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RESEARCH PAPER

Antioxidant properties of Lemongrass leaves aqueous extract and its effect on fish balls oxidative stability during refrigerated storage

Amer H.H. Alzobaay**,**
Food Sci. Dep, Baghdad Uni.
Baghdad, Iraq
amirhussin@coagri.uobaghdad.edu.iq

Baidaa H. Kadhim,
Food Sci. Dep, Baghdad Uni.
Baghdad, Iraq
yara_azoz@yahoo.com

Rawaa M. Abdul Al-wahid
Agricultural Research Office
Baghdad, Iraq
ralshyraida@gmail.com

A B S T R A C T:

Lemongrass *(Cymbopogon citratus)* which belongs to the Gramineae family. Lemongrass leaves were extracted by water. Several methods were used to evaluate total antioxidant capacity for *C.citratus* leaves aqueous extract by estimation (reduction power and free radical inhibiting DPPH (-1diphenyl-2-picrylhydrazyl) effect compared with synthetic antioxidant, Propyl gallate (PG) ,butylated hydroxy toluene (BHT) and ascorbic acid. *C.citratus* leaves aqueous extract were showed superior to reduction power registered 135.33% than PG and BHT were 99.2% and 134.93% respectively at 50mg/ml. Aqueous extract showed highest effectiveness against free radicals reached 92.32 % at 500 µl/ml. Antioxidant properties of fish balls treated with (5 ,10, 15, 20) % C.*citratus* leaves aqueous extract were determined for (1,3,6,9,12,15) days under refrigerated conditions. A significant reduction in moisture and increasing in protein, fat, ash and carbohydrate contents as compared with control (untreated fish balls). Treatment Lg3 (treated with 15% aqueous extract) had the best chemical properties in thiobarbituric acid (TBA) and Peroxide value (PV) during refrigerated storage.

KEY WORDS: Lemongrass leaves; aqueous extract; fish balls; Antioxidant; PV; TBA.
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1. INTRODUCTION:

Spices and herbs, used in food to improve color and flavor, are well known for their antioxidant properties, turmeric, curry leaf, lemongrass and torch ginger are spices and herbs which are widely used as essential ingredients in preparation of different cuisines and medicinal treatments in South and Southeast Asia. It has been reported that they are rich in antioxidant components, namely polyphenols, which are responsible for their medicinal properties (Sepahpour *et al.*, 2018). Lemongrass had gained interest as a nutritional supplement and is widely used in human foods in tropical countries. Lemongrass herb has been reported to have antibacterial, antioxidant and anti-hyper ammonia-producing ruminal bacterial activities (Kholif *et al.*, 2017). Lemongrass has been evaluated for its antioxidant properties and found that Lemongrass extracts containing efficient vehicles acting as an antioxidant linked (Balakrishnan *et al*., 2015). Pezeshk *et al*., (2015) noted that phenolic components of Lemongrass were most active as natural antioxidants in seafood. These components delayed the chemical changes, retarded the microbial growth, maintained the sensory characteristics, and extended the shelf-life of seafood during storage. The essential oil and plant extracts can be utilized as safe methods for the preservation of fish and seafood during storage.Fish balls were the popular food product in the world. Fish balls were the staple food in many parts of Asia (Hoque & Begum, 2016). The aim of this Study to evaluate the antioxidant activity of *C.citratus* leaves aqueous extract on fish balls under refrigerated storage.

* **Corresponding Author:**
Amer H.H. Alzobaay,
E-mail: amirhussin@coagri.uobaghdad.edu.iq
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2. MATERIALS AND METHOD

2.1 Aqueous Extraction of C.citratus leaves

Aqueous extraction was done according to (Balakrishnan *et al.*, 2014) with slight modifications, as follows: 100g of *C.citratus*

powder was diluted in1000 ml distilled water. The mixture was shaked for 8 h at 40°C and filtered by Whatman No.1. The extract was poured in Petri dishes and placed in electric oven at 40°c until entire dried and stored at refrigeration until use.

2.2 Antioxidant activity of Lemongrass extracts

Reductive potential of Lemongrass extracts was determined according to Benzie and Strain (1996), different concentrations of *C.citratus* aqueous extract (10-50 mg/ml) were added to 2.5 ml of 0.2 M sodium phosphate buffer (pH 6.6) and 2.5ml of 1% potassium ferricyanide [$K_3Fe(CN)_6$]. The mixture was vortex well and incubated at 50°C for 20 min. 2.5 ml of 10% trichloro acetic acid was added to the mixture and centrifuged at 4.000 rpm for 10 min. The supernatant (2.5 ml) was mixed with 0.5ml of deionized water and 1ml of 0.1% ferric chloride and the absorbance was measured at 700 nm using PG, BHT as a standard. Control solution was prepared at the same conditions but without plant extracts. The reduction power was calculated by equation:

$$Reducing\ power = [(B - A)/ B] \times 100$$

A: The absorbance of the sample extract, *B:* The absorbance for the control sample.

Scavenging activity of the 2,2-diphenyl-1-picrylhydrazyl (DPPH) for free radicals was measured according method described to (Liu *et al*., 2014). Different concentrations of the extract sample and standard (ascorbic acids) were prepared in (50, 100, 200, 250, 500) µg/ml respectively. DPPH solution (300µl, 0.1mM) was added to 1ml solution of extract of *C.citratus* and stand after 30 min incubation period at room temperature in the dark. The absorbance of the resulting mixture was measured at 518 nm. The blank was prepared by adding ethanol to DPPH solutions, the inhibition using the formula:

$$\%\ Inhibition = (Abs_{blank} - Abs_{sample} / Abs_{blank}) \times 100$$

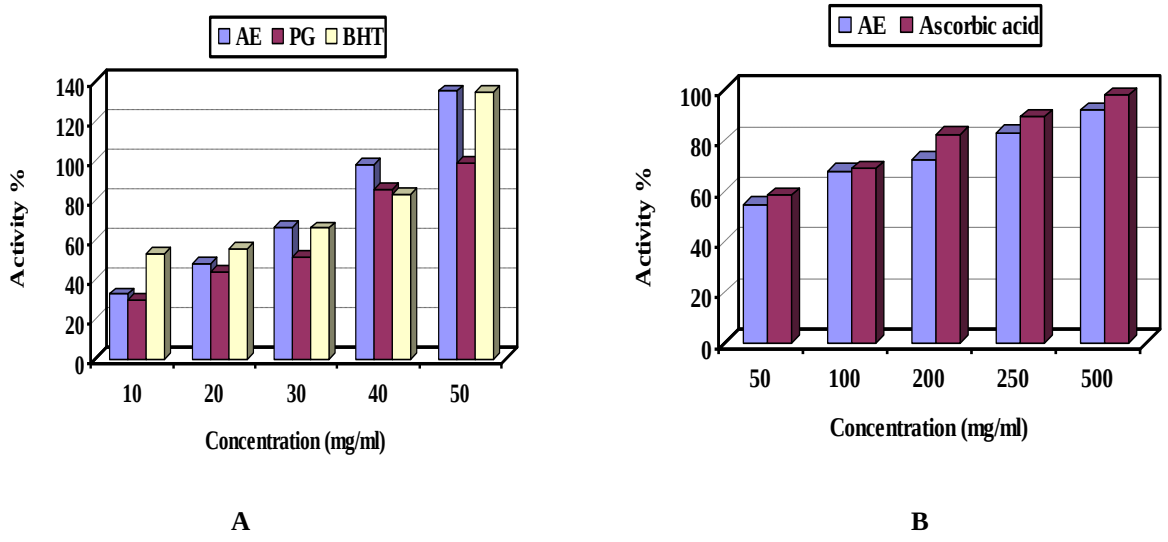
2.3 fish balls preparation

Attended Mixture the fresh minced meat obtained from common carp *(Cyprinus carpio)* fish, salt, pepper powder, coriander, love, cumin, garlic and breadcrumbs were (100, 1.5, 0.25, 025, 0.25, 0.25, 0.4 and 10)% respectively, with each concentration of *C.citratus* leaves aqueous extract (5, 10, 15, 20)% and mixing them by hand using medical gloves to ensure mixing and homogenization of all ingredients mix was used for the preparation of fish balls, the products stored at 4°C until were subjected for quality evaluation (Bavitha, 2016; Omar, 2003). The fish balls content of moisture, protein, fat, ash, carbohydrate contents and peroxide value (PV) were determined according to AOAC (2008). pH was determined by(Capita *et al.*, 2006). Thiobarbituric acid value (TBA) was determined according to (Witte *et al*., 1970).

3.RESULTS AND DISCUSSION

3.1 C.citratus aqueous extract antioxidant activity

Fig1. (A) showed the reducing power assay of Lemongrass aqueous extract compared with synthetic antioxidants PG, BHT. Aqueous extract exhibited higher reducing ability was 135.33% at 50µg/ml than PG, BHT which recorded 92.2 and 134.93 % respectively at the same concentration. Reductive capabilities were found to increase with increasing of concentration in extract antioxidant, (Geetha & Geetha, 2016). Fig 1. (B) mentioned DPPH free radicals scavenging activity of *C.citratus* aqueous extract compared with ascorbic acid. Aqueous extract showed high antioxidant activity trough aqueous extract concentration increasing. 50 µg/ml aqueous extract had showed 54.8% inhibition while 92.32% at 500 µg/ml.



A **B**

Figure 1. (A) Reducing ability of AE PG and BHT; (B) Free Radical Scavenging Activity DPPH of AE and Ascorbic acid. AE= *C.citratus* aqueous extract leaves

Balakrishnan *et al*., (2015) pointed essential oil of *C.citratus* has promising antioxidant activity property since it reduces free radicals. El-Shennawy & Abozid (2017) showed the highest antioxidant activity of Lemongrass essential oil compared with sage and thyme essential oils (Lemongrass, sage and thyme). Polyphenols are very important plant constituents because of their scavenging ability on free radicals due to their hydroxyl groups. The antioxidative properties of polyphenols arised from their high reactivity as hydrogen or electron donors. The important of the polyphenol was derived from its ability to scavenge radicals by stabilize and delocalize the unpaired electron and also from their potential to chelate metal ions (Sah *et al*., 2012).

3.2 Fish balls chemical components

Fig 2. presented moisture, protein content in fish balls treated with aqueous extract of Lemongrass. Results showed reduced of moisture content in all samples of fish balls with *C.citratus* leaves aqueous extract and sample control during refrigerated storage 4±2 $^{\circ}$C for 15 days. It noted slow reduction of moisture in sample Lg_3 (treated with aqueous extract 15%) reached 78.02 % at end storage, compared with Control sample reached 73.22% after 6th day of storage. Naturally, the duration of the storage period decreases the moisture content and increases the proportion of dry matter, which includes protein, fat and ash, and this low moisture content indicated that the fish balls have the tendency to be very stable.

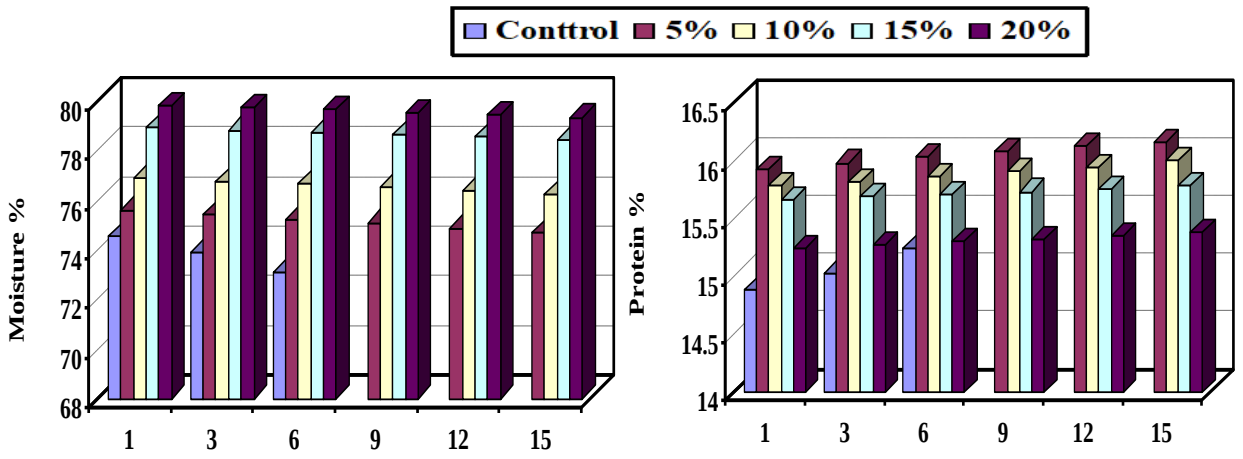


Figure 2. Moisture, protein content of fish balls treated with *C.citratus* leaves aqueous extract during refrigerated storage 4±2°C

Slow increasing (P <0.05) protein content in samples treated with *C.citratus* aqueous extract after 15 days storage. Treatment Lg_1 (treated with aqueous extract 5%) showed raising protein content from 15.83% to 16.32%, treatment Lg_2 (treated with aqueous extract 10%) from 15.80% to 16.43% after 15 days of refrigerated storage. The protein content of fish balls treatments showed an increasing trend treated with aqueous extract during refrigerated storage, the higher protein content may be attributed due to the addition of ingredients.

Results observed significant increase (P <0.05) in fat ratio of control treatment from first day was 4.64% to 5.22 % in sixth days during storage and a significant incurring in other treatments in 15 days compared with control treatment fig 3.

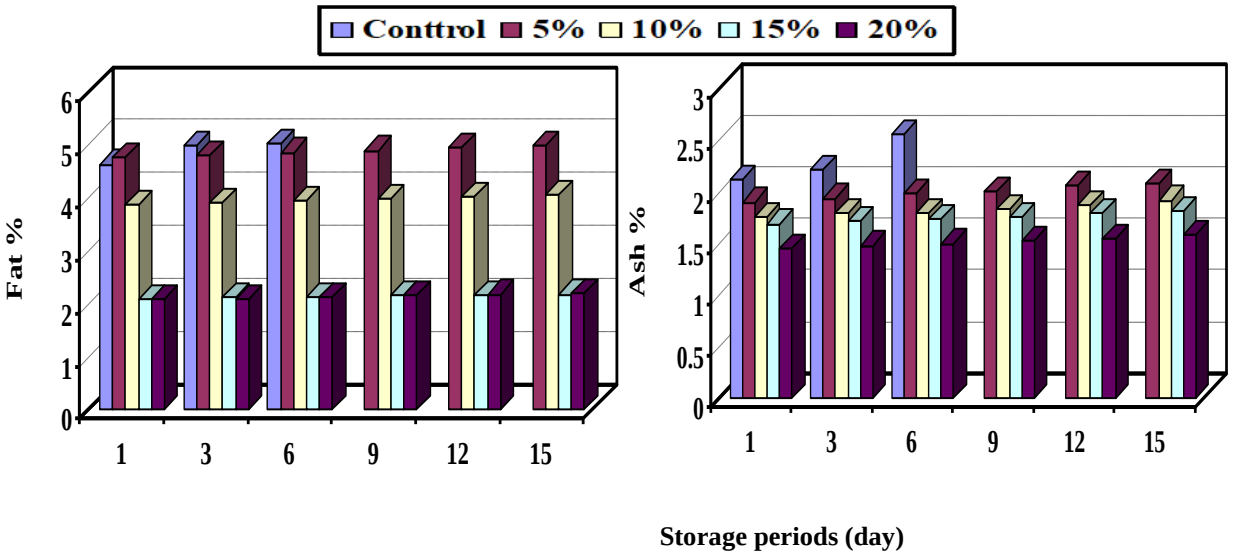


Figure 3. Fat, Ash content of fish balls treated with *C.citratus* leaves aqueous extract during refrigerated storage 4±2°C

Ash ratio showed a slow decrease in treatments containing *C.citratus* aqueous extract compared with sample control which raised ash content during refrigerated storage at end of storage fig 3.

Results observed carbohydrates content in Samples treated with *C.citratus* leaves aqueous extract. Control treatment was recorded 4.1% after 6 day during refrigerated storage.

pH values observed in models treated with *C.citratus* leaves aqueous extract compared with control sample, which reported 6.47 after 1day during refrigerated storage. Our results were agreement with the findings of Iheagwara, (2013)

who used ginger extract smoked mackerel fish stored at 28 ±2°c was determined over 20 days , Protein , Fat and Ash contents of the ginger extract treated samples had marked 27.02 to 20.09 % increase compared to the control, the moisture of the samples significantly decreased. Bavitha *et al*., (2016) observed moisture content reduction in fish burger treated the ginger extract decreased from 55.25 % to 50.84 % after 7 to 10 days of storage and an increase fat content throughout storage period 13.24 to 17.89 % of 19 days at 4°C. AL-Dhaheri, (2012) mentioned height fat content in control treatment compared with treatments (adding with marjoram extract) from 6.57 to 8.09 at refrigerated storage. An increased in total carbohydrates during storage which may be due to decreased moisture and increased concentration of total solids including carbohydrates (Omer,2003). Alsaiqali *et al*., (2016) observed that 1.2 % essential oils of (thyme, cumin and parsley) in beef burger stored at 4±1°C for 4 days reduced the pH.values.

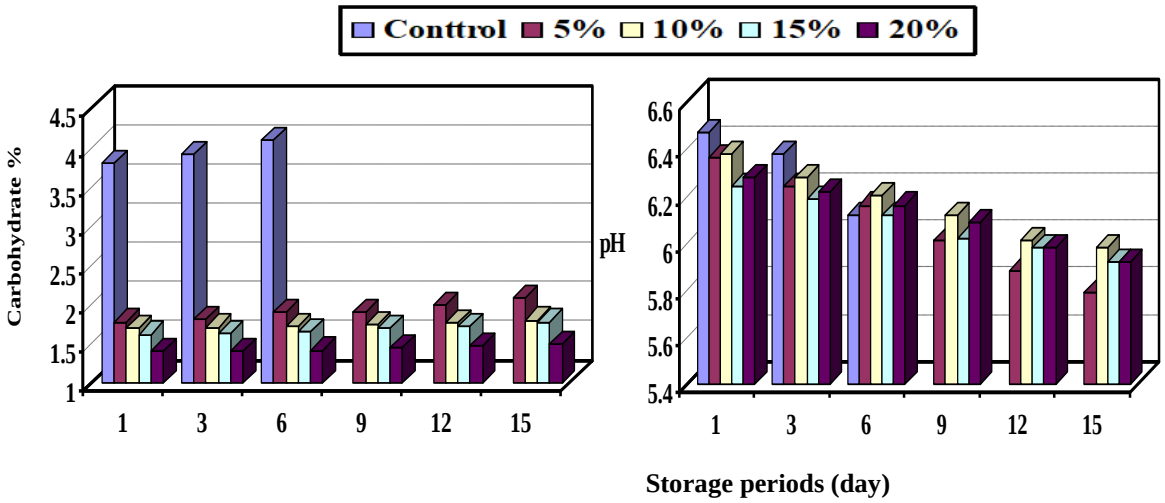


Figure 4. Carbohydrates content and pH value in fish balls treated with *C.citratus* leaves aqueous extract during refrigerated storage 4±2°C

PV value significantly increased in all the samples treated with *C.citratus* leaves aqueous extract compared with control sample at the beginning of storage during the 6 days storage, and there were not significant differences ($P \leq 0.05$) among the samples fig 5.

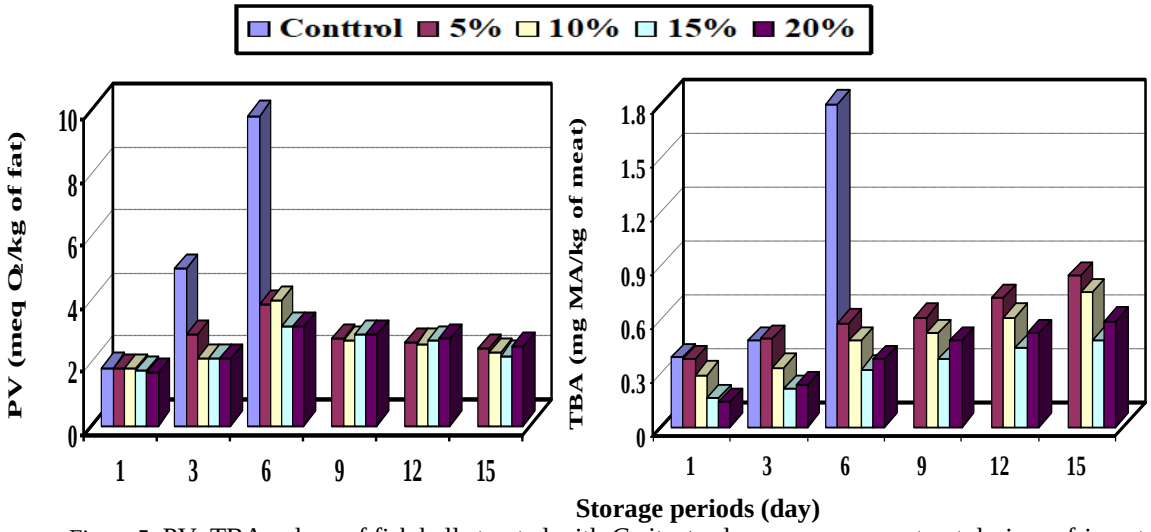


Figure 5. PV, TBA values of fish balls treated with *C.citratus* leaves aqueous extract during refrigerated storage 4±2°C

Whereas the highest value recorded in control sample was 9.81mEq/kg during 6th day of refrigerated storage, while the lowest value 2.22mEq/kg was observed in sample (treated with 15% aqueous extract. Also, it was observed that PV content decreased progressively after 6th day of refrigerated storage in all samples treated with *C.citratus* aqueous extract. Results were in agreement with Iheagwara, (2013) which showed that ginger extract is effective in retarding rancidity in of smoked mackerel fish stored at 28±2°c. Bavitha *et al*.,(2015) found in initial stage of storage period, PV of Sample of fish burgers were 4.80 meqO_2/kg fat and increased to 7.26 meqO_2/kg fat after 5th day of storage but subsequently decreased from 5.23 to 5.12 meqO_2/kg of fat at the end of 19th day of storage period for treated samples. The antioxidant properties of Lemongrass extract are mainly attributed to phenolic compounds. *C.citratus* leaves extracts were known also contains several other important compounds such as flavonoid, tannin, and phenolic compound Sari *et al.*, (2017). Ordialez *et al*., (2016) observed Lemongrass extract made no changes in PV of the deboned milkfish (*Chanos chanos*) during 30 day of frozen storage registered 6.8 ± 0.684 meq/kg fat acceptability limit in fish oil was 7-8 meq/kg not exceeding to 20 meq/kg and not more than ≤ 5.0 meq/kg as maximum level for fish products

TBA value showed a significant increase (P <0.05) in all the samples treated with *C.citratus* leaves aqueous extract during refrigerated storage. The highest TBA value was 1.88 mg/MA /kg recorded for control sample during 6th day of refrigerated storage, which excluded due to exceeds the maximum limits of criteria for 2 mg /MA /kg while the lowest value 0.48 mg/MA/kg was observed in the sample(treated with 15% *C.citratus* leaves aqueous extract) in end of storage. The US standard states that the maximum value of TBA is 2mg /MA/kg of proposed meat (FSIS), 2000. Nearly similar results were obtained by Amany *et al*., (2010) noted that TBA values decrease as the concentration of Lemongrass oil gave the best effectiveness in beef samples extend the shelf life of the treated samples compared the control samples. The results indicated that *C.citratus* leaves aqueous extract was effective in retarding lipid oxidation. This antioxidant activity had been mainly attributed to flavonoids and ascorbic acid in citrus fruits (hesperidin, neohesperidin and eriocitrin) while the lowest significant incremental rate was recorded in samples treated with 1.5% Lemongrass oil in refrigerated minced beef. Clove oil (*Eugenia caryophyllata*) on sliced smoked rainbow trout fillets were evaluated during storage at 2 °C that showed significantly higher values of TBA in control sample (Çoban & Patir, 2013). Zulfa *et al*., (2016) mentioned that *C. citratus* leaves extract had potent antioxidant activity due to its polyphenolic content.

4. CONCLUSIONS

Lemongrass (*Cymbopogon citrates*) leaves aqueous had antioxidant effectiveness in the reduction power and inhibiting free radical effect DPPH. Treatment Lg_3 (treated with aqueous extract 15%) Showed the best properties in maintain PV and TBA properties during 15 day of refrigerated storage.

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