younis, M. (2016). Onion, Orange and Prickly Pear Peel Extracts Mixed with Beef Meatballs Ameliorate the Effect of Alloxan Induced Diabetic Rats. American Journal of Pharmacology and Phytotherapy, 1(1), 15 .

El-wardany, N. A. (2016). Utilization of Wastes Resulted From Juice Production in the Fortification of Some Bakery Products for the Protection of Cardiovascular Diseases. *Alexandria Science Exchange Journal, 37*(October-December), 851-866 .

Galanakis, C. M. (2012). Recovery of high added-value components from food wastes: conventional, emerging technologies and commercialized applications. *Trends in Food Science & Technology, 26*(2), 68-87 .

Ghareeb, M. A., Hussein, A. H., Hassan, M., Laila, A., Mona, A., & Amal, M. (2014). Antioxidant and cytotoxic activities of Tectona grandis linn leaves. *International Journal of Phytopharmacology, 5*(2), 143-157 .

Ghareeb, M. A., Refahy, L. A., Saad, A. M., Osman, N. S., Abdel-Aziz, M. S., El-Shazly, M. A., & Mohamed, A. S. (2015). In vitro antimicrobial activity of five Egyptian plant species. *J Appl Pharm Sci, 5*, 045-049 .

Ghareeb, M., Ahmed, W., Refahy, L., Abdou, A., Hamed, M & ،.Abdel-Aziz, M. (2016a). Isolation and characterization of the bioactive phenolic compounds from Morus alba leaves growing in Egypt. *Pharmacology online, 3*, 157-167 .

Ghareeb, M. A., Saad, A. M., Abdou, A. M., Refahy, L. A.-G., & Ahmed, W. S. (2016b). A new kaempferol glycoside with antioxidant activity from Chenopodium ambrosioides growing in Egypt. *Orient J Chem, 32*(6), 3054-3061 .

Ghasemi, K., Ghasemi, Y., & Ebrahimzadeh, M. A. (2009). Antioxidant activity, phenol and flavonoid contents of 13 citrus species peels and tissues. *Pak J Pharm Sci, 22*(3), 277-281 .

Gotmare, S., & Gade ،J. (2018). Orange peel: A potential source of phytochemical compounds. ***Int. J. ChemTech Res, 11*****, 240-243 .**

Huang, Y.-W., Liu, Y., Dushenkov, S., Ho, C.-T., & Huang, M.-T. (2009). Anti-obesity effects of epigallocatechin-3-gallate, orange peel extract, black tea extract, caffeine and their combinations in a mouse model. ***Journal of Functional Foods, 1*****(3), 304-310 .**

Huber, R., & Schoenlechner, R. (2017). Waffle production: influence of batter ingredients on sticking of fresh egg waffles at baking plates—Part I: effect of starch and sugar components. ***Food science & nutrition, 5*****(3), 504-512 .**

Ibrahim, H. M., & Hamed, A. A. (2018). Some physicochemical and functional properties of lemon and orange peels. ***International Journal of Current Microbiology and Applied Sciences, 7*****, 4871-4885 .**

Iqbal, H. M. N., Kyazze, G., & Keshavarz, T. (2013). Advances in the valorization of lignocellulosic materials by biotechnology: an overview. ***BioResources, 8*****(2), 3157-3176 .**

Khalaf, O. M., Abdel-Aziz, M. S., El-Hagrassi, A. M., Osman, A. F., & Ghareeb, M. A. (2021). ***Biochemical Aspect, Antimicrobial and Antioxidant Activities of Melaleuca and Syzygium Species (Myrtaceae) Grown in Egypt.*** **Paper presented at the Journal of Physics: Conference Series.**

Kim, H., Moon, J. Y., Kim, H., Lee, D.-S., Cho, M., Choi, H.-K., & Cho, S. K. (2010). Antioxidant and antiproliferative activities of mango (Mangifera indica L.) flesh and peel. ***Food Chemistry, 121*****(2), 429-436 .**

Kirkpatrick, L., & Feeney, B. (2012). A simple guide to IBM SPSS: for version 20.0: Nelson education: USA.

Kris-Etherton, P. M., Hecker, K. D., Bonanome, A., Coval, S. M., Binkoski, A. E., Hilpert, K. F., & Etherton, T. D. (2002). Bioactive compounds in foods: their role in the prevention of cardiovascular disease and cancer. ***The American journal of medicine, 113*****(9), 71-88 .**

M'hiri, N., Irina, I., Cédric, P., Ghoul, M., & Boudhrioua, N. (2017). Antioxidants of maltease orange peel: comparative investigation of the efficiency of four extraction methods. ***J Appl Pharm Sci, 7*****(11), 126-135 .**

Madkour, H. M., Ghareeb, M. A., Abdel-Aziz, M. S., Khalaf, O. M., Saad, A. M., El-Ziaty, A. K., & Abdel-Mogib, M. (2017). Gas chromatography-mass spectrometry analysis, antimicrobial, anticancer and antioxidant activities of n-hexane and methylene chloride extracts of Senna italica. ***J. Appl. Pharm. Sci, 7*****, 23-32 .**

Manjarres-Pinzon, K., Cortes-Rodriguez, M., & Rodríguez-Sandoval, E. (2013). Effect of drying conditions on the physical properties of impregnated orange peel. Brazilian Journal of Chemical Engineering, 30, 667-676 .

Marina, Z., & Noriham, A. (2014). Quantification of total phenolic compound and in vitro antioxidant potential of fruit peel extracts. International Food Research Journal, 21 .(٥)

Nandini, K., Gaur, A., & Sundari, S. K. (2013). The suitability of natural tannins from food and agricultural residues (FAR) for producing industrially important tannase and gallic acid through microbial fermentation. *Int J Agric Food Sci Technol, 4*(10), 999-1010 .

Negro, V., Mancini, G., Ruggeri, B., & Fino, D. (٢٠١٦). Citrus waste as feedstock for bio-based products recovery: Review on limonene case study and energy valorization. *Bioresource technology, 214*, 806-815 .

Oikeh, E., Oriakhi, K., & Omoregie, E. (2013). Proximate analysis and phytochemical screening of *Citrus sinensis* fruit wastes. *The Bioscientist Journal, 1*(2), 164-170 .

Pereira, I. M. C., Matos, J. D., Figueiredo, R. W., Carvalho, J. D. G., Figueiredo, E. A. T. d., Menezes, N. V. S. d., & Gaban, S. V. F. (2020). Physicochemical characterization, antioxidant activity, and sensory analysis of beers brewed with cashew peduncle (Anacardium occidentale) and orange peel (*Citrus sinensis*). *Food Science and Technology, 40*, 749-755 .

Praptiwi, R. M., Wulansari ،D., Fathoni, A., & Agusta, A. (2018). Antibacterial and antioxidant activities of endophytic fungi extracts of medicinal plants from Central Sulawesi. *Journal of Applied Pharmaceutical Science, 8*(08), 069-074 .

Prieto, P., Pineda, M., & Aguilar, M. (1999). Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. *Analytical biochemistry, 269*(2), 337-341.

Sagar, N. A., Pareek, S., Sharma, S., Yahia, E. M., & Lobo, M. G. (2018). Fruit and vegetable waste: Bioactive compounds, their extraction, and possible utilization. *Comprehensive reviews in food science and food safety, 17*(3), 512-531 .

Sharma, K., Mahato, N., Cho, M .H., & Lee, Y. R. (2017). Converting citrus wastes into value-added products: Economic and environmently friendly approaches. *Nutrition, 34*, 29-46 .

Sharoba, A. M., Farrag, M., & Abd El-Salam, A. (2013). Utilization of some fruits and vegetables waste as a source of dietary fiber and its effect on the cake making and its quality attributes. *Journal of Agroalimentary Processes and Technologies, 19*(4), 429-444 .

Shawky, B. T., Nagah, M., Ghareeb, M. A., El-Sherbiny, G. M., Moghannem, S. A., & Abdel-Aziz, M .S. (2019). Evaluation of Antioxidants, Total

Phenolics and Antimicrobial Activities of Ethyl Acetate Extracts From Fungi Grown on Rice Straw. *Journal of Renewable Materials, 7*(7), 662-677 .

Taveira, M., Silva, L. R., Vale-Silva, L. A., Pinto, E., Valentao, P., Ferreres, F., & Andrade, P. B. (2010). Lycopersicon esculentum seeds: an industrial byproduct as an antimicrobial agent. *Journal of agricultural and food chemistry, 58*(17), 9529-9536 .

Vaishnav, P., & Demain, A. L. (2011). Unexpected applications of secondary metabolites. *Biotechnology Advances, 29*(2), 223-229 .

Watts, B. M., Ylimaki, G., Jeffery, L., & Elias, L. G. (1989). Basic sensory methods for food evaluation: IDRC, Ottawa, ON, CA

Williamson, S ،.Block, L. G., & Keller, P. A. (2016). Of waste and waists: The effect of plate material on food consumption and waste. *Journal of the Association for Consumer Research, 1*(1), 147-160 .

Yashaswini, Y. (2018). Arvind: Antimicrobial Properties of Orange (Citrus reticulata var. Kinnow). Peel Extracts against Pathogenic Bacteria. *Int J Curr Microbiol App Sci, 7*(3), 737-746 .

Zaker, M., Sawate, A., Patil, B., Sadawarte, S., & Kshirsagar, R. (2017). Utilization of orange (Citrus sinesis) peel powder as a source of dietary fibre and its effect on the cake quality attributes. *International Journal of Agricultural Sciences, 13*(1), 56-61 .

Zhang, J., Tang, Q., Zimmerman-Kordmann, M., Reutter, W., & Fan, H. (2002). Activation of B lymphocytes by GLIS, a bioactive proteoglycan from Ganoderma lucidum. *Life sciences, 71*(6), 623-638 .

Zoair, A., Attia, S., Abou Garbia, A., & Youssef, M. (2019). Utilization of orange, banana and potato peels in formulating functional cupcakes and crackers .