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Effects of Electrospinning on Antifungal Properties of Thyme and Cardamom Oils

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Abstract

Thyme and cardamom oils are known to be various health benefits along with their antimicrobial properties are used in foods as additives or condiments. Such functional properties of these essential oils (EOs) stem from bioactive and volatile components that are very sensitive to ambient conditions. Encapsulation of EOs provides protection of core material. Electrospinning is an easy, effective and relatively low-cost encapsulation method. There is no clear statement on how electrospinning affect the functional properties of active material that is to be encapsulated. The aim of this study was to investigate the effects of uniaxial and coaxial electrospinning on antifungal effects of thyme and cardamom oils, which had proven their antifungal effects, against isolates of *Aspergillus carbonarius* 35-03X1 and 39-04X4. The uniaxial and coaxial electrospinning encapsulated thyme oil exhibited less antifungal effect against 39-04X4 compared to non-encapsulated thyme oil. No antifungal effect of encapsulated cardamom oil was detected. Electrospinning inhibited the antifungal effect of cardamom oil. The outcomes of this study can help effect of electrospinning on functional properties of active materials.

Keywords: Thyme oil, cardamom oil, antifungal, electrospinning, encapsulation, *Aspergillus carbonarius*

Research article
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INTRODUCTION

Essential oils (EOs) composed of various bioactive agents are strong, bitter and volatile. These oils are obtained by distillation from seeds and tissues of plants (Singh et al., 2002). Most of EOs are generally considered as GRAS (Generally Recognized as Safe) by the Food and Drug Administration (FDA) due to fact that they are not phytotoxic or harmful for environment (Da Cruz Cabral et al., 2013; Holley and Patel, 2005). EOs have antimicrobial effects on microorganisms because of their aromatic contents; they can be used as sedative and analgesic as well (Bakkali et al., 2008). These oils have antibacterial and antifungal phytochemical ingredients such as thymol, eugenol, menthol, carvacrol, benzoic acids, phenolic acids and flavone (Souza et al., 2005). The antimicrobial activity of EOs is affected by various parameters. For instance, high oil concentrations have generally more antimicrobial activity. The solubility and the diffusion ability of EOs should be taken into account as well. In addition, antimicrobial effect is stronger as exposure time to EOs increases (Seow et al., 2014).

In other words, antimicrobial activity of EOs depends on type of oil and microorganism (Friedman et al., 2004), concentration of EOs, exposure time, pH, temperature and other ambient conditions (Seow et al., 2014). Some species of black *Aspergillus* species produce mycotoxins like ochratoxin (Abarca et al., 2003; Abarca et al., 2004; Cabaňes et al., 2002) and fumonisin (Frisvad et al., 2007; Frisvad et al., 2011, Perrone et al., 2011). *Aspergillus carbonarius, Aspergillus niger, Aspergillus acidus, Aspergillus brasiliensis and Aspergillus ibericus* are common species of black *Aspergillus* (Nielsen et al., 2009). *A. carbonarius* can be isolated from several foods such as grapes, coffee, spices, cereal and cereal products (Joosten et al., 2001; Kapetanaku et al., 2011; Nakajima et al., 1997). Most of *A. carbonarius* isolates are highly ochratoxigenic. At tropical or semi-tropical areas, *A. carbonarius* is source of ochratoxin A (Joosten et al., 2001; Nakajima et al., 1997; Patino et al., 2005; Pitt, 2000). The maximum growth rate of *A. carbonarius* is observed at a_w 0.95 between 0.8-0.95 and 30°C between 15-35˚C at a study. Results showed that *A. carbonarius* cannot grow at below 0.85 a_w (Romero et al., 2007). Ochratoxin A is produced at 0.86-0.94 a_w values by *A. carbonarius* (Esteban et al., 2006). Some environmental factors such as temperature, pH and aw affect fungal growth and ochratoxin synthesis (Gürhayta and Çağrındı, 2016). EOs and their terpenoid components can easily dissolve in lipids. Generally, EOs damage the biological membrane and microbial membrane-catalyzed activities like, e.g. respiratory pathway due to their lipophilic properties. Therefore, they can act as antimicrobial and antifungal agent by their components (Knobloch et al., 1989). There are various studies on the antifungal affects of EOs. The summary of some of the studies were given at Table 1. EOs should be protected from ambient conditions through processing and storage for exhibiting their desirable effects. Encapsulation can isolate and immobilize sensible bioactive components, therefore the substance is protected from the surrounding environmental conditions. Simultaneously, the controlled release and stabilization of bioactive materials can be provided by the matrix. In addition, core material can be added to foods without affecting taste, aroma and texture of food (Augustin and Hemar, 2009).

Table 1. Various studies on the antifungal affects of EOs.

Essential Oil	Mold	Concentration	Medium	Effect	MIC or MFC*	Reference
Thyme (*Thymus serpyllum* L)	*A. carbonarius*		Potato dextrose agar (PDA)	Antifungal effect	1.25 µl/ mL	Sokolic-Mihalak et al. (2012)
Boldo, poleo, clove, anise and thyme	*A. niger aggregate and A. carbonarius*	500 µL/L	Peanut meal extract agar	*A. carbonarius* inhibition: 52.8 %		Passone et al. (2012)
Cuminum cyminum, *Ziziphora clinopodioides* and *Nigella sativa*	*A. parasiticus*				*C. cyminum*: MIC: 1.6 mg/mL MFC: 3.5 mg/mL	Khosravi et al. (2011)
Marjoram	*A. niger*, *A. carbonarius* and *A. wentii*	2.5 mL/100 mL	Czapek yeast autolysate agar (CYA)	*A. carbonarius* inhibition: 95.6 %		Kocić-Tanackov et al. (2012)
Cinnamon, clove, anise, cardamom	*A. flavus*	10 mg/mL		Cardamom and anise: no antifungal effect, Cinnamon and cloves: 100% inhibition	MIC of cinnamon: 4 mg/mL, MIC of clove: 2 mg/mL	AikoMehta (2013)

Basil (*Ocimum basilicum* L.)	*Alternaria spp., A.flavus, Botrytis cinerea, Cladosporium herbarum, Eurotium amstelodami* and *E. Chevalieri*			*E. chevalieri* most sensitive, *A. flavus* most durable		Jakowienko et al. (2011)
Lippia rugosa, Plectranthus glandulosus, Clausena anisata and *Vepris heterophylla*					*Aspergillus spp*.: *L. rogusa*: 0.5 mg/mL, *P.glandulosus*:1.5 mg/mL *Fusarium* spp.: *L. rogusa*: 0.3 mg/mL, *P.glandulosus*:1.2 mg/mL *Penicilium* spp.: L. rogusa: 0.6 mg/mL, *P.glandulosus*:2.0 mg/mL	Aoudou et al. (2010)
Origanum vulgare L. (Lamiaceae)	*Aspergillus, Penicillium* and *Fusarium spp.*	80 and 40 µl/mL		Prevention of spore formation	80- 20 µl/mL	Carmo et al. (2008)
Thymus mastichina L. ssp. mastichina	*Aspergillus species (A. flavus, A. parasiticus, A. terreus, A. ochraceus, A. fumigatus ve A. niger)*				MIC: 1500-2100 µg/mL MFC: 2.0-2.4 mg/mL	Fraternale et al. (2003)
Thyme, cinnamon, marigold, spearmint, basil and quyssum	*Fusarium species*	500-3000 ppm	Potato dextrose agar (PDA)	Thyme: fungistatic: 250 ppm, fungicidal: 500 ppm Basil: fungistatic: 2000 ppm, fungicidal: 3000 ppm		Soliman and Badeaa (2002)
Marjoram (*Origanum vulgare*)	*A. flavus, A. parasiticus, A. ochraceus* and *F.moniliforme*	1000 ppm	Yeast extractsucrose (YES) broth	antifungal		Basílico and Basílico (1999)
Cardamom	*A. ochraceus*	0.05 %		Prevention of *A. parasiticus* growth		Badei (1992)

*MIC: minimum inhibition concentration; MFC: minimum fungisidal concentratio

When capsule size is less than 100 nm, capsules are named as nanocapsules (Augustin and Hemar, 2009). The nanoencapsulation is an encapsulation technique that uses nanotechnology processes such as nanoemulsification and nanostructuration. Nanoencapsulation provides final product functionality including controlled release of the core which is expected to be maintained during storage (Quintanilla-Carvajal et al., 2010). Nanocapsules present several potential advantages such as better encapsulation efficiency, protection, distribution for the bioactive ingredients compared to microcapsules. Furthermore, the improved bioavailability or overcoming incompability can be handled by nanocapsules (Xiao et al., 2013).

Donsi et al. (2011) nanoencapsulated terpenes and D-limonene and tested on *Escherichia coli*, *Lactobacillus delbrueckii* and *Saccharomyces cerevisiae*. Nanoencapsulation of terpenes decreased the minimum inhibition concentration (MIC) and minimum bactericial concentration (MBC) values or they were equal to the values of the unencapsulated mixture, although the nanoencapsulation of D-limonene reduced just the MIC. Low concentrations of the nanoencapsulated terpenes retarded the microbial growth (1.0 g/l terpenes) or inactivated the microorganisms (5.0 g/l terpenes) by adding to them into the fruit juices. In addition, they caused minimal organoleptic changes in fruit juices (Donsì et al., 2011). In an another study, thymol and carvacrol oils were encapsulated by the zein nanoparticles using the liquid-liquid dispersion method. After that, the nanoparticules were tested against *E. coli*. 0.8-1.8 log CFU/ml decrease was reported (Wu et al., 2012). Zhaveh et al. (2015) encapsulated *Cuminum cyminum* by chitosan (CS)–caffeic acid (CA) nanogel and tested it against *Aspergillus flavus*. The MICs of encapsulated and non-encapsulated *C. cyminum* were 350 and 650 ppm against *A. flavus*, respectively (Zhaveh et al., 2015). In a similar study, thyme was encapsulated by using CS and benzoic acid (BA) nanogel to improve its antifungal properties and half-life. Under sealed condition, the MIC of the CS-BA encapsulated EO was 300 mg/l, although the free thyme extract prevented the growth of *A. flavus* at 400 mg/l. Under non-sealed condition, higher concentration of encapsulated thyme oil (500 mg/l) was needed to fungi inhibition and free oils could not provide the complete inhibition, even at concentrations as high as 1000 mg/l. It was found that the nanogel encapsulation significantly increased half-life and the anti-fungal properties of thyme essential oil (Khalili et al., 2015).

Electrospinning is an encapsulation method, which can produce fibers with a diameter of 2 nm to few micrometers obtained uniaxially or coaxially. Electrospinning process is performed at room conditions, i.e. ambient temperature and pressure, which may be more suitable for encapsulation of sensitive bioactive components. In uniaxial electrospinning one feed solution, containing wall polymer and active material is present, whereas at coaxial electrospinning, there are two immiscible co-flowing fluids that are fed by two different capillary tubes. One of them carries inner fluid whereas the other one delivers the shell fluid. At the end of capillary tubes, electrical charge is applied on co-fluids and coaxial jet moves to the collector (Díaz et al., 2008; Bhardwaj and Kundu, 2010).

Wen et al. (2016) produced electrospun polyvinyl alcohol/cinnamon essential oil/β-cyclodextrin (PVA/CEO/b-CD) and applied on *Staphylococcus aureus* and *E. coli*. The MIC and MBC values were approximately 0.9-1 mg/mL and 7-8 mg/mL, which is a strong antimicrobial activity against the *S. aureus* and *E. coli* (Wen et al., 2016).

Using high voltage, i.e. over 10 kV, the electrospinning may have some undesirable effects on the active material that is to be encapsulated. Or, during electrospinning process, active materials which are sensitive to ambient conditions such as light, temperature, humidity may have been affected inversely or lost their functions. Eventhough, electrospinning encapsulation produce high yields in terms of efficiency, this point should be investigated. In this study, we used thyme and cardamom oil, which was proven that they have antifungal effect, to investigate the influence of electrospinning on their antifungal properties. Therefore, the aim of this study was to investigate the effects of electrospinning encapsulation of thyme and cardamom oils on their antifungal properties against *Aspergillus carbonarius* 35-03X1 and 39-04X4 isolated from dried figs from Aegean Region.

MATERIALS AND METHODS

Materials

At this study, *A. carbonarius* 35-03X1 and 39-04X4 which were isolated from dried figs from Aegean Region, were used to determine antifungal activity (Karbancıoğlu-Güler, 2008). Thyme (0.864 g/mL) and cardamom (0.899 g/mL) oils were supplied from IFF Inc., (International Flavors and Fragrances Inc., Kocaeli, Turkey) and were stored in original packages at 4ºC until further analysis. Gelatin and acetic acid were purchased from Sigma-Aldrich, USA.

Encapsulation of EOs

All details of the electrospinning and the concentration of feed solutions were given in elsewhere (Arıkan et al., 2016). Electrospun mats were cut as 1x1 cm or 2 x 2 cm. Electrospun samples of uniaxial (U) and coaxial (C) encapsulated thyme (T) and cardamom (C) oils were named as UT-1, UT-2, CT-1, CT-2, UC-1 and UC-2 based on their dimensions in the petri dishes (code 1 is for 1x1 cm and code 2 is for 2x2 cm), respectively. The gelatin electrospun mats without EOs were used as control.

Preparing mold isolates and spore suspensions

To prepare mold isolates and spore suspensions, the methods given in (Yavuz, 2015) were applied. In this study, *Aspergillus carbonarius* 39-04X4 and 35-03X1 isolates were used. Spore concentration of moulds were adjusted $1x10^6$ spore/mL in distilled water with 0.05% Tween 80.

Antifungal effects of electrospun EOs

The antifungal effects of electrospun mats were determined by using disc diffusion method against *A. carbonarius* (Maruzzella and Liguori, 1958). 100 µL spore suspension was inoculated onto MEA by spreading plate method. Electrospun mats were sterilized under UV irradiation for 2 h (each side for 1 h). Sterilized electrospun mats were placed on the inoculated media. After incubation at 30 °C for 48 h, the antimicrobial properties of the mats were assessed by measuring the inhibition zone diameter (including nanofiber mat) in each inoculated plate. All analysis were carried out in triplicate for each mat.

RESULTS and DISCUSSION

Electrospinning of EOs

Thyme oil encapsulated by uniaxial and coaxial electrospinning whereas cardamom oil can only be encapsulated by uniaxial electrospinning (Arıkan et al., 2016). The uniaxial electrospun nanofibers of gelatin without EOs were also obtained as a control sample.

Antifungal properties of electrospun EOs

The uniaxially nanofiber encapsulated cardamom oil (UC-1 and UC-2) exhibited no antifungal effect on *A. carbonarius* isolates. This may be because of the morphology of the electrospun samples with cardamom oil, which were not smooth and continuous as a fiber form (Arıkan et al., 2016). Probably, the volatile active components of cardamom oil evaporated during electrospinning. In this case, then, for volatile component of cardamom oil should be solubilized in the feed solution (the solvent was a mixture of acetic acid:water) by addition of a proper material or a surfactant for the electrospinning to obtain full and homogenous solution with all volatiles are present. Another point is that, Yavuz (2014) reported that the antifungal effect of cardamom oil was low compared to thyme oil against *A. carbonarius* 3904-X4.

The petri dishes, which include uniaxial and coaxial nanofibers of thyme oil, and uniaxial nanofibers of gelatin against 39-04X4 were given at Figure 1, 2 and 3, respectively. As it can be clearly seen from Fig 1 and Fig 2, uniaxially (UT-1 and UT-2) and coaxially (CT-1 and CT-2) nanofiber encapsulated thyme oil had an antifungal effect compared to the control, because there were clear and distinct inhibition zones in the petri dishes. The inhibition zones for UT-1 and UT-2 were 1.1-0.9 mm and 2.5-2.9 mm, respectively. This antifungal effect was more profound for the uniaxially encapsulated samples, probably due to that thyme oil in these samples was readily to show its effect. On the other hand, the inhibition zones were smaller for the CT-1 (1.0-0.8 mm) and CT-2 (1.2-0.9 mm) compared to the inhibition zones of UT-1 and UT-2. This may be attributed to that thyme oil encapsulated in the coaxial geometry of the nanofiber system. Therefore, the exhibiting its effect compared to uniaxial geometry was probably extended.

Yavuz (2015) reported that non-encapsulated thyme oil at 1:10 dilution had the inhibition zone of 16 mm against 39-04X4. It appears that uniaxial electrospinning of thyme oil (2.5 and 2.9 mm for UT-2) decreased the inhibition zone almost 6.4 and 5.5 times compared to the inhibition zone of non-encapsulated thyme oil. This was that even the ratio of thyme oil to gelation solution in the uniaxial electrospinning feed solution was 1:9 (Arıkan et al., 2016) whereas thyme oil was diluted 1:10 given by Yavuz (2015). This decrease was probably due to that possible matrix effect of encapsulation system or the high applied voltage during electrospinning. However, it may be concluded that electrospinning decreased the antifungal effect of thyme oil.

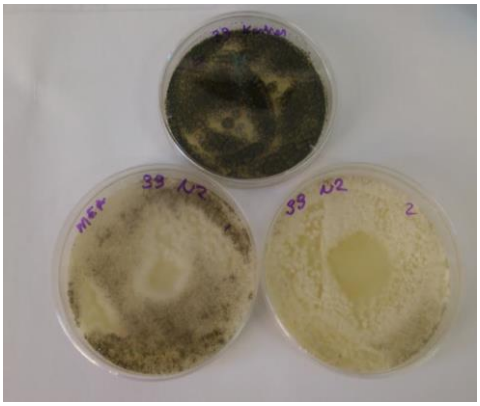

Figure 1. The petri dishes of control (up) and UT-1 (uniaxial electrospun thyme oil, 1 cm x1 cm) (on the left) and UT-2 (uniaxial electrospun thyme oil, 2 cm x 2 cm) (on the right) against 39-04X4

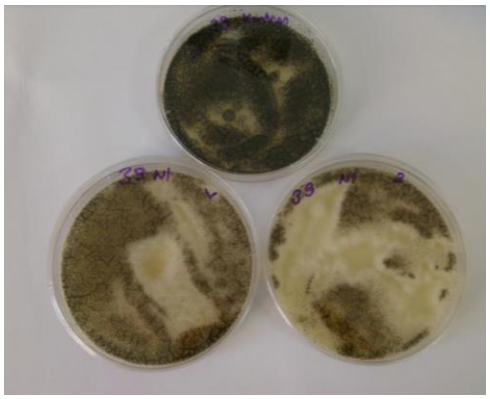

Figure 2. The petri dishes of control (up) and CT-1 (coaxial electrospun thyme oil, 1 cm x1 cm) (on the left) and CT-2 (coaxial electrospun thyme oil, 2 cm x 2 cm) (on the right) against 39-04X4.

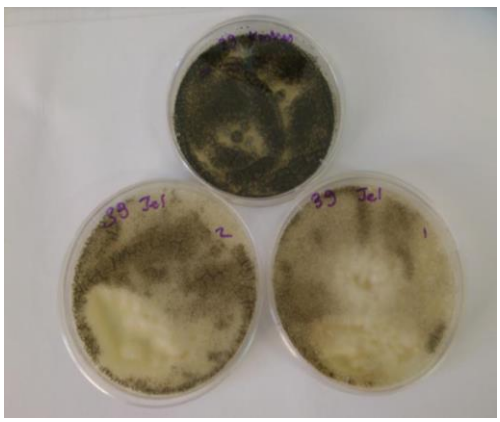

Figure 3. The petri dishes of control (up) and uniaxial electrospun gelatin with 2 cm x2 cm (on the left) and uniaxial electrospun gelatin with 1 cm x 1 cm (on the right) against 39-04X4

The petri dishes, which include uniaxial/coaxial nanofibers of thyme oil and uniaxial nanofibers of gelatin against 35-03X1 were given at Figure 4, 5 and 6, respectively.

As it can be clearly seen from Fig 4 and Fig 5, uniaxially (UT-1-X1 and UT-2-X1) and coaxially (CT-1-X1 and CT-2-X1) nanofiber encapsulated thyme oil had an antifungal effect compared to the control, because there were clear and distinct inhibition zones in the petri dishes. The inhibition zones for UT-1-X1 and UT-2-X1 were smaller than 2.0 mm and 2.5-2.4 mm, respectively. This antifungal effect was more profound for the uniaxially encapsulated samples, probably due to that thyme oil in these samples was readily to show its effect. On the other hand, the inhibition zones were smaller for the CT-1-X1 (1.0-1.0 mm) and CT-2-X1 (1.4-2.0 mm) compared to the inhibition zones of UT-1-X1 and UT-2-X1. This may be attributed to that thyme oil encapsulated in the coaxial geometry of the nanofiber system. Compared to the effect on 39-04X4, coaxial nanofibers with thyme oil had more effect on 35-03X1.

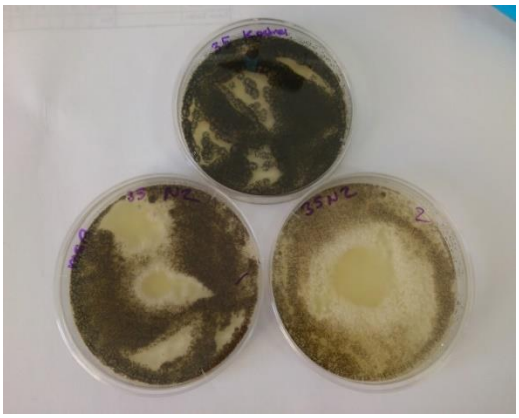

Figure 4. The petri dishes of control (up) and UT-1-X1 (uniaxial electrospun thyme oil, 1 cm x1 cm) (on the left) and UT-2-X1 (uniaxial electrospun thyme oil, 2 cm x 2 cm) (on the right) against 35-03X1

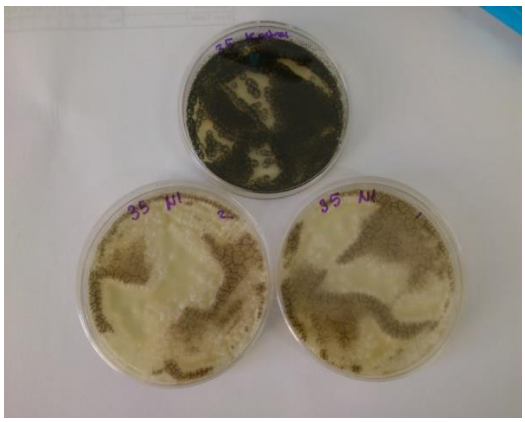

Figure 5. The petri dishes of control (up) and CT-1-X1 (coaxial electrospun thyme oil, 1 cm x1 cm) (on the left) and CT-2-X2 (coaxial electrospun thyme oil, 2 cm x 2 cm) (on the right) against 35-03X1

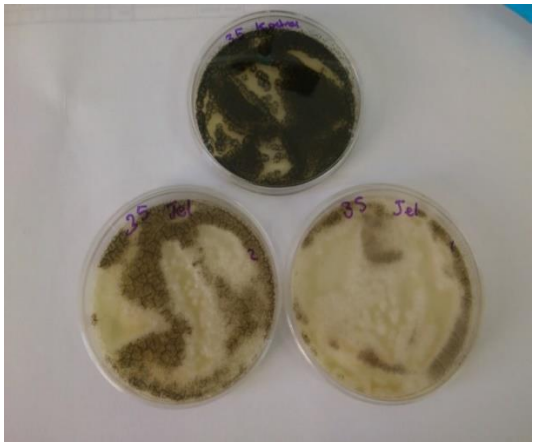

Figure 6. The petri dishes of control (up) and uniaxial electrospun gelatin with 2 cm x2 cm (on the left) and uniaxial electrospun gelatin with 1 cm x 1 cm (on the right) against 35-03X1

In order to evaluate the antifungal property of encapsulated thyme oil; the same protocol was applied to gelatin nanofibers, which had no antifungal effect itself (Fig 3 and Fig 6). As seen from Fig 3 and Fig 6, there was no distinct inhibition zone in the petri dishes for gelatin nanofibers. However, it can be seen a delay for spore growth, meaning that gelatin may contain peptide part or parts, which may have antifungal effect at the nanoscale to take effect in the petri dishes. Actually, the petri dishes contained uniaxial and coaxial nanofiber encapsulated thyme oil exhibited inhibition zone with the delay for spore growth as well. This may be due to gelatin nanofiber, which was used as wall material in the encapsulation system.

CONCLUSIONS

The outcomes of this study suggested that uniaxially nanofiber encapsulated thyme oil can be used as an antifungal. On the other hand, coaxially encapsulated thyme oil has an extended antifungal effect due to the geometry of the coaxial electrospinning encapsulation system. The electrospinning decreased the antifungal effect of uniaxial and coaxial encapsulated thyme oil. The nanofiber encapsulation of cardamom oil should be investigated in detail, because it has an antifungal effect without encapsulation. It is interesting to study the effect of electrospinning on antimicrobial properties of essential oils which may help to understand the encapsulation mechanism during electrospinning and antimicrobial effect of encapsulated essential oils. Furthermore, their release from the encapsulation system can be tailored as needed.

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Development of a Locust Bean Fermentation Bin

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Abstract

Locust bean is an important food condiment in Africa; it is rich in protein, carbohydrates, fat, and oil. Fermentation of the cooked bean (which is carried out traditionally) is one of the major and the last operation in the processing of the seed to obtain the condiment. A fermentation bin for fermenting locust beans was designed, fabricated, and evaluated in this study. The fermentation bin simulated the principle of the traditional fermentation operation by enhancing the conditions necessary for the complete fermentation of locust beans. The bin consists of an inner chamber (made of stainless steel) where fermentation takes place, the lagging material (made of fibreglass) to prevent heat loss in the closed system, and the outer chamber (made of metal sheet) which serves as housing for the bin. The bin was evaluated based on locust bean loading (2, 4, 6, 8, 10, 12, 14, and 16 kg) and the time (48 hours), which was kept constant throughout the experiment. The results showed that an increase in the mass of locust beans in the fermentation bin led to a reduction in the overall rating of the output. For the above-listed loading of locust bean, the efficiency of the fermentation bin was 80%, 80%, 70%, 70%, 60%, 60%, 30%, and 20%, respectively. This result provides an insight into how to improve the efficiency of the device.

Keywords: Locust bean, sensory analysis, fermentation operation, fermentation bin

Research article
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1. INTRODUCTION

African locust bean (*Parkia biglobosa*) is common around villages in the Savannah areas of West Africa where it is left standing when land is cleared or sometimes planted and trees are individually owned (Dalziel, 1937). It is popular for the production of soup condiments (Akande et al., 2010). Fermented legumes, oilseeds, and nuts are commonly used in condiments. Some examples are *iru* from the locust bean, ogiri from the castor seed, and soy sauce from the soybean. *Iru* or *dawadawa* is a condiment used in many African dishes especially in Nigeria. The African locust bean and dawadawa are particularly useful sources of protein to the poorer sections of the community (Campbell, 1980). Locust beans are commonly found in Tropical Africa and the Mediterranean. It was estimated that about 200,000 tonnes of Africa locust beans seeds are gathered each year in Nigeria alone, as well as large quantities are produced in the savannah region of Southwest, Nigeria (Diawara, 2000).

In addition, a large quantity is produced in the savannah region of West Africa. More than 100 million people in West Africa use *iru* as a foodstuff (Odunfa, 1981). All parts of the crop are useful. It is used as a food condiment and it is a good substitute for meat, Maggi, and all other canned seasonings because it is high in protein, fat, and vitamins and it is rich in tannin and mineral content. The pods are used for the production of locust bean gum. This gum is used around the world as a thickening agent and stabilizer in many food products such as mayonnaise and within the textile industry as a print thickener (Glasson Grain Ltd, 2006). The fermented bean pulp waste contains protein 11.75 %; ash, 15.86 %; crude fiber, 21.55 %; starch, 32.14 %; dry matter, 93.5% and moisture, 6.5 % while the unfermented pulp contains protein 10.13 %; ash content, 14.14%; crude fiber 22.63%; starch, 28.20%; dry matter, 92.5% and moisture, 7.5%. The unfermented locust pulp waste exhibited a stronger binding effect than corn starch after 12 weeks of storage (Akegbejo-Samsons, 2004). Traditional boiling is carried out for cooking the beans with the hull for 8 to 10 h using firewood as fuel (Oyewole and Odunfa, 1990). After cooking, fermentation operation is carried out with the use of a basket and plantain leaves compacted in an enclosed system. The bean being fermented is left for 48 hours after which a sweet smell is perceived from the compacted enclosed system indicating the end of fermentation.

2. MATERIALS and METHOD

2.1. Description of the fermentation bin

The fermentation bin simulates the traditional way of enclosing the cooked bean in a controlled system, whereby a basket, cloth, or banana leaf is used to create the enclosure. This stage is important because it's the last stage that precedes the finished product. The design as shown in Figure 1 consists of an inner chamber, the lagging material, and the outer chamber. The inner chamber makes up the fermentation bin; this is where the dehulled locust bean seeds to be fermented are placed. The inner chamber is made up of cylindrical stainless steel to accommodate the locust bean. The lagging material is made up of fibreglass placed in-between the inner and the outer chamber to conserve the heat required. The outer chamber is made up of sheet metal to form the body of the fermentation bin. The external section makes up the housing chamber that insulates around the internal chamber as shown in Figs 2 and 3.

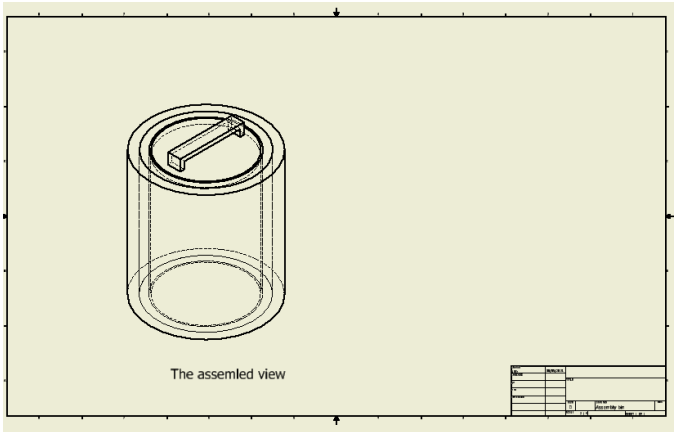

Figure 1. CAD design of the fermentation bin

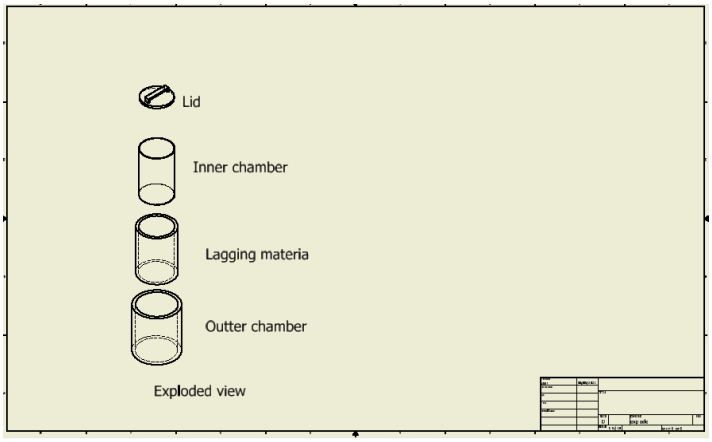

Figure 2. Exploded view of the fermentation bin

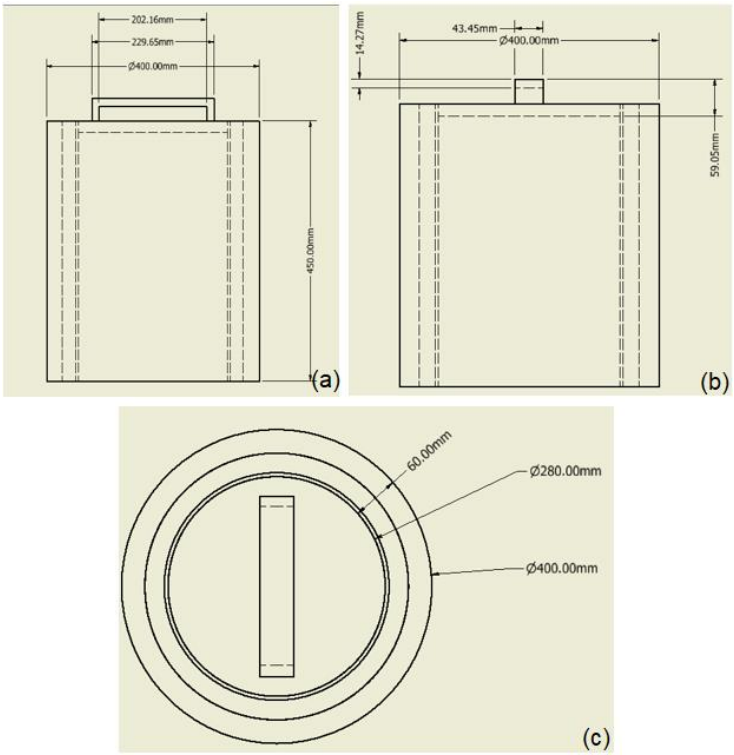

Figure 3. (a) Front view, (b) Side view, (c) Plan view

Figure 4. Fabricated fermentation bin

2.2. Specification of the Component Parts of Bin

The parts of the device and the materials used for their fabrication are shown in Table 1. Stainless steel was selected for the inner chamber because of its non-corrosive nature, due to the high moisture present in the parboiled locust bean it can corrode ordinary metal hence the need for stainless steel at the inner chamber, fibreglass was selected as a lagging material due to its ability to absolve heat and prevent heat loss and, mild steel was chosen for the outer chamber due to its ability to resists corrosion.

Table 1. Material Selection for the Design of Fermentation Bin

Parts of the devices	**Materials of construction**	**Specifications**
Lid	Mild steel	59.05 x 280 mm
Inner chamber	Stainless steel	450 x 280 mm
Lagging material	Fibreglass	450 x 60 mm
Outer chamber	Mild steel	450 x 400 mm

2.3. Design of the inner chamber

The inner chamber which determines the volume of the fermentation bin was designed based on the data obtained from the literature. The bulk density of locust beans is 538.02 kg/m^3 (Ogunjimi et al., 2002). For the maximum load of locust bean expected to fill the fermentation bin, the volume, height, and diameter of the bin were determined as follows.
Using 16 kg of a locust bean seed.

$$\text{Density } (\rho) = \frac{Mass\ (m)}{Volume\ (v)} \quad (1)$$

$$\text{Volume} = \frac{Mass\ (m)}{\text{Density } (\rho)} \quad (2)$$

$$\text{Volume of locust bean seed} = \frac{16}{538.026} \quad (3)$$

$=0.029\text{m}^3$

$$\text{Recall; Volume} = \pi r^2 h \quad (4)$$

The height is calculated from formula 4 as:

$$\text{Height} = \frac{volume}{\pi r^2} \quad (5)$$

$h = 0.45\text{m}$

The radius is calculated from formula 4 as:

$$r^2 = \frac{volume}{\pi h} \quad (6)$$

$$r = \sqrt{\frac{volume}{\pi h}} \quad (7)$$

r=0.14m=14cm

Therefore, the height of the fermentation bin required to process a 16 kg locust been was found to be 0.45 m with a diameter of 0.14 m.

2.4. Analysis of heat transfer across the walls of the fermentation bin

Each section of the fermentation bin wall behaves as a composite wall (Fig.4). Firstly, the temperature of the fermentation chamber after being filled appropriately with boiled dehulled locust bin is designatedT_{s1}. The heat as a result of the temperature circulates by radiation and convection on the internal stainless steel. The heat is transferred by conduction through the stainless steel wall to T_{s2}. The heat is transfered through a distance of L_A to the lagged material and then through L_B by conduction in the fibreglass from T_2 toT_3, to the external mild steel and by a distance of L_C heat is lost to the surrounding environment as shown in Fig 4. Heat lost to the environment from the external mild steel is by convection and radiation.

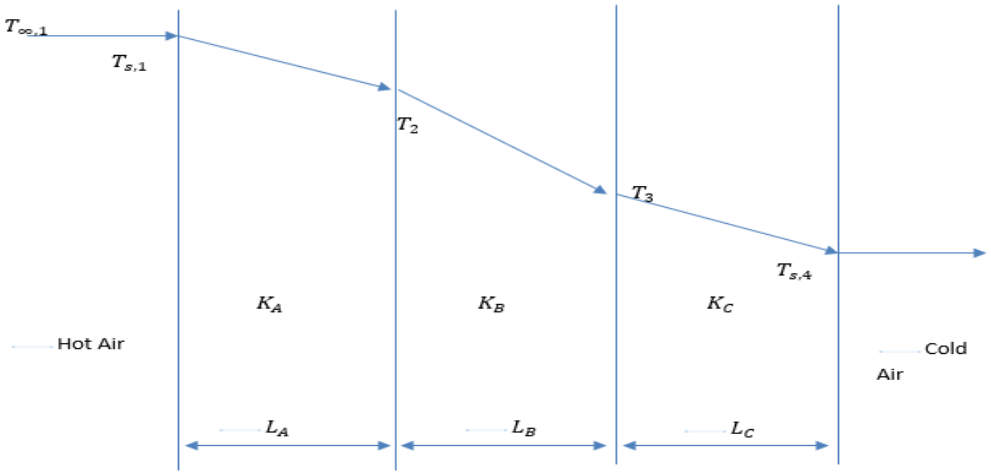

Figure 4. Graphical representation of heat transfer in the fermentation bin

2.5. Performance evaluation of the fermentation bin

The cooked locust bean was loaded in the inner chamber at different loading rates (2, 4, 6, 8, 12, 14, and 16kg), covered, and left for 48 hours. At the end of 48 hours, the condiment brings out a sweet smell indicating the process is completed.

To evaluate the quality of the fermented locust beans and the performance of the fermentation bin, ten (10) member panellists were selected (Sadiku, 2010) to carry out a sensory evaluation. These members consist of workers and staff of different eateries on the Obafemi Awolowo University campus; they were selected based on their experience and familiarity with locust beans. Parameters used in accessing the quality include aroma, colour, appearance, and mouthfeel. Their opinions were collated and analysed and their judgment was based on a standard scale Table 2.

3. RESULTS and DISCUSSION

The result obtained from the fermentation of the locust bean (Table 2) shows that fermentation is affected by the loading mass. The perception of the panellist revealed that the fermentation of the locust beans was done appropriately and pleasingly to taste except for loading of 14 and 16 kg. This result obtained for 14 and 16 kg is similar to the report of (Leito et al., 2006) who reported that a very high organic loading rate leads to a decrease in bioreactor performance due to disruption in microbial community structure.

Table 2. Physical quality assessment of the fermented locust bean.

Properties	**Samples**							
	2kg	4kg	6kg	8kg	10kg	12kg	14kg	16kg
Colour	3	3	1	2	1	1	5	5
Mouthfeel	3	3	1	1	1	1	5	4
Aroma	3	3	1	1	1	1	5	4
Appearance	3	3	3	3	1	1	4	5

Normal (1), Good (2), Very good (3), Bad (4), Very bad (5)

3.1. The effect of loading mass on the appearance of the fermented locust bean

Fig.5 shows the result of the physical quality assessment of the fermented locust bean. Loading the fermentation bin with 2 kg of locust bean gave an appealing appearance. However, there was a fluctuating increase in the appearance of the fermented locust bean as the loading mass increased until the load increased to 10 kg; the appearance was steady from 10 to12 kg

The phenomena declined when loaded with 14 and 16 kg. This implies that loading the fermentation bin with locust bean weight between 14 and 16 kg will give the locust bean an unfriendly appearance as a result of the decrease or disruption in performance due to disruption of microbial community structure in the fermentation bin (Leito et al., 2006)

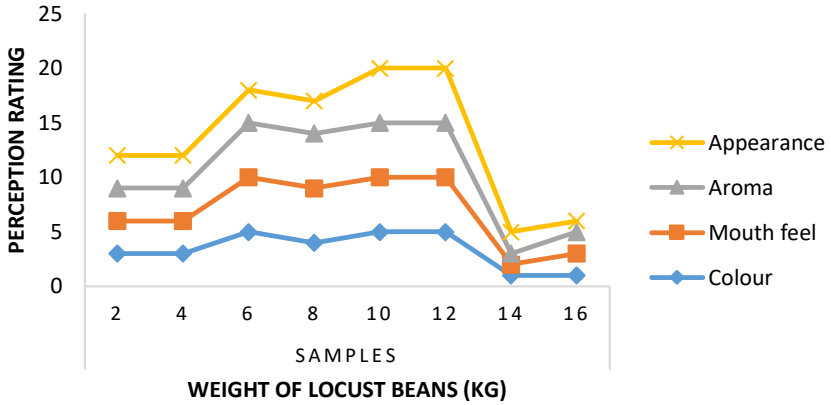

Figure 5. Graphical representation of the physical quality assessment of the fermented locust bean

3.2. The effect of loading mass on the aroma of the fermented locust bean

It was observed that 2 and 4 kg of the fermented locust bean produced a sweet aroma which is the expected characteristic of *dawadawa* aroma. Increasing the mass of the fermented condiment resulted in a slight increase in the perception of the aroma. The aroma was perceived to be less favourable as the mass of the fermented locust beans increased from 6 to 8 kg, while the aroma of 8 and 10 kg remained acceptable. This was similar to (Gernah et al., 2006) reports, starting that the characteristics of *dawadawa* aroma proceed out of the traditional fermentation when gmelina and banana leaves were used as fermentation material. However, loading the bin with 14 and 16 kg produced a bad aroma indicating that the bin at the specified geometry is ineffective when loaded with locust bean more than 12 kg (Fig 5). This is also similar to (Leito et al., 2006)] report on the ineffectiveness of a bioreactor when overloaded.

3.3. The effect of loading mass on the mouthfeel of the fermented locust bean

The trend of the analysis of the acceptability of the fermented locust bean based on mouthfeel is similar to other sensory test parameters considered for this study (Fig 5). When experimenting with the effectiveness of the fermentation bin with respect to the mouthfeel, it was observed that 2 and 4 kg samples of the condiment produced the best mouthfeel. However, compacting the locust bean in the fermentation bin up to 10 kg gave a good mouthfeel. The assertion is similar to mouthfeel is similar to the report of (Sadiku, 2010) who reported a sweet mouthfeel for fermented locust bean when fermentation was carried out properly therefore, recommending the control method as the best for fermentation. Any attempt to increase the load inside the fermentation bin beyond 12 kg resulted in poor taste. Hence, for an optimal feel of taste, the fermentation bin could be loaded with locust beans was between 10 to 12 kg.

3.4. The effect of loading mass on the colour of the fermented locust bean

Generally, the colour of the fermented locust bean does not appeal to all the observant (Fig 5). Conversely, it was preferred in some circumstances. The colour of the fermented locust bean is less appealing when the bin was loaded with 14 and 16 kg. It gave a cool brown colour when loaded with 2 and 4 kg of locust bean.

Moreover, the colour appears better when loaded with 6, 10, and 12 kg, respectively. The fermentation bin produced locust beans that maintained a stable colour with 10 and 12 kg of loading mass. This also implies that an effective colouration could be obtained with the fermentation bin when the loading mass does not exceed 12 kg. The brown colour obtained is similar to the report of (Sadiku, 2010) in his steam sample experiment, which reveals that good fermentation brings about a creamy brown colour. Also, (Gernah et al., 2006) reported that when fermentation is carried out traditionally using gmelina and banana leaf, a brownish colour was observed.

3.5. Acceptability and overall rating of the fermented locust beans

From Fig.6, it is evident that the fermentation bin was able to produce good quality fermented locust beans that met human satisfaction. However, the condiment has highest the acceptance when it was processed with less compaction in the fermentation bin. This could be a result of the effective migration of heat within the fermentation bin. Overloading the bin with locust bean tends to reduce the effect of fermentation leading to rejection of the fermented locust bean. Similarly, Fig 7 showed that the product of the fermentation was disliked when the locust bean did not ferment as expected. This implies that any attempt to increase the mass of the product being fermented will always lead to poor performance of the device and a decline in the quality of the product. Since the product is expected to satisfy human beings, therefore, it is important to limit the application of the volume of the fermentation bin between 2 and 12 kg.

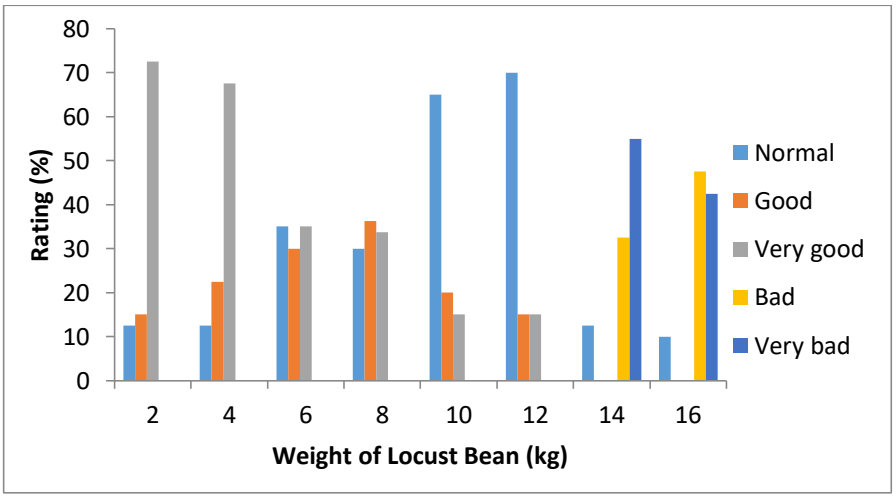

Figure 6. Graphical representation of the acceptability level

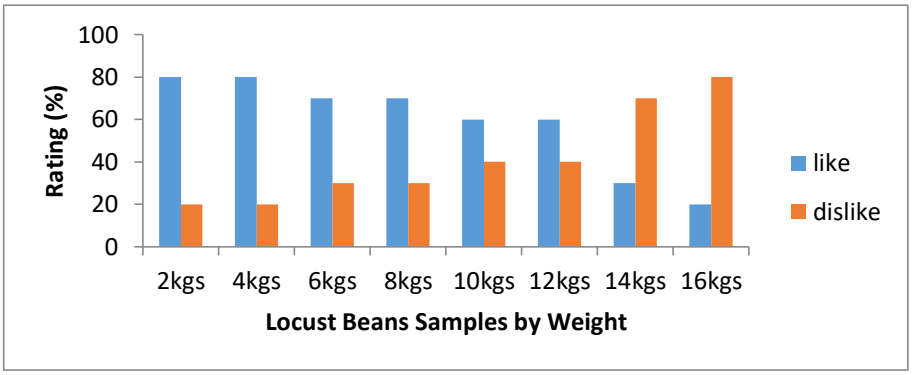

Figure 7. Graphical representation for the overall rating

4. CONCLUSION

It has been shown that the processing of locust bean into *Iru* will give very good and high nutritive quality if it is processed with the right device which simulates the traditional way of processing the condiment. It also shows that without the application of chemical substances as processing catalysts (like wood ash), additives, or preservatives, the condiment could be processed at a shorter time compared to the waiting time using the traditional approach. The fermentation bin performed better when the locust bean was not compacted. This allowed the migration of moisture and the circulation of heat required to facilitate fermentation and enhance the quality of the product. From the loading of 2 to 12 kg, the fermentation bin produced fermented locust beans with relatively better physical qualities than 14 and 16kg in terms of aroma, colour, taste, and texture. Since the bin simulates the traditional way of fermentation and performed better using a physical comparison and sensory evaluation approach.

Therefore, the fermentation bin is recommended to replace the traditional way of fermentation. The results obtained coupled with the desirable products of the bin as compared with the traditional way of fermentation will encourage large production of fermented locust beans and products that meet human satisfaction.

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Environmentally Friendly Bacterial Cellulose Films for Food Packaging

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Abstract

Use of biodegradable polymer films such as polyhydroxybutyrate (PHB), chitosan and cellulose as packaging materials in food storage has become an important issue in the storage of food. The aim of this study was to assess the effect of bacterial cellulose films in terms of extending the shelf life of food storage will be determined compared to other petrochemical materials. Bacterial cellulose films were formed by *Gluconacetobacter hansenii* HE1 strain. The cellulose layer was dried, sterilized and used as packaging material. Cling wrap was used as a positive control. Sausage specimens purchased from the market were kept in the fridge at 0, 2, 4 and 6 days at +4ºC without wrapping, wrapped with a cling wrap, wrapped with bacterial cellulose film. Colony counts for microbial load were determined by the arithmetic mean, with 3 replicates among each sample group. At the end of the sixth day, the microbial load of the sausage wrapped with bacterial cellulose was found to be as $1{,}2x10^4$ cfu/mL. However, the microbial load of the sausage wrapped with cling wrap was found to be as $2{,}7x10^5$ cfu/mL while the microbial load of the non-wrapped sausages was found to be as $1{,}0x10^6$ cfu/mL. It has been observed that microbial contamination with airborne filtration through porous, thin, web-like structure, which bacterial cellulose has, can filtrate air-borne contamination better than petrochemical-derived cling wrap. For this reason, bacterial cellulose can be used as a packaging material to store foodstuffs and to extend shelf life.

Keywords: Bacterial cellulose, food packaging, cling wrap, microbial load

Research article
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INTRODUCTION

It is an important issue for people to be able to take and consume food products in a safe and sufficient amount in order to maintain their lives and physical development in a healthy way. The processes of food from the manufacturing stage to the marketing stage are as important as the microbiological problems that may arise during the waiting times of the food by the consumer in the refrigerator (Brooks and Flint, 2008).

Food safety is known as taking precautions by obeying the necessary rules during the acquisition, production, processing, storage, transportation, distribution and consumption of food raw materials in order to ensure healthy and flawless food production. In food safety, it is very important that the food does not lose its nutritional value, that the food is clean and intact in physical, chemical and biological terms. Foreign particles such as soil, wood, metal, glass parts, hair, nails, flies, insects that should not be present in food are physical hazards.

Chemical hazards are the presence of metals such as mercury, lead, cadmium, detergent wastes and pesticides in foods. These chemical hazards are mainly caused by containers or petrochemical-legal bags in which food is stored or packaged. Among the biological hazards, they are the bacteria that threaten food safety the most and cause food poisoning (Borchers et al., 2010).

Many methods have been developed such as heat treatment and cold application, drying, salting and sealing protection, vacuuming protection, from the manufacture of food to consumption. Especially in recent years, it is very common for food to be vacuumed and delivered to consumers in this way. By vacuuming, the contact of the food with air is prevented and the growth of microorganisms on the food is prevented. However, even if a vacuumed food package is opened and stored in the refrigerator, it tends to deteriorate after a certain period of time. If the foods such as salami, sausage, soudjouk and cheese purchased in vacuumed packages are wrapped with materials such as refrigerator bag, cling film and stored in storage containers after the package is opened, it does not prevent food spoilage (Arvanitoyannis and Kotsanopoulos, 2014).

Petrochemical origin plastics such as polyethylene, polyester, polyolefin, and polyamides, which we use to store the remaining part of vacuumed foods in the refrigerator after opening, have been used a lot in recent years. Because such materials have a high tension and high tear strength, they also have important features in terms of air tightness, liquid leakage and heat retention. However, components such as styrene, 1,3-butadiene, melamine, formaldehyde, acrylamide, di-2-ethylhexyl phthalate, di-2-ethylhexyl adipate, vinyl chloride and bisphenol which may be present in petrochemical-based food packages can cause serious problems on human health (Durusoy and Karababa, 2011; Raheem, 2012). Besides, there is very low evaporation transition from such materials, which causes moisture to form in the food package and cause microorganism reproduction. In addition, since petrochemical-based materials used as food packaging materials don't have a biodegradable property, the waste remains in the environment for many years and causes serious ecological problems (Siroli et al., 2017). Therefore, the use of biodegradable polymer films such as polyhydroxybutyrate (PHB), chitosan and cellulose as packaging materials in food storage has become an important issue in the storage of food. Bacterial cellulose also has a biodegradable, flexible, porous structure that can be used as environmentally friendly packaging material (Aider, 2010; Khosravi-Darani and Bucci, 2016; Wu et al., 2016; Atares and Chiralt, 2016).

Cellulose and its derivatives are also used as packaging material in foods, including textile, paper and food industry. Although it is structurally similar to plant cellulose, the fact that bacterial cellulose is completely pure, very fine-pored and flexible is an important feature and has many industrial uses (Shah et al., 2013; Keshk, 2014).

In this study, we aimed the use of bacterial cellulose films as food packaging material. Therefore, we determined the microbiological load formed in different medium at specific time intervals.

MATERIAL AND METHODS

Material

Sausage samples sold in vacuum packages were used as material. Bacterial cellulose and cling wrap were used to store the food after the food sample was unpacked. *Gluconacetobacter hansenii* HE1 strain used for bacterial cellulose production was provided from Aydın Adnan Menderes University Microbiology Laboratory stock cultures.

Production and purification of cellulose

G. hansenii HE1 strain was inoculated in HS (Hestrin-Schramm) (2% glucose, 0.5% yeast extract, 0.5% polypeptone, 0.675% Na_2HPO_4, 0.115% citric acid) broth and was allowed to produce cellulose after an incubation period of 10 days at 30ºC (Hestrin and Schramm, 1954). 4% NaOH and 6% acetic acid solutions were used for purification and dried by lyophilisation (Çoban et al., 2017; Çoban et al., 2020).

Preparation of bacterial films as packaging material

The cellulose layer used as packaging material was sterilized at 121ºC for 20 min. Stretch films were used as a positive control. Stretch films were sterilized by standing under UV for 3 hours. Sausage specimens purchased from the market were kept in the fridge at 0, 2, 4 and 6 days at +4ºC without wrapping, wrapped with a cling wrap, wrapped with bacterial cellulose film.

Microbiological Analysis

A 10 g of sausage samples were aseptically added with 90 mL of sterile 0,85 % NaCl (physiological salt water) solution. After, the mixture was homogenized in a Stomacher for 2 min. Then, serial dilutions up to 10^{-4} were prepared and carried out following standard methodologies (Ferna´ndez-Lo´pez et al., 2008).

A 0,1 mL sample from each dilution was plated onto selective media. Plate Count Agar (Merck) was used to determine total mesophile aerobes at dilutions of 10^{-1}-$10^{-4.}$ PCA plates were incubated aerobically at 30°C for 72 h. Violet Red Bile Agar (VRBA) (Merck) was used to determine coliform bacteria at dilutions of 10^{-1}-$10^{-4.}$ VRBA plates were incubated aerobically at 37°C for 24 h. Baird Parker Agar (Merck) was used to determine *Staphylococcus aureus* at dilutions of 10^{-1}-10^{-4}. BPA plates were incubated aerobically at 37°C for 24-48 h. Selenite Cystine Broth (SCB) (Merck) was used to detect the presence of Salmonella as an enrichment medium at dilutions of 10^{-1}-10^{-2}. SCB tubes were incubated aerobically at 37°C for 18-24 h. A 0,1 mL sample from each SCB tubes was plated onto Bismuth Sulfite Agar (BSA) (Merck) for Salmonella. BSA plates were incubated aerobically at 37°C for 24 h. Rose-Bengal Chloramphenicol Agar (RBCA) (Merck) was used to determine microfungi at dilutions of 10^{-1}-10^{-4} . RBCA plates were incubated aerobically at 25°C for 5 days (Drosinos et al., 2005; Ferna´ndez-Lo´pez et al., 2008).

Following the incubations, colonies were counted as colony morphology and microbial load calculated as cfu/mL. Colony counts for microbial load were determined by the arithmetic mean, with 3 replicates among each sample group.

RESULTS and DISCUSSION

Production of purification of bacterial cellulose

Bacterial cellulose was obtained by *Gluconacetobacter hansenii* HE1 strain and purified and dried (Fig. 1a, b).

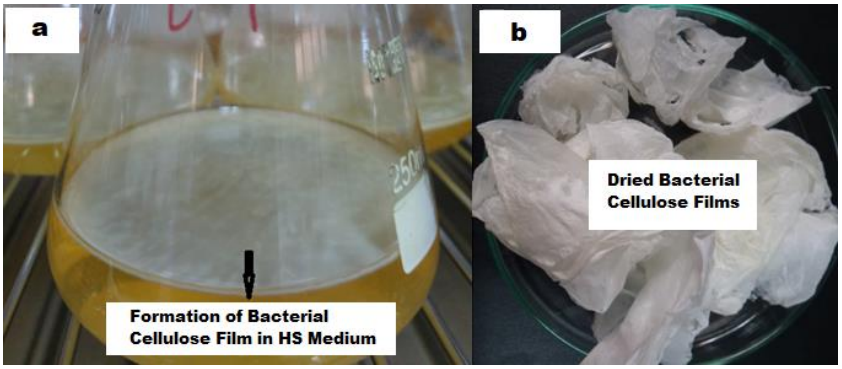

Figure 1. a. Formation bacterial cellulose film in HS Medium **b.** Dried bacterial cellulose films

Microbiological Analysis

Plate Count Agar (PCA) medium for the general viability count, Violet Red Bile Agar (VRBA) medium for the coliform bacteria count, Baird Parker Agar (BPA) for the *Staphylococcus aureus* count, Selenite Cystine Broth and Bismuth Sulfite Agar (BSA) for the presence of Salmonella and Rose-Bengal Chloramphenicol Agar for the microfungus count were used in the study.

According to study results, no microorganism growth was observed in the Violet Red Bile Agar (VRBA), Baird Parker Agar (BPA), Bismuth Sulfite Agar (BSA) and Rose-Bengal Chloramphenicol Agar (RBCA) media on day 6th (Fig. 2a, b, c; 3a, b, c; 4a, b, c). However, microorganism growth was seen on Plate Count Agar (PCA) medium (Fig. 5a, b, c).

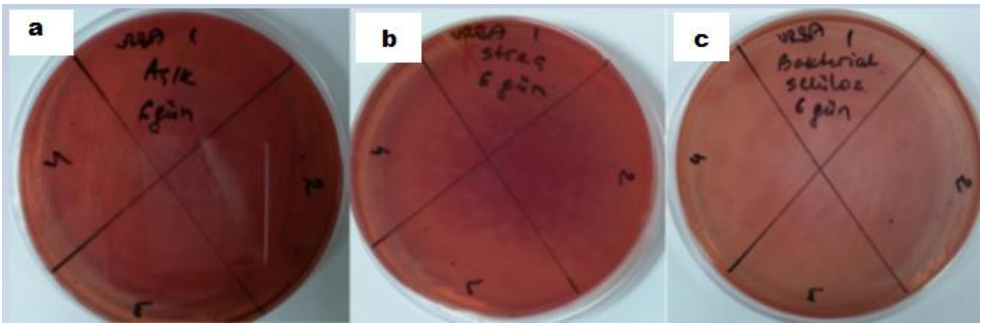

Figure 2. Bacteria growth in Violet Red Bile Agar Medium on day 6th **a.** without wrapping **b.**wrapped with a cling wrap **c.** wrapped with bacterial cellulose film (No growth)

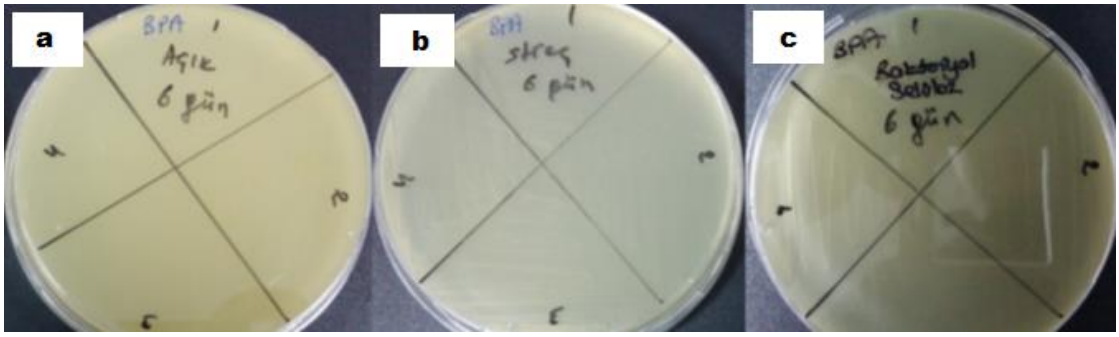

Figure 3. Bacteria growth in Baird Parker Agar Medium on day 6th **a.** without wrapping **b.** wrapped with a cling wrap **c.** wrapped with bacterial cellulose film (No growth)

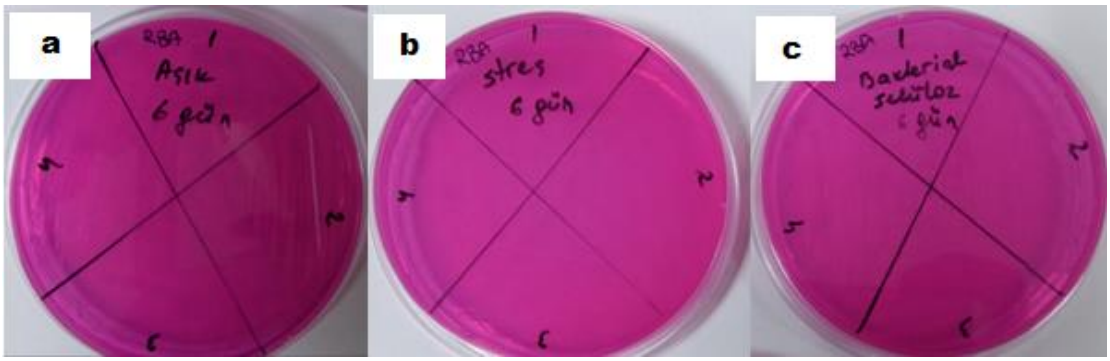

Figure 4. Bacteria growth in Rose-Bengal Chloramphenicol Agar Medium on day 6th **a.** without wrapping **b.** wrapped with a stretch film **c.** wrapped with bacterial cellulose film (No growth)

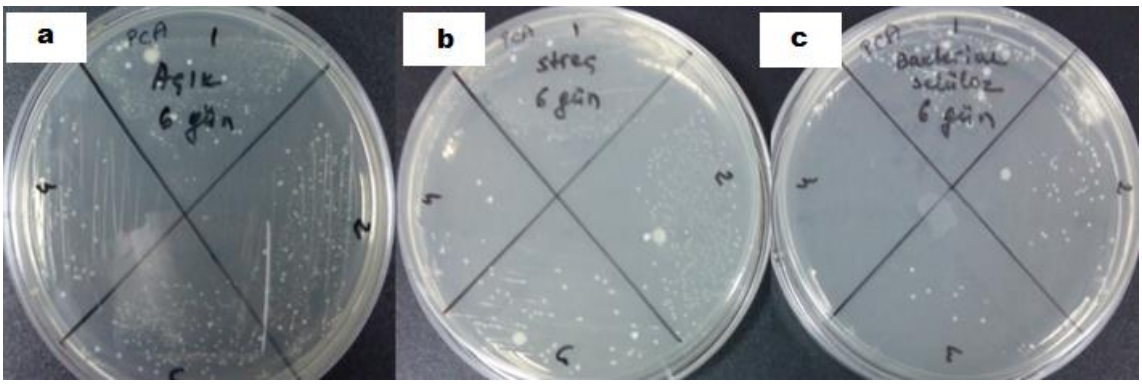

Figure 5. Bacteria growth in Plate Count Agar Medium on day 6th **a.** without wrapping **b.** wrapped with a cling wrap **c.** wrapped with bacterial cellulose film

The colonies formed on days 0, 2, 4, and 6th in PCA medium were counted and the results were given as cfu/mL in Table 1.

Table 1. Colony counts and microbial loads of microorganisms in PCA medium on days 0, 2, 4 and 6th

Medium	Dilution rates				Microbial load (cfu/mL)
	10^{-1}	10^{-2}	10^{-3}	10^{-4}	
PCA	Colony Count				
Without wrapping					
0. day	-	-	-	-	-
2. day	62	15	1	-	$6,2x10^3$
4. day	≥300	86	12	-	$8,6x10^4$
6.day	≥300	≥300	102	24	$1,0x10^6$
Wrapped with a cling wrap					
0. day	-	-	-	-	-
2. day	23	6	-	-	$2,3x10^3$
4. day	≥300	55	6	-	$5,5\ x10^4$
6.day	≥300	270	53	2	$2,7\ x10^5$
Wrapped with bacterial cellulose film					
0. day	-	-	-	-	-
2. day	20	2	-	-	$2,0x10^3$
4. day	85	11	-	-	$8,5\ x10^3$
6.day	117	38	3	-	$1,2\ x10^4$

The sausages were kept in the refrigerator in 3 different ways for 6 days. At the end of the sixth day, the microbial load of the sausage wrapped with bacterial cellulose was found to be as $1,2x10^4$ cfu/mL. However, the microbial load of the sausage wrapped with cling wrap was found to be as $2,7x10^5$ cfu/mL while the microbial load of the non-wrapped sausages was found to be as $1,0x10^6$ cfu/mL.

Kuswandi et al. (2020) developed an edible pH sensor based on immobilized red cabbage anthocyanins into bacterial cellulose membrane for intelligent food packaging. It was reported that the edible pH sensor can distinguish fresh milk from spoilage, making it suitable to be used in an intelligent packaging system as a freshness sensor (Kuswandi et al., 2020). It showed that use of bacterial exopolysaccharides and their potential application are possible as food packaging materials, including edible coatings, intelligent films, and thermo-insulated aerogel packaging (Neši´c et al., 2020). Bandyopadhyay et al. (2020) researched use of bacterial cellulose based polymeric films for food packaging. They found that bacterial cellulose films preserved the freshness of fruits and vegetables for a long time (Bandyopadhyay et al., 2020). In another study, it was expressed that nanocomposites of bacterial cellulose nanofibrils and zein nanoparticles are useful for food packaging (Li et al., 2020). In a same study, it reported that bacterial cellulose is appropriate as a raw material for food and food packaging applications (Azeredo et al., 2019).

Padrao et al. (2016) showed that bacterial cellulose films modified by bovine lactoferrin. The films were applied as edible antimicrobial packaging on meat products such as fresh sausage. Bacteriocidal effect of the films were examined against *Escherichia coli* and *Staphylococcus aureus.* As a result, the films significantly hindered the specific growth rate of both bacteria (Padrao et al., 2016).

Also, Abdul Khalil et al. (2016) reviwed that the demand for exploring advanced and eco-friendly sustainable packaging materials with superior physical, mechanical and barrier properties is increasing. Therefore, it has been emphasized that cellulosic nanofibers can be used in food packaging applications (Abdul Khalil et al., 2016). In another review, it was remarked that use of natural polymers such as protein based edible films and films from cellulose and its derivatives are available as cling films for food packaging (Malhotra et al., 2015). Besides, Pawar and Purwar (2013) reported that bacterial cellulose can be used as a biodegradable food packaging material in the protection and storage of foods from microbial contaminants.

CONCLUSION

Bacterial cellulose has a porous, thin, reticulated structure. With this structure, it filters the air and keeps small particles such as dust, microorganisms and fungus spores that may be present in the air. However, microorganism growth is observed more in foods wrapped with petrochemical-derived stretch film and food spoilage happens more quickly.

Therefore, it is appropriate to use bacterial cellulose films as food packaging material in the storage of foods and extend their shelf life. We think that it should be evaluated as a new and alternative food packaging material of biological origin in the food industry. The fact that bacterial cellulose is non-toxic, compatible with living tissue, edible and environmentally friendly shows that it can be used safely in the food industry. Therefore, the use of bacterial cellulose as food packaging material has a high added value.

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CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

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Challenges of Food Storage and Preservation in Restaurants in Katsina Metropolis, Katsina State, Nigeria

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Abstract

Restaurants produce the greatest amount of food waste and loss in the food supply chains of most developing economies including Nigeria. Reducing all food losses will result in a more secure global food system and it is important for us to show how restaurants services can reduce food waste in their domain. Food waste is one of the major challenging issues facing most restaurants as well as food services operators as it post many negative economic and environmental health impacts. Thus this study examines challenges of food preservation in restaurants, providing data and insights with a case study on Katsina city. Primary and secondary sources of data were adopted as a method for the data collection. From the finding of the study, most restaurants in the study area conduct an inventory checks on a daily basis, to stock up on required materials and gather understanding on which items are being used. In addition, restaurants keep a regulated check to ensure minimum possible preparations on certain items need to be prepared in excess. It was also found that, fresh foods purchased have a reported 5.8-fold greater food waste compared to frozen food purchases in most restaurants in Katsina. Obtaining such data is a challenge future research into food waste and preservation will need to address so that it can be transferred to food product development operations for maximum impact. It concluded that Katsina restaurants do not recognize the need to have a scientific approach for the treatment of their surpluses and mitigation of the same. The true solution lies in creating heightened levels of awareness in the restaurant industry about correct waste management practices and stringent measures need to be taken to ensure that these are adequately followed.

Keywords: Food waste, Food preservation, Restaurants and Katsina Metropolis

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INTRODUCTION

Food plays a central role in our lives, not only providing fuel, nutrients and sustenance, nor merely being central to many of our social interactions, but also feeding into our sense of identity (White et al., 2009). However, across the world one-third of all the food produced is wasted which amounts to a staggering $400 billion annually (Nixon, 2015). Food waste has attracted the attention of researchers, the media, politicians and others, largely because of its far-reaching effects on the economy and the environment (Buzby et al., 2014). Food wastage and food losses combined together constitute the issue at hand resulting in food shortage across the world besides other negative implications (Lipinski et al., 2013).

This issue is fast assuming grave dimensions in Katsina, Nigeria. Restaurants produce the greatest amount of food waste and loss in the food supply chains of most developing economies including Nigeria (Lipinski et al., 2013). Reducing all food losses will result in a more secure global food system and it is important for us to show how restaurants services can reduce food waste in their domain. Although the safety of foods served in restaurants in Nigeria has been an ongoing concern. At the same time, restaurants play an important socioeconomic role in meeting food and nutritional requirements of city consumers at affordable prices to the lower and middle income groups and are appreciated for their unique flavours and convenience (Buzby et al., 2014)).

In the last few decades, increased intra-urban mobility of Katsina dwellers due to urban expansion, coupled with the increased engagement of women in the wage labour market, have left most working class people in Katsina with less time to cook and eat meals at home. This resulted in changes in their eating patterns and habits, and in an increased demand for ready-to-eat food out of the house. Many people have thus taken the chance to start their catering business to meet such demand: brick-and-mortar sit-down restaurants (eg. luxury, mid-range, ethnic/foreign), traditional "*Bukas*" or "*Mama puts*" (street kiosks, small diners, and hole-in-the-wall restaurants), as well as modern American-style fast food outlets have mushroomed in most cities including Katsina.

Food waste is one of the major challenging issues facing most restaurants as well as food services operators as it post many negative economic and environmental health impacts. Economically, they represent a wasted investment that can reduce restaurant owners' incomes and increase consumers' expenses. Environmentally, food waste inflicts a host of impacts, including unnecessary greenhouse gas emissions and inefficiently used water and land. "Food waste" refers to the edible parts of plants and people do not ultimately consume animals that are prepared in city restaurants for human consumption but that. In particular, "food loss" refers to food that spills, spoils, incurs an abnormal reduction in quality before or after food being prepared in the restaurants, before it reaches the consumer. "Food waste" refers to food that is of good quality and fit for human consumption but that does not get consumed because it is discarded either before or after it spoils. Food waste is the result of negligence or a conscious decision to throw food away (Haq, 2016).

This study examines challenges of food preservation in restaurants, providing data and insights with a case study on Katsina city, to appraise the operational efficiency of the system and contributes to understanding of food waste dynamics. Through in-depth interviews of 63 restaurant owners/managers, the paper also explores the strategies adopted by restaurants in Katsina to reduce challenges in food preservation and recommends ways to reduce waste amounts.

Food Loss and Food Waste

According to the Food and Agriculture Organization (FAO, 2016), roughly one-third of the food produced in the world for human consumption every year, approximately 1.3 billion tons, gets lost or wasted. Food losses and waste amount to roughly US$ 680 billion in industrialized countries and US$ 310 billion in developing countries. Studies have pointed out repeatedly that the proportion of food wasted in India is almost one-third the amount of its annual production. Around 67 million tons of food is wasted in India every year, which has a value of more than more than US$14 billion (Haq, 2016).

Statistics provided by the United Nations Development Program states that India wastes 40 percent of the food it produces. The causes of food losses and waste in low-income countries are mainly connected to financial, managerial and technical limitations in harvesting techniques, storage and cooling facilities in difficult climatic conditions, infrastructure, packaging and marketing systems (Food and Agriculture Organization, 2011).

Food Preservation

Food preservation involves the action taken to maintain foods with the desired properties or nature for as long as possible. The process is now moving from an art to highly interdisciplinary sciences that need to be known to restaurants operators. In most countries, innovation, sustainability, and safety have become the main foci of modern industry and economy. The United Nations World Commission on Environment and Development defined sustainable development as “meeting the needs of the present generation without compromising the ability of future generations to meet their own needs.” A sustainable way of designing and developing food products stands to appeal to consumers, and provides a point of differentiation from competitors in an urban setting and a perfect platform for a range of positive public relations activities (Biswa et al., 2010).

Previous food waste reduction initiatives have typically focussed outside of this restaurants service arena and they have focussed on manufacturing and retail food losses. They have been successful at designing out food waste using the right-weighting of food products (portion control) and light-weighting of packaging (material resource efficiency). Their success has been made possible through cooperative actions across the food industries that have developed joint responsibility for food waste. It is essential that these initiatives now act to reduce the food that consumers purchase but do not eat (Mena et al., 2011).

Food Handling in Restaurant

Food handling is indeed a crucial (but not the sole) factor that can pose serious challenge in food preservation as it can cause food contamination. Several authors found that only a small percentage of food handlers in restaurants and or vendors in major cities in Nigeria including Katsina attended training workshops on food hygiene and safety before engaging into the food business. This gap, though, does not seem to prevent most of the handlers/vendors to show a good knowledge of safe food handling practices (e.g. hand washing). However, such knowledge does not always translate into good practices: for instance, while almost all food handlers are found to wash their hands with soap and water after going to the toilet, only a few wash their hands after touching dirty materials and body parts (Smith et al., 2010).

Food Storage in Restaurants

Food storage practices are another crucial issue. About one third of the street food vendors in Katsina usually sell leftovers, that is, food prepared and cooked more than 24 hours before selling. Given that storage facilities in many low-class households (e.g. fridge) are missing or become almost useless due to the frequent power outages plaguing Katsina, leftovers could serve as a breeding ground for microorganisms that could lead to food poisoning. It is important to note that even when food handlers have knowledge about safe food storage, handling, and preparation practices, they do not necessarily know the factors and mechanisms that cause food contamination, nor the effects of such contamination. For instance, more than one third of street food vendors in cities like Kano, Lagos and alike are not aware that food contaminated by pathogenic germs can cause serious damage to health.

MATERIAL and METHOD

Data collection and analysis

A qualitative inquiry has been used for this study. Primary and secondary sources of data were adopted as a method for the data collection. Primary data was collected using interview method and questionnaire survey; whereas the secondary data involves use of already publish papers, journals and article as well as other internet sources on food preservation and related areas.

The restaurant selection was undertaken with care. The research population for this study consisted of 63 restaurant owners/managers of "**A**" grade and "**B**" grade "**C**" evenly distributed across Katsina city whose establishments had been at least three years in the business with a minimum of five (5) employees each. The restaurants selected include all income range options ranging from higher end fine dining, fast food joints, cafes, mid-range restaurants, street food vendors, etc. Initially 78 restaurants had been shortlisted of which 15 did not wish to participate in the survey citing various constraints. Both closed- and open-ended questions were employed. In-depth face-to-face discussions held with these restaurant owners/managers in various matters of interest to this study. A research protocol was prepared to combine a systematic data collection from the 63 restaurants, which helped to address the research questions, guide the interviews and provide the structure.

In order to ensure accurate information collection, only restaurant managers, in case the owners were not available, who had complete information pertaining to operations, kitchen guidelines, rules and regulations and waste audit procedures have been interviewed for this research project. Also formal and informal discussions were held with a few employees subject to the opportunity and availability.

RESULTS and DISCUSSION

Food Surplus materials in Restaurants

Although the different restaurants used for the purpose of this study adopt different practices about food waste and surplus food management, some common threads have been observed (Figure 1).

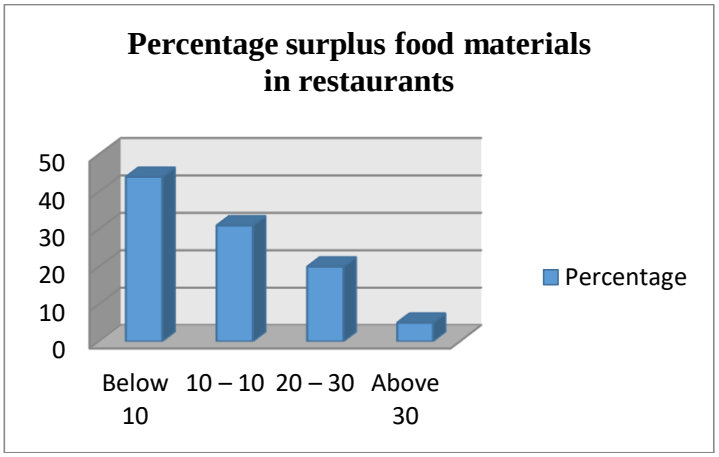

Figure 1. Percentages of surplus food materials in restaurant

The results show that 75 percent of the restaurants have 10-20 percent extra preparation that they claim is a safety margin, to enable them to cater to additional crowds. In total, 5 percent keep a margin of above 30 percent. It found that the high-end fine-dining restaurants make additional preparations as compared to the other types of restaurants. In-depth interviews with restaurant owners and managers reveal that after years of being in the restaurant business, several of them can estimate the requirements on specific days of the week and they only make what is required for the day, so generally food was not cooked greatly in excess.

Food Wasted in Restaurants

Although most restaurants keep a regulated check to ensure minimum possible preparations, certain items need to be prepared in excess. Also, several of the restaurants managers claimed not to re-use food and claim they are able forecast the footfall with a fair degree of accuracy. Some managers affirmed that they would rather declare a “sold out” than have to discard unsold stock at the end of the day (Figure 2).

Table 1. Food wasted in Restaurants in Katsina

Percentage food wasted in Restaurants	Standard restaurants	Street vendors	Fast food restaurant
Fresh food wasted	9.3	10	5
Frozen food wasted	2	8	7
Cooked food wasted	11	15	4

Source: Field work, 2019

Inventory check in Restaurants

Most restaurants conduct inventory checks on a daily basis, to stock up on required materials and gather understanding on which items are being used. Others conduct daily inventory only for perishables like dairy products, poultry items, etc., and weekly inventory checks for other materials like grains, sauces, etc. (Figure 2).

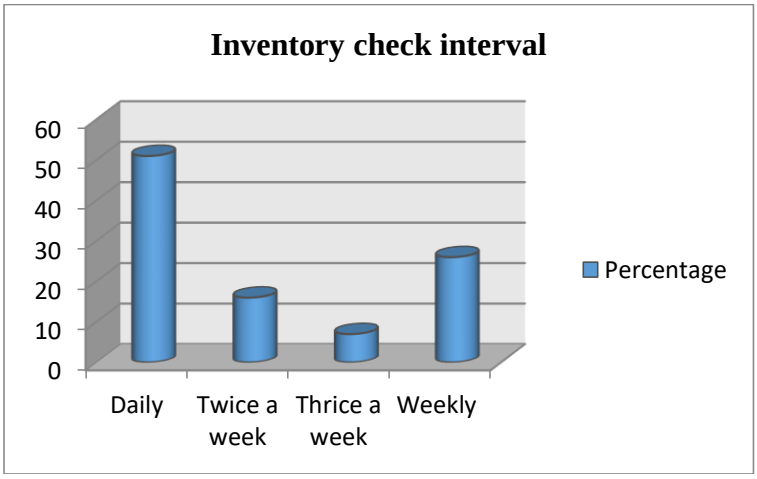

Figure 2. Inventory check interval among Restaurants managers

Preparatory leftover in restaurants

In Figure 3, high percentage (43 percent) of the participating restaurants immediately refrigerates these. In certain cases, if food chicken, fish or vegetables are in excess in the early stages of cooking, these are refrigerated and are reused. The treatment varies depending upon the item as well. Vegetables rot faster and are disposed of whereas meat can be refrigerated. However, 18 percent of the restaurants surveyed claimed to have a complete dispose of policy whereby everything prepared in the kitchen that has not been used till the end of the business day must go into garbage disposal. With the new laws in place the waste has to be segregated. However, all restaurant managers complain that though they have the waste divided as wet and dry, these procedures are not pursued at further stages so it is of no use to take all the trouble.

In all, 30 percent of the restaurants re-use these preparatory leftovers in some way. Three of the restaurants attached to five star hotels have made their own arrangements for waste handling (Figure 3).

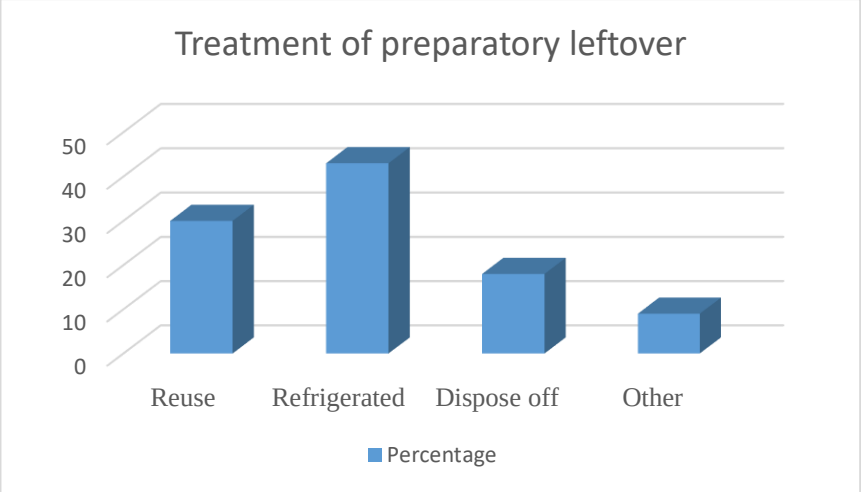

Figure 3. Treatment of preparatory leftover among restaurants in Katsina

Out of the 63 restaurants surveyed, only 2 had a tie-up with a food bank to donate the leftovers. Some of the luxury restaurants have a have tie-ups with NGOs to distribute the leftovers while some prefer not to do so due to bad experiences in the past. Some of the restaurants spoke highly of distributing the surplus the less privilege people living in a close settle zones.

Storage Temperature in Restaurants

Few restaurant surveyed has invested in a "public fridge" which has been mounted a few feet away from the restaurant, but majority used own fridge mounted in their restaurants. Destitute can pick up the surplus food from this fridge. One restaurant owner narrated that in the previous year a very complicated matter came up as the donee claimed that they felt sick after consuming the food, demanding massive compensation (Figure 5).

Table 2. Showing cold storage temperature for food in restaurants

Cold storage temperature food in restaurants	**Percentage**
Below 36^{o}F (2.222^{o}C)	64
36 - 45^{o}F (2.22^{o}C – 7.222^{o}C)	30
Above 45^{o}F (7.222^{o}C)	6

Source: Field work, 2019

Fresh/Frozen Food Wasted in Restaurants

Table 4, shows fresh foods purchased have a reported 5.8-fold greater food waste compared to frozen food purchases in most restaurants in Katsina. Obtaining such data is a challenge future research into food waste and preservation will need to address so that it can be transferred to food product development operations for maximum impact. The findings of this study correlated with Austrian research as, reported that the fresh food thrown away per household per person for 288 samples was 37.48 kg each year while the frozen food thrown away per household per person was 6.46 kg and per year. The nutritional losses associated with food waste despite employing preservation methods have yet to be fully characterised but they are an important component of food waste projections (Halloran et al., 2014).

Table 3. Showing fresh/frozen food purchase wasted

Percentage of fresh food purchase wasted		**Percentage of frozen food purchase wasted**	
Fruits	9.8	Fruits	2.5
Vegetables	6.7	Vegetables	3.4
Potatoes	3.9	Potatoes	1.0
Bread	5.5	Spinach	0.5
Pasta	1.9	Pasta	0.4
Meat	2.8	Meat	1.6
Fish	0.6	Fish	0.7
Snacks	5.8	Snacks	3.7

Source: Field work, 2019

Mitigation of Food Waste Among Restaurants

A good proportion of consumers in Katsina especially males have a tendency to fill their plates at buffets and cannot finish most of the food. A mid-range restaurant exhibited a sign put up at two strategic places at the buffet table "You are welcome to come back as many times as you want." In total, 74 percent of the restaurants use 32 cm plates while the remaining use 27 cm plates. Larger plates might contribute to people serving and consuming more food due to visual illusions that lead to biased perceptions of how much food is served or consumed (Van Ittersum and Wansink, 2012).

Several of the managers complained the customers send food back as it has gone cold, in keeping with the Nigerian mentality that food must be served piping hot. Fine-dining restaurants are the ones more receptive to participating and also sensitive toward importance of waste management. Three of the fine-dining restaurants take efforts to ensure that the kitchen staffs chops vegetables correctly, does not over trim meat or add excessive ingredients thereby mitigating food waste.. Though preventing use of material-intensive equipment is a good idea, for instance pasta makers used by most restaurants have higher wastage of the pasta materials while there are other alternatives, perhaps due to the cost and effort involved this alternative did not find many takers among the restaurant owners and managers.

Table 4. Showing mitigation of food waste among restaurants

Mitigation of waste	**Percentage**
Redesigning menu	31
Portion related guidance	8
Waste Audit team	5
Single dinner option	13
Use of material intensive equipments	11
Reusing leftover	32

Source: Field work, 2019

DISCUSSIONS and IMPLICATIONS

The causes of food waste in restaurants are numerous and diverse. Food losses and waste are currently at the heart of academic debates, civil society initiatives and political agendas (Falasconi et al., 2015). A number of strategic challenges arise from the observations and inferences outlined in the previous section. Waste management and effective waste disposal practices are measures that have become essential for restaurants not only to reduce wastage of resources but also to ensure an economic business. Restaurants need to have complete information of various methods for effective disposal of surplus food and recycling options and in particular about the processes. Restaurants need to adopt strategies to translate the vision of zero food loss and wastage of food into concrete action.

As per this study, the hospitality sector is not keen on government interference to reduce avoidable the challenges of food preservation and would rather solve the issue at their level and through their associations. There is opposition to taxes on avoidable food waste. The larger establishments are frustrated by the subjective evaluations adopted by food administration inspectors and resent the fact they have to frequently bribe these inspectors. Experienced staff needs to be assigned the job of checking orders from distributors so that no substandard quality inputs enter the chain in the first place. Buffet restaurants could learn from the mid-sized restaurant in our sample and put up signage that it is socially acceptable for guests to help themselves several times. This might help to reduce the amount left over (Wansink, 2006).

Restaurants should be encouraged to use 27 cm plates and this could be enforced through the associations to ensure uniformity. The average consumer consistently serves more onto relatively larger dinnerware than onto relatively smaller dinnerware (Wansink, 2006). This is thought to occur because of the Delboeuf illusion, which makes a meal appear smaller than it really is when more white space surrounds the food (Van Ittersum and Wansink, 2012).

This means that a large plate can make a meal look smaller and this causes guests to order meals they will never land up finishing. Thus using 27 cm plates would reduce the amount of food waste. Restaurants could be provided with some incentives like subsidies or lesser taxes for minimizing wastages. Seminars on food waste management can be conducted by food regulatory associations to create awareness amongst the kitchen staff.

At the time of primary data collection, negligence and indifference of restaurant staff in relation to waste and preservation was observed so they should be offered certain incentives to minimize the same. Taking a cue from the fine-dining restaurants in this study, the kitchen staff should be properly trained in chopping and trimming and made more conscious about the effects of food wastage. The staffs also needs more training in employing greater innovation of converting leftovers into dishes as two of the restaurants in the sample are doing. The menu could be rearranged to delete less popular items or the recipe could be readjusted. Proper systems to use perishables in a timely manner using the "first in, first out," system would help prevent spoilage.

In the study by Falasconi et al., (2015), side dishes had the highest percentage of food waste so reducing the amount served of side dishes might be of help. From their experience, kitchens should learn to stagger meal preparation so dishes are ready at the same time and do not go cold before they are serve.

It would be immensely useful for these restaurants to allocate adequate resources toward formation of a Food Waste Audit and this should be made mandatory by law to reduce the challenges in food preservation. Prevention alone is not sufficient to achieve an optimal allocation of resources; it should be integrated with recovery mechanisms to intervene and adjust the additional failures of the catering system (Falasconi et al., 2015). This study recommends a three way strong digital communication between restaurants, NGOs/food banks and food regulatory governing bodies for quick transfer of information and leftovers. In addition, if the collection agencies undertake the responsibility of enforcing strict necessary quality checks on the donated food, the restaurants would be more receptive to the idea of donating it to the poor. More restaurants should tie up with reputed organizations. Currently, the checks conducted to determine whether restaurants are following the dictated waste management norms (preservation) are minimal. These should be conducted at intervals that are more regular and in a more comprehensive manner. In addition, integrity among the inspectors conducting checks on restaurants is vital. Waste management regulation in Katsina and Nigeria at large needs to be strengthened to include correct cold storage norms and recycling mandates. Both these are not given the importance they deserve in the Katsina as well as Nigerian food industry.

CONCLUSION

This paper has analyzed the challenges of food preservation in Katsina restaurants, attempted to assess the operational efficiency of the system and given recommendations to minimize challenges attached to food preservation. Katsina restaurants do not recognize the need to have a scientific approach for the treatment of their surpluses and mitigation of the same. The true solution lies in creating heightened levels of awareness in the restaurant industry about correct waste management practices and stringent measures need to be taken to ensure that these are adequately followed.
Every restaurant needs to work harder to monitor and minimize food wastage, for the sake of the community and the bottom line, though some do have a good grip on it. Following the observation that there is a weak association between waste prevention attitudes and behaviours there could be enormous gains between pro-environmental behaviours and values. It is just a matter of finding the strategy that works for the restaurant. The restaurateurs affirm that the solution to Katsina's restaurant waste management lies in micro management (and a laudable initiative by one of the restaurants is the "public fridge") rather than large scale plans. While this may be true, there is also a need for the government to amend and strictly enforce the laws on the same. The Nigerian government including Katsina should also speed up research in nanotechnology to invent eco-friendly and healthy food preservation applications to preserve food for longer periods of time and to keep farm produce fresh. It is easy to argue that food loss when saved can feed the hungry Nigerians but food loss and waste reduction should then become a natural part of national and regional strategy for food security. It is important to consider that food loss, waste reduction is a mechanism to improve food security, poverty alleviation, economic development, environmental health, and interventions should focus on the largest possible gains.

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Gastroprotective and Antioxidant Effects of the *Cinnamon, Cumin, Sumac* on Indomethacin Induced Gastric Ulcer in Rats

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Abstract

The gastro-protective effect of water extracts spice of cinnamon, cumin and sumac in indomethacin induced ulcer model in rats was investigated, in vivo and biochemical mechanism of spice extracts were monitored by measuring their antioxidant. For each cumin, cinnamon and sumach extracts 50, 100, 200 and 400 mg/kg, positive control famotidine (FAM) 25 mg/kg and negative control indomethacin (IND) 25 mg/kg dosage form was orally administered. After that catalase (CAT), superoxide dismutase (SOD), myeloperoxidase (MPx) enzyme activities and glutathione (GSH), lipid perxidase (LPO) amounts were measured. In IND induced ulcer group, It is determined that each four dosage form of spices and positive control FAM group significantly decreased the ulcer area ($p<0.05$). In addition to this, CAT, SOD, MPx enzyme activities and GSH, LPO amounts were measured in spice extract administered gastric damaged rat stomach tissues in order to explain the effects of antioxidant defense system on antiulcerogenic activity. In IND induced tissues, while LPO, MPx and CAT increased GSH and SOD decreased with respect to the (healthy) control group. It is claimed by the measured enzyme activity differences that these spices have antiulcerogenic activity.

Keywords: Antioxidant, cinnamon, cumin, sumac, rat

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INTRODUCTION

The use of nonsteroidal anti-inflammatory (NSAID) drugs is very common in cases of pain and fever. However, these drugs have some side effects, especially on the gastrointestinal tract. Reactive oxygen species (ROS) play an important role in gastric lesions resulting from inhibition of prostaglandin synthesis. (Elliott and Wallace, 1997; Kaplan et al., 2012; Tanas et al., 2010). Indomethacin-induced ulcer models are a good source of reactive oxygen species. These play a creative role in gastric injury, neurodegeneration and arthritis through various processes, cancer, atherosclerosis (Miura et al., 2012; Halici et al., 2008; Kandaz et al., 2009; Karaca et al., 2009; Kumtepe et al., 2010).

Indomethacin is a commonly used analgesic in humans but in many studies, showed pro-oxidant activity and provided the production of ROS. Thus leading to the onset of lipid peroxidation and the confrontation of the mucosal cells with antioxidant systems (Miura et al., 2012; Halici et al., 2008; Kandaz et al., 2009; Karaca et al., 2009; Kumtepe et al., 2010; Takeuchi et al., 1991; Odabasoglu et al., 2006a; Odabasoglu et al., 2006b; Albayrak et al., 2010). Especially, ROS such as hydrogen peroxide (H_2O_2) can be seen as the cause of many illnesses, especially gastric damage. (Karaca 2013; Kumtepe et al., 2010) So, researchers have concentrated more on oxygen-derived free radicals in recent years (Mates et al., 1999; Cadirci et al., 2010a and b) ROS attacks unsaturated fatty acids, destroy the structure of membrane lipids and initiate lipid peroxidation. The enzyme activity, permeability, and cell activation are reduced in the damaged membrane proteins. Therefore, antioxidant defense systems are important to prevent the toxic effects of free radicals which cause many diseases. Oxygen-handling cells contain antioxidant enzymes, the enzymatic and non-enzymatic, that can protect them against oxidative stress (Odabasoglu, 2006; Koc et al.,2008). These antioxidants such as glutathione (GSH), vitamin-A and C, α-tocopherol, β-carotene also play an important role in the prevention of gastric damage.

Plants are used, all over the world for hundreds of years, in improving the taste and aroma of foods, (Shelef, 1983) eliminating unwanted odors in foods (Giese, 1994) and most importantly for therapeutical reasons. Spices that are known as aromatic herbal products are used for different purposes in food and beverages. Some parts of plants can be used as spices. Root, tuber, rhizome, onions, straw, bark, leaf, flower, fruit, seeds and secretion are parts of plants that can be used as a spice (Pruthi, 1980; Aran, 1988; Akgül, 1997). Cinnamon is genus of evergreen trees aromatic odor where is mostly found in South and Southeast Asia (Gunther, 1959). Due to its antioxidant properties provides a wide usage to cinnamon as a food product (Shan et al., 2005). Cinnamon bark extract has been reported that it has a good free radical scavenging effect and also depending to dose, it inhibits superoxide radicals in different models *in vitro* (Mathew and Abraham, 2006). The Motherland of cumin is Eastern Mediterranean and the Middle East. It is also found in northern and central Europe. Cumin has been reported that it is used for gas removed, milk enhancer, retarding the periodic bleeding, relieve diarrhea, muscle pain reliever (analgesic and myorelaksan effect), treatment of rheumatism (anti-inflammatory effect), dental pain (analgesic and anti-inflammatory action), pharyngitis (anti-inflammatory effect), abdominal pain (antispasmodic effect), the diuretic and urinary tract congestion on (anti-inflammatory effect) (Baytop, 1983; Pamuk, 1998). Sumac (*Rhus coriaria* L.) is called genus *Rhus* in the family of Anacardiaceae. *Rhus coriaria* is common in Turkey which is grown in different regions of the world around 150 types (Davis, 1967). Sumac is commonly used as spice in Turkey and the Middle East. Chemical compounds contained in sumac plant have antioxidant and antimicrobial properties (Wildman, 2001; Kosar et al., 2007).

Active oxygen forms are occurred with the encouragement of some factors during the normal process of oxygen usage in human metabolism. If active oxygen forms are not inhibited, they may cause structural corruption in DNA, protein, carbohydrates and lipids. Thus, Active oxygen forms bring about degenerative disease by corrupting both structure and functions of cell membrane (Katiyar ve Mukhtar, 1997; Sivritepe, 2000). Antioxidants inhibit degenerative disease formation and oxidation induced cell damages by either inhibiting active oxygen formation or inactivate the active oxygens E and C vitamins, karetenoids and phenolic compounds are the most important antioxidants for human health (Baublis ve ark., 2000; Sivritepe, 2000).

Their many beneficial effects emerge when many diseases are presented to related with oxidative stress (Luximon-Ramna et al., 2003, Toyokuni et al., 2003, Caia et al., 2004 and Romani et al., 2004). The antioxidants taken as daily nutrients can be said to be more effective in preventing diseases caused by oxidative stress. (Ames et al., 1995; Kaur and Kapoor, 2001). There is increasingly growing market for nutraceuticals and functional food. Products containing nutraceuticals have reached a worldwide estimated value of $65 billion (Lachance, 2002).

MATERIALS and METHODS

Chemicals

All chemical materials used in the experiments Sigma Chemicals Company (Germany) were obtained from.

Plant material

Spices (cinnamon, cumin and sumac) was used as working material in this research were obtained from the country's leading industrial companies and one of the most important spice producers ‘Bagdat Baharat’.

Extraction of plant materials

Examples of spices were pulverized with treated liquid nitrogen in a mortar. 100 g of species sample was weighed and was placed in a Soxalet device flask. They were extracted in a shaker water bath for two days. The extracts were filtered and the solvent content is also removed in rotary evaporator (the evaporator) by low pressure and low temperature. The extracts were lyophilized in a 5 mm-Hg pressure. % yields of extracts (g lyophilizate / 100 g of spices) was determined by weigh.

Antioxidant activity assays

The antioxidant activities of spice samples were measured by thiocyanate method. (Mitsuda et al., 1996). Briefly, each species sample (1mg) in 1 ml distilled water was mixed with 5 ml linoleic acid emulsion (0.02M, pH 7.0) and 5ml phosphate buffer (0.2M, pH 7.0). Linoleic acid emulsion was prepared by mixing 0.5608 g of linoleic acid with 0.5608 g of Tween 20 as emulsifier, and 100 ml phosphate buffer (0.2M, pH 7.0). The obtained mixture was homogenized and was incubated at 37 ^{0}C.

According to the thiocyanate method, the degree of oxidation was measured by sequentially adding 4.7 ml ethanol (75%), 0.1 ml ammonium thiocyanate (30 %), 0.1 ml sample solution, and 0.1 ml ferrous chloride (0.02 M, in 3.5 % HCl). After three minutes of waiting, the peroxide value was assigned by reading the absorbance at using a UV (ThermoSpectronic-HEλIOS β). Trolox and ascorbic acid solutions were used as positive control. While a separate linoleic acid was measured as a control group without extracts (Tables 2,3 and 4). Inhibition % was calculated by using the equation:

I = (1-absorbance of sample at 500 nm/absorbance of control at 500 nm) x 100

Table 2. The antioxidant activities of cinnamon species extract in different doses

Extract	Dose	Total Antioxidant Activitiy	Inh.	Reducing The Amount of Power	Phenolic Compounds	C Vitamin 1mg/ml (Inhibition %)	Trolox 1mg/ml (inhibition %)	Control (Water)
	mg/ml	Avg. Absorbance	%	Avg. Absorbance	mg GAE/g lyophilizate	Avg. Absorbance	Avg. Absorbance	Avg. Absorbance
WECN	1	0.423±0.001**d**	80.0	0.417±0.014**a**	0.746±0.008**a**	0.951±0.001**g** (55.0)	0.165±0.02**b** (92.2)	2.114±0.01**e**
	5	0.260±0.004**d**	87.7	1.500±0.012**b**	1.393±0.001**b**			
	10	0.204±0.007**b**	90.4	2.371±0.002**c**	1.790±0.003**c**			
EWECN	1	0.654±0.005**d**	43.4	0.391±0.003**a**	0.555±0.001**a**	0.413±0.008**e** (64.3)	0.109±0.001**c** (90.6)	1.160±0.014**f**
	5	0.321±0.002**c**	72.2	1.305±0.002**b**	1.293±0.001**b**			
	10	0.183±0.001**a**	84.1	2.194±0.001**c**	1.944±0.009**c**			
MECN	1	0.913±0.001**f**	45.4	0.079±0.002**a**	0.252±0.008**a**	0.570±0.006**e** (66.0)	0.184±0.003**b,c** (89.0)	1.671±0.001**d**
	5	0.830±0.003**e**	50.4	0.125±0.001**b**	0.351±0.001**b**			
	10	0.831±0.003**d**	50.3	0.151±0.002**c**	0.650±0.006**c**			
CECN	1	1.268±0.005**h**	26.7	0.089±0.008**a**	0.250±0.005**a**	0.631±0.01**g** (63.6)	0.308±0.006**b,c** (82.2)	1.731±0.003**h**
	5	0.988±0.002**f**	42.9	0.102±0.009**a**	0.352±0.001**b**			
	10	0.995±0.005**g**	42.5	0.142±0.008**b**	0.501±0.002**c**			

Table 3. The antioxidant activities of cumin species extract in different doses

Extract	Dose	Total Antioxidant Activitiy	Inh.	Reducing The Amount of Power	Phenolic Compounds	C Vitamin 1mg/ml (Inhibition %)	Trolox 1mg/ml (inhibition %)	Control (Water)
	mg/ml	Avg. Absorbance	%	Avg. Absorbance	mg GAE/g lyophilizate	Avg. Absorbance	Avg. Absorbance	Avg. Absorbance
WECM	5	0.226±0.001**b**	89.3	0.499±0.002**a**	0.652±0.001**a**	0.951±0.001**g** (50.0)	0.165±0.002**b** (92.2)	2.114±0.001**e**
	7.5	0.203±0.003**b**	90.4	0.948±0.003**b**	0.979±0.001**b**			
	10	0.184±0.002**b**	91.3	1.667±0.001**c**	1.509±0.002**c**			
EWECM	1	0.310±0.002**d**	74.2	0.243±0.001**a**	0.699±0.008**a**	0.227±0.003**c** (81.1)	0.111±0.002**a** (90.1)	1.201±0.001**g**
	5	0.208±0.002**a**	82.7	0.673±0.001**b**	1.401±0.009**b**			
	10	0.17±0.002**a,b**	85.9	1.023±0.001**c**	2.316±0.004**c**			
MECM	1	0.444±0.005**d**	73.4	0.273±0.005**a**	0.421±0.009**a**	0.568±0.006**e** (66.0)	0.184±0.003**b,c** (90.0)	1.671±0.001**d**
	5	0.33±0.003**c,d**	80.5	0.306±0.002**b**	0.680±0.001**b**			
	10	0.260±0.002**a**	84.5	0.384±0.002**c**	0.850±0.005**c**			
CECM	1	1.244±0.003**i**	28.2	0.129±0.008**a**	0.181±0.001**a**	0.631±0.001**g** (63.6)	0.308±0.006**b,c** (82.2)	1.731±0.004**h**
	5	1.244±0.003**i**	28.2	0.152±0.003**a**	0.203±0.008**b**			
	10	1.112±0.003**g**	35.7	0.132±0.001**a**	0.240±0.005**c**			

Table 4. The antioxidant activities of sumac species extract in different doses

Extract	Dose	Total Antioxidant Activitiy	Inh.	Reducing The Amount of Power	Phenolic Compounds	C Vitamin 1mg/ml (Inhibition %)	Trolox 1mg/ml (inhibition %)	Control (Water)
	mg/ml	Avg. Absorbance	%	Avg. Absorbance	mg GAE/g lyophilizate	Avg. Absorbance	Avg. Absorbance	Avg. Absorbance
WES	5	1.075±0.008**e**	49.2	0.516±0.003**a**	0.844±0.001**a**	0.951±0.001**g** (55.0)	0.165±0.002**b** (92.2)	2.114±0.001**e**
	7.5	0.958±0.006**f**	54.7	0.992±0.001**b**	1.442±0.008**b**			
	10	0.596±0.006**d**	67.1	1.344±0.01**c**	2.203±0.009**c**			
EWES	2.5	0.22±0.003**d,e**	82.6	0.641±0.005**a**	1.403±0.003**a**	0.300±0.001**e** (76.1)	0.124±0.001**b** (90.1)	1.255±0.01**g**
	10	0.186±0.001**e**	85.2	1.741±0.008**b**	2.401±0.001**b**			
	20	0.183±0.001**d**	85.4	2.561±0.008**c**	2.733±0.001**c**			
MES	2.5	0.598±0.006**e**	64.2	1.076±0.001**a**	1.602±0.001**a**	0.566±0.006**e** (66.0)	0.194±0.001**c** (88.4)	1.671±0.001**d**
	10	0.410±0.005**e**	76.0	2.618±0.009**b**	2.901±0.005**b**			
	20	0.323±0.001**c**	80.7	3.750±0.002**c**	3.402±0.008**b**			
CES	2.5	1.279±0.002**f**	26.2	0.174±0.008**a**	0.424±0.002**a**	0.631±0.001**g** (63.6)	0.308±0.006**b,c** (82.2)	1.731±0.003**h**
	10	1.190±0.004**h**	31.3	0.184±0.009**a**	0.531±0.001**b**			
	20	1.00±0.003**e,f**	42.0	0.221±0.001**b**	0.608±0.002**c**			

Animals

The 90 Wistar rats, weighing 180–200 g, were obtained from Experimental Animal Laboratory of Ataturk University, Experimental Animal Teaching and Researcher Center. The animals were kept under the same conditions (Care 1993). The experiment protocol of the Ethics Committee on Experimental Animal Use and Care was approved throughout the research (B.30.2.ATA.0.23.85-9/58).

Indomethacin-induced gastric damage

The gastroprotective effect of species was determined in comparison with famotidine. The animals were fasted for 24 hours. They are divided into groups to be practiced. 50, 100, 200, 400 mg/kg doses of cinnamon, cumin, sumac and 20 mg / kg doses of famotidine orally administrated to the rats. After five minutes, indomethacin was administered to induce damage. It was waited for 6 hours. At the end of 6 hours, the animals were sacrificed and the stomachs removed. The stomaches was washed and ulcer areas were identified on the millimeter paper (Halici et al., 2008; Karakus et al., 2009).

Biochemical investigation of stomach tissues

The biochemical enzymes such as catalase, myeloperoxidase, superoxide dismutase and the amounts of GSH, LPO were determined after the macroscopic analysis. The stomach tissues were ground to prepare the tissue homogenates with liquid nitrogen in a mortar. Then, 0.5 g tissue was kept under 4.5 ml of appropriate buffer. Ultra-turraks homogenizer were used to homogenize the stomach tissues. Filtration and homogenization process were carried out at 4°C. Then, these supernatants were used in order to determine enzymatic activities and amounts of GSH, LPO. All biochemichal assays were analyzed by using a UV–VIS spectrophotometer.

Catalase (CAT) activity

Decomposition of H_2O_2 in presence of catalase was at 240 nm (Aebi, 1984). Catalase activity was defined as the amount of enzyme required to decompose 1 nmol of H_2O_2 per minute, at 25°C and pH 7.8. Results were expressed as mmol/min/mg tissue.

Myeloperoxidase (MPx) activity

According to the modified method of myeloperoxidase activity was measured (Bradley et al., 1982). The homogenized samples were frozen and thawed three times, and centrifuged at 1500 g for 10 min at 4°C. Myeloperoxidase activity in the supernatant was determined by adding 100 ml of the supernatant to 1.9 ml of 10 mmol/l phosphate buffers (pH 6.0) and 1 ml of 1.5 mol/l o-dianisidine hydrochloride containing 0.0005% (w/v) hydrogen peroxide. The changes in absorbance at 450 nm of each sample were recorded on a UV–VIS spectrophotometer. Myeloperoxidase activity in tissues was expressed as μmol/min/mg tissue.

Superoxide dismutase (SOD) activity

SOD activity was measured according to the generation of superoxide radicals produced by xanthine and xanthine oxidase, which react with nitro blue tetrazolium (NTB) to form formazan dye (Sun et al., 1988). SOD activity was then measured at 560 nm by the degree of inhibition of this reaction.

Total glutathione (GSH) determination

The amount of GSH was measured in the appropriate method (Sedlak and Lindsay, 1968) with minor changes. The stomach tissues were homogenized in 2 ml of 50 mM Tris–HCl buffer containing 20 mM EDTA and 0.2 M sucrose (pH 7.5). The homogenate was centrifuge at 4200 rpm for 40 min at 4°C. The supernatant was used to determine GSH using 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB). Absorbance was measured at 412 nm. The results of the GSH level in the gastric mucosa were expressed as nmol/g tissue.

Lipid peroxidation (LPO) determination

The level of LPO was determined by estimating MDA using the thiobarbituric acid test (Ohkawa et al., 1979). The stomach weighed and homogenized in 10 mL of 100 g/L KCl. The homogenate (0.5 mL) was added with a solution containing 0.2 mL of 80 g/L sodium laurylsulfate, 1.5 mL of 200 g/L acetic acid, 1.5 mL of 8 g/L 2-thiobarbiturate and 0.3 mL distilled water. The mixture was incubated at 98°C for 1 h. Upon cooling, 5 mL of n-butanol: pyridine (15:l) was added. The mixture was vortexed for 1 min and centrifuged for 30 min at 1875 x g. The absorbance was measured at 532 nm. The standard curve was obtained by using 1,1,3,3-tetramethoxypropane. The recovery was over 90%. The results were expressed as nanomol MDA per gram tissue (nmol/g tissue).

Statistical analyses

Statistical calculations were done by using SPSS 12.0 software. To be able to determine the statistical significance of AA, one-way variance analyses (ANOVA) was applied, which showed that there was a statistically significant difference ($p<0.05$) Multiple comparisons were performed Duncan test.

RESULTS

Gastroprotective effect of cinnamon, cumin and sumac on indomethacin-induced gastric damage

The gastroprotective effect of 50, 100, 200 and 400 mg/kg doses of species on IND-induced gastric damage in rats was macroscopically determined. Their inhibitory effects are showed in Table 1 and Figure 1. There was a significant improvement in treatment and FAM groups when there was a strong injury in the IND applied rats. As indicated in the table, when the ulcer areas were compared with indomethacin, the treatment groups were as effective as the famotidine group and provided the necessary healing. According to these results, FAM and all doses of species significantly protective against gastric damage caused by IND.

Table 1. Effects of different doses of species extracts and single dose of famotidine (FAM) on indomethacin (IND)-induced gastric damage in rats.

Treatment	N	Ulcer index (mm^2/rat)[a]	% Inhibition[b]
IND+Cinnamon (50mg/kg)	6	21.4±0.7i	48
IND+Cinnamon (100mg/kg)	6	20.3±0.3ı	50.6
IND+Cinnamon (200mg/kg)	6	19.1±0.1h	54
IND+Cinnamon (400mg/kg)	6	15.1±0.1d	63
IND+Cumin (50mg/kg)	6	20.1±0.1ı	51
IND+Cumin (100mg/kg)	6	18.1±0.1g	56
IND+Cumin (200mg/kg)	6	17.2±0.1f	58
IND+Cumin (400mg/kg)	6	14.1±0.1c	66
IND+Sumac (50mg/kg)	6	21±0.2i	49
IND+Sumac (100mg/kg)	6	19.1±0.1h	54
IND+Sumac (200mg/kg)	6	17.2±0.1f	58
IND+Sumac (400mg/kg)	6	16.1±0.1e	61
FAM (25 mg/kg)	6	7.1±0.1b	83
IND (25 mg/kg)	6	41.1±0.04j	0
Healthy[c]	6	0±0a	-

Means in the same column by the same letter are not significantly different to the Duncan test p<0.05).
a Mean damage index ± SE of six animals in each group.
b % Inhibition in ulcer index in relation to indomethacin group.
c Nothing administrated. N: The number of rats.

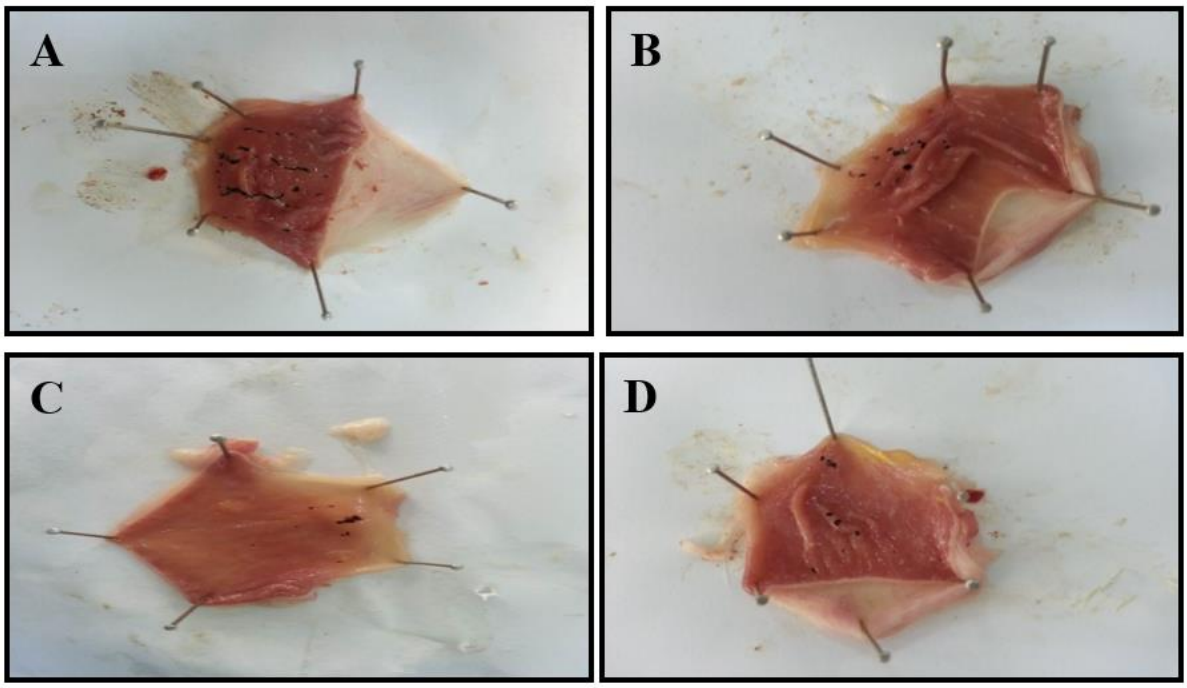

Figure 1. Ulcerous areas in the gastric tissues of indomethacin (IND)-induced rat by orally administrated (A). Sections of the gastric tissues after IND-administration were obtained from some experimental groups. The B, C and B sections show some ulcerative areas: B, the cinnamon group (400 mg/kg body wt.); C, Cumin group (400 mg/kg body wt.) and D; sumac group (400 mg/kg body wt.)

Comparison of enzymes' activities in rats' stomach tissues

The enzyme activities were measured to demonstrate the function of the antioxidant defense system in the prevention of ulcer formation and ulcers in rat gastric tissues. The results are presented in figures and tables shows that IND administration increased the LPO level compared to healthy rat tissues. In contrast to IND, all doses of species and positive control drug, famotidine, reduced the LPO level in rat stomach tissues. These results showed that species has a reducing effect on LPO in tissues Similarly, MPx and CAT enzyme activities were increased in the IND administration rats (Fig. 4,5,6).

Figure 4. Effects of different doses of species extracts and single dose of famotidine (FAM) on the catalase (CAT) enzyme in rat's indomethacin (IND)-induced gastric tissue. Means in the same column by the same letter are not significantly different to the Duncan test ($p<0.05$). Results are means ± SE of six measurements.

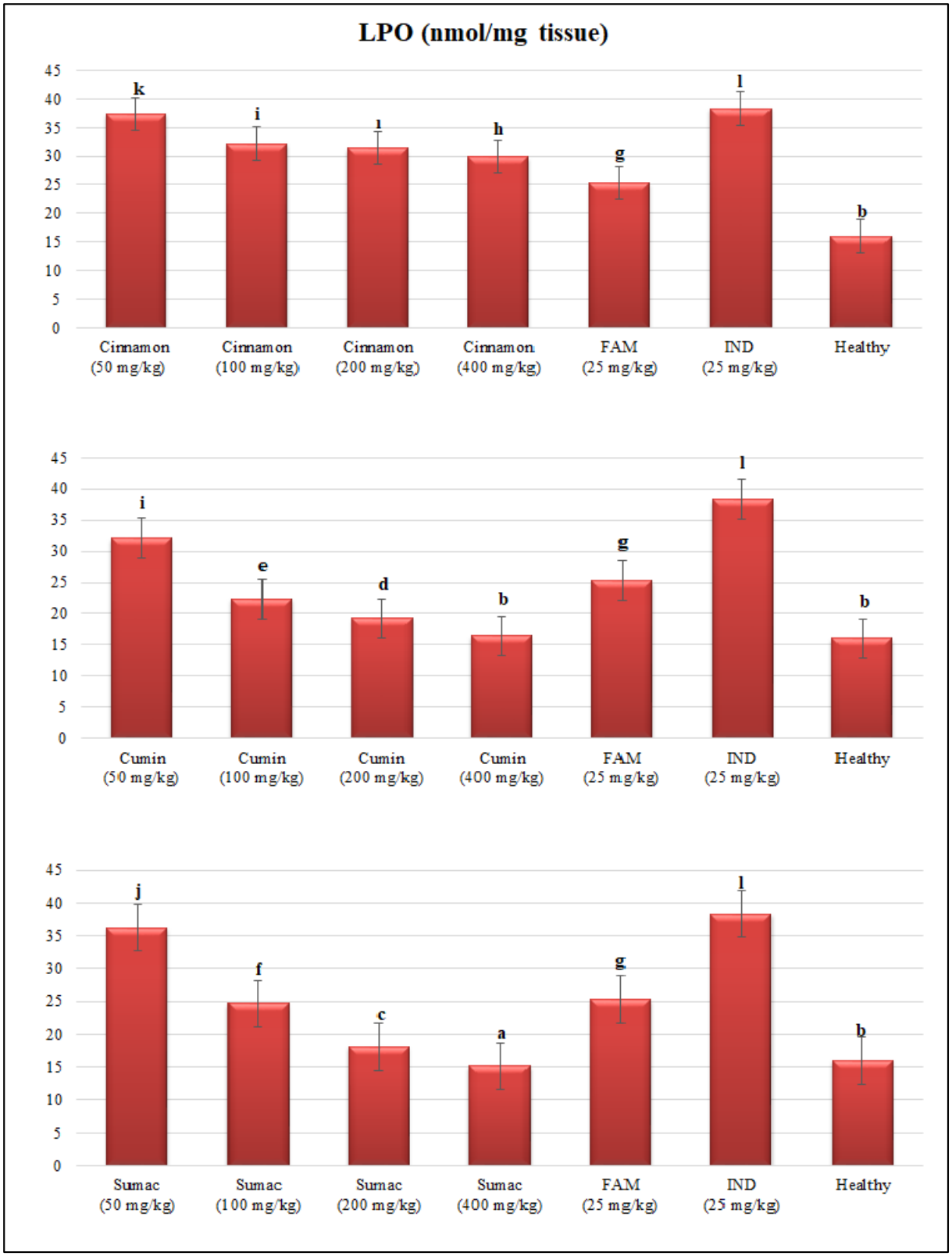

Figure 5. Effects of different doses of species extracts and single dose of famotidine (FAM) on the amount of lipid peroxidation (LPO) in rat's indomethacin (IND)-induced gastric tissue.Means in the same column by the same letter are not significantly different to the Duncan test ($p<0.05$). Results are means ± SE of six measurements.

Figure 6. Effects of different doses of species extracts and single dose of famotidine (FAM) on the myeloperoxidase (MPx) enzyme in rat's indomethacin (IND)-induced gastric tissue. Means in the same column by the same letter are not significantly different to the Duncan test ($p<0.05$). Results are means ± SE of six measurements.

This increase was reduced to the healthy group level owing to the spice samples applied at different doses and famotidine (Fig. 4 and Fig. 6). On the other hand, indomethacin increased SOD enzyme activity and glutathione levels in contrast to healthy tissues ($p<0.05$) (Fig. 2 and 3). This increase, caused by indomethacin, brought the applied spice extracts close to nearly healthy tissue. These results prove to be as protective as famotidine, a positive drug.

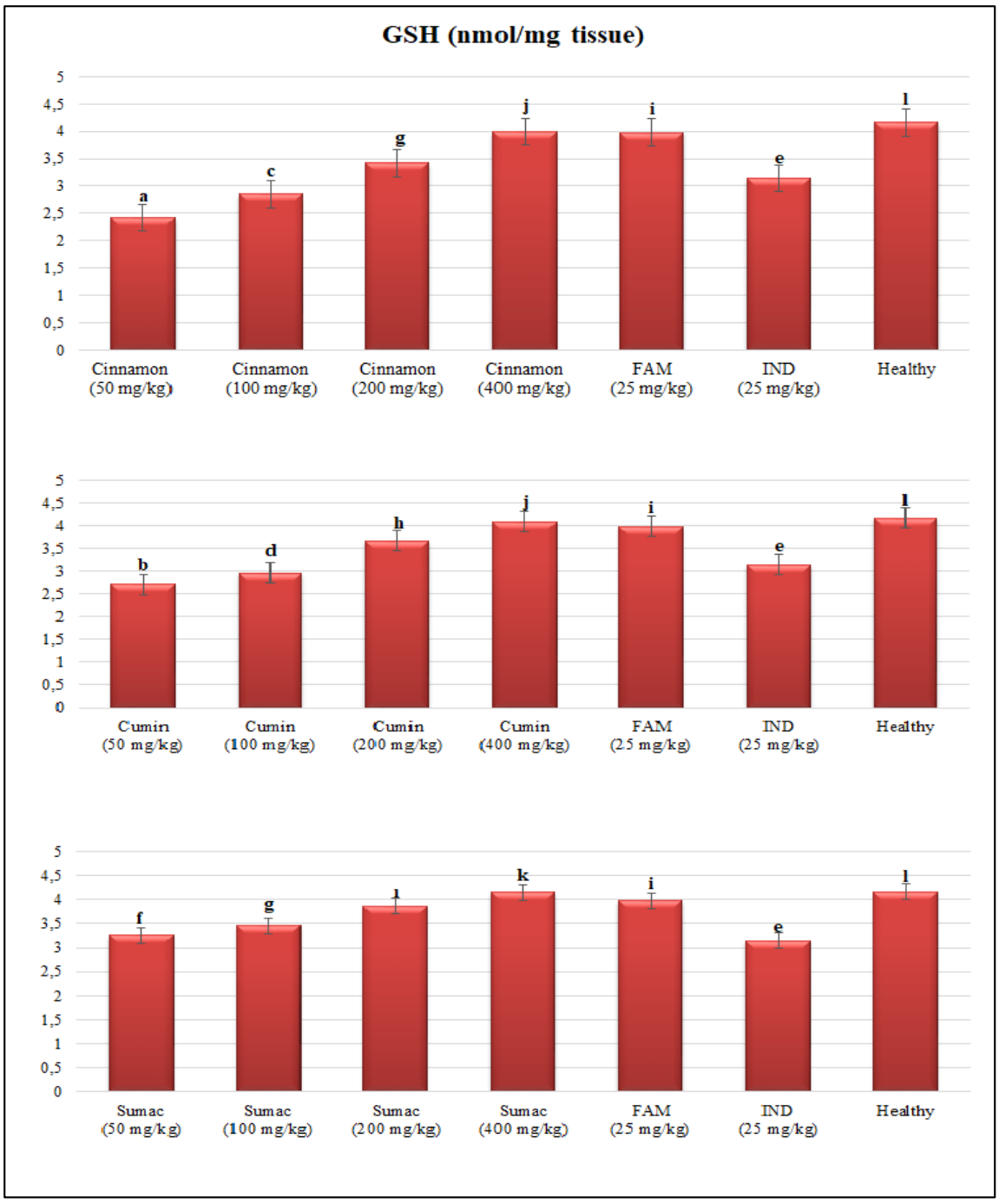

Figure 2. Effects of different doses of species extracts and single dose of famotidine (FAM) on the amount of glutathione (GSH) in rat's indomethacin (IND)-induced gastric tissue. Means in the same column by the same letter are not significantly different to the Duncan test ($p<0.05$). Results are means ± SE of six measurements.

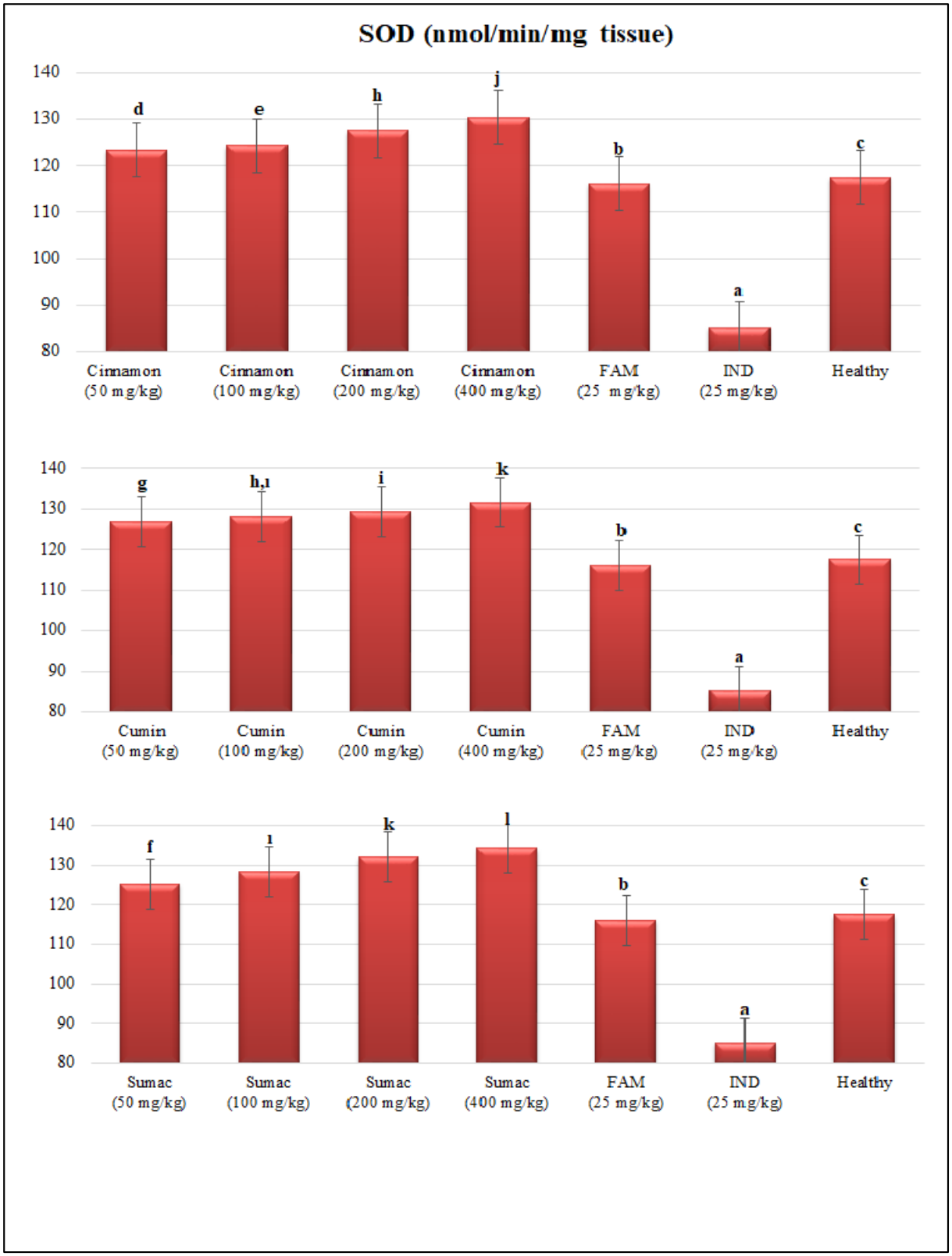

Figure 3. Effects of different doses of species extracts and single dose of famotidine (FAM) on the superoxide dismutase (SOD) enzyme in rat's indomethacin (IND)-induced gastric tissue. Means in the same column by the same letter are not significantly different to the Duncan test ($p<0.05$). Results are means ± SE of six measurements.

DISCUSSION

The organism gives a physiological response to the immunological mechanisms in arousal, infection or trauma. This response demonstrates that the level of arachidonic acid is highly active. Thus, enzymes such as cyclo-oxygenase, 5-lipoxygenase, cytochrome P450 hydroxylase and epoxygenase provide for the formation of prostaglandins. (Odabasoglu et al., 2011). NSAIDs such as indomethacin, which is frequently used in daily life, interfere with the synthesis of prostaglandin and inhibit its release. It also increases the reabsorption of the hydrogen ions, thereby increasing the acid release. (Whittle, 1981).

The pathogenesis of NSAID-induced peptic ulcers is quite complex and many factors are connected. Recent advances in cellular and molecular biology have highlighted the importance of various prostaglandin-independent mechanisms. So, pharmacogenetic studies will be much more useful in elucidating damage.

In addition to adding flavor to the food, spices can be used as antimicrobial (garlic, mustard, thyme, red currant, cinnamon, clove), antioxidative (rosemary, sage, thyme, sumac, clove), blood pressure lowering (garlic) It is also used as donor (cement), aphrodisiac (vanilla), painkiller (clove) and soothing (sage) (Yıldız and Kılınc, 2010; Gurib, 2006). There are very few studies on the anti-ulcerogenic effects of cinnamon, cumin and sumac on the spices used widely in our country and in the world in the literature reviews made. There is little information about the mechanism of action in these studies. The current work is planned to determine whether the spices used so widely are effective on the digestive system, the organ system of which food is first, and will also shed light on the future use of these plants. So, the present study examined the gastroprotective effects of four doses of species (cinnamon, cumin and sumac) (50, 100, 200 and 400 mg/kg body wt.) on IND-induced gastric damage in rats (Table 1). Experiments were carried out using four different dosages of extracts from cinnamon, cumin and sumac spices. As a control group, rats given tap water were used. Naturally, there was no damage in the stomach of rats used as control group. Ulcerative hemorrhages were significantly detected in the IND rats used as a negative control group (Table 1) (41.1 / mm^2); the damage to the rat stomach was very low (7.1 / mm^2) in the FAM group given as positive control. For the extracts supplied with IND, it was determined that the damages were inhibited at different levels and that the damaged areas were reduced by doses. It was also found that gastric damage was reduced more effectively when given at a dose of 400 mg / kg for all three spice extracts. The current results show that all three spice varieties inhibit gastric damage, indicating that the most effective gastroprotective spice in these spices is also caraway (Table 1 and Figure 1). Orally administered IND (25 mg/kg body wt. dose) has caused gastric damage. This damage is caused by the inhibition of prostaglandins synthesized by COX-1 and COX-2. But it is much less active against COX-2 than against COX-1 (Whitle, 1981; Dengiz et al., 2007; Karakus et al., 2009; Tanas et al., 2010; Odabasoglu et al., 2011). However, in most of the studies have shown that ROS also play an important role in the mucosal damage caused by IND. (Elliot and Wallace, 1998; Miura et al., 2002; Karakus et al.,2009).

The IND carries out mucosal damage with the pro-oxidant property. It produces LPO by initiating ROS and interferes with endogenous antioxidant systems. (Miura et al., 2002; Tanas et al., 2010). Recent studies have shown that pro-oxidants expeditiously block cells' antioxidant systems, which causes ROS formation. As a result of this process, oxidative damage occurs (Elliot and Wallace, 1998; Miura et al., 2002; Dengiz et al., 2007).

As indicated by this mechanism, the level of LPO is shown to be greatly increased in tissues where IND is applied. But this increase in the treatment groups, the spice extracts and the FAM group, was found to be reduced to a level close to healthy tissue ($P<0.05$) (Sandip et al., 2000; Odabasoglu et al., 2006).

Living organisms have both enzymatic and nonenzymatic antioxidant defense systems to neutralize oxidative stress. The most important enzymatic defense mechanisms include SOD, CAT and glutathione metabolism enzymes (GPx, GST and GR). GSH, tocopherols (E-vitamin), ascorbic acid (C vitamine), A-vitamine and phenolic substances are non-enzymatic antioxidant defense system components (Atalay et al., 2016; Odabasoglu et al., 2006; Halici et al., 2011).

A number of studies have reported that many NSAIDs, such as IND, reduce SOD activity in the stomach (El-Missiry et al., 2001; Mizoguchi et al., 2001; .de la Lsatra et al., 2002; Tanas et al., 2010). SOD is the most important enzyme that disables superoxides. The CAT enzyme is another enzyme that converts H_2O_2 into water. GSH is an antioxidant molecule found in all tissues that electron transfers different free radicals. It has been shown that GSH protects cell membranes from oxidative damage in gastric tissues (Kaplan et al., 2012; Atalay et al., 2016; Odabasoglu et al., 2006; Halici et al., 2011).

In addition to neutralizing GSH, H_2O_2 and its oxygen radicals, it also plays a role in the stimulation of prostaglandin synthesis in gastric tissues. As the radical oxygen molecules in organisms multiply, the level of GSH and other endogenous antioxidants decreases. At the level of these, the hypothalamus weakens the gastric mucosa against oxidative damage. This means; The decrease in the amount of SOD and GSH is indicative of an excessive increase in the amount of oxygen radicals such as LPO level. This judgment has been recorded in the literature with a number of studies (Sakurai and Yamasaki, 1994; Yoshikawa et al., 1997; Hiraishi et al., 1994; Atalay et al., 2016; Odabasoglu et al., 2006; Tanas et al., 2010).

SOD plays an important role in eliminating gastric damage by partially preventing oxidative damage. SOD destroys the highly reactive radical O_2 ($O_2^{-\cdot}$) by converting it into the less reactive H_2O_2 that can be destroyed by the CAT reaction. In Figure 3 we seemed that IND reduced SOD. This means that superoxide radicals could not convert to H_2O_2 by SOD. But CAT activity was increased in IND administrated tissues. The increase in CAT activity means that there is an increase in the amounts of H_2O_2, but how? It is reported that superoxide radicals spontaneously convert to H_2O_2 and peroxil (HO_2^-) radicals in acidic media (Mahadik and Scheffer, 1996). This spontaneous dismutation is fastest in pH 4.8. In addition, superoxide and perhydroxil radicals react with each other which causes and oxidation and a reduction. As a result of this dismutation, H_2O_2 and O_2 occurs (Weiss and Lobuglio, 1982). Thus, CAT activity in IND-administrated tissues might be increased because of this spontaneously dismutation (Figure 4).

CAT is a highly reactive enzyme that reacts with H_2O_2 to form water and molecular oxygen, and can also form methanol, ethanol, formic acid or phenols by donating hydrogen. (Sedlak and Lindsay, 1968; Elliot and Wallace, 1998). In the present study we established that all doses of species extract and FAM decreased CAT activity (Figure 4), which was increased by IND in rat gastric tissues. Our study found that SOD activity was reduced by IND ($P<0.05$), and that all doses of species extract and FAM increased SOD activity to near control group levels ($P<0.05$) (Figure 3). If species increases the activity of SOD, why CAT activity decreases in the same tissues?

In the presence of the Fe and Cu metals, H_2O_2 reacts with superoxides which results with the most reactive and damager free oxygen radical, hydroxyl radical (Weiss and Lobuglio, 1982). This reaction is called as Haber – Weiss reaction. Haber – Weiss reaction occurs in the presence of catalyst or without catalyst. The reaction without catalyst is very slow. The second way catalyzed by Fe^{3+} is very fast. Hydrogen peroxide could be changed to hydroxyl radicals via Fenton reaction in the presence of this iron. The mechanisms of these reactions were demonstrated by Figure 3 (Freeman and Crapo, 1982; Szabo, 1987).

On the other hand, Chen et al. (1998) suggested that CAT stimulates the expression of mRNA and the protein for COX-2 in rats' aortic smooth muscle cells, despite not affecting the expression of either mRNA or the protein for COX-1. That is, CAT exerted a biphasic effect on prostaglandin synthesis and enhanced prostaglandin production at low concentrations. This suggests that, at low concentrations, increased CAT activity may cause inflammation as reflected by increased COX-2 activity. One of the factors causing the IND-induced gastric ulceration process is possibly an augmentation of CAT activity, which was ascertained in the results of the present experiment (Figure 4).

The MPO assay has had widespread use as an index of neutrophil infiltration in various gastric injuries (Karakus et al., 2009; Tanas et al., 2010). As shown in Figure 6, the MPO activity in IND-administrated rat stomach tissues increases in comparison with that occurring in the tissues of healthy rats ($P<0.05$). The increase in this enzyme activity level may be associated with increases in the levels of neutrophil infiltration and H_2O_2 in those gastric damaged tissues administered with IND. The activity level of this enzyme was alleviated by each dose of species, acting counter to the IND. Similarly, FAM also decreased MPO activity. It has been reported that the release of MPO from gastric cells is another indication of the degree of ulceration, with NSAIDs such as IND also exerting their effects via inhibition of MPO pathways (Karakus et al., 2009; Tanas et al., 2010). Tissue MPO activity is a sensitive and specific marker of acute inflammation and reflects polymorphonuclear cell infiltration into the parenchyma. The effect of species on decreasing MPO activity may be related to its gastroprotective ability.

In conclusion, this experiment showed that IND successfully induced ulcers in rat stomachs. The species extract reduced ulcers at a greater magnitude when compared to FAM. The levels of MPO, anti-oxidant system enzymes (SOD and CAT), LPO and GSH were adversely affected by ulcer induction. Administrated drugs (FAM and species extract) alleviated the adverse effects of ulceration on these parameters. The gastroprotective properties of species extract could be related to its positive effects on the antioxidant system and MPO activity in IND-induced gastric ulcers in rats.

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DISCLOSURE STATEMENT

All authors declare that there are no conflicts of interest.

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Effects of Climate Change on Aquaculture Production

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Abstract

Aquaculture continues to develop at a rapid pace, it's the fastest-growing food production sector in the world. However, the sector's long-term viability is challenged by the consequences of climate change. Climate change effects on aquaculture production are expected to be both direct and indirect. Various factors of a changing climate have been considered in this review, including rising temperatures, sea-level rise, changes in rainfall patterns, the unpredictable supply of external inputs, changes in sea surface salinity, and extreme climatic events. The effects of climate change will be persistent and likely to be irreversible, resulting in severe consequences on the economy of those engaged in the sector. Wherefore, more effort must be made by the fisheries authorities to understand the dimensions of the impact of climate on aquaculture and prepare for its possible consequences and to assess the types of consequences and develop an appropriate response to manage them.

Keywords: Aquaculture, Fisheries, Food Production. Climate Change

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INTRODUCTION

Global climate change, the industrial revolution of the then mankind atmosphere to release the carbon dioxide, methane, ozone and nitrogen oxides as gases are very quickly heat the earth by the greenhouse effect that occurred as a result of the increase is a result of an increase above normal (Bağdatlı and Bellitürk, 2016a). Increasing or decreasing changes in climatic values affect living things negatively and cause a decrease in productivity, especially in agricultural production (İstanbulluoğlu et al., 2013). Aquaculture is the fastest-growing food-production technique, now accounting for more fish biomass than catch fisheries (Edwards, et al., 2019) and more overall biomass than beef on a worldwide scale. The sector is distinguished by the fact that the organisms produced are all poikilotherms, making it the most diversified of all farming systems in terms of the number of taxa farmed. It may be found in fresh, brackish, and marine waterways, as well as in temperate and tropical climates (De Silva, 2013). Aquaculture's contribution to world fish output has increased, now accounting for 82.1 million tons (46 percent) of total production of 179 million tons. Furthermore, aquaculture's proportion of world fish output is predicted to increase from 46 percent now to 53 percent in 2030 (FAO, 2020).

The majority of this progress has occurred in the previous 50 years, and as a result, sustainability, particularly environmental sustainability, has become a major concern. Increased attention has been paid to raising environmental awareness and, as a result, implementing strategies to lessen aquaculture's environmental imprint. Environmental degradation was not considered the most pressing worry of the sector five decades ago, but it is now a critical focus point, whether academic, governmental, productive, or market-based. It is no longer arguable whether aquaculture output should be managed in an ecologically responsible and sustainable manner in today's society (*Engle, C., and D'Abramo, L. 2018.).* Sustainability has progressed from obscurity to the forefront of the factors that influence aquaculture business management, legislation, public image, and product marketing (Boyd et al., 2020). The most pressing question, however, is whether the industry can develop sustainably and quickly enough to satisfy future predicted demand, which is being worsened by a rapidly rising human population and a changing environment. Climate change is currently seen as a serious danger to world food supply, both in terms of quality and quantity (Hamdan et al., 2015; Myers et al., 2017). The expected impacts of climate change are putting food security, particularly availability to dietary protein, under growing jeopardy (Kandu, 2017). Increasing world population, changing climate conditions and economic activities are growing with each passing day makes it more important than water (Bağdatlı and Bellitürk, 2016b).

AQUACULTURE and CLIMATE CHANGE RISK

World has been threatened by climate change under the effect of increased carbon emission and greenhouse gas. Carbon is one of the basic elements of life and shows search without being fixed. The amount of CO_2 reduces the protective use of the bard layer. With this effect, it causes irregular precipitation and excessive temperature increases (Bağdatlı and Arıkan, 2020).

Despite all of the debates and controversies, a global consensus has emerged that climate change is a reality and that it will have an impact on food production systems, global biodiversity, and overall human well-being in a variety of ways, including increased global temperature, sea level rise, more frequent occurrence of extreme weather events, changes in weather patterns etc. and Aquaculture is no exception (De Silva, 2013).

Population growth rate along with the climate change phenomenon will cause lots of problems for worldwide food supply and we will face numerous nutritional problems in the near future. By gradually reaching to the 8 billion population on the earth, the mankind is really in challenge to provide the growing population food needs (Bağdatlı et al., 2015)

The majority of contemporary research in aquaculture indicates that some climatic changes, such as rising temperatures, altering precipitation patterns, and increased frequency of some extreme events, have already had an impact on water supplies, while others are still emerging (Fleming et al., 2014; Blanchard et al., 2017; Troell et al., 2017; Zolnikov, 2019).

Because of the sector's substantial contribution to global food security, nutrition, and livelihoods, climate change implications on aquaculture sustainability have recently attracted a lot of attention (Blanchard et al., 2017; Dabbadie et al., 2018; FAO, 2020).

The effects of climate change will be persistent and likely to be irreversible, resulting in severe consequences on the economy of those engaged in the sector, with extreme effects projected on poorer communities (IPCC, 2013, 2014; Holmyard, 2014; Barange et al., 2018; Dabbadie et al., 2018). At both the regional and global levels, the impacts of climate change on aquaculture have been thoroughly researched and evaluated (De Silva and Turchini, 2009; Yazdi and Shakouri, 2010; Clements and Chopin, 2016; Bueno and Soto, 2017; Chung et al., 2017; Ellis et al., 2017; Froehlich et al., 2017; Handisyde et al., 2017; Harvey et al., 2017; Klinger et al., 2017; Beveridge et al., 2018; Dabbadie et al., 2018; Maulu et al., 2021). Climate change effects on aquaculture production are expected to be both direct and indirect (Handisyde et al., 2006; De Silva and Turchini, 2009). Direct effects include influencing the physical and physiology of finfish and shellfish stocks in production systems, while indirect effects include changes in ecosystem productivity and structure, input supplies, and product prices, fishmeal and fish oil costs, and other goods and services required by fishers and aquaculture producers (Handisyde et al., 2006; De Silva and Turchini, 2009; Freeman, 2017; Adhikari et al., 2018). Aquaculture production, it is widely agreed, does not take place in a vacuum; it is intertwined with other food production systems (De Silva and Turchini, 2009; Troell et al., 2014).

Furthermore, (Blanchard et al., 2017) pointed out that in order to fulfill the ever-increasing demand for aquatic goods in a sustainable manner, it is necessary to identify the strong links that exist within and between the aims of fisheries, aquaculture, and agriculture systems. The difficulties will differ greatly depending on the weather conditions. The biggest issues in the tropics will be farming operations that take place in deltaic zones, which also happen to be aquaculture centres. Sea level rise will have the greatest impact on aquaculture in tropical deltaic areas, resulting in greater salty water intrusion and lower water flows, among other things. Extreme weather conditions, increased upwelling of deoxygenated waters in reservoirs, and other factors could affect inland cage culture and other aquaculture activities elsewhere in the tropics, necessitating increased vigilance and monitoring, as well as the readiness to relocate operations to more conducive areas in a waterbody. Similarly, analogous effects will be seen in the culture of species whose culture is dependent on natural spit collecting, such as many cultured molluscs. Global warming might elevate temperatures in the temperate area to the upper tolerance limits of some cultured species, rendering such culture systems susceptible to high temperatures. Increases in water temperature may cause new or previously non-pathogenic organisms to become virulent, exposing the sector to new, previously unmanifested, or little known diseases (De Silva, 2013; Brange et al., 2018).

Impacts on production of fish species utilized for reduction, which in turn provide the foundation for aquaculture feeds, particularly for carnivorous species, will be one of the most important indirect consequences of climate change. In all climate regimes, these indirect impacts are expected to have a significant impact on several critical aquaculture operations. Limited supplies of fishmeal and fish oil, as well as the accompanying excessive price spikes for these commodities, would likely lead to more imaginative and pragmatic component substitution options for aquatic feeds, which might be a beneficial effect of a severe necessity to keep a significant industry afloat (Naylor et al., 2000; De Silva and Turchini, 2009; De Silva, 2013).

There are changes in the water surface in the world due to global warming. This is the effect of evaporation in water resources and irregularity in the current precipitation regime due to climate change (Albut et al., 2018). Climate change and global warming are reducing the available water resources almost everywhere in the world (Uçak and Bağdatlı, 2017). Excessive increase and decrease of temperatures negatively affect the life of living things. It will be difficult to find clean water in the future as the increase of temperatures will increase the evaporation level. Increasing or falling temperatures will cause climate change (Bağdatlı and Can, 2020).

Food patterns throughout the world have evolved over time. Food safety and quality, backed up by ecolabelling, are now top priorities; this was not the case 20 years ago. In the not-too-distant future, consumer awareness will require that all farmed foods branded goods contain the green house gas (GHG) emissions per unit of produce. Aquaculture is, without a doubt, a viable tool for reaching these objectives. (De Silva, 2013; Robb et al., 2017). Given that around 70% of all finfish and nearly 100% of all mollusks and seaweeds release very little of green house gas, aquaculture may be promoted as the most green house gas-friendly food source. The industry might adapt to such needs and continue to service the growing demand for global food fish supplies. However, a paradigm shift in our seafood consumption habits will be required to achieve this (Halwart, 2020; De Silva, 2013).

CONCLUSION

This review has addressed important aspects of climate change and aquaculture production; where highlighted the potential effects of climate change on aquaculture production. Human-caused climate change is posing a growing danger to the aquaculture industry, which is both a present and future reality. These impacts on aquaculture are projected to be both beneficial and negative, but the negative consequences are likely to outnumber the favorable ones. Furthermore, while climate change is a worldwide food production concern, the hazards associated with aquaculture are predicted to vary by geographical or climatic zones, national economy, water environment, production techniques, production size, and aquaculture producers' cultivated species.

Therefore, Aquaculture producers must adapt to the available choices and mitigate the consequences by making essential modifications in their production processes to create resilience and sustain output in a changing environment. As the continued growth of the aquaculture sector and the increase in the risks of climate change, there is a need to develop research and conduct field studies to reduce the risks related to climate change and its impact on aquaculture.

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Optimization of Extraction Parameters and Effect of Different Solvent Systems on The Omega-3 Fatty Acids Content of Algal Oil *(Nannochloropsis sp.)*

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Abstract

Ultrasonically-assisted algal oil (*Nannocholoropsis sp*.) extraction (UAE) was optimized using Response Surface Methodology (RSM) and hexane. Extraction variables were determined as extraction time, temperature, and solvent:biomass ratio. Optimization was made by aiming both maximum oil yield and omega-3 fatty acid (ω-3 FA) content at the same time. The optimum conditions were determined to be 44.30 ℃, 62.46 min, 19.9:1 g/ml. The extraction time and temperature significantly affected the yield and ω-3 FA content, whereas solvent: biomass ratio did not affect the range of values tested for each of the variables ($p<0.05$). Then, under these optimum conditions, UAE was applied using selected solvents of different polarities (hexane, chloroform, methanol, ethanol and 2-propanol). The effects of different solvents on the oil yield, ω-3 FA content, oxidation, and nutritional properties of algal oil were investigated. Methanol was found to be more efficient than other solvents considering oil yield and ω-3 FA, especially eicosapentaenoic acid (EPA) content (14.46%). Although methanol and chloroform are widely used in extraction, their toxicity limits their use in the food industry. Considering the oil yield and ω-3 FA content of non-toxic solvents, it was determined that 2-propanol was more preferable due to its high ω-3 FA content.

Keywords: Edible algal oil, Ultrasonic assisted extraction, RSM optimization, Solvent polarity, Omega-3 fatty acid

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INTRODUCTION

Microalgae are called single-celled, photosynthetic, and aquatic organisms ranging in size from 1 to 100 μm (Saber et al., 2016). Although it varies according to the species, microalgae are good sources of proteins, lipids, carbohydrates and vitamins. Owing to these properties, they are widely used in cosmetics, pharmaceutical, animal feed, biofuel, and food industries (Gong et al., 2011; Rebolloso-Fuentes et al., 2001; Spolaore et al., 2006). *Nannochloropsis sp*., a type of microalgae, is used in the production of algal oil as an alternative to fish oil due to its abundant content of polyunsaturated fatty acids (PUFA), especially omega-3 (ω-3) fatty acids (FA) (Ryckebosch et al., 2014b).

Eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA), which are among the ω-3 FA, are important fatty acids with many health-beneficial properties. These essential fatty acids, which the body cannot produce and must be taken by foods, are important in the regulation of physiological functions, including blood pressure, brain and eye development, and in the prevention of some diseases such as cardiovascular diseases, hypertension, and diabetes (Fedorova-Dahms et al., 2011; Joumard-Cubizolles et al., 2017; Kaushik et al., 2015).

Fish oils are the primary sources of ω-3 FA. However, negative factors such as unwanted fishy odor, risk of heavy metals, and fat-soluble contaminants limit the consumption of fish oil (Khozin-Goldberg et al., 2011; Li et al., 2019; Oterhals et al., 2010). Due to the popularity of diets belonging to different diet groups such as vegan or vegetarian, there have been studies in recent years on the use of microalgae as an alternative ω-3 source to fish oil (Katiyar and Arora, 2020; Ryan and Symington, 2015). In addition, since microalgae are the primary producers of ω-3 in the marine environment, they can be considered the key to a sustainable PUFA source (Ryckebosch et al., 2014).

Some studies have already been performed on the lipid extraction from microalgae, although most of them focused on biodiesel production (Archer et al., 2019; Li et al., 2019; Wang et al., 2016). Traditionally, the extraction of various bioactive compounds from natural products has been carried out many industries using different methods and solvents. In the lab-scale, conventional extraction methods such as maceration and soxhlet extraction have many disadvantages like a large amount of solvent utilization, long extraction time, and lower extraction yield (Bermúdez Menéndez et al., 2014).

The ultrasound-assisted extraction (UAE) method is an inexpensive and simple substitute for traditional extraction techniques (Dey and Rathod, 2013). UAE uses acoustic cavitation for producing cavitation bubbles that implode resulting in high shear forces. This helps in disrupting the cell wall allowing the solvent to penetrate the material and increases the contact surface area between the solvent and compound of interest, resulting in increased mass transfer along with good mixing (Vinatoru et al., 1997). Extraction temperature, time, along with other factors such as solvent concentrations, pH, solid-liquid ratio, particle size which affect the extraction of bioactive compounds (Ma et al., 2008, Maran et al., 2017). Response surface methodology (RSM) can be employed to optimize extraction conditions for algal oil where extraction temperature, time, and solvent:biomass ratio serve as independent variables that influence responses in a given set of experiments (Myers and Montgomery, 2002; Belwal et al., 2016).

The aim of this study was to determine the optimum extraction conditions for independent oil extraction variables (extraction temperature, time, and solvent:biomass ratio) and validate the optimized conditions based on the combination of algal oil yield and its ω-3 FA content. Furthermore, polar and non-polar solvents including hexane, methanol, ethanol, chloroform, and 2-propanol were tested to increase further extraction yield and oil quality under optimum extraction condition from *Nannochloropsis sp.*

MATERIALS and METHODS

Materials

Freeze-dried *Nannochloropsis sp.* powder were obtained from BluebioTech Int. (Kaltenkirchen, Germany). All chemicals were purchased from Merck (Merck Chemicals Co. (Darmstadt, Germany) and Sigma (Sigma-Aldrich GmbH, Sternheim, Germany). Chemical properties of solvents (hexane, methanol, ethanol, chloroform, and 2-propanol) were given in Table 1.

Table 1. Chemical properties of solvents used in extraction

Solvent	Polarity Index	Dipole Moment	Dielectric constant	Boiling point	Viscosity (40°C)
Hexane	0.1	0	1.9	68.9	0.26
Chloroform	4.1	1.1	4.8	61.7	0.47
Methanol	5.1	1.70	32.6	65	1.70
Ethanol	4.6	1.70	24.3	78.5	0.83
2-propanol	3.9	1.66	18.3	82.4	1.35

Methods

Chemical composition

The total protein content of *Nannochloropsis sp.* was determined according to the method of A.O.A.C. (AOAC, 1984); moisture and ash contents were determined according to the methods of Helrich (Helrich, 1990). Total lipid content was analyzed using the method of Bligh and Dyer (AOAC, 1984). The oil yield (% of the dry algae biomass) was quantified gravimetrically.

Experimental design

The test ranges of extraction temperature, time, and solvent: biomass ratios on the algal oil yield and ω-3 FA content were chosen based on our preliminary work. They were 25-65 °C for extraction temperature, 30-90 min for extraction time, and 10:1-30:1 (ml:g) for solvent (hexane): biomass ratio. Optimization was made by targeting both maximum oil yield and maximum omega-3 content at the same time. These three variables at three levels were applied to Box-Behnken Design (Box et al., 1960) to generate a second-order polynomial Equation 1.

$$Y = \beta 0 + \sum \beta i\, Xi + \beta ii\, X2i + \sum \beta ij\, Xi\, Xj, \quad (1)$$

In the equation *Y* stands for the experimental response; β0, βi, βii, and βij are constants and regression coefficients of the model; and *Xi* and *Xj* are uncoded values of independent variables. The experimental design and responses (oil yield and its ω-3 FA content) are given in Table 2.

The responses obtained from the experimental design were subjected to multiple nonlinear regressions using the software Design-Expert 9.0. (Topuz et al., 2016). Optimum conditions were determined by the computer program by entering the maximum and minimum points of the variables.

Table 2. Box–Behnken experimental design and responses

	Experimental design						Responses	
Test	Max-min. points			X_1 (g: mL)	X_2 (°C)	X_3 (min.)	Oil yield (%)	Omega-3 fatty acid* content (%)
A_1	-1	-1	0	1:10	25	60	18.26±1.13	33.04±0.43
A_2	0	-1	-1	1:20	25	30	18.67±0.86	33.40±0.65
A_3	+1	-1	0	1:30	25	60	18.81±0.78	32.59±0.56
A_4	0	-1	+1	1:20	25	90	18.89±0.82	32.06±0.74
A_5	+1	0	+1	1:30	45	90	20.44±1.05	31.04±0.60
A_6	+1	0	-1	1:30	45	30	19.61±0.63	31.76±0.43
A_7	0	0	0	1:20	45	60	19.88±0.67	31.75±0.84
A_8	0	0	0	1:20	45	60	19.88±0.93	31.60±0.66
A_9	-1	0	-1	1:10	45	30	18.98±0.79	31.78±0.72
A_{10}	0	0	0	1:20	45	60	20.33±1.03	31.45±0.53
A_{11}	-1	0	+1	1:10	45	90	20.41±0.45	31.40±0.44
A_{12}	0	+1	-1	1:20	65	30	20.90±0.56	30.92±0.75
A_{13}	0	+1	+1	1:20	65	90	21.60±0.71	30.30±0.56
A_{14}	+1	+1	0	1:30	65	60	21.19±0.84	30.61±0.34
A_{15}	-1	+1	0	1:10	65	60	21.05±0.58	30.81±0.52

X_1: biomass:solvent (hexane) ratio; X_2: Extraction temperature; X_3: Extraction time
*Omega-3 fatty acids: C18:3 (alpha-linolenic acid, ALA), C20:3, C20:5 (Eicosapentaenoic acid, EPA), C22:6 (Docosahexaenoic acid, DHA).

Ultrasound-assisted extraction

UAE of algal oil was performed using an ultrasonic homogenizer (Sonopuls HD 4200, 200W, Bandelin GmbH & Co, Berlin, Germany) equipped with a 13 mm diameter tip at a frequency of 20 kHz. Hexane was used as a solvent in the optimization step of the study. And ethanol, methanol, chloroform, and 2-propanol were selected as additional extracting solvents to be evaluated. The extraction was carried out at the required temperature and time according to the experimental design. After the extraction, the mixture was centrifuged and filtered through Whatman No. 1. The solvent was evaporated with a rotary evaporator (Heidolph, HeiVAP Advantage, Schwabach, Germany) at 50°C. Then, nitrogen was injected into the flask and oils were stored at amber-colored flasks at -45 °C.

Fatty acid composition

The fatty acid methyl esterification procedure was carried out according to Ozogul (Ozogul and Ozogul, 2007). The algal oil (10 mg) dissolved in 2-mL n-heptane was mixed with 4-mL 2-M methanolic KOH and centrifuged at 4,000 rpm for 10 min. The upper layer was injected into a gas chromatograph. The fatty acid composition analyses were performed in duplicate and the results were given in chromatography area % as mean values.

Gas Chromatography Conditions: Gas chromatography (Perkin Elmer, Clarus 500, Waltham, MA, USA) equipped with a BPX70 silica column (50 m x 0.22 mm, film thickness 0.25 µm; SGE Inc., Victoria, Australia) and a flame ionization detector was used. The oven temperature was started at 140 ℃ for 5 min, raised to 200 ℃ at a rate of 4 ℃/min and ended at 220 ℃ with an increase of 1℃/min. The injection temperature was 220℃ and the carrier gas, Helium flow rate, was 1.0 ml/min. The detector temperature was set at 280 ℃ with a split ratio of 1:50.

Oil oxidation analysis

Peroxide value

Peroxide analysis was performed using the method described by A.O.A.C (A.O.A.C, 1990). The oil was dissolved by adding chloroform: acetic acid (1:1.5) solution and 1 ml of potassium iodide on the sample of algae (1 g). After standing for 5 minutes in the dark, some water, and 1% starch solution were added as an indicator. The mixture was titrated with 0.01 N sodium thiosulfate conjugate. The obtained results are calculated according to the formula given below.

$$PV\ (meq/kg) = (V\text{-}B \times Nf / W) \times 1000 \quad (2)$$

V: volume of sodium thiosulfate;
B:used sodium thiosulfate,
W: sample weight;
Nf: the normality of sodium thiosulfate.

Para-anisidine value (p-Av)

The value of *p*-Av, the indicator of secondary oxidation product, was determined according to the methods of Frankel (1984). 0.5 g algal oil was dissolved in 25 ml n-hexane (A1). 5 ml of the solution was taken and 1 ml *p*-Av standard (Merck 800458, Germany) was added and kept at room temperature for 10 minutes in the dark (A2). The *p*-Av value of the samples was determined by the following formula with the aid of the absorbance values obtained by spectrophotometric measurements at 350 nm. Due to the dark color of the algal oil, 1/10 dilution was applied in the *p*-Av analysis based on the colorimetric measurement.

$$p\text{-}Av = 25\ (1.2 \times (A2 - A1)) / \text{sample weight} \quad (3)$$

Nutritional properties

The effect of algae oil extracted with solvents selected from *Nannocholoropsis sp*. on nutritional quality and chronic heart health was evaluated by two separate indexes given below. These indexes were calculated using the equations described by Ulbricht (1991).

$$\text{Atherogenicity index (AI)} = \frac{[(C12{:}0 + (4 \times C14{:}0) + C16{:}0)]}{(\Sigma\text{MUFA} + \Sigma\omega\text{-}6 + \Sigma\omega\text{-}3)} \quad (4)$$

$$\text{Thrombogenicity index (TI)} = \frac{(C14{:}0 + C16{:}0 + C18{:}0)}{[(0.5 \times \Sigma\text{MUFA}) + (0.5 \times \Sigma\omega\text{-}6) + (3 \times \Sigma\omega\text{-}3) + (\Sigma\omega\text{-}3 / \Sigma\omega\text{-}6)]} \quad (5)$$

C12:0 (Dodecanoic acid); C14:0 (Tetradecanoic acid); C16:0 (Hexadecanoic acid); C18:0 (Octadecanoic acid); C18:1ω9 (9-octadecanoic acid); C18:2ω6 (9,12-octadecadienoic acid); C18:3ω3 (9,12,15-octadecatrienoic acid); C20:4ω6 (5,8,11,14-eicosatetraenoic acid); C20:5ω3 (5,8,11,14,17-eicosapentaenoic acid); ω-3 (Omega-3); ω-6 (Omega-6).

STATISTICAL ANALYSIS

The findings were subjected to analysis of variance using the Design Expert Statistical Program (Stat-Ease Inc. Minneapolis, USA) and SAS 9.0 (Statistical Analysis System, Cary, NC, USA) packet programs and significant differences were determined by the Duncan Multiple Comparison Test. All analyses were performed in duplicate.

RESULTS and DISCUSSION

The composition of dried *Nannochloropsis sp.* algae biomass was found to contain 35.7% carbohydrate, 31.3 % protein, 23.1% oil, 9.9% ash, and 5.7% moisture. In a similar study, Babuskin et al. (2014) was found the oil and protein contents of *Nannochloropsis oculata* as 22.1 and 36.0, respectively. Total oil and protein contents of *Nannocholoropsis sp.* in the study are compatible with the literature.

Optimization and verification of algal oil extraction conditions

Dried *Nannochloropsis sp.* algae were extracted using hexane under the conditions in Table 2. determined by the Design Expert program, and oil yield and ω-3 FA contents were examined. The optimum extraction conditions were determined as 44.30 °C extraction temperature, 62.46 min extraction time and 1:19.9 g/ml biomass:solvent ratio. Under these conditions, the computer program calculated both the oil yield and ω-3 FA content to be highest as 19.79% and 32.03%, respectively. To verify the predicted result with the practical value, extractions were performed using the obtained optimal conditions.

The experimental results had no significant difference according to the predicted results ($p<0.05$). The mean values of oil yield (19.96%) and ω-3 FA content (32.21%) obtained from real extractions demonstrated the validity of the RSM model for maximal oil yield and ω-3 FA content (Table 3).

Table 3. Predicted and experimental optimum conditions for maximum yield and omega-3 fatty acid content

	Optimum conditions			Responses	
	X_1 (g/ml)	X_2 (°C)	X_3 (min)	Oil yield (%)	Omega-3 fatty acids* content (%)
Predicted**	1/19.9	44.30	62.46	19.79[a]	32.03[a]
Experimental***	1/19.9	44.30	62.46	19.96±0.71[a]	32.21±0.44[a]

X_1: Biomass: solvent (hexane) ratio; X_2: Extraction temperature; X_3: Extraction time,
* Omega-3 fatty acids: C18:3 (ALA), C20:3, C20:5 (EPA), C22:6 (DHA),
**Predicted using ridge analysis of response surface quadratic model,
*** The values represent means ± standard deviation, *n*:3. The different letters in the same column show the values were significantly different according to Duncan's multiple range test ($p<0.05$).

The results of the ANOVA test according to the quadratic equations of Design Expert for the extraction oil yield and ω-3 FA are given in Table 4. According to the Analysis of variance (ANOVA) probability values in the model, it was found that while extraction temperature and time had a significant effect on oil yield and ω-3 FA, solvent:biomass ratio was ineffective in the range of values tested for each of the variables ($p<0.05$).

Table 4. Analysis of variance (ANOVA) probability of oil yield and ω-3 FA.

Source	**Sum of squares**		**Df**		**Mean square**		**F-value**		**p-value**	
	Oil yield	ω-3 FA	Oil yield	ω-3 FA	Oil yield	ω-3 FA	Oil yield	ω-3 FA	Oil yield	ω-3 FA
Model	14.46	10.46	6	6	2.41	1.73	39.62	46.44	<0.0001	<0.0001
X_1 (g/ml)	0.2278	0.1326	1	1	0.2278	0.1326	3.75	3.55	0.089	0.0962
X_2 (°C)	12.78	8.93	1	1	12.78	8.93	210.08	239.08	<0.0001	<0.0001
X_3 (min)	1.26	1.17	1	1	1.26	1.17	20.78	31.35	0.0019	0.0005
$X_1 X_2$	0.0420	0.0156	1	1	0.042	0.0156	0.6910	0.4185	0.4299	0.5358
$X_1 X_3$	0.0900	0.0289	1	1	0.0900	0.0289	1.48	0.7741	0.2585	0.4046
$X_2 X_3$	0.0576	0.1296	1	1	0.0576	0.1296	0.9471	3.47	0.3590	0.0994
Residual	0.4865	0.2987	8	8	0.0608	0.0373	-	-	-	-
Pure error	0.1350	0.0450	2	2	0.0675	0.0225	-	-	-	-
R^2	0.976	0.9721	6	6						
Adjusted R^2	0.943	0.9512	1	1						
Predicted R^2	0.873	0.8703	1	1						
Adeq precision	19.72	21.80	1	1						

X_1: Solvent: biomass ratio (g/ml), X_2: Temperature (°C), X_3: Time (min.).
$p<0.05$ indicate model terms are significant.
Df: Degree of freedom.

The Predicted R^2 of oil yield (0.8733) is in reasonable agreement with the Adjusted R^2 (0.9430). The Predicted R^2 of ω-3 FA (0.8703) is in reasonable agreement with the Adjusted R^2 (0.9512), too; i.e. the difference is less than 0.2 (Design-Expert 8.7.1).

Adeq Precision measures the signal-to-noise ratio. A ratio greater than 4 is desirable. The calculated ratio for oil yield (19.72) and ω-3 FA (21.80) indicates an adequate signal. This model can be used to navigate the design space (Design-Expert 8.7.1).

Effect of extraction variables on yield and ω-3 FA content of algal oil

The effect of solvent:biomass ratio, extraction temperature, and extraction time on oil extraction yield and ω-3 FA content *Nannocholoropsis sp.* are shown in Figure 1. The extracted total lipid yield of biomass varied between 18.26 and 21.60%. The total ω-3 FA content ranged between 30.30 and 33.40%.

As can be seen in Figure 1, increasing the temperature by fixing the extraction time at 60 min resulted in a linear increase in oil yield (Fig. 1a) and a decrease in ω-3 FA (Fig. 1d). The increase in the solvent: biomass ratio slightly increased the oil yield and the ω-3 FA content much in the range of values tested for each of the variables. Increasing both variables together increased the oil yield up to 21.6.

At the fixed temperature (45 °C), increasing extraction time increased the oil yield (Fig. 1b). the increasing rate of The solvent:biomass, slightly increased the oil yield and ω-3 FA (Fig.1b/1e). At the fixed solvent:biomass ratio (1:20), the increase in extraction temperature increased the oil yield (Fig. 1c) in contrast to the ω-3 FA content (Fig.1f). When both variables increased together, a positive acceleration was observed in the oil yield increase.

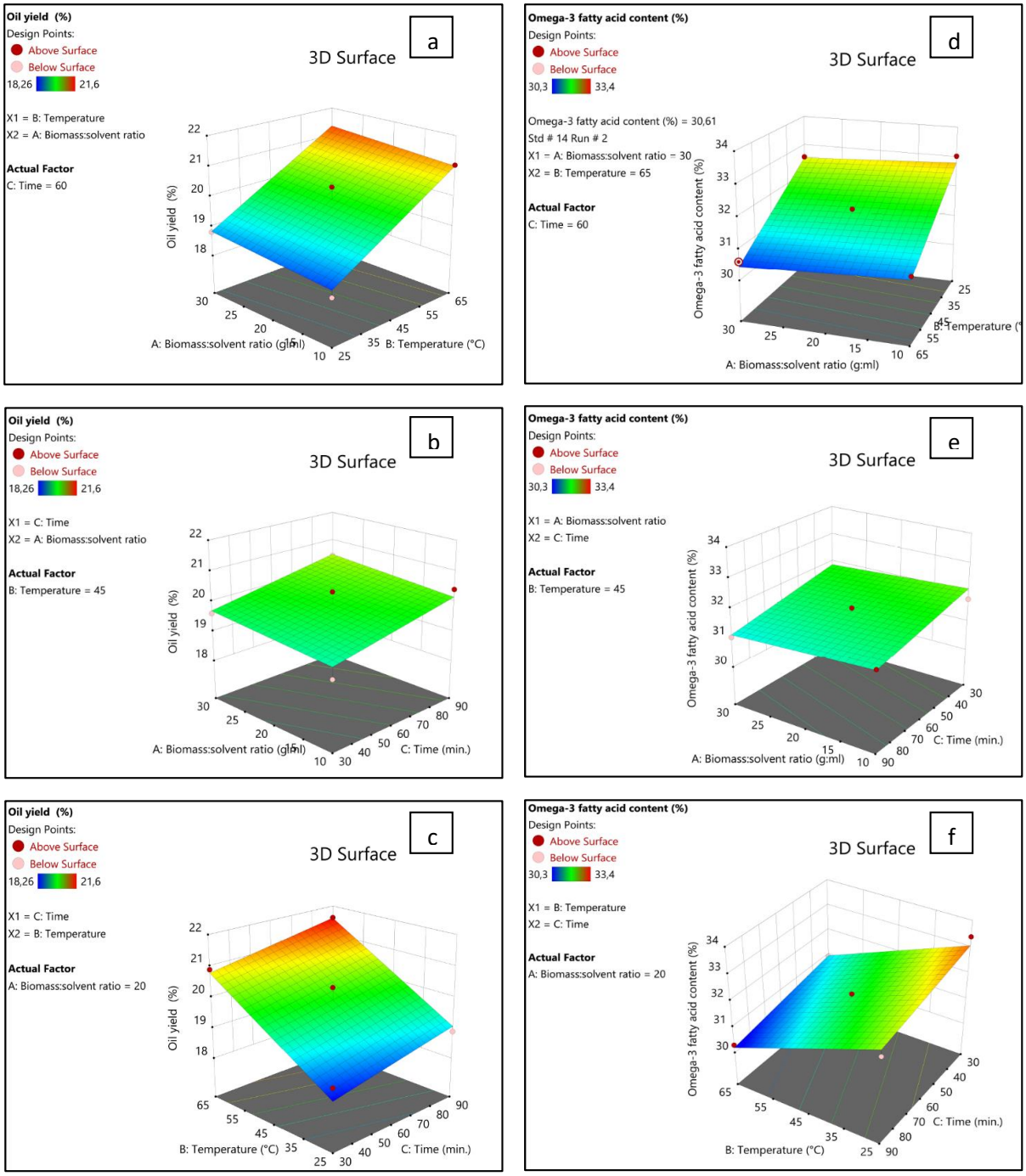

Figure 1. Response surface plots for maximum oil yield (a, b, c) and ω-3 FA content (d, e, f) of algal oil

Effect of solvent type on the yield of algal oil

The oil extraction yields of *Nannocholoropsis sp.* at optimum conditions are shown in Table 5. The solvent type significantly ($P<0.05$) affected the yield of algal oil. Oil yields of extractions made using the solvents having different polarity, varied between 19.40 and 27.61%. Methanol had the significantly highest oil yield while, ethanol had the lowest.

Also, there was no significant difference between the oil yield of hexane and ethanol. In addition, chloroform had a significantly higher oil yield than 2-propanol. Although methanol and chloroform were effective solvents, especially the toxicity of chloroform limiting its use in the food industry. However, these solvents are used in pharmacology and cosmetic technology.

Table 5. Effect of solvent polarity on the oxidative stability, yield and ω-3 FA of algal oil

Solvents	Oil yield (g/100g)	ω-3 FA (g/100g)	PV (meq O_2/kg)	p-Av
Hexane	20.12±0.35^{d}	30.75±0.49^{c}	2.12±0.20^{c,b}	1.40±0.05^{a}
Chloroform	25.62±0.26^{b}	34.93±0.25^{a}	1.45±0.03^{c}	1.61±0.04^{a}
Methanol	27.61±0.12^{a}	30.45±0.39^{c}	2.11±0.00^{c,b}	1.15±0.68^{a}
Ethanol	19.4±0.41^{d}	34.23±0.21^{b}	2.32±0.05^{b}	1.29±0.11^{a}
2-propanol	23.73±0.21^{c}	34.81±0.23^{a}	3.97±0.43^{a}	1.56±0.53^{a}

The values represent means ± standard deviation. Means with different letters (a, b, c) in the same columns are significantly different ($p<0.05$).

There are different opinions in the literature about the use of solvents for oil extraction one by one or as a mixture. In a study conducted by Balasubramanian, it was stated that the use of polar and apolar solvent mixtures provides higher oil yield than using only polar or only apolar solvents (Balasubramanian et al., 2013).

However, in another study, it was reported that a mixture of hexane and ethanol (1: 1, v / v) provided less lipid yield than extraction using only hexane as solvent (Shen et al., 2009). Mixed solvents were not used in this study, but it was found that oil yield and ω-3 FA content did not show a linear increase with the polarity index.

Effect of solvents polarity on the oxidation of algal oil

The effects of selected solvents on the peroxide (PV) and para anisidine (*p*-Av) values of algal oil extracted at optimum conditions are shown in Table 5. Oxidative stability can be assessed by analyzing primary and secondary oxidation products (measured by PV and anisidine *p*-Av, respectively) (Balboa et al., 2014). The solvent type significantly ($p<0.05$) affected PV values of algal oil whereas, had not affected the *p*-Av values The highest PV was obtained with 2-propanol, while the lowest PV was obtained with chloroform. PV values of hexane, methanol and ethanol, there was no significant difference ($p<0.05$). Both PV (1.45-3.97 meq/kg) and *p*-Av (1.15-1.61) oxidation values were lower than reported by Liu et al. (2018) (7.23-9.06 meq/kg and 1.9-7.9, respectively). It can be said that methanol, chloroform and hexane relatively retards oil oxidation compared to the other solvents.

Effect of solvent polarity on the fatty acid composition of algal oil

The FA profile of algal oils extracted with the solvents selected under optimum conditions is shown in Table 6. As it was shown in Table 6, there was no significant difference between both of the SFA and MUFA contents of hexane and chloroform ($p<0.05$). It was also found that hexane and chloroform had significantly higher SFA and MUFA content than other solvents. In this study, similar to Schambach et al. (2020), it was obtained that a high amount of palmitic (16: 0) and palmitoleic acid (16: 1) as well as minor amounts of myristic acid (14:0). The Oleic acid (18:1) content was relatively higher in this study.

Table 6. Effect of solvent polarity on the fatty acid profile of algal oil

Fatty acids (%)	**Solvents**				
	Hexane	**Chloroform**	**Methanol**	**Ethanol**	**2-propanol**
C14:0	3.28±0.25	4.44±0.03	5.09±0.04	4.47±0.04	3.76±0.05
C15:0	0.34±0.07	0.45±0.01	0.44±0.01	0.46±0.00	0.43±0.01
C16:0	37.89±0.52	37.93±0.12	33.49±0.15	34.57±0.25	33.85±0.19
C17:0	0.41±0.08	0.42±0.03	0.30±0.05	0.45±0.00	0.32±0.00
C18:0	1.41±0.08	1.25±0.12	0.35±0.05	1.26±0.01	1.11±0.02
ΣSFA	***44.42±1.17^{a}***	***45.31±0.00^{a}***	***40.04±0.28^{b}***	***42.27±0.28^{b}***	***41.03±0.28^{b}***
C16:1	29.36±1.02	30.48±0.12	29.25±0.26	28.72±0.24	27.79±0.24
C17:1	0.54±0.06	nd	nd	nd	nd
C18:1 n-9	11.78±1.57	12.35±0.13	9.64±0.36	10.11±0.04	11.01±0.04
ΣMUFA	***41.67±0.85^{a}***	***42.83±0.01^{a}***	***38.89±0.14^{b}***	***38.83±0.39^{b}***	***38.80±0.19^{b}***
C18:2 n-6	0.94±0.11	1.08±0.07	1.36±0.01	0.82±0.01	1.05±0.07
C18:3 n-6	0.45±0.11	0.67±0.00	0.69±0.06	0.48±0.01	0.73±0.06
C18:3n-3	1.21±0.06	0.78±0.01	0.51±0.06	2.52±0.01	2.72±0.03
C20:3 n-3	2.50±0.02	1.32±0.49	2.81±0,02	2.54±0.01	3.93±0.06
C20:4n-6	2.08±0.16	nd	nd	nd	nd
C20:5 n-3(EPA)	5.83±0.07	7.19±0.08	14.46±0.23	10.41±0.31	9.09±0.19
C22:6 n-3(*DHA*)	0.21±0.01	0.27±0.02	0.76±0.03	0.43±0.02	0.32±0.02
ΣPUFA	***13.23±0.33^{c}***	***11.30±0.22^{d}***	***20.57±0.08^{a}***	***17.19±0.19^{b}***	***17.83±0.01^{b}***
Σn-6	3.47	1.75	2.05	1.3	1.78
Σn-3	9.75	9.56	18.54	15.9	16.06
n-6/n-3	0.35	0.18	0.11	0.08	0.10
AI	0,92	1,03	0,91	0,94	0,86
TI	0,78	0,77	0,45	0,5	0,5
Undefined	0.68	0.56	0.50	1.71	2.34

The values represent ± standard errors, *n*:3 per experimental replicate; Means within the same row (a, b, c, d) with different letters are different ($p<0.05$).

SFA: Saturated fatty acids, MUFA: Monounsaturated fatty acids, PUFA: Polyunsaturated fatty acids, AI: Atherogenicity index; TI: Thrombogenicity index.

PUFA contents of the samples varied between 11.30 and 20.57. While there was no significant difference between ethanol and 2-propanol, a significant difference was observed between other solvents ($p<0.05$). *Nannochloropsis sp*. is one of the potential genera of microalgae having higher EPA (C20:5), biomass, and lipid productivities (Chua et al., 2020). EPA, which is one of the valuable ω-3 FA found in algal oil, was the major PUFA in all oil samples in the study. The EPA content varied between 5.83% and 14.46%. Figueiredo, in his study investigating the effect of different extraction methods on the ω-3 FA content of *Nannochloropsis oceanica*, obtained the highest EPA content (18.4%) from the dichloromethane/methanol mixture (Figueiredo et al., 2019).

In this study, methanol was found to be the most effective solvent for the extraction of EPA and ω-3 FA (14.46 ±0.23 EPA/g dry biomass). In addition, it was determined that the algae oil extracted with methanol (20.57%) had the highest PUFA content, while the oil extracted with chloroform (11.30%) had the lowest PUFA content. However, the use of methanol in the food industry is not safe. Therefore, it is considered more appropriate to use ethanol and 2-propanol, which do not differ statistically, in the production of ω-3 FA.

Nutritional properties of algal oil

AI and TI values provide information about the nutritional quality of FA and the effect of chronic heart health. Algal oil, AI and TI values and PUFA/SFA and n6/n3 ratios are given in Figure 2. It is reported that long-chain PUFA, especially EPA and DHA, have protective effects against heart diseases. In a clinical study of over a thousand patients, it was reported that supplying patients with 1g/day of ω-3 FA resulted in a large reduction in death, cardiovascular disease, and heart failure by 20, 30, and 45%, respectively (Punia et al., 2019). World health organization and food and agriculture organization experts stated daily intake of EPA+DHA as at least 250 mg, while the American Heart Association reported 500 mg/day for adults (Martins et al., 2013). It is recommended to occur. N6/n3 ratio should be less than 4, n3/n6 should be greater than 6, and PUFA / SFA should be greater than 0.4 (Topuz et al., 2017).

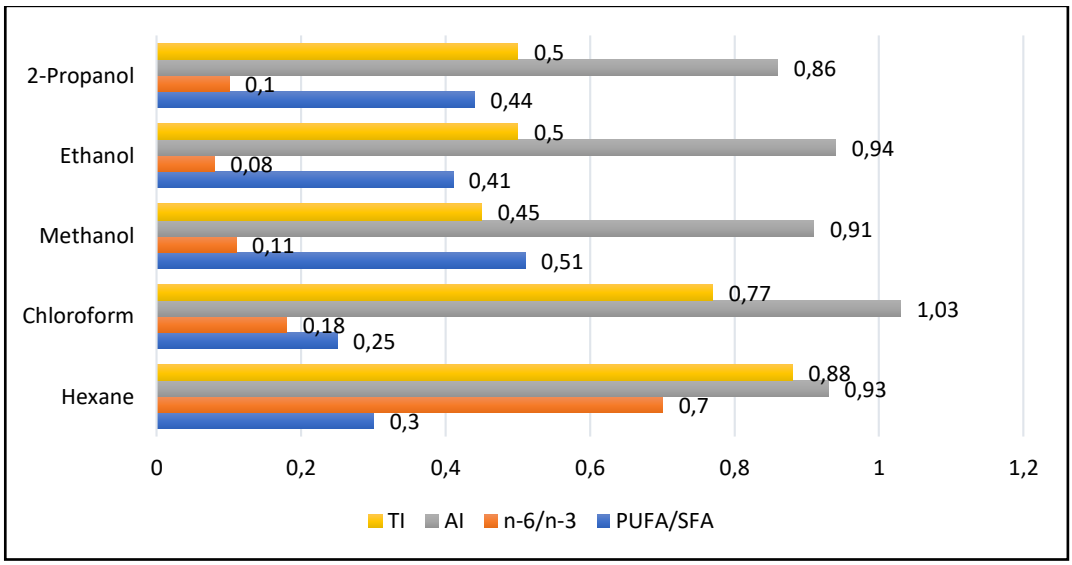

Figure 2. Nutritional properties of algal oil extracted using different polarity solvents
TI: Thrombogenicity index, AI: Atherogenicity index,

n-6: ω-6 FA, n-3: ω -3 FA, PUFA: Polyunsaturated fatty acid, SFA: Saturated fatty acid

The PUFA/SFA ratio as a measure of the propensity of diet to influence the incidence of coronary heart disease should be replaced by the atherogenic index (AI) and thrombogenic index (TI) (Ulbricht, 1991). The algal oils extracted with methanol and hexane, which had relatively high amounts of EPA, led to the desired AI and TI values of 1.43 and 0.31; followed by 1.40 and 0.29, respectively. These findings in accordance with the results of the previous study performed by Mitra (Mitra and Mishra, 2019).

It is seen that the oil extracted with methanol has the highest PUFA content (20.50%), and the lowest (11.30%) of the oil extracted with chloroform. When Figure 3 is examined, it is seen that the solvents in accordance with the specified reference values are methanol, ethanol and 2-propanol. When algal oil and fish oil were compared in terms of essential fatty acid, it was observed that there was no significant difference, and the EPA content was higher in *Nannocholoropsis sp.* than in fish oil.

Currently, there are many studies on ω-3 FA from fish (especially EPA and DHA). However, there are relatively few studies on the beneficial effects of algal oil on metabolism, and the use of algal oil as part of the modern diet is gaining acceptance. As supported in the literature, algal oil has a high EPA content and is an alternative source of ω-3 FA for daily consumption in terms of nutritional quality (Chen et al., 2007; Kumari et al., 2013; Mitra et al., 2015).

CONCLUSION

The optimization of ultrasound/hexane-assisted extraction conditions of edible algal oil from *Nannocholoropsis sp.* was performed employing RSM and using hexane. Extraction temperature (44.30℃) and time (62.46 min) affected the oil yield and its ω-3 FA content, whereas the solvent:biomass ratio (19.9:1 g/ml) did not affect the range of values tested for each of the variables. The oil yield and ω-3 FA content were highly dependent on the solvent, used in oil extraction. Methanol had the highest oil yield , whereas the highest ω-3 FA content were obtained in oil samples extracted with 2-propanol and chloroform. Although chloroform/methanol is the most frequently lipid solvent mixture used due to its fast and quantitative extraction, its biggest disadvantage is the high toxicity of chloroform. When the oil yield and ω-3 FA content of non-toxic solvents (hexane, ethanol and 2-propanol) were compared, it was found that 2-propanol was more suitable for use in the food industry owing to its higher ω-3 FA content. The results from this study may help extract ω-3 FA (especially EPA)-rich edible oil from microalgae adequately and efficiently.

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Quality Characteristics and Sensory Evaluation of Cakes Produced from Composite Blends of Wheat (*Titricum Aestivum L.*) and Finger Millet *(Pennisetum Glaucum)* Flour

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Abstract

The proximate composition and functional properties of flour blends from wheat and millet at 0%, 10%, 20%, 30% and 40% were determined using AOAC standard method. The products were coded as A(100% Wheat flour as control), B(90% Wheat flour and 10% Millet flour), C(80% Wheat flour and 20% Millet flour), D(70% Wheat flour and 30% Millet flour), E(60% Wheat flour and 40% Millet flour). Cakes were prepared using these blends and their sensory properties assessed. Results obtained revealed that the protein content of the flour increased with increase in millet flour addition from 9.36–13.74%. Significant differences ($p < 0.05$) were observed in the fat (12.38–20.35%), ash (0.69–1.28%), crude fibre (1.78–3.85% and carbohydrate (51.65–55.48%) respectively. The bulk density, water and oil absorption capability, emulsion ability, and foaming capacity had significant variations ($p<0.05$) in all the flour samples. The cake with 40% millet flour (D) was most accepted amongst the samples produced. The composite cake samples recorded a significant difference ($p < 0.05$) between the control in most parameters assessed. The study concluded that millet flour could be used to supplement wheat flour up to 40% in baking to minimize wheat flour imports while also increasing millet flour production and its value.

Keywords: Millet flour, flour blends, composite cake, functional properties acceptable, sensory

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INTRODUCTION

The nutritional adjustment of food products has gained popularity in recent years as consumers' interest in healthy eating has grown (Shandilya and Sharma, 2017). Cake is a popular and widely consumed cereal-based dish that offers key nutrients like carbohydrate, fat, protein, fibre, vitamins, and minerals (Bagdi et al., 2016; Callejo et al., 2016).

It is a sweeter confectionary product than other confectionery goods. Flour, sugar, oil, eggs, flavour, and leavening are used to make it.Cakes are one of the most popular confectionary products since they are seen as expensive gifts for adults and children, particularly on birthdays, and because someone birthday is celebrated every day, this makes it even more popular (Shameena Beegum, 2016).

People are more anxious about what they eat these days due to health concerns, thus, many additional or more nutritive elements are utilized to fortify cakes. Wheat flour is basic ingredient in bread and cakes preparation. It contains starches and glutens that aid in the baking of leavened aerated bread and batters, although it is low in fat and balanced amino acids (Goesaert et al., 2005). However, much of the imported wheat with high gluten functionality is unsuitable for tropical climes. The performance of cassava flour with soybean flour used in wheat bread and cakes has been documented, with a surge in interest in locally based food components to partially replace wheat flour in cakes and bread preparation (Ayele et al., 2017). Due to the high price of wheat on the global market, partial substitution of wheat flour with flour from other crops such as root and tuber could be a useful method for developing nations to solve wheat shortages and also enhance the nutritional value of the food as well as diversifying the use of underutilised crops (Mitiku et al., 2018). To address the needs of individuals who consume wheat products, it is critical to employ locally available affordable crops like finger millet (FM) for baking.

Millets are small-seeded subsistence cereal plants in the *Poaceae* family and it is one of the main drought-resistant crops and the world's sixth most-produced cereal grain. They are a staple food for a vast number of poor people in Africa, East Asia, and the Indian subcontinent (Chandrasekara et al., 2012). In comparison to other grains, millet possesses pest and disease tolerance, a short growing season, and high yield during droughts, therefore are an important part of many developing countries' economies. (Devi et al., 2011). Millets are high in phytochemicals and micronutrients (Mal et al., 2010; Singh et al. 2012). It is high in carbohydrate, protein, dietary fibre, vitamins B complex, and minerals like calcium, phosphorus, magnesium, and manganese (OkwudiliUdeh et al., 2017), as well as phenolic compounds (OkwudiliUdeh et al., 2017; Shahidi and Chandrasekara, 2013). These phenolic have been linked to several potential health benefits including cancer prevention, cardiovascular disease prevention, and blood pressure reduction (Saleh et al., 2013). Finger millets have a higher dietary fibre and mineral content than wheat and rice (Ramashia et al., 2018).

For human consumption, finger millet is used to make chapatti, dumpling, and porridge. A prior study shows that finger millet flour could be mixed with wheat flour in various quantities for making cakes, biscuits, and snacks (Gavurnikova et al., 2011). The iron and calcium content of composite cake increases when wheat flour is partially replaced with flour from locally grown cereal grains (Oladele and Aina, 2009). Jensen et al., (2015) and Begum et al. (2011) conducted previous research in which wheat flour was replaced with 30 and 20% cassava flour respectively, and created satisfactory composite bread with negligible differences when compared to 100% wheat flour bread. Nowadays, confectionery industries have the difficulty of making meals that contain useful components in order to meet the nutritional needs of all people, including those with unique health concerns, which is why millet flour has been added to wheat flour for cakes making.

As a result, the goal of this research was to determine the quality characteristics of cakes that was partially substituted with finger millet (*Eleusine corocana*) flour in order to produce a composite cake from wheat and Finger Millet flours to improve the nutrients and diversify the use of an underutilized crop.

MATERIALS AND METHODS

Source of raw materials

Finger millet grain was procured from Kumasi Central Market in the Ashanti Region of Ghana. Other ingredients such as wheat flour, margarine, eggs, sugar, salt, vanilla essence and baking powder were purchased from the same Market in the Ashanti Region of Ghana.

Sample Preparation
Finger Millet

To eliminate foreign contaminants and sand, 3 kg of grains were washed in distilled water and dried for 24 hours at 50°C in a hot air oven drier (Apex, Royce Ross Ltd). In a Quaker City Crushing Co, Model 4-E, Phoenixville, Dad mill. It was then milled into flour using the Jideani (2005) process. The flour was sieved, packed and sealed in a polythene bag for preparation of composite flour.

Formulation of composite flour

Table 1 shows how composite flours were made from wheat and Finger Millet. The flour samples were prepared with the incorporation of 0, 10, 20, 30 and 40% finger millet flour (FMF). As a control (sample A), one hundred percent (100%) wheat flour was used. 90% wheat flour and 10% Finger Millet flour were used in Sample B, 80% wheat flour and 20% Finger Millet flour were used in Sample C, while Sample D was made up of 70% wheat flour and 30% Finger Millet flour, then 60% wheat flour and 40% Millet flour were used in Sample E. To achieve uniform blending, the mixes were carefully blended in a blender (Aboshora et al., 2016).

Table 1. Formulation of ingredients for cake making

Ingredients	**A**	**B**	**C**	**D**	**E**
Soft wheat flour (g)	100	90	80	70	60
Millet flour	0	10	20	30	40
sugar (g)	100	100	100	100	100
Margarine (g)	100	100	100	10	10
Eggs (large size)	10	10	10	10	10
Baking powder (g)	10	10	10	10	10
Salt (g)	1	1	1	1	1
Vanilla essence (ml)	5	5	5	5	5

A(100% Wheat flour), B(90% Wheat flour and 10% Millet flour), C(80% Wheat flour and 20% Millet flour), D(70% Wheat flour and 30% Millet flour), E(60% Wheat flour and 40%Millet flour)

Method of preparation

For the production of the various cake samples, Tanya SNC (2016) approach was followed with minimal changes. In a medium sized mixing bowl, sugar (100 g) and margarine (100 g) were creamed together until light and fluffy. Ten grams (10 g) of eggs were beaten into the mixture and gradually mixed. Wheat flour (200 g), baking powder (10 g), salt (1 g), and nutmeg powder (1 g) were combined in a separate bowl with a food mixer.

The combined dried ingredient was gradually added to the wet ingredients and gently mixed until there were no lumps. The mixture was then spooned onto a prepared 6 x 2 inch loaf pan and baked in a preheated electric oven (De'Longhi Kenwood A.P.A Ltd, M0746, Hong Kong) at 180 °C for 30 min until a toothpick inserted in the center comes out clean (approx.30-32 min). The cake samples were allowed to cool for 10 min, which was then packaged in a sealed polyethylene bags and stored at 4°C until analyzed.

Proximate composition

To ascertain the nutritional quality of the cake samples, they were evaluated. Moisture, ash, protein, fat, and carbohydrate content were all assessed using established procedures by AOAC (2015).

Moisture content and total solids: Oven Drying Method

Five grams (5g) of the cake sample was transferred to the previously dried and weighed dish. The Dish was placed in an oven and thermostatically controlled at 105 degrees for 5 hours. Dish was removed and placed in a desiccator to cool to room temperature and weighed. It was then dried again for 30 minutes, cooled down again and weighed. Drying, cooling and weighing were repeated until a constant weight was reached. (Alternatively, sample could be dried in a thermostatically controlled oven for at least 8 hours where a constant weight would be achieved). The determinations were duplicated and the average found.

Calculations

$$\% \text{ Moisture (wt/wt)} = \frac{\text{wt } H_2O \text{ in sample}}{\text{Wt of wet sample}} \times 100$$

$$\% \text{ Moisture (wt/wt)} = \frac{\text{wt of wet sample-wtof dry sample}}{\text{Wt of wet sample}} \times 100$$

$$\% \text{ Total solids (wt/wt)} = \frac{\text{wt of dried sample}}{\text{Wt of wet sample}} \times 100$$

Where wt= Weight of sample/spread

Ash content

Five grams 5g sample was weighed into a tarred crucible and was pre-dried. Crucibles were placed in cool muffle furnace using tongs, gloves and protective eyewear. The crucibles Ignited for 2 hours at about 600 degrees Celsius. Muffle furnace was turned off and opened when temperature dropped to at least 250^{o}C preferably lower. The door was carefully opened to avoid losing ash that may be fluffy. Safety tongs was used to transfer crucibles to a desiccator with a porcelain plate and desiccant. Desiccator was closed and allowed crucibles to cool prior to weighing.

Calculations

$$\%Ash = \frac{\text{wt of ash}}{\text{Wt of sample}} \times 100$$

$$\%Ash = \frac{(\text{wt of crucible+ ash}) - \text{wt of empty crucible}}{(\text{wt of crucible+ sample}) - \text{wt of empty crucible}} \times 100$$

Where wt= Weight of sample/spread

Fat content: soxhlet extraction

Previously dried (air oven at 100°C) 250 ml round bottom flask was weighed accurately. 5.0g of dried sample to 22 ×80mm paper thimble or a folded filter paper was weighed. A small of cotton or glass wool was placed into the thimble to prevent loss of the sample. 150ml of petroleum spirit B.P 40-60°C was added to the round bottom flask and assembled the apparatus. A condenser was connected to the soxhlet extractor and reflux for 4 - 6 hours on the heating mantle. After extraction, thimble was removed and recovered solvent by distillation. The flask and fat/oil was heated in an oven at about 103°C to evaporate the solvent. The flask and contents were cooled to room temperature in a desiccator. The flask was weighed to determine weight of fat/oil collected.

$$\%\ \text{Fat (dry basis)} = \frac{\text{fat/oil collected} \times 100}{\text{Weight of sample}}$$

$$\%\ \text{Fat (dry basis)} = \frac{(\text{wt of flask + oil}) - \text{wt. of flask} \times 100}{\text{Weight of sample}}$$

Crude fibre determination

Two grams (2g) of the sample from crude fat determination was weighed into a 750ml Erlenmeyer flask. Two hundred milliliters (200ml) of 1.25% H_2SO_4 was added and immediately flask was set on hot plate and connected to the condenser. The contents were boiled within 1 minute of contact with solution. At the end of 30 minutes, flask was removed and immediately filtered through linen cloth in funnel and washed with a large volume of water. Filtrate (containing sample from acid hydrolysis) was washed and returned into the flask with 200ml 1.25% NaOH solutions.

Flask was connected to the condenser and was boiled for exactly 30 minutes. It was then filtered through Fischer's crucible and washed thoroughly with water and added 15ml 96% alcohol. Crucible and contents was dried for 2 hour at 105 °C and cooled in desiccator and it was weighed. Crucible was ignited in a furnace for 30 minutes and after that it was cooled and reweighed.

$$\% \text{ Crude fibre} = \frac{\text{weight of crude fibre} \times 100}{\text{Weight of sample}}$$

$$\% \text{ Crude fibre} = \frac{\text{wt of crucible + sample (before – after) ashing} \times 100}{\text{Weight of sample}}$$

Where wt= Weight of sample/spread

Protein Determination

Digestion Method

Two grams (2g) of sample and a half of selenium –based catalyst tablets and a few anti-bumping agents were added to the digestion flask. Twenty five milliliters (25ml) of concentrated H_2SO_4 was added and the flask was shaken for the entire sample to become thoroughly wet. Flask was placed on digestion burner and heated slowly until boiling ceased and the resulting solution was clear. The sample was then cooled to room temperature and digested sample solution was transferred into a 100ml volumetric flask and made up to the mark.

Distillation Method

To flush out the apparatus before use, distilled water was boiled in a steam generator of the distillation apparatus with the connections arranged to circulate through the condenser, for at least 10 minutes. The receiving flask was lowered and continued to heat for 30 seconds in order to carry over all liquid in the condenser. 25 ml of 2% boric acid was pipetted into 250ml conical flask and 2 drops of mixed indicator added. The conical flask and its contents were placed under the condenser in such a position that the tip of the condenser is completely immersed in solution. 10ml of the digested sample solution was measured into the decomposition flask of the Kejdahl unit, fixed it and add excess of 40% NaOH (about 15-20ml) to it. The ammonia produced was distilled into the collection flask with the condenser tip immersed in the receiving flask till a volume of about 150ml– 200ml is collected. Before distilling another sample and on completion of all distillations, the apparatus was flushed as in step 1 above. Steam was allowed to pass only until 5ml of the distillate is obtained.

Titration Method

The Distillate with 0.1N HCL solution was titrated. The acid was added until the solution became colourless. Any additional acid added made the two solutions become pink. The nitrogen content was determined in duplicate, and a blank determination was run using the same amount of all reagents as used for the sample. The blank was meant to correct for traces of nitrogen in the reagents and included digestion as well as distillation methods.

Calculation

% Total nitrogen = $\frac{100 \times (Va-Vb) \times NA \times 0.01401 \times 100}{W \times 10}$

Where:
Va- volume in ml of standard acid used in titration
Vb- volume in ml of standard acid used in blank
NA- normality of acid
W- Weight of sample taken

Carbohydrate content

The calculation of available carbohydrate (nitrogen-free extract-NFE) was made after completing the analysis for ash, crude fibre, ether extract and crude protein. The calculation was made by adding the percentage values on dry matter basis of these analysed contents and subtracting them from 100%.

Calculation

Carbohydrate (%) =100 - (% moisture +% fat +% protein +% ash)

x. Calculation for dry basis = $\frac{(100-\%\ \text{moisture}) \times \text{wet basis}}{100}$

Functional Properties

Water and oil absorption capacity

The water/oil absorption capacity and emulsion stability of the various flours were determined using the method described by Chandra et al. (2015). One gram (1g) of the flour sample was dispersed in 10ml of oil and vortex the suspension for 5 minutes. The suspension obtained was centrifuged at 3500 rpm for 30min. it was then decanted and measured with supernatant in a 10ml graduated cylinder. The density of the oil, and calculated oil absorption capacity were determined using the formula;

Oil absorption capacity (% OAC) = $\frac{(y-z)\times d}{x} \times 100$

Where
y= initial volume of oil added
Z= volume of supernatant collected
X= initial weight of (dried) sample taken
d= density of oil
y-z =volume of water retained by the sample after centrifugation

Water absorption capacity

One gram (1g) of the sample in 10ml distilled water was dispersed and vortex the suspension for 5 minutes. The suspension obtained at 3500 rpm for 30min was centrifuged. It was then decanted and measured with supernatant in a 10ml graduated cylinder. Density of water was taken as 1.0gcm^{-3}, and calculates water absorption capacity as

Water absorption capacity (% WAC) = $\frac{y-z}{x} \times 100$
Where y= initial volume of water added
Z= volume of supernatant collected
X= initial weight of (dried) sample taken
y-z =volume of water retained by the sample after centrifugation

Bulk density

The bulk density (BD) of the flours was determined using Amandikwa et al (2015) method. An amount of 100g of the sample was weighed directly into 250ml capacity graduated cylinder and tap the measuring cylinder 10 to 15 times until no change in volume is observed.
Bulk density = weight of sample (g)
Volume of sample after tapping (ml)

Foaming capacity and foaming stability

Foaming capacity and foaming stability were determined as described by Narayana & Narsinga Rao (1982) with slight modifications. Five millilitre (5ml) of sample was weighed and mixed in 40ml distilled water and homogenized for 5min at high speed using a homogenizer with a suitable stirrer. The volume of foam separated was noted. For stability, the collapse in foam if any at the end of a specific time was measured (e.g. 1 minute, 2 minutes, 4 minutes and 5 minutes).

Calculation of the capacity and stability is as follows:

%foaming capacity=$\frac{(volafterhomogenization)-(volbeforehomogenization)}{volbeforehomogenization} \times 100$

%foam stability= $\frac{foamvolumeaftertime(t)}{initialfoamvolume} \times 100$

Sensory Evaluation

The cakes were put through a sensory evaluation utilizing the Larmond method (1977). A total of 50 semi-trained panelists took part in the evaluation. Colour, taste, flavour, texture and overall acceptance were among the qualities evaluated. In discrete cubicles with adequate lighting, the coded samples were served in clean plastic plates at room temperature. The panelists were given a random sample presentation. Panelists were asked to sample the items and rate them on a five-point hedonic scale (5-like very much, 4-like much, 3-neither like nor dislike, 2-dislike much, and 1-dislike very much).

Statistical Analysis

Data were subjected to Analysis of Variance (ANOVA) and Tukey Test was used to determine significant difference among the various samples in duplicates. Data were analyzed using the software, Statistical Package for Social Science (SPSS) version 22.00 (SPSS inc., Chicago), IL, USA at the 0.05 level of significance.

RESULTS AND DISCUSSION

Table 2 shows the proximate composition of cake samples produced from wheat-millet flour blends. The moisture content of the cake samples ranged from 31.25% to 36.26% with cake sample produced from wheat flour having the highest moisture content (36.26 ± 0.46) while cake sample 'E' made of 60% wheat flour and 40% millet flour had least moisture content. The moisture contents of the composite cake samples decreased significantly ($p < 0.05$) with increasing levels of Finger Millet flour substitution. Besbes et al., (2016) have reported similar moisture content in bread produced from millet and wheat composite flour. The decrease in moisture content of composite cake could be attributed to protein denaturation, which resulted in greater contacts between proteins and polysaccharides. This resulted in the formation of an intermolecular network, water entrapment, and a decrease in free water content, all of which are linked to a drop in food moisture content (Zhang et al., 2016). Moisture is required for cake to maintain its quality, and excessive moisture has a negative impact on bread storage stability.

Adeleke and Odedeji (2010) found comparable findings using wheat and sweet potato flour blends in their bread. The ash contents ranged from 0.69 ± 0.05 to 1.28 ± 0.27 with the control cake sample having the least ash content while cake sample produced from 60% wheat flour and 40% finger millet recorded the highest ash content. The ash content increased significantly ($p < 0.05$) with increasing levels of Finger Millet flour. Because Finger Millet grains are a good source of calcium, phosphorus, magnesium, and iron, the increased ash concentration in the composite cakes indicates that Finger Millet flour contains more minerals than wheat flour. Our findings support Mitiku et al. (2018) for wheat-sweet potato flour composite bread.

The protein content of samples A to E ranged from 9.36 to 13.74 g/100 g, and was substantially different ($p <0.05$) from one variety to the next (Table 2). There was a substantial difference in protein levels among all millet kinds in sample BCDE. The protein content of these varieties was close to Ali et al., (2003), who reported 12.5-13.6 percent protein content, but greater than Saleh et al., (2013), who reported 7.7-12.1 g/100g protein content for diverse millet varieties. With a protein level of more than 7%, these types could be useful in combating protein-energy malnutrition, particularly among youngsters in places where the crop is produced. The use of Finger Millet flour may have increased the protein content of the composite cake, resulting in a high protein level (Ijah et al., 2014). Amandikwa et al. (2015) reported similar results for wheat-yam flour composite bread, and Mitiku et al. (2018) reported similar results for wheat-sweet potato flour composite bread.

With increasing quantities of Finger Millet flour substitution, the fat content of the cakes increased significantly from 12.38% (Sample A) to 20.35% (Sample E). This could be due to the fact that Finger Millet contains roughly 1% to 3% fat, which could have contributed to the increase of the fat level. Furthermore, fat functionality, such as emulsifier capacity, has an impact on cake texture and bubble formation. The capacity to make a cake from composite flour blends without using any shortening could be explained by the high fat content of the composite flour samples (Menon et al., 2015). Because fat promotes food palatability, composite cake samples with a significantly ($p < 0.05$) higher fat content will be more palatable (Bolarinwa et al., 2019).

These results are in support of Man et al. (2015) on incorporation of chickpea flours to bread. As the quantity of of Finger Millet flour increased, it caused the fiber content to increase significantly ($p < 0.05$), ranging from 1.78% to 3.85% for Sample A (10%) and Sample E (40%) Finger Millet flour composite cakes. When compared to the control sample (100% wheat cake), it was realized that cakes with finger millet incorporation had higher fiber content. The crude fiber content of the 100% wheat cake exceeded the maximum permitted fiber value of 1.5% (Oluwamukomi et al., 2011). The fiber content of the cakes was found to rise as the amount of finger millet flour increased. The findings of this study matched those of a study conducted by by Henshaw and Agunbiade, 2004).

Carbohydrate content increased when the percentage of Finger Millet flour substituted increased from 51.65% (Sample A) to 55.48% (Sample E). The changes in carbohydrate content between control and composite cakes may be attributable to variances in other components including protein, fat, and ash. The high carbohydrate content of composite cakes is advisable because starch granules swell and create a gel when heated in the presence of water, which is vital for bakery product structure and texture (Inyang and Asuquo, 2016).

Table 2. Proximate composition of cake

Sample	**Moisture**(g/100g)	**Ash**(g/100g)	**Protein**(g/100g)	**Fat**(g/100 g)	**Fibre**(g/100 g)	**CHO**(g/100 g)
A	36.26 ± 0.46^{e}	0.69 ± 0.05^{e}	9.36 ± 0.40^{e}	12.38 ± 1.49^{e}	1.78 ± 0.03^{e}	51.65 ± 0.03^{e}
B	34.73 ± 0.22^{d}	0.95 ± 0.30^{d}	10.64 ± 0.19^{d}	14.98 ± 0.83^{d}	1.94 ± 0.01^{d}	52.66 ± 0.25^{d}
C	33.73 ± 1.04^{c}	1.20 ± 0.21^{c}	11.36 ± 0.23^{c}	16.74 ± 0.04^{c}	2.52 ± 0.02^{c}	53.13 ± 0.23^{c}
D	32.71 ± 0.06^{b}	1.25 ± 0.08^{b}	12.47 ± 0.28^{b}	18.65 ± 0.03^{b}	2.56 ± 0.07^{b}	54.15 ± 0.12^{b}
E	31.25 ± 0.28^{a}	1.28 ± 0.27^{a}	13.74 ± 0.30^{a}	20.35 ± 0.02^{a}	3.85 ± 0.07^{a}	55.48 ± 0.15^{a}

Values represent means and standard deviation replicate readings for various parameters. Values in the same column with different superscripts are significantly different (p>0.05). Keys: A(100% Wheat flour), B(90% Wheat flour and 10% Millet flour), C(80% Wheat flour and 20% Millet flour), D(70% Wheat flour and 30% Millet flour), E(60% Wheat flour and 40%Millet flour)

Table 3 shows the results of the functional attributes of the flour blends. Bulk density, water and oil absorption capability, emulsion ability, and foaming capacity all had significant variations ($p<0.05$). For all flours, the water absorption ranged from 108.00g to 134.87g. The highest value was 134.87g for control sample (100% wheat flour), while the lowest was 80.61g for composite sample D (60% wheat flour and 40% finger millet flour).

The results revealed that using millet blended flour instead of 100% wheat flour affected the amount of water absorbed. It was discovered that increasing the millet flour substitution resulted in lower water absorption for the composite flours. Previously, similar reports had surfaced (Kaushal et al., 2012).

Oil absorption capacity refers to the ability of flour protein to physically bind fat through capillary attraction and is potentially useful in structural interactions in food, particularly in flavour retention, palatability improvement, and shelf life extension of bakery or meat products, doughnuts, baked goods, pancakes, and soups where fat absorption is desired (Iwe, et al., 1999, Aremu et al 2007).

The oil absorption capacity ranged from 72.63g to 96.44g, the highest oil absorption capacity was found in flour sample A (100% wheat flour), whereas the lowest oil absorption capacity flour sample C(70% wheat flour and 30% millet flour). The oil absorption capacity of the composite flours (B–E) rose dramatically as the quantity of millet flour increased. High water and oil absorption capacities are advantageous, especially in bakery items such as cakes and cookies, where hydration and shortening are desired to ease handling. They may also affect how foods taste and feel in the mouth (Okezie and Bello, 1988). The bulk density ranged from 0.58 to 0.69 g/m3. The bulk density of the control sample A (100% wheat flour) was 0.58 g/m3, while the bulk density of the flour sample B (90% wheat flour and 10% millet flour) was 0.69 g/m3. The flour's emulsion ability ranged from 11.57 to 62.25 g/g, with A (100% wheat flour) having the highest value at 62.23 g/g. Significant differences ($p>0.05$) were found between flour blends B (90:10), C (80:20), D (70:30), E (60:40) and the control (100%).

Table 3. Functional Properties of wheat/finger millet flour blends

Sample	WAC	OAC	BD	EA	FC
A	134.87 ± 0.66^{e}	96.44 ± 3.01^{a}	0.58 ± 0.03^{a}	62.25 ± 1.77^{e}	13.05 ± 0.00^{a}
B	120.97 ± 0.88^{d}	69.00 ± 1.48^{b}	0.69 ± 0.01^{e}	11.57 ± 1.69^{a}	2.43 ± 0.00^{b}
C	94.64 ± 1.50^{c}	72.63 ± 0.29^{b}	0.68 ± 0.00^{d}	12.42 ± 1.27^{b}	2.43 ± 0.00^{b}
D	92.10 ± 1.69^{b}	74.21 ± 1.90^{b}	0.65 ± 0.00^{c}	15.73 ± 1.18^{c}	2.43 ± 0.00^{b}
E	80.61 ± 2.29^{a}	75.21 ± 1.52^{b}	0.63 ± 0.05^{b}	18.16 ± 0.69^{d}	2.43 ± 0.00^{b}

Values represent means and standard deviation replicate readings for various parameters. Values in the same column with different superscripts are significantly different ($p>0.05$). WAC = Water Absorption Capacity, OAC = Oil Absorption Capacity, BD = Bulk Density, EA = Emulsion Ability, FC = Foaming Capacity

Table 4 shows the sensory evaluation results of cakes made using wheat/millet flour mixtures. The sensory evaluation revealed significant differences ($p<0.05$) between the samples in colour, taste, flavour, texture, and overall acceptability (Table 4) as the amount of millet flour in the cakes increased. The sensory evaluation of the current investigation revealed a pattern that differed from that described by Sukhcharn et al., (2008). The distinctive baking quality of millet flour (Okoye, Nkwocha and Ogbonnaya, 2008; Adeyeye and Akingbala, 2015) and the varying rates of preference and acceptable values of panelists may explain the different directions of score patterns.

The judges gave sample A (100% wheat flour) cake the highest colour rating, yet it was substantially different ($P<0.05$) from the composite cake. The cakes' colour shifted from light brown to dark brown, and the mean scores began to decline. It is possible that the darker colour comes from the Maillard reaction between lowering carbohydrates and protein (Dhingra and Jood, 2000).

Taste is the most important component in determining a product's acceptability, and it has the greatest impact on the product's market success. Cake made with 60% wheat flour and 40% millet flour tasted sweet, while those made with 100% wheat flour received the second highest mean ratings. Texture is one of the most essential criteria associated with product quality, since it is the sensory indicator of the structure of food and the manner in which the structure reacts to applied force (Jean-Xavier and Rossella, 1996).

Texture analysis is the process of determining the factors that influence how a food feels in the mouth. The texture of composite cakes and the control cake were significantly different ($p<0.05$) (100% wheat flour). The control sample had the highest texture score, while cake sample E (60% wheat flour and 40% millet flour) had the highest texture mean value (8.64). The overall acceptability, which is an important metric in organoleptic estimate, includes several implications. In terms of general acceptability, the cake sample made with 60% wheat flour and 40% millet flour had the greatest mean value (10.59) and therefore was preferred by the panellists' followed by Sample A (100% wheat flour cake). Nwaojigwa et al., (2007) reported similar findings, stating that biscuits prepared from sweet potato-wheat flour were satisfactory up to a 40% supplementation level based on sensory qualities.

Table 4. Sensory attributes of the composite cake

Sample	Colour	Taste	Flavour	Texture	Overall Acceptance
A	8.63 ± 0.95^{d}	8.77 ± 1.13^{d}	9.62 ± 1.17^{c}	8.50 ± 0.98^{a}	9.63 ± 0.73^{d}
B	7.85 ± 0.70^{c}	8.70 ± 0.97^{c}	9.83 ± 1.00^{d}	8.50 ± 1.21^{b}	9.12 ± 0.80^{b}
C	5.61 ± 0.72^{b}	8.67 ± 0.76^{b}	8.73 ± 0.82^{b}	8.50 ± 1.06^{c}	9.54 ± 0.88^{c}
D	4.56 ± 1.00^{a}	9.15 ± 0.72^{e}	10.96 ± 0.82^{e}	8.64 ± 1.18^{e}	10.59 ± 0.67^{e}
E	4.35 ± 0.80^{a}	7.81 ± 1.02^{a}	8.48 ± 1.07^{a}	7.60 ± 1.26^{d}	8.87 ± 0.92^{a}

Values represent means and standard deviation replicate readings for various parameters. Values in the same column with different superscripts are significantly different ($p>0.05$). Keys: A(100% Wheat flour), B(90% Wheat flour and 10% Millet flour), C(80% Wheat flour and 20% Millet flour), D(70% Wheat flour and 30% Millet flour), E(60% Wheat flour and 40%Millet flour)

CONCLUSIONS

The use of millet flour to supplement wheat flour in baking could minimize wheat flour imports while also increasing millet flour value. The millet/wheat flour blends had enough protein, ash, fats, dietary fibre, and carbohydrate contents. As a result, combining it with wheat flour to make cakes would be nutritionally beneficial. When compared to the cake made with 100% wheat flour, the cake made with wheat flour supplemented with 40% millet flour was highly acceptable. The result revealed that Millet flour could be a better substitute for wheat flour in preparation of flour products.

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Determination of Antioxidant Capacity and Phenolic Content of Tunceli Garlic Extracts (*Allium Tuncelianum*) by Different Solvents

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Abstract

Free radicals are wastes that come into play when using oxygen in the body. These materials have high destructive ability and could break the structure of molecules in contact. Free radicals are primarily eliminated or destroyed by the natural antioxidant defense systems in the body. When the activity of free radicals is more intense than the body's antioxidant activity, metabolic imbalance and oxidative stress in the cells. As a result, diseases such as premature ageing, heart disease and cancer can be observed. Many carcinogens, such as chemicals in the air, additives in the kitchen, drug remnants, sunlight, exhaust fumes, increase the need for antioxidants. The amount of antioxidant produced by the body can be enough. Therefore, it is necessary to consume antioxidant-rich foods in the diet. In this study, total phenolic content, DPPH and ABTS radical reduction percentage and total flavonoid content were determined of extracts obtained by different solvents (water, ethanol and methanol) with ultrasound-assisted from Tunceli garlic. In addition, the total antioxidant capacity was determined with CUPRAC and DPPH method. While TPC was found 25.09-30.08 mg GAE/100 g, TFC was found 18.92-25.54 mg QE/100 g. DPPH, ABTS were found 48.09-90.23%. CUPRAC was determined 44.58-111.12 mg CAE/100 g.

Keywords: *Allium tuncelianum*, antioxidant activity, total phenolic content, ultrasound

Research article
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INTRODUCTION

Free radicals are essential for every biochemical process and are an essential part of aerobic life and metabolism. Free radicals are constantly produced by oxygen metabolism. There is a dynamic balance between the number of free radicals that naturally occur in the body and the antioxidants that remove or destroy them and protect their bodies from their harmful effects. It is known that many diseases can occur if the free radical-antioxidant balance is disturbed in the body.

Antioxidants interact with free radicals and neutralize them. This way eliminates the harmful effects of free radicals. So antioxidants are also known as 'free radical scavengers' (Galano & Raúl Alvarez-Idaboy, 2019). Antioxidants are either produced naturally by the body (endogenous antioxidants) or taken externally (exogenous antioxidants). Exogenous antioxidants are also commonly referred to as dietary antioxidants. Fruits, vegetables and grains have high dietary antioxidants (Diplock, 1998; Karabulut and Gülay, 2016).

The adverse effects of free radicals on human life and the role of antioxidants in eliminating these harmful effects are better understood day by day. Plant-based antioxidant-rich foods traditionally composed of the bulk of the human diet. Descriptive epidemiological studies show that people with high fruit and vegetable intake have a low risk of epithelial cancer, especially in the upper gastrointestinal regions and in the respiratory tract (Møller & Loft, 2006). Studies on the protective effects of fruits and vegetables rich in natural antioxidants against these diseases are ongoing.

In our country, some of the endemic plant species grow in the Tunceli region surrounding the Munzur mountains, and one of the most important is Tunceli garlic (Allium tuncelianum). Tunceli garlic is a plant with a single leaf with white and purple flowers and creamy-white onions. It is endemic in eastern (Sarikamiş et al., 2010). Tunceli garlic carry fertile black seeds that can be used for propagation (Kiralan et al., 2013). Bulbs are propagated aseptically. However, their propagation and fertilization are relatively slow (Kızıl et al., 2009).

Tunceli garlic is an endemic species and is widely found around Tunceli province, especially Ovacık and Pülümür districts and on the skirts of Munzur mountains and is called mountain garlic (Firat, 2015; Koyuncu & Güvenç, 1994). Since the 1980s, the plant has been removed and traded, with an estimated annual figure of 15-20 tons (Firat, 2015). A. tuncelianum, which was defined as a subspecies of A. macrochaetum by (Kollman, 1983) was later defined as a different species (Özhatay et al., 1997). A. tuncelianum, also known as Tunceli garlic, is in the genus of 2n=16 chromosomes, the Monocotyledonea (monocotyledons) class, the Liliflore order, and the Allium genus of the Liaceae family (Özhatay, 2002).

Garlic has a wide range of uses, especially in the herbal therapy and food industry, and its benefits have been shown in many studies. It protects to human against heart disease by lowering high blood pressure, high cholesterol and triglyceride levels. They can destroy carcinogens that cause tumor formation by activating the immune system with the help of allinase and some other substances in their structure. Some of the sulfur-containing compounds of garlic have been found to have a positive effect on both high and low blood sugar cases, as they can specially regulate sugar metabolism. It is a strong and natural antiseptic because it destroys microbes (Gün, 2018). In addition, it has been determined that Tunceli garlic has an anti-parasitic effect (Aykur et al., 2020). In this study, Tunceli garlic was extracted with different solvents and the antioxidant capacity of the extracts was determined.

MATERIAL and METHOD

Material

In this study, Tunceli garlic was purchased from local markets in Tunceli province of Turkey in 2016. The provided garlic was washed, sorted and stored in plastic bags at -20 °C until analysis. All chemicals used were provided by Merck (Darmstadt, Germany) and Sigma (St. Louis, USA). Ultrapure water was used to prepare chemicals for analysis.

Extraction of Samples

5 g of the samples were weighed, then 25 mL of solvent (water, ethanol, methanol) was added. The mixture homogenized with ultraturrax (IKA, T18, Staufen,Germany)It was treated in an ultrasonic water bath (Lab Companion, UC 10, Boston, USA) for 30 minutes.

It was centrifuged at 8500 rpm for 20 minutes at 4 °C in a homogenized refrigerated centrifuge (Centurion Scientific K3 Series, Chichester, UK).). And then, the supernatant was collected and filtered with 0.45 µm filters. These extracts prepared for total phenolic content (TPC), total flavonoid content (TFC), DPPH radical scavenging activity, 2, 2-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid; ABTS) radical scavenging capacity and cupric reducing antioxidant capacity (CUPRAC).

Total Phenolic Content (TPC)

Total phenolic compounds were determined using the Folin-Ciocalteu reagent Singleton et al., (1999) method with modification. The Folin-Ciocalteu method is based on the measurement of absorbance according to the color intensity formed by the reagent that gives. Briefly, 1 mL of garlic samples and 45 mL water was added to a 100 mL flask. 1 mL of Folin-Ciocalteu reagent was added to it. After 3 minutes, 3 mL of 3% Na2CO2 solution was added. The resulting mixture was shaken at room temperature in the dark for 2 hours. Finally, the absorbance of the samples against pure water at 720 nm was measured in the UV spectrophotometer (Shimadzu, Japan). Gallic acid was used as the standard phenolic compound. The results are given as Gallic acid equivalent.

Total Flavonoid Content (TFC)

TFC was detected Woisky & Salatino (1998) method with minor modification. 1 g of garlic extract was completed to 10 mL with the solvents used in the study. 1 mL of this solution was taken, and 4 mL of 2% ethanolic AlCl3 solution was added thereto. After 1-hour incubation at room temperature, the absorbance was read at 420 nm. All values were expressed as mg quercetin equivalents (QE) per 100 g fresh matter of garlic sample.

DPPH Free Radical Removal Capacity

Determination of DPPH free radical scavenging capacity (DPPH) of garlic samples was performed by modifying the method of Mercan et al., (2018). 1 mL of garlic extracts was taken, and 2 mL of DPPH solution was mixed. After the mixture was incubated in the dark for 30 minutes at room temperature, its absorbance against the solvent was determined at 517 nm in the UV spectrometer. Results are given as inhibition (%).

ABTS Radical Scavenging Capacity

In this method, solutions of 2.45 mM K2S2O8 and 7 mM ABTS (2,2'-azino-bis (3-ethylbenzthioazoline-6-sulfonic acid) were mixed at a ratio of 1:1 and incubated for 16 hours at room temperature in the dark. The absorbance of prepared ABTS radical solution was diluted with ethanol until 1.850 ±0.05 abs in 734 nm. This absorbance was used as the control absorbance. Then 4 mL of this radical solution was taken into test tubes. 100 µL of plant extracts were added onto these tubes and incubated for 2 hours at room temperature and in the dark. At the end of this period, the absorbance of the samples was recorded at 734 nm against the curd consisting of PBS (Phosphate Buffer, pH = 7.4) (Wu et al., 2009) Decreasing absorbance gives the amount of ABTS radicals removed from the medium (Keser et al., 2013). Results are given as inhibition (%).

Cupric Reducing Antioxidant Capacity (CUPRAC)

CUPRAC values of Tunceli garlic extracts were determined by modifying the method of (Apak et al., 2004). First, 1 mL of 10 mM Cu (II), 7.5 mM neocuprine and 1 mL NH4Ac (1 M, pH 7) were added to the test tubes. Then 1 mL of garlic extract was added. After the mixture was kept in the dark for 30 minutes, its absorbance at 450 nm was measured. Cafeic acid was used as standard.

Statistical Analysis

All analyzes in the study were done in triplicate. One-way variance analysis was used to examine the statistical differences between the groups. Duncan multiple comparison test was used to determine among which groups the differences were. Results were analyzed statistically by SPSS 24.0 package program.

RESULTS and DISCUSSION

TPC were found in water extract (35.08 ± 1.95 mg GAE/100 g), methanol and ethanol extracts was determined 20.58 ±1.44 mg GAE/100 g and 27.42 ± 1.12 mg GAE/100 g, respectively. The differences between the TPC amount of all extracts are statistically significant ($P < 0.05$). The graph of gallic acid used as a standard and equation are given in Figure 1.

Ağbaş et al., (2013) compared the chemical properties of Tunceli garlic and commercial garlic. In the study, the TPC amount of the ethanol extract of Tunceli garlic was 16.21 mg GAE/g and the water extract was 54.25 mg GAE/g, while the TPC amount of the ethanol and water extracts of commercial garlic was 49.47 mg GAE/g and 4.01 mg GAE/g, respectively. Karaaslan et al., (2019) investigated the TPC amount of Tunceli garlic using acidified solvents. This study, they determined the TPC amount of acidified water, acetonitrile, methanol and ethanol extracts between 0.206±0.012-0.537±0.027 mg GAE/g Fresh weigh Gün (2018) determined the TPC amount of ethanol extract of Tunceli garlic as 16.72 µg GAE/ml.

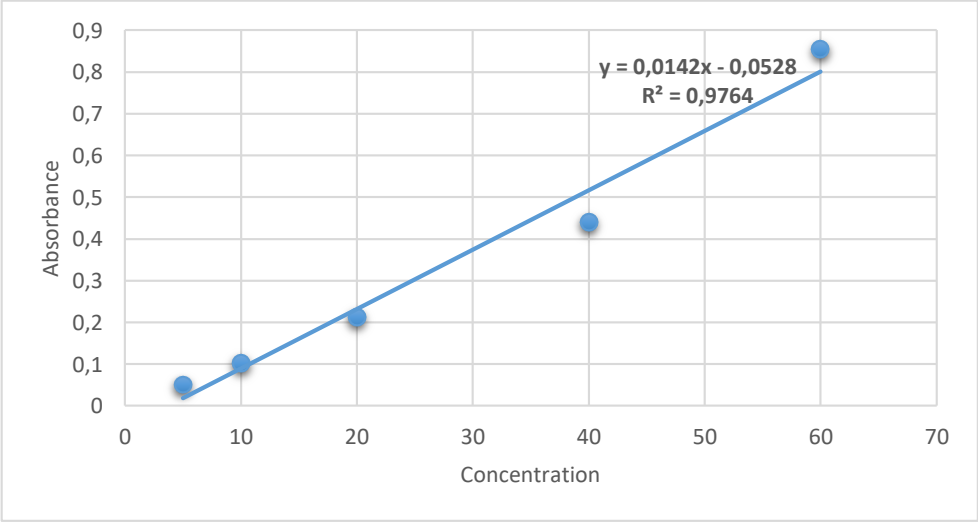

Figure 1. Gallic acid standard curve and equation

TFC amount of Tunceli garlic is shown in Table 1. TFC detected methanol (25.54±1.83 mg QE/100 g), water (21.59 ± 1.95 mg QE/100 g) and ethanol extracts (18.92±1.44 mg QE/100 g) It has been detected in extracts. While the difference between methanol and ethanol extracts is statistically significant ($P < 0.05$), the differences with water extract are not statistically significant ($P > 0.05$). Gün (2018), the TFC amount of the ethanol extract of Tunceli garlic was determined as 6.73±0.11 µg QE/ml. Bozin et al. (2008) reported that 80% methanol extracts of the garlics they produced were detected in TFC between 4.16-6.99 11 µg QE/g.

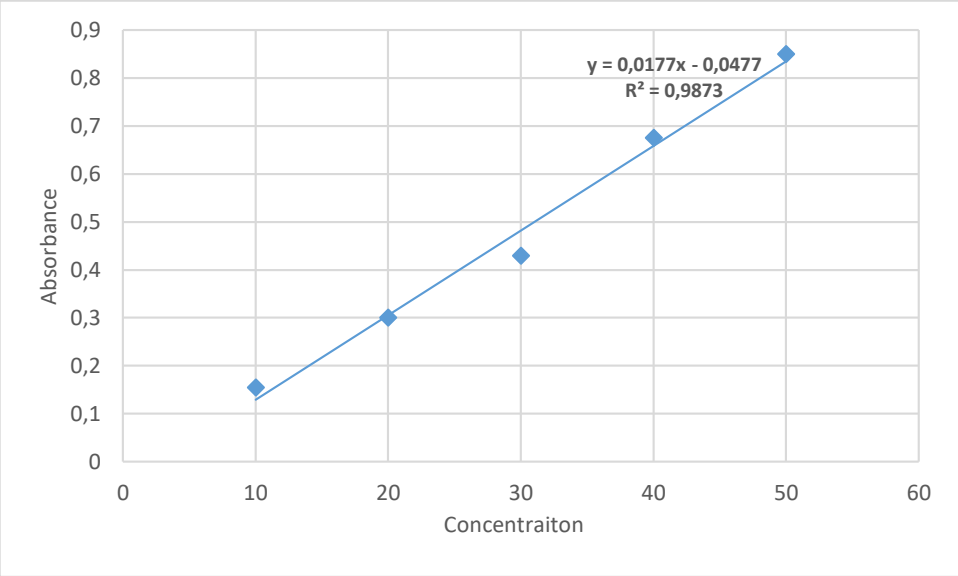

Figure 2. Quercetin standard curve and equation

Table 1. TPC, TFC, DPPH *, ABTS * and CUPRAC values of different solvent extracts of Tunceli garlic (*Allium tuncelianum*)

	Water	Ethanol	Methanol	BHT
TPC (mg GAE/100g)	35.08a	20.58c	25.09b	
TFC (mg QE/100g)	21.59ab	18.92b	25.54a	
DPPH (500 ml/L	48.09c	74.75b	90.23a	75.02b
ABTS (100 ml/L)	63.03c	86.98a	70.45b	69.89b
CUPRAC (mg CAE/g)	44.58c	30.97b	111.12a	

DPPH was the lowest inhibition in water extract (48.09±1.26%), followed by ethanol (74.75±2.54%) and BHT (75.02 ± 2.6%) extracts. The highest inhibition was determined in the methanol extract (90.23±2.79%). Ağbaş et al., (2013) compared the chemical properties of Tunceli garlic and commercial garlic in their study. In the study, they determined the DPPH activity of the ethanol extract of Tunceli garlic as 83.69% and the water extract as 59.44%, while the DPPH activity of the ethanol and water extracts of commercial garlic was 80.35% and 45.70%, respectively. Gün (2018) determined the DPHH capacity of the ethanol extract of Tunceli garlic between 19.4 ±0.7-89.7 ±0.3% in study.

ABTS capacity was determined as the lowest inhibition in water extract (63.03±1.50%), followed by methanol (70.45±1.50%) and BHT (69.89±0.93%) extracts. The highest inhibition was determined in the methanol extract (86.98±1.60%). ABTS test results in boiled garlic samples were determined as 5.5±2.5, 37.2±3.6, 57.3±5.2, 66.3±6.3 and 17.6±1.7, 30.8 ± 3.3, 47.5±5.1, 56.2±5.9 percent inhibition (Gorinstein et al., 2006). Kang et al., (2012) optimized the extraction conditions of black garlic in their study and determined the ABTS value of black garlic as 75.02 inhibition percentage.

Karaaslan et al., (2019) determined ABTS activities of acidified water, acetonitrile, methanol and ethanol extracts as 0.202, 0.255, 0.260 and 0.203 mg TEAC/g, respectively. Ağbaş et al. (2013) determined the ABTS activity of the ethanol extract of Tunceli garlic as 90.47% and the water extract as 48.54%, while the ABTS of the ethanol and water extracts of commercial garlic was 40.84% and 46.75%, respectively.

The standard curve of cupric reducing antioxidant capacity (CUPRAC) is shown in Figure 4. CUPRAC capacity was determined in the lowest ethanol extract (30.97±0.89 mg CAE/100 g), while the highest was determined in the methanol extract (111.12±3.06 mg CAE/100 g) (Table 1). Karaaslan et al., (2019) found ABTS activities of acidified water, acetonitrile, methanol and ethanol extracts as 33, 53, 88 and 45 mg CAE/g respectively.

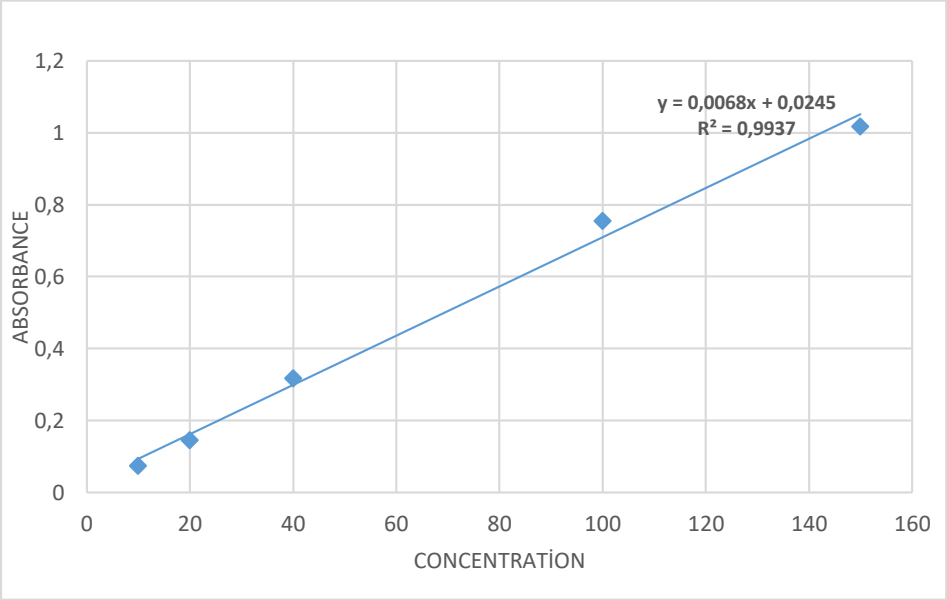

Figure 3. Caffeic acid standard curve and equation

CONCLUSION

In the study, the antioxidant capacities of the extracts of Tunceli garlic in different solvents and ultrasonic water bath, which is an endemic species for Tunceli, were determined using TPC, TFC, ABTS, DPHH and CUPRAC methods. According to the results obtained, the change of extraction solvent had a statistically significant effect on the results.

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Effects of Climate Change on Food Production

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Abstract

Food production has been adversely influenced through unstable switch in climate conditions, there is currently rise in the requirement of food as a result of higher global population. One of the important factors that influence the occurrence of greenhouse gas to weather condition swift is carbon dioxide (CO_2), there is overall clear outcome on crops development. when carbon dioxide level rises, also the level of photosynthesis and carbon absorption rise and this process is termed as carbon dioxide enrichment, and this situation leads to various environmental issues that affects food production. Accessibility to moisture, atmospheric contamination and soil potency have tremendous effects on agricultural production. An unstable rapid change in climate conditions in the face of global food insecurity that occurred through extreme adverse climate condition which have influence on food production, several food systems have been influenced negatively, and hereby, putting the food production system at risk. Yield of several crops have been dwindling and low output recovered in some nations as a result of drought, heat wave, livestock and fisheries are likewise tremendously affected in terms of disease outbreak, low productivity in yield. Food availability and accessibility is the major and essential climatic -linked issues, due to the fact that global poverty is increasing day by day and food would become scarce and unaffordable. The way to prevent shortage in food production is to mitigate the effects of climate change and also to adopt techniques and approach to sideline its impacts and enhance food productivity.

Keywords: Climate change, carbon dioxide, crop yield, food production, food security, livestock yield.

Review article
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INTRODUCTION

Food production has been adversely influenced though the unstable switch in climate conditions (Arunanondchai et al., 2018). there is currently rise in the requirement of food as a result of higher global populace. Accessibility to moisture, atmospheric contamination and soil potency has tremendous effects on agricultural production (Noya et al., 2018). When there is a rapid switch in climate conditions. There is always an increased adverse effects with high magnitude as a result of intentional and unintentional impacts of non-living stress which occurred from the constant desertification and over- used of fossil fuel, Carbon dioxide level has risen higher to 280 μmol^{-1} to 400 μmol^{-1} in the air. There is projection that carbon dioxide shall rise to double, meaning up to 800 μmol^{-1} towards the ending of the centenary. farming is tremendously determined by temperature and precipitation. One of the important factors that influence the occurrence of greenhouse gas to weather condition swift is CO_2 (Masson-Delmotte et al., 2018).

Increasing the necessary studies and measures to minimize the emissions of carbon emissions should be taken all over the world and measures that will minimize the greenhouse gas effect will play an important role in reducing the effects of global warming (Bağdatlı and Arıkan, 2020).

There is overall clear outcome on crops development. when carbon dioxide level rises, also the level of photosynthesis and carbon absorption rises and this process is termed as carbon dioxide enrichment (Wang et al., 2020).

Photosynthesis is tremendously responsive to environmental pressure like drought, increased hot climate, isothermal layer, as a result of suspension that occur to photosynthesis heat transmission from their effect, and this action negatively influenced photosynthesis metabolism procedures, and this occurrence resulted in destruction of thylakoid, covering layer and organelle system (Ainsworth et al., 2012; Sieber et al., 2016).

Switch or changes in climatic situation could result in higher outputs of few produced in few areas. However, in order to achieve these values, there are some factors that should be available such as quantity of the nutrient in the soil, ground precipitation, accessibility of aqua. The swifts in the prevalence and intensity of dryness and surge have capacity to constitute problems to the farm producers and husbandman and this situation invariably pose a threat to food security (Ziska et al., 2016).

EFFECTS of DROUGHT and TEMPERATURE on CROPS

Climate change has become the focus of constant attention of living things and civilizations take into account the climatic parameters determined their lifestyles. Climate increasing or decreasing in changes affect living things negatively. Decrease in productivity, especially in agricultural production causes (İstanbulluoğlu et al., 2013)**.**

World effects of global warming caused by changes in the climate system of the highest peaks, ocean depths, is felt throughout much of the world from the equator to the poles. The polar ice caps are melting, sea level is rising and soil losses are experienced in coastal areas. Sea level due to melting of glaciers Increasing the temperature rose from 10 to 20centimeters (Bağdatlı and Bellitürk, 2016).

As a matter of facts, rise in level of hot climate in the atmosphere could decrease outputs of produce within 6 -25%, though this is determined by the area or location of planting such produce (Sieber et al., 2016; Zhao et al., 2017). Nonetheless, dryness in the air is one of the main environmental pressure which hinder plant produce (Lesk et al., 2016; Zipper et al., 2016) as a result of photosynthesis restraints enforced through pore and non-pore procedure (Dahal et al., 2014).Dryness in the air has been predicted to negatively influenced losses of 1820 million tons of wheat or grain produced over the past four decennary (Lesk et al., 2016).The incidence and harshness of dryness in the air was predicted to increase, expanding the hazard of produce wastage for about 24% of soya bean, 21%corn, 18%rice, and 20%grain (Leng and Hall 2019). Global climate change affects the world negatively day by day and reveals negative results in agricultural product yield. In particular, it is inevitable to evaluate the regional temperatures and to review the product pattern in parallel with the increasing global climate change (Bağdatlı et al., 2014).

Farming is always affected by the unstable atmospheric condition, nevertheless, a prompt switch in atmospheric condition subject farming activities to be sensitive in few zone or locations, a temperate hot atmosphere could cause produce outcome to be high. Generally, the effects of atmospheric condition switch on farming are projected to be adverse which leads to decrease in food productions and thereby, increase food costs (Nelson et al., 2009).

Some countries are currently experiencing difficulty that occurred as a result of increased level of faming also some areas in sub-Sahara Africa and southern part of Asia, was forecasted that there will be tremendous reduction in food yields (Nelson et al., 2009; Gornall et al., 2010). High concentrations of air carbon dioxide was likewise predicted to decrease to low percentage of zinc, iron, and more essential minerals in produce (Dietterich et al., 2014).

The unstable switch in precipitations order subjected crops producer to experience double risks arising out of floods and air dryness. This two environmental conditions could damage grains. When floods occur manure and productive soils are washed out, this productive soil are assumed by the crop producer to help higher yields of crops, thereafter, air dryness dried it up, it becomes easy to be carried out by wind. Increased hot atmospheric conditions cause increment of grains moisture requirement, which then subject the crops to be susceptible when there is season of dryness (Nelson et al., 2009).

Some groups of plants, arthropods, and many pests derived gain from increased hot atmospheric condition and elated carbon dioxide, raising their ability to destroy grains and causes difficulty in finance of the crop growers. switch or change in atmospheric condition likewise contributes to the spread of pests to farmland that are yet to be cultivated. Increased atmospheric condition also caused the global glaciers to diminish which leads to negative effects on the crop growers that relied on the frozen moisture to dissolve into liquid and be applied for watering crops (Field and Barros, 2014).

Elevated oceans height, at the same time, elated flooding poses threat on the seaside farmland, also leads to heightened marine interference into the seaside which resulted into salt water and makes it impossible to be applied for watering of the crops (Backlund et al., 2008).

Switch atmospheric condition likewise are projected to influence environment and the functions they rendered for farming activities, like propagation and regulation of pest through nature carnivores. Most of the trees genus in their natural habitat are incorporated for the purpose of fertilization, and this wild animal experience risks of destruction (Jarvis et al., 2008).

EFFECTS of CLIMATE CHANGE on DIFFERENT CROP YIELD

Crop anatomy are tremendously affected through climate instability in many ways. Ecological excesses and atmospheric instability improve the possibility of many pressures on crops (Thornton et al., 2014). As the soil temperature decreases, plants that are not suitable for climatic conditions and resistant to cold will be affected by root and cause drying. As a result, a constantly increasing soil temperature will adversely affect plant life. It will decrease the efficiency (Bağdatlı and Ballı, 2020).

There are three major ways switch atmospheric condition impacts cultivation of grains which are as follows direct, indirect and socio-economic impacts. The work of Boyer revealed that atmospheric conditions caused decrease in the output of plant yield of about 70% from over three decades (Boyer, 1982). Based on the report of (Van Velthuizen, 2007).

They reported that every farmland where farming had been carried out globally experienced the impacts of climate switch, except 3.5% of regions are free from ecological barriers. The pressures from non-living holds tangible impacts on grains produce, though, this is greatly determined based on the depth of loss to overall regions that are been utilized for planting crops (Tebaldi and Lobell, 2018; Bonan and Doney, 2018).

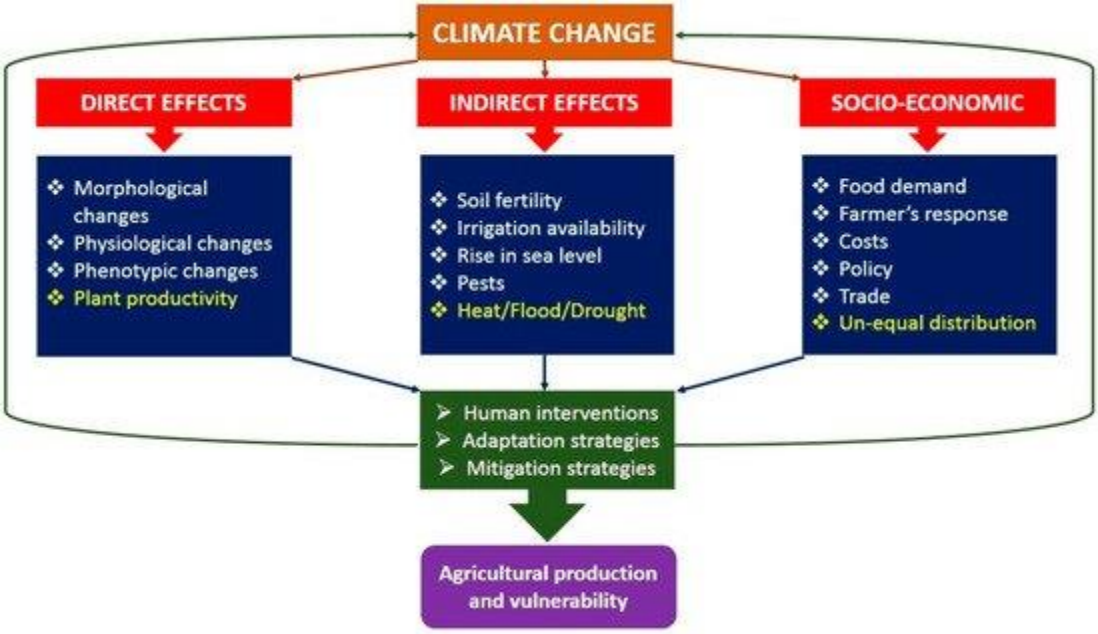

Figure 1. Direct, indirect and socio-economic impacts of climate change on food production (Raza et al., 2019).

Grain yield is extremely impacted through high temperature that occurred as a result of switch in atmospheric condition in more regions and could decrease produce output for about 6% representing an increase in degree of the temperature (Winkel et al., 1997). Dryness (drought) also increased temperature and are the major pressure elements with the highest effects on grains output. The inner enzyme of photosynthetic experienced limitations when the temperature rises from 35%^{o}C and cause alteration on the procedure that involves photosynthesis. A contradictory impact from heat waves on enzymes available in Zea mays (Gong et al., 1997).

The dual influence of heat and dryness leads to pressure on grains output was analyzed in sorghum, maize and barley. Observation on the dual impacts of heat and drought pressure was identified as self- pressure. as a result of switch in atmospheric conditions, precipitation shortfall and adverse hotness affects the propagation stage of crop development. There was record that the flowering and boom are adversely influenced due to the lack of precipitation in grains cultivation,

Likewise, a rise in temperature to around 30^{O}C while the process of boom growth set in, this lack of precipitation could lead to infertility in grains (Saini and Aspinall, 1982).

When grains, such as rice, and wheat are undergoing cell division, they deteriorate of about 35-75% because of moisture loss (Saini and Aspinall, 1982; Saini and Aspinall, 1981). Dryness tremendously affect rice during the procedure of propagation and efflorescence as a result of precipitation shortfall the yield rate is decreased to 60% (Garrity and O'Toole, 1994).

The production of Cocoa output was potentially decreased through the main dryness period in West Africa when there was occurrence of El Niño period (Hellin et al., 2014). The soybean produce was greatly affected as a result of dry period and there was 42% decreased in the soybean output when the phase of dressing was carried out (Maleki et al., 2013).

EFFECTS of CLIMATE CHANGE on ANIMAL PRODUCTIVITY

Animal production is important supply of nourishment for consumption of growing global populace. Livestock produce such as milk, yoghurts, eggs, milk, fabric, and feathers are greatly relied on by people. Animals produce are tremendously negatively influenced through several excesses of atmospheric situations (Koirala and Bhandari, 2019).

The influence of climatic switch is obvious and greatly felt on animals in several forms. Climatic switch is anticipated to results in rising in atmospheric linked phenomenal dangers and excessive climatic situations, like dryness, heat, deforestation, insect's occurrence (Khanal, 2010). Climate condition that persist for several years could influence later years livestock such as seas creature, ranches, parks, and deserts (Khanal, 2011).

Changing climate conditions will be an important factor in the current situation and the problems that may arise in the coming years. For this reason, solutions are needed for global warming and reduction of greenhouse gases that cause climate change (Bağdatlı and Arslan, 2020).

Warm and wet atmospheric conditions, as a result of climatic switch could leads to rise in the threat and incidence of livestock infections, some specific groups of animals are known as disease carriers, like insects that bites and tick, have the ability to live throughout the year. Several infections are posed as threat when the weather is hot. climatic switch through which the ecological conditions are susceptible for infections to develop and multiply like bacterial and viruses and the carrier of the virus or bacteria would be vulnerable with ease (Koirala and Bhandari, 2019).

High Temperature could leads to heat pressure in land and water animal, which results in decreased development, reduced yield and decreased immunity of livestock, When the weather is hot, livestock are likely to decrease their forage consumptions and the level at which their feeds are converted becomes lower and this conditions leads to projection to provide warmth or cooler environment for the livestock when the climatic condition is at the climax, and this results in addition in the expenses incurred in production. Significant climatic switch and instability like increase in temperature, humidity and unstable down pour of rain order has resulted to death of larger quantity of animal and invariably increased the cost of production, also influenced food safety negatively. Climatic switch also affects development of foraging crops and how animals graze (Koirala and Bhandari, 2019).

Heat waves also resulted in the reduction of yield and constituents of milk production. And this could lead to a swift dwindling in the milk output of about 40%. The stresses that occur as a result of heat cause a surge in the temperature of animal and attack the fatty composite of ductless gland and constituents such as fatty portion (%), solid-non-fatty, protein, casein and lactose constituents are affected. The occurrence of heat waves in cattle that are used for milk production leads to surge in the udder temperature and result in breast disease called mastitis (Pragna et al., 2017).

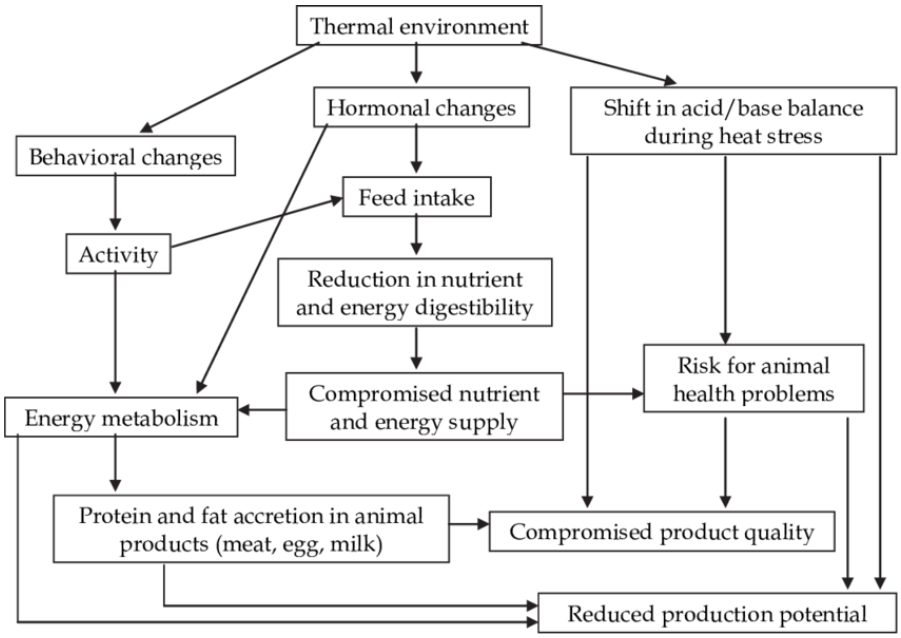

Figure 2. Schematic representation of the possible activities of inconvenient thermal surrounding on the production ability and products qualities of animals (Babinszky et al., 2011).

EFFECTS OF CLIMATE CHANGE ON FISHERIES PRODUCTIVITY

When climatic switch occur some groups of fish and shellfish migrate from warm location to cooler water part of the seas. Nonetheless, this movement from warm water part to cooler part poses threat to the group of fish and shellfish and both migrated fish and other group begins to compete for food. Switch in climatic condition was found to be in association with many seafoods infection eruption, Also, the warmth in the Arctic during cold period has influence on the infections in salmon in Bering oceans and this leads to dwindling in salmon quantity. Also, warm temperature has led to surge in infections in corals, eelgrass, and abalone (Ziska et al., 2016; Doney et al., 2014).

The decrease over time of the changes in the surface of the water is noticeable. This also shows itself as the effect of disorder in the vaporization and current precipitation regime in the water sources dependent on climate change (Albut at al., 2018).

Alterations in temperature and periods could influence the time of breeding and movement. several activities inside the water with organism life span are regulated through switch in climatic conditions. For instance, warm oceans temperatures could alter the life span of salmon and extend the possibility of infections. Coupled alongside of climatic influences, and this alteration is expected to result in more dwindling in salmon populace (Melillo et al., 2014).

Also, the seas acidity level steadily becomes high as a result of rise in the concentration of carbon dioxide in the atmosphere. when there is rise in acid level of the oceans, the high acid can endanger the crustacean through causing weakness in their carapace, this occur through elimination of calcium from marine body. Acidification pose threats to the water system of marine that are responsive, and many fisheries produce depend on them (Melillo et al., 2014).

Many shellfish are excessively susceptible to seas acidification, when there influence of climate change as a result of excess carbon dioxide. These mollusks are projected to have adverse effects on the finance of a nation, for instance, annually, United State of America reserves mollusks such as oysters, clams, and scallops of around 170 million pound of Seafood that worth $400 million.

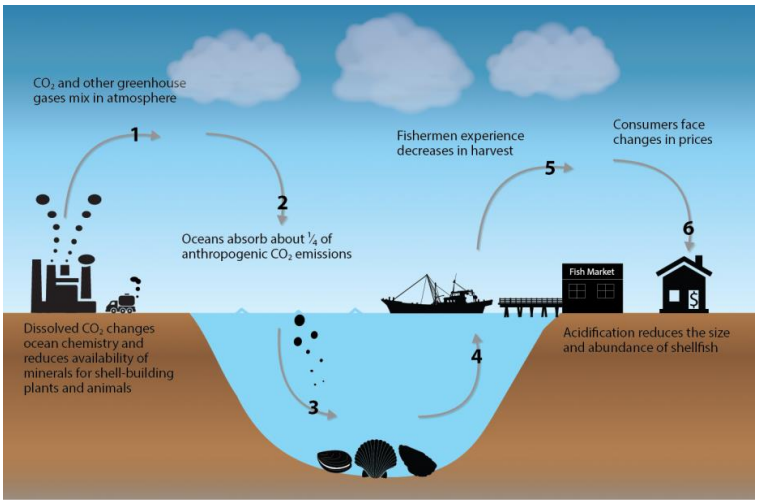

Figure 3. Ocean Acidification Impact Pathway for Shellfish (The diagram represented the influence pathway of carbon dioxide emissions on the shellfish market (US Environmental Protection Agency, 2015).

CONCLUSION

Gradually decreasing rainfalls due to climate changes endanger the living habitat. As a precaution, precise solutions are needed to reduce carbon dioxide in the air and slow down global warming and eventually end it. In this way, greenhouse effect and global warming can be prevented (Bağdatlı and Can, 2019). Climate change generally controls all activities of food production, from planting to processing. It will invariably affects regions and communities which relied on fishing for their source of income and survival as this will be interrupted by climate change, Also the effects of heat waves renders animal infertile and likewise subject them to infections, cattle are also affected by heat waves and all this will lead to low production in milk from dairy cattle.

The change in atmospheric conditions would affect the development of economy, threaten many nations food security and more impoverishment, Food availability and accessibility is the major and essential climatic -linked issues, due to the fact that global poverty is increasing day by day and food would become scarce and unaffordable. The way to prevent shortage in food supply is to mitigate the effects of climate change and also to adopt techniques to sideline its impacts for instance in aspect of drought, low precipitation, lack of rainfall, Irrigation can be employed.

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