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# Extraction and Applications of Grapefruit (*Citrus paradise*) Peel Oil Against *E. coli*

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**Abstract:** Phytochemicals has got special attention of researchers because of their value in food preservation and value addition due to consumer preference. Grapefruit (*Citrus paradisi)* is one of important member of family Rutaceae (citrus family). Oil was extracted through solvent extraction method using soxhlet's apparatus. The nuggets were prepared by incorporating the grapefruit peel oil at different levels 0 (control), 5, 10, 25, 50, 100% and stored for duration period (0, 7 and 14 days). Peel oil refractive index, specific gravity, pH, acid value, iodine values 1.47, 0.85, 3.67, 0.81, 99.4 were respectively calculated. The peak values for proximate analysis (protein, moisture, fat, ash contents) were 62.32, 15.29, 14.0 and 2.76 respectively were recorded in $T_1$ (5% grapefruit peel oil) at all storage periods. In color, water activity and texture analysis were 117.77, 0.94 and 1691.7 respectively in $T_1$ at all storage durations. Similarly in sensory evaluation of color, taste, flavor and overall acceptability found statistically that $T_3$ (25% grapefruit oil) treatment is highly significant at all storage periods and gave maximum value for these parameters and $T_5$ (100% grapefruit oil) gave minimum value for its effectiveness. Grapefruit peel oil was checked for its antimicrobial activity test e.g., total plate count, disc diffusion method and minimum inhibitory action against *Escherichia coli* on chicken nuggets. In antimicrobial analysis MIC (Minimum Inhibitory Concentration) decreased with increasing the concentration of grapefruit peel oil and in disc diffusion method, its values increased with increasing grapefruit peel oil.

**Key words:** Grapefruit peel oil, antimicrobial activity, chicken nuggets

## INTRODUCTION

Citrus is considered the most important fruit in the world and grapefruit has greater value in human diet. There are many options available from broad family of citrus e.g., kinnow, sweet orange, lemon, etc., Grapefruit (*Citrus paradisi*) is one of important member of family Rutaceae (citrus family), It is subtropical in origin and well known for its bitter taste (initially cultivated in Jamaica around 18th century and now grown in all parts of the world). It has yellow-orange skin and varies in diameter from 10-15 cm. The flesh of grapefruit is segmented and acidic, varying in color depending on the cultivars including white, pink and red pulp of different sweetness (Malik, 1994). Approximately one third of total citrus production is used for treatment. This proportion is greater in the case of oranges, over 40% globally oranges produced are used for treatment. The proportion of use for the treatment of grapefruit is similar to that of the oranges. However all small citrus-tangerine-like are intended for use in the fresh market. Lemon and lime are somewhat different, since they are typically consumed with other foods. They are grown primarily for fresh market and its juice is mainly used as a flavoring in drinks. The peels of citrus fruits are rich source of bioactive molecules and the albedo layer is a good source of pectin (Richard, 1994).

The varieties of grapefruit are also categorized by the pigmentation of the fruit, state of ripeness and genetic bent. The most popular varieties cultivated presently are red, white and pink. Double-red flesh grapefruit varieties are also popular in the USA, Israel, Europe, Mexico and are increasing in Asia (Isgro *et al*., 2001). Arritt (2007) conducted experiments and found that citrus peel contains dry matter 16.8%, ash 3.7%, crude protein 6.8% crude fat 1.9%, crude fiber 6.2% and carbohydrates 81.4%. Citrus is the second largest fruit being cultivated in Pakistan. The essential oils produced by different plant genera are in many cases biologically active, endowed with antimicrobial, allopathic, antioxidant and bio-regulatory properties. The antimicrobial abilities of essential oils, among which citrus oils are also shown to be a particularly interesting field for applications within the food industries (Caccioni *et al*., 1998). Preparation from peel, flowers and leaves of bitter orange (*Citrus aurantium L*.) are popularly used in order to minimize central nervous system disorders (Pultrini *et al*., 2006). The peel of Citrus fruits is a rich source of flavones and many polymethoxylated flavones which are very rare in other plants (Ahmed *et al*., 2006). The results showed that the EO of grapefruit peel had a wide spectrum of antimicrobial activities against *Staphylococcus aureus*, *Enterococcus faecalis*, *Staphylococcus epidermidis*, *Escherichia coli*, *Salmonella typhimurium*, *Serratia marcescens* and *Proteus vulgaris* with their inhibition zones ranging from 11 to 53 mm.

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*Escherichia coli* O157 is an uncommon but serious cause of gastroenteritis. This bacterium is noteworthy because a few but significant, number of infected people develop the hemolytic uremic syndrome which is the most frequent cause of acute renal failure in children in the Americas and Europe. In Pakistan, whole scenario of fruit cultivation is dominated by citrus with an area (183.8 thousand hectare) and production (1943.7 thousand tons) (FAO, 2007-08).

Grapefruit (*Citrus paradisi)* has been used as a folk medicine in many countries as antibacterial, anti-fungal, anti-inflammatory, antimicrobial, antioxidant, antiviral, astringent and preservative. It has also been used for cancer prevention, cellular regeneration, lowering cholesterol, cleansing, detoxification, heart health maintenance, arthritis and weight loss. This may help the scientists in designing new drugs with varied activities in a single formulation.

## MATERIALS AND METHODS

The grapefruit was collected in December 2011. The fruit albedo layers peeled off carefully and discarded (Kirbaslar *et al*., 2006). Grapefruit peel essential oils extracted from the peels of Grapefruit by using solvent extraction method as described by Bayramoglu *et al*. (2008). Refractive index was determined by the Abbe's method (Greenwood *et al*., 2004). Specific gravity of oil sample was determined at room temperature with specific gravity bottle (Pycnometer). Iodine value and pH value was determined by using the methods of AOAC (2000). Acid value was measured by AACC (2000) standard procedure No. Cd (3a-63). In preparation of media for microbiological analysis Nutrient agar used at the concentration of 27.8 g was dissolved in a liter of distilled water and autoclaved for 15 minutes at 121°C. The antimicrobial effect of EO will be determined against *E. coli* by Disc diffusion method, where a filter disc will be impregnated with the antimicrobial agent, placed on the surface of inoculated agar plates and inhibition of *E. coli* growth will be observed (Fisher and Phillips, 2006). MIC will be determined by Agar Dilution Method as described by Ortuno *et al*. (2006). The proximate analysis determined by AACC (2000) method. Color analysis was analyzed by colorimeter and water activity determined by hygrometer. The nuggets prepared by using the essential oil will be assessed by a panel of judges on the basis of seven point scale at National Institute of Food Science and Technology, University of Agriculture, Faisalabad recommended by (Carpenter *et al*., 2000).

## RESULTS AND DISCUSSION

In this study chicken nuggets were prepared with the addition of different levels of grapefruit peel oil and these nuggets were evaluated for the antimicrobial activities incurred by the above mentioned active material. Raw samples of grapefruit peel oil were also evaluated for the inherent antimicrobial activity *in vitro*. All the procedures from procurement to laboratory examination were carried out in line with the best sanitary and hygienic conditions.

Table 1: Characterization of grapefruit peel oil

| Properties | Values |
|---|---|
| Refractive Index | 1.47 |
| Specific gravity | 0.85 |
| pH | 3.67 |
| Acid value | 0.81 |
| Iodine value | 99.4 |

Table 2: Effect of grapefruit peel oil on physico-chemical properties of chicken nuggets (Mean ± SE)

| Parameters | Control | Optimized product |
|---|---|---|
| Water activity | 0.972±0.0026 | 0.974±0.0026 |
| Moisture | 62.031±0.192 | 62.037±0.238 |
| Protein | 15.354±0.422 | 15.173±0.405 |
| Fat | 13.093±0.310 | 12.783±0.125 |
| Total ash | 2.764±0.041 | 2.730±0.033 |

Table 3: Mean value for bacterial stain (Minimum Inhibitory Concentration and Zone Inhibition)

| | *E. coli* | |
|---|---|---|
| Treatment | MIC | ZI |
| $T_0$ | None | None |
| $T_1$ | 12.46±0.06a | 7.37±0.01e |
| $T_2$ | 11.90±0.08b | 8.94±0.05d |
| $T_3$ | 10.55±0.06c | 10.86±0.04c |
| $T_4$ | 8.78±0.03d | 12.64±0.03b |
| $T_5$ | 7.22±0.03e | 14.44±0.03a |
| Means | 10.18 | 10.85 |

**Refractive index:** Physical parameters are widely quoted to evaluate purity and quality of essential oils. In trading of these oils, any impurity or adulteration, if practiced, can also be detected easily by matching the physical characteristics of essential oils with the already established required standards (Guenther, 1955). Refractive index of grapefruit oil was 1.47, respectively.

**Specific gravity:** The peel oil of grapefruit was subjected to analysis for specific gravity as a first physical parameter. The results revealed that the specific gravity of grapefruit oil was 0.855, respectively.

**pH and acid value:** pH and acid value of grapefruit peel oil was determined 3.67 and 0.81, respectively.

**Iodine value:** The iodine value of grapefruit peel oil in vitro is same as describe in the following method. The value of the iodine number 99.4 places the oil in a position in-between the nondrying and semi-drying oil. By implication, the oil can be easily converted into semidrying oil for use in the production of paints and vanish, while it may also be used as nondrying oil in the lubricant industry.

**Product analysis:** Table 2 indicated that incorporation of grapefruit peel oil in chicken nugget formulation caused slight increase in moisture content and water activity, whereas a slight decrease in protein, fat and total ash content were observed after incorporation. Chemical composition was also determined.

**Antimicrobial activity *in-vitro*:** The modern world has seen an outbreak of new food borne diseases caused by microbes of pathogenic nature. This has brought with it a whole new change in the acceptability criteria and attitude from the consumer side. Increasing use of artificial antimicrobial compounds chemical preservatives to curb the growth or inactivation of these micro-organisms has been observed (Zancan, 2002). Antimicorbial compounds have been in commercial use for decades now and their efficacy against pathogenic bacteria has been established for long. Natural antimicrobials are an alternate to synthetic antimicrobial compounds as they are more environment and consumer friendly. These are derived either from animals, plants or other micro-organisms (Raybaudi, 2008).

$T_0$ = 0% Peel oil $T_3$ = 25% Peel oil
$T_1$ = 5% Peel oil $T_4$ = 50% Peel oil
$T_2$ = 10% Peel oil $T_5$ = 100% Peel oil

With reference to the mean value of bacterial strain, Analyze the results with different treatments. $T_0$ is control treatment so give zero value. But $T_1$ in which the oil concentration is 5% its value is 12.46. $T_2$ value is 11.90 in which grapefruit peel oil concentration is 10%. Similar type of results in remaining treatments. So I find that with increasing the concentration of grapefruit peel oil minimum inhibitory concentration decrease. For example when oil concentration of $T_5$ is 100% then the MIC was 7.22. The antibacterial activities of the extracts, individually and in combination were determined using agar diffusion method and the Minimum Inhibitory Concentration (MIC) carried out by agar dilution technique. The plant materials possessed antimicrobial activity with greater efficacy when used synergistically on the test organism. A high activity of *Citrus paradisi* (grapefruit) oil to *P. larvae* with MIC 385.0 mg/L has also been reported. The *Citrus paradisi* and Ficus carica were tested against pathogenic microorganisms; *S. aureus, E. coli, K. pneumoniae, B. subtilis, M. luteus* and *Candida albicans*. The extracts tested exhibited good antimicrobial activity against all the clinical isolates when compared with standard. The different extracts showed remarkable inhibitory action against various Gram positive and Gram negative bacteria and two fungal species. The methanolic, petroleum ether, chloroform, ethyl ether, ethanol extract of *Citrus paradisi* was screened for its antimicrobial activity. Antimicrobial activity was detected by observing the growth response of different organisms to the methanolic extract. It was generally based on the inhibition of growth of microorganisms which were measured with a desired concentration of the plant extract of *Citrus paradisi* to be examined with the standard concentration preparation (Sharma, 2005).

**Conclusion:** It is concluded by this study that grapefruit peel oil has good antioxidant properties. Substantial quantities of antioxidant compounds were reported which in turn exhibited strong inhibition against the free radicals. Their inclusion in chicken nuggets also proved effective in radical scavenging and retarding of microbial growth. $T_5$ and $T_8$ are reported to be promising candidates for further evaluation as alternate sources of antioxidants. It is concluded that grapefruit peel which showed higher potential as compared to grapefruit fruit powder can be utilized as an economically feasible as well as natural source of antioxidants in food products in order to enhance the shelf life of food products while at the same time reducing the risks associated with the use of synthetic compounds.

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