Eurasian Journal of Food Science and Technology

Volume 5, Issue 1, June 2021

e - ISSN: 2667 – 4890

https://dergipark.org.tr/en/pub/ejfst

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INTERNATIONAL INDEXING

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Traditional Dried and Salted Nile Fish Products in Sudan: A review

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Abstract

The objective of this review is to check the common methods used in drying and salting of fish preservation in Sudan and their effect on sensory, chemical and microbial indicators. Drying and salting of fish are very common in Sudan, when the other methods of preservation are not available in fish production areas. salted product (Fassiekh) are important sources of nourishment, they contain great amount of a high quality protein, the chemical and microbiological characteristics of Fassiekh shown slight differences in most of their proximate components, mineral contents and microbial characteristics, the salt concentration level resulted in an increase in crude protein and ash content than fresh fish. The sun-dried product (Kejeik) of Nile fish is a good source of proteins, minerals as well as energy, which means Kejeik has high nutritional value for healthy diet. Therefore, it becomes clear that the drying and salting process is safe to some extent and does not have a negative effect on the quality of fish products.

Keywords: Drying fish; salting fish, traditional foods.

Review article
Received date: 24 December 2020
Accepted date: 28 April 2021

INTRODUCTION

Fish constitute in Sudan it is a very important component of the diet and often provides the much needed nutrient for Sudanese people (Mohamed et al., 2010). Sudan has the presence of a number of large water reservoirs, which contains a huge wealth of fish of severalty species and the estimated wealth was more than 110 thousand tons of fish (FAO, 1989). The Blue Nile, White Nile, River Nile, lake reservoirs behind dams and irrigation canals and the red sea these are the main sources of fish in Sudan. (FAO, 2019).

Fish are important substances in global food markets, they contain omega-3 fatty acids, high-quality protein, essential amino acids, minerals and vitamins (Kim, 2015). Fish is an extremely perishable food item it required preservation for future uses because fish is susceptible to damage as soon as it harvested, especially due to the high temperatures in Sudan and the lack of storing and distributing facilities of fresh fish in production area. many methods are followed over the world for preserving fish to extend its shelf-life, like drying, salting and smoking (Reza et al., 2007; Abolagba and Melle, 2008). Dry fish is a very favourite food item and used as a substitute of fish at the scarcity of fresh fish in many countries. (Oduor et al., 2010)

A Little is known about the nutritional quality of the dried and salted Nile fish products that is why the objectives of the present work include a review of the prevailing drying and salting methods of fish. In this review we are going to explain the methods and challenges during process in this kind of preservation methods.

Drying

Drying is one of the oldest methods used to preserve food products. Drying removed water from the foodstuff, which reduces the moisture percentage to the extent that it is difficult for organisms to live to occur damage and stop the enzymes in the events of changes of chemical spam in foods (Alasod, et al 2000). Natural solar drying, manufactured solar dryers, electric ovens and microwave ovens were used for preservation. The natural solar drying method was accompanied by changes in the dried foodstuff associated with changes and different weather conditions, pollution microorganisms, dust, and insects, which may leads to a reduction in their nutritional value (Eskander, 2020). Natural solar drying method requires a large area and long drying time (Darvishi and et al 2013). Majeed and Al Halphi (2007) concluded that the decrease in moisture percentage for fish and meat was higher by using the solar dryer compared with the using natural sun drying. Dried fish and meat using the solar dryer, it was isolated from the outer environment and did not affect by any change in environmental conditions.

Sun-drying and Salting is most of the traditional methods employed to preserve fish in Sudan. Sun drying is a low cost method and the product plays an important role particularly in providing nutrition to the people in Sudan, especially in remote areas with few rainfall and seasonal water streams. The process of sun-drying a fish consists of simply laying whole fillets or strips of fillets on drying racks directly under the sun, in the open air using solar energy to evaporate the water content in the fish, and the dry product is known as "Kejeik". (Elagba, 2010).

Sulieman (2012) studied the Quality Characteristics of Dried Fish Obtained from Eldeim Area, Central Sudan: where he prepared the fish for drying, for this, the head and fins were removed and the body was cut along the abdomen. All the viscera including the gonads were removed. Then the fish was cut along the back and the backbone and as far as possible all the ribs were removed and the meat and fat were carefully cleaned of skin. The fish drying started after preparation. After gutting the fish, it was either dried whole, or split along the spine leaving the tail connected. The fish was hung on the flakes from March -June. The fish flakes were subjected to vacuum packaging. After two months of hanging on the flakes, the fish was then matured for another month indoors in a dry and airy environment. During the drying, about 85% of the water in the fish disappeared. His results showed that the chemical analyses indicated the similarity of most of the tested chemical components of the fresh and dried fish products. For microbiological analyses, the dried fish samples were safe microbiologically.

Elegba (2013) studied the nutrient value of traditional sun dried fish in Sudan: Where she bought Sun-dried strips of fish fillet from the central fish market in Khartoum, crushed and grinded. Samples of powder of each species were weighed and freeze dried by Freeze Dryer model 230 to -40°C and the surrounding pressure was reduced to 110, using (MODULYOD) to remove water from the powder, until the samples had constant weights, The proximate constituents of the dry samples were determined by the method of the Association of Official Analytical Chemists (AOAC, 2005). The results showed that the dry product (Kejeik) of Nile fishes is of high nutritional value and good source of proteins, minerals as well as energy.

The results also indicated that dried fish contains minimal amount of heavy metals and is a useful food source for maintaining human health.

Zahra (2014) studied Nutritional Value of Kejeik: A Dry Fish Product of the Sudan: she mentioned that: during the period January-April, The fish samples had been produced by natural fermentation. In this process the fisher men are used to prepare Kejeik from fresh water Nile fish, the fish were split longitudinally, gutted and beheaded, the split fish were then hung on ropes or spread on rock or tree branches, out in the open air, under the direct sun.

When the drying process was over, the large pieces of fish are stacked together on mats, covered with another set of mats and trodden check on by fishermen, to flatten and pack the dry fish more compactly, further shade drying them follows after which the fish products were ready to be transported to the local markets. She concluded that the analyses indicated that there was non-significant difference between the Blue Nile and White Nile Kejeik samples in most of the chemical components of Kejeik samples prepared from three fish types. All Kejeik samples free from toxic metals such as mercury, arsenic and cadmium.

Sulieman (2012) studied Microbial Safety of Dried Fish Meat (Kejeik) Produced in Sudan: his results pointed that Kejeik is free from harmful bacteria (pathogenic and spoilage bacteria) Acidophile bacteria such as *Escherichia coli* (E. coli), *Staphylococcus aureus*, *Salmonella*, *Listeria Monocytogenes*, *Vibrio parahaemolyticus* and *Vibrio cholorea*. The absence of harmful and pathogenic bacteria indicated safety of the Kejeik products, The variations in microbial counts of Kejeik samples from different markets and seasons could be attributed to a lack of proper procedures adopted by the Kejeik processor and/or improper hygienic in some of these areas.

Salting

Salting is a physical and chemical process as a result of salt penetration of the fish's body with the forces of moisture to leave the muscles, resulting in a change in weight and is intended to preserve the fish (Eskander, 2020). The salting process leads to loss of part from the protein reducing worked lipolysis enzymes and also salting works to the decrease of numbers lipolysis bacteria through the decrease of free fatty acids after salting (Aldouri and et al, 1990). Salting by salt solution gives more regularly salting compared to dry salting if the high moisture content of the products will need more salt compared to dry products (Hilderbrand, 1999).

Salted fish (Fassiekh) are manufactured in southern Sudan and parts of White Nile, fish are grouped into boat and washed well and cut from abdomen to chest and washed for second time with water and salt within limits of 2 kg salt in order to prevent from flies exposure during salting, and then placed a few salt within the abdomen , after wounded all fish sprinkle a little salt and stir and then thrown into plastic sacks, such as sugar plastic sacks for 4 - 7 days after sprinkle amount of salt in the range of 10%, while after reaching save stage, packaging's again empty and covers floor by linoleum and then sprayed a another quantity of salt per hundred kilograms amount of 10 kilograms of salt ,and kept for another 10 days , then discharged for the second time and placed in wooden boxes intended for drying, after three days can be discharged and placed in Dryer to ventilation for 6 hours and are weighed in plastic packaging's (Hafiz, 2010).

Manufacturing Fassiekh as powder and paste in the same last manner, so Fassiekh fish is cleaned of crust , spinal and head of up party and wash and then displays to full ventilation to dry water and then hashes and also placed for ventilation for a full day or half-day as ventilation, and same way in manufacture of powder when pulp dried completely, so that it almost dry and then hashes for the second time and dried for a week in a place not exposed to dust or sun so as not to be affected by unpalatable taste. (Hafiz, 2010)

Onaheed and et al., (2018). Studied the Quality appraisement of Fassiekh manufactured by using different fish species in El-Dueim locality, Sudan: he subjected sensory analysis (color, flavor, texture and overall acceptability) and chemical analysis (moisture, protein, ash, crude fat and fatty acids composition for Fassiekh, The score sensory evaluation of salted fish (Fassiekh) was found to be affected by the type of fish and all species of fish used in manufacturing of Fassiekh were highly accepted by the panelists. The chemical composition of Fassiekh is deviated in association with the fish species. The content of omega-6 fatty acids was high in all species compared to omega-3.

Sulieman (2012) Studied the Effect of Antimicrobial Properties of Pepper Fruits on Some Spoilage Organism of Sudanese Wet-Salted Fermented Fish (Fassiekh) Product, he concluded that, the pepper fruits can play as antimicrobial agent in conserving the Fassiekh product by lowering the total viable count, killing or inhibiting some organism that related to Fassiekh spoilage and recommend that, the hot pepper can be used in small amounts in preparation and production of Fassiekh.

The unhygienic practices of Fassiekh making and the higher number of bacterial load and staphylococci count may pose hazards to human health, the consumers should take more consideration to paste Fassiekh available in the retails, due to the higher count staphylococci species which are considered as food poisoning organism, as well as the conclude that the paste Fassiekh is more contaminated than the wet-salted than the dried salted Fassiekh.(Goja, 2013).

The salt concentration level on studied fish species resulted in an increase in crude protein and ash content than fresh fish and produced well wet-salted fermented product with reasonably long storage shelf life (Sulieman, 2012).

CONCLUSION

Through this study, it appears that the use of drying and salting for fish preservation is highly effective, as is the high nutritional value and good source of proteins, minerals and energy of the dry product (Kejeik) and the salted product (Fassiekh) of Nile fish. Studies have also shown that dried fish contains small amounts of heavy metals and is a valuable source of food for the protection of human health.

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Peroxide Value in Oils Used for Chicken Frying in Selected Restaurants in Khartoum City- Khartoum State-Sudan

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Abstract

The Increased peroxide value more than the recommended cause several health problems such as liver, cancers and cardiovascular diseases. This study was designed to estimate the amount of peroxide value of used oils in frying chicken, since some restaurants repeatedly reuse the oil for frying in order to reduce the cost Peroxide value of oils used for chicken frying in selected restaurants in Khartoum was measured. The study aim to assess the peroxide value of the oils at different frying times as follows: Fresh oil before frying-at the middle of frying and -at the end of frying. The study showed variations in the peroxide value in the oil before frying (fresh oil). All restaurants showed values within the recommended value except one restaurant showed high level (20.9meq/kg). The peanut oil showed lower peroxide value than palm olein. Several variations in the peroxide value during frying were observed. The changes were obvious in two restaurants where the values increased from 1.5 to 5.1mqv/kg and from 2.9 to 4.0 respectively at the middle of frying, then decreased to0.07and to 1.6 meq/kg at the end of frying. It is advisable to use freshly produce oil in frying process. It is not suitable to reuse the same oil for frying more than once plus check internationally, this may cause some health risks. Monitoring and inspection of oil should be used for frying before, during and at the end of frying process.

Keyword: Frying chicken, oil, peroxide value

Research article
Received date: 17 January 2021
Accepted date: 28 April 2021

INTRODUCTION

Detection of peroxide gives the initial evidence of rancidity in unsaturated fats and oils. Other methods are available, but peroxide value is the most widely used. It gives a measure of the extent to which an oil sample has undergone primary oxidation, extent of secondary oxidation may be determined from p-anisidine test (Chakrabarty, 2016).The peroxide values is used for estimating the peroxides expressed as milliequivalent/kg of oil or milliequivalent active oxygen per kg of fat. high peroxide values point that lipid oxidation has occur. It is measured as reactive oxygen in terms of milliequivlent per kg fat. These oxidation is correlated with reduced flavor scores or paint flavor defects. Peroxide value is often used as indicator of freshness and quality related to oil oxidation (Gunstone, 2002). Fried chicken restaurants are the most popular restaurants in Sudan and fried chicken is the one of the most popular dishes preferred by all consumers worldwide (Talpur et al., 2009).

An independent positive association between the risk of hypertension and intake of heated frying oil had been proved to increase the risk of cancer and cardiovascular diseases (Rossouw, 2002). Deep frying is the most popular and one of the oldest methods of food production worldwide (Rani et al., 2010). Fried foods have desired flavor, color, and crunchy texture, which make deep-fat fried foods very populist to consumers. Frying is a process of immersing food in hot oil, in atmospheric pressure, and at a high temperature of 150º to 190ºC (Choe and min, 2006). When food is fried, the frying oil replaces part of the moisture content and during deep frying, the oil is continuously or repeatedly being exposed to temperature between 150and 1800 ºC in the presence of the substrate, air and water. Under these conditions, complex series of reactions take place, namely hydrolysis, oxidation, polymerization and cyclic compounds, resulting in the both volatile and non-volatile products (Mariod et al., 2006). It is important to understand what happen to the temperature, moisture and oil content of the product during the frying process to determine safe temperature and time of frying oil (Farah, 2004).

Shakak (2007) also reported that the chemical changes in the frying oils result in changes in their physical characteristics. The darkness of color of oil increases with increasing content of polymers, viscosity increases, and greater foaming of the oil occur. Some of the more volatile component, such as free fatty acids, accumulated to the point where the smoking point is decreased. The aroma and flavor also change with increased frying time, as do the color and flavor of the food fried. Deep fat frying is a complex phenomenon where water, oxygen and heat are the main factors, which determine the kinetics of oxidation, hydrolytic and polymerization processes. Beside the radical mechanism for the lipid peroxidation and polymerization of triglyceride another non-radical mechanism for formation of non-oxidized dimmers and cyclic triglycerides was proposed (Gertz and Kochar, 2001).

Also, it was declared that common antioxidant including tocopherols, butyrate hydroxyl anisole and propyl gallate, retard oxidation at ambient temperature, but they become substantially less effective or even inactive when subject to frying temperature (Nor ,2012). When the oil heated to 2000 ºC the peroxide will break down to aldehydes and ketones (Ali, 2002). With the continues of oxidation the peroxide will breakdown to aldehydes, ketones and acids and all had law molecular weight saturated or unsaturated and also had strong odors, non-toxic and bad flavor. Oxidation also cause stress in the oil color change to dark color and increased viscosity (Osman, 2007).

The final products of lipid oxidation - aldehydes, furans, ketones, alcohols, epoxides, and others - are responsible for well-known flavors and odors of oxidized lipids. However, these products are difficult to detect because many of these products are not stable, they are present at very low concentrations, present in complex mixtures that are difficult to separate, and they are reactive, so transform to other products and complex with food molecules, particularly proteins (Steltzer,2012). It is evident that the fatty acid composition of a particular fat is more important that it's absolute concentration regarding these diseases. High intakes of saturated fats and Trans fatty acids have been recognized as a risk factor for coronary heart disease (Ghidurus et al., 2010).

A huge consumption of frying chicken led owners of these restaurants to reduce the cost of oil by using the oil more than once for frying. This may lead to many health problems. Therefor regulations for food control are very important. This paper aims to determine hydrogen peroxide value in oils used for chicken frying in selected restaurants in Khartoum - Sudan.

MATERIALS and METHODS

Fried chicken oils Samples

Ten frying chicken restaurants were selected from Al-Amarat – Khartoum- Sudan, three oil samples were collected from each restaurant for determination of peroxide value as follows: At zero frying time (fresh oil), at middle of frying time, at the end of frying time before changing the oils.

Note: 8 restaurants were using peanut oils while two restaurants using palm olin oil.

Peroxide value determination

Peroxide value was determined according to method describe by A.O.A.C, (2005). Where five grams ±0.05 g were weighted and placed into 250 ml Erlenmeyer flask. Thirteen ml of acetic acid-chloroform (3:2) was added to samples and shacked to dissolve. 0.5 ml of saturated potassium iodide (KI) was added and left to stand with occasional swirled for one minute. Thirteen ml distilled water were added, 0.5 ml of 1% soluble starch indicator was added which gave blue color. Sodium thiosulfate ($Na_2S_2O_3$) 0.01 N were titrated with the Erlenmeyer flask contents and shacked until the color changed to light yellow. The titration was continued with continues shacked till the end point which is indicated by a faint blue color. Sodium thiosulfate ($Na_2S_2O_3$) was added dropwise until the blue color disappears. The peroxide value was calculated as meqg of peroxide value /Kg of oil according to the following equation.

$$PV=(S-B) *M*1000w$$

Where: S = volume (ml) of sodium thiosulphate in test active solution.

B = volume (ml) of sodium thiosulphate in blank solution

M = 0.01the concentration of the $Na_2S_2O_3$ solution.

W = Wight of oil sample in gram

RESULTS and DISCUSSION

Table 1. peroxide value in fresh oils, middle of frying and the end of frying of chicken.

Restaurant No	peroxide value in fresh oils Pv1(meq/kg)	peroxide value at the middle of frying Pv2(meq/kg)	peroxide value at the End of frying Pv3(meq/kg)
1	1.4	0.7	0.0
2	1.5	0.0	0.2
3	2.1	1.1	0.3
4	2.2	1.4	0.7
5	2.3	1.9	0.7
6	2.4	2.1	0.8
7	2.9	2.2	0.9
8	3	3	1.0
9	3.9	4	1.0
10	20.9	5.1	1.6

Table 1 shows that, the initial values for peroxide value of fresh, middle, and the end used oil in 10 restaurants before starting frying process. High value was recorded in restaurant no.10 (20.9meq/kg) while restaurant number 5 showed lower value of 2-3 meq/kg although the two restaurants were using palm oil .These differences in peroxide value between the 2 restaurants 5 and 10 may be due to differences of the source of palm oil , date of production and storage condition. On the other hand, the oil produced from peanut showed lower values of peroxide value. The peroxide value ranged from 1.4 to 2.2 meq/kg in restaurants No.1- 2- 3and 4 while restaurants no. 7- 8and 9 showed high values of 2.9- 3 and 3.9 meq/kg of oil respectively although this restaurants peanut oil .This variation may be due to oil processing techniques used for extraction of the oil, date of production and storage condition. The lower value 1.4meq/kg was recorded in restaurant no.1 .while restaurant no. 9 showed high value of 3.9meq/kg. All restaurants showed lower values below 10meq/kg except restaurant no. 10 which was higher than others.

The results of peroxide value (PV) at the middle of frying process are presented. The peroxide values decreased in all restaurants except in restaurants no. 2 and no. 7 were pv value increased from 1.5to 5.1meq/kg and from 2.9 to 4meq/kg respectively. The results of pv at the end of frying process .It could be noted that pv were decreased throughout in all restaurants. This is may be attributed to the fact that during frying process, food placed in hot oil will be heated quickly where water is evaporated, and the resulting steam causes a boiling action in the oil. This boiling action increases aeration in the oil, which results in increased oxidation of oil with the formation of hydro-peroxide, the primary oxidation product. All restaurants from where the sample of frying oil were taken are using high temperature of 190 to 200^0C which will cause breakdown of pv to aldehydes and ketones. These results are in agreement with what reported by Shakak (2007) and Ali, (2002). These results were further confirmed by darkness of color and high viscosity observed in all oils at the end of frying process. These changes in physical characteristics color darkening, increased viscosity and formation of foam will increase with increase of polymers content. To avoid formation of such physical characteristic using oil more than once should be avoided in order to avoid occurrence of such products which have some health hazards, This findings agreed with what reported by Choe and Min (2006) who reported in the chemical changes in the frying oils resulted in change in their physical characteristics. The color of oil darkness with increasing content of polymers, viscosity increased, and greater foaming of the oil occurred. The outcome of this study showed that the peroxide value increased initially and decreed at the end of frying process especially when the oil is repeatedly used for frying which may produce some chemical products that may be considered as health hazards. This has been confirmed by chi-square statistical test which showed that the peroxide value is greater than 0.05(>0.05) indicate that oil should not be used more than once in frying process.

CONCLUSIONS

Edible oils differ in their thermal susceptibility to frying .it is advisable to use freshly produced oil as possible and it is also advisable to chick the peroxide value before and during frying process. The quality of oils used for frying deteriorated at the end of frying time producing some unhealthy components such as ketons and aldeheds therefore it is not advisable to reuse the same oil for frying more than once. Monitoring, evaluation and inspection of oil used for frying should be carried out before and during frying process, the reuse of frying oil in chicken frying restaurants should be avoided and it is recommended that the oils used for frying of other products should be checked for peroxide value periodically.

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Vacuum Frying of Selected Shellfish Products

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Abstract

Vacuum frying of frozen prawn, shrimp and mussel was carried out at 80°C frying temperature, 0.4 kPa chamber pressure and 50 minutes frying time. The vacuum fried (VF) cooked prawn and shrimp had the lowest final moisture content while the VF raw prawn had the highest. The VF cooked (VFC) whole and half mussels had the highest product yields and the VFC shrimp had the lowest. The fat contents of the VF shellfish products were very close to each other. The VFC half mussel had the lowest fracturability while the VF raw prawn had the highest. The VF raw prawn had the highest chroma value while the VFC whole mussel had the lowest. The VFC whole mussel had the highest hue angle value but the VFC shrimp had the lowest. The VFC shrimp had the highest rehydration rate and ratio while the VFC prawn had the lowest values.

Keywords: Vacuum frying, prawn, shrimp, mussel, physicochemical properties, texture, colour

Research article
Received date: 20 January 2021
Accepted date: 28 April 2021

INTRODUCTION

Seafoods include fish, whether from freshwater, estuarine or marine habitats, and also shellfish which include crustaceans and mollusks. The crustacean comprises of crayfish, crab, shrimp/prawn and lobster, while mollusks could be bivalves such as mussel, oyster and scallop, univalve creatures such as abalone, snail and conch, and cephalopods which include squid, cuttlefish and octopus (Venugopal, 2005). The world's annual catch of fish and shellfish is approximately 100 million metric tons, from which only 20% is processed for food (Shahidi, 2012). Fish and shellfish are important sources of protein for some people in some areas of the world.

Shellfish such as shrimp, prawn and mussel are popular because of their unique taste, absence of fishy smell and high calcium content. Steamed green lipped mussel has a protein content of 18.8g/100g sample and calcium content of 173mg/100g sample (Lesperance, 2018). The raw and cooked shrimps have protein contents of 20.10 and 22.78g/100g sample, respectively. On the other hand, the raw and cooked shrimps have calcium contents of 64 and 91mg/100g sample, respectively (USDA-ARS National Agricultural Library, 2020). The calcium contents of these cooked shellfish products are much higher than popular cooked table fish like flounder, salmon, snapper and tuna (Lesperance, 2018).

There are different ways to process shellfish into stable products including freezing, salting, smoking, fermenting, canning and drying. Ready-to-eat shellfish products such as chilled cooked shrimp, prawn and mussel are very popular however these products need refrigerated storage. At the present, there are no available ready-to-eat shellfish products that can be stored at ambient conditions. There is therefore a need to explore other methods of producing ambient shelf stable shellfish products and one possible way is to use the vacuum frying process. In addition, the vacuum fried shellfish products can be used as ingredients for instant noodles with shellfish flavor.

Vacuum frying is an efficient method to reduce the oil content in fried foods, maintain product nutritional quality and reduce oil deterioration. It is a technology generally that can be used to obtain products from fruits and vegetables (Da Silva & Moreira, 2008) and seafoods (Andres-Bello, Garcia-Segovia & Martinez-Monzo, 2010; Pan, Ji, Liu & He, 2015) with the necessary degree of dehydration without excessive darkening or scorching of the products.

The vacuum frying process required the heating of oil to the required temperature. Then the sample to be processed was placed in the basket inside the frying chamber but suspended above the hot oil. The pressure inside the vacuum frying chamber was reduced to the required pressure. The sample was then lowered into the hot oil for the required duration and then the basket was raised above the oil and then centrifuged within the chamber for the required speed and time. The fried product can also be taken out of the chamber and centrifuged using a separate machine or stood in the frying chamber to drain the surface oil (Diamante, Shi, Hellmann & Busch, 2015). In comparison to atmospheric deep-fat frying, this technique works under pressures well below atmospheric levels, preferably below 6.65 kPa thereby decreasing the boiling point of water considerably below 100°C (Garayo & Moreira, 2002). Hence, moisture evaporation of food during frying can happen far below the 160°C temperature that is commonly used for atmospheric frying.

At the present, vacuum frying has been used mostly in different plant origin foods, including apples, apricot, beans, carrots, blue potato, jackfruit, gold kiwifruit, mangoes, potato, pineapple and sweet potato (Diamante, Savage & Vanhanen, 2013; Diamante, Savage, Vanhanen and Ihns, 2012; Yang, Park, Kim, Choi, Kim, & Choi, 2012; Yagua & Moreira, 2011; Dueik, & Bouchon, 2011; Diamante, 2009; Da Silva & Moreira, 2008; Perez-Tinoco, Perez, Salgado-Cervantes, Reynes, & Vaillant, 2008; Fan, Zhang & Mujumdar, 2005) and lately on animal origin foods such as breaded shrimp, gilthead sea bream fillets, chicken nugget (Pan et al., 2015; Teruel, Chen, Zhang & Fang, 2014; Garcia-Segovia, Martinez-Monzo, Linares & Garrido, 2014; Andres-Bello et al., 2010). Presently there are no literatures on vacuum frying of shellfish such as shrimp, prawn and mussel, hence this study.

The objectives of the study were: a) to carry out preliminary experiments on vacuum frying of a representative frozen cooked shellfish sample; b) to determine the effect of frying time on the final moisture content of vacuum fried raw and cooked prawn, shrimp and mussel; c) to conduct vacuum frying at constant conditions on selected frozen raw and cooked shellfish samples; d) to analyze and compare the physicochemical properties of the resulting vacuum fried raw and cooked shellfish products; and e) to come up with recommendations on the vacuum frying conditions for processing of selected frozen raw and cooked shellfish samples.

MATERIALS and METHODS

Materials

Survey of the local supermarkets in Christchurch, New Zealand revealed the availability of the following frozen samples including raw peeled prawn, cooked peeled prawn, cooked peeled shrimp and deshelled green-lipped mussel. Five kilograms each of frozen samples of raw peeled prawn, cooked peeled prawn and cooked peeled shrimp while 10 kilograms of deshelled green mussel were purchased from the supermarkets. Both the frozen raw peeled and cooked peeled prawn had an average length of 4.5 cm and a thickness of 1.5 cm. The frozen cooked peeled shrimp had an average length of 2.0 cm and a thickness of 0.75 cm while the deshelled whole mussel had an average length of 3.5 cm and a thickness of 1.00 cm. The samples were stored in a chest freezer at -20°C for about one month until all the experiments were done. The sample for the cooked half mussel was obtained by cutting the cooked mussel crosswise. Three hundred liters of canola oil were obtained from an ingredients company in Christchurch, New Zealand and stored at room temperature (15 to 20°C) for about one month until all were used up.

Vacuum frying system

The equipment used for the experiments consisted of a sealable fryer vessel connected to a condensation unit and a vacuum pump as shown in Fig. 1. The heating of the oil was done using band heaters on the fryer walls and the condenser was cooled using a refrigeration system. Inside the vessel, a frying basket was located, which can be rotated within the chamber. For every trial, 20 liters of canola oil (Seafrost, Kuala Lumpur, Malaysia) were poured in the frying vessel and heated up to the target temperature. This took approximately one hour.

The content of a bag of frozen sample (500±10 g) was loaded into the frying basket. After closing the vessel lid, the valve to the vacuum pump was opened. When a pressure of 0.4 kPa was reached, the basket with the samples was immersed into the hot oil. From this moment, the time was started, the temperature as well as pressure was recorded. With the help of the vacuum pump and condensation unit, the escaping steam was taken out of the vessel. Because of high steam generated at the beginning, the pressure increased for a short period and dropped down again to about 0.4 kPa. The temperature fluctuated with decreasing amplitudes and settled down to the required temperature due to the temperature controller. When the required frying time was reached the basket was completely brought out of the oil and centrifuged using 670 rpm for 4 minutes still at the same chamber pressure to enhance the removal of residual oil from the sample surface. After this procedure, the system was pressurised back to atmospheric pressure. The product was removed out of the basket, cooled down to room temperature, placed inside aluminium laminated bags and then stored at room temperature until analyses.

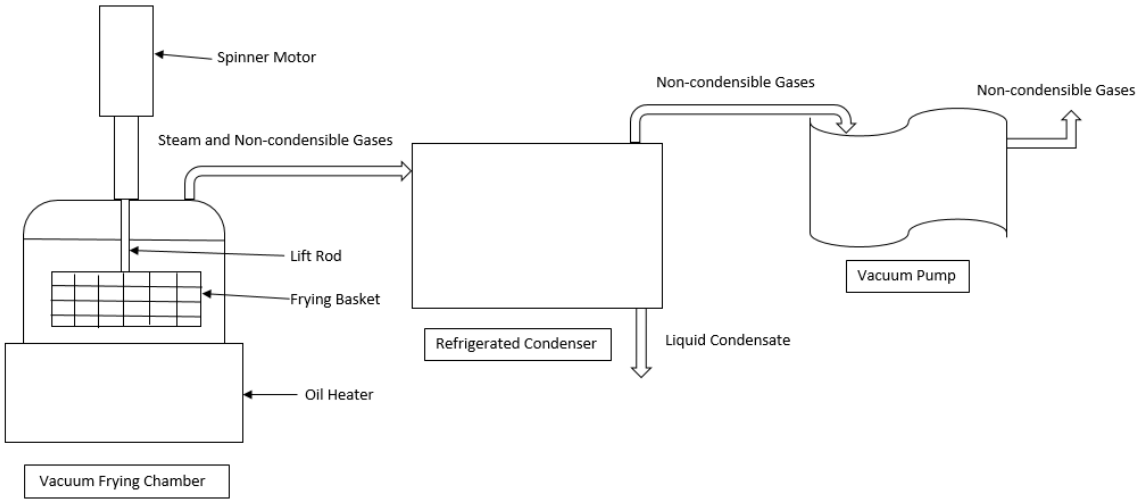

Figure 1. Schematic diagram of the vacuum frying system for the experiments (Diamante et al., 2015).

Moisture Content Determination

The moisture contents of the initial and final samples from each trial run were determined using the air oven method. The samples were dried at a constant temperature of 105°C for exactly 16 hours after that time a constant weight was reached (Diamante, Durand, Savage, & Vanhanen, 2010). The weight of samples was determined in an analytical balance with an accuracy of 0.0001g (Mettler Toledo, Greifensee, Switzerland) before and after drying in the air oven (Watson Victor Ltd, Clayson Laboratory Apparatus Ltd, NZ) using triplicate measurements. The moisture content was calculated by using the equation,

$$M_{WB} = \frac{B-C}{B-A} * 100 \quad (1)$$

where: M_{WB} = moisture content calculated on % wet basis

A = weight of container [g]

B = weight of container and sample before drying [g]

C = weight of container and sample after drying [g]

Product Yield Calculation

The product yield of the vacuum fried samples wa obtained from its initial and final weights. The amount of frozen sample was determined using a weighing balance with an accuracy of 0.01g (Mettler Toledo, Greifensee, Switzerland). After vacuum frying, the product was cooled down before weighing. The product yield was determined using the following equation,

$$\text{Product Yield} = \frac{\text{Weight of the vacuum fried product}}{\text{Weight of the frozen sample}} \times 100 \quad (2)$$

Fat Content Determination

The fat content of the ground vacuum fried samples was determined gravimetrically by solvent extraction using the Soxhlet technique as described in Bouchon *et al.* (2003). The fat content of the samples was calculated on a percent dry basis and the average value of the triplicate measurements were used.

Fracturability Analysis

Texture properties of the vacuum fried products were determined by measuring the fracturability of the sample using a texture analyzer (Texture Analyser Model: TA-XT plus, Serial No: 10781, Stable Micro Systems, Surrey, UK) equipped with a 5 kg load cell. Fracturability measures the highest peak on the force versus time curve. When the fracturability value is low, the product easily breaks up indicating a crunchier product. A ball probe (5 mm diameter) was used to penetrate the samples at a constant speed rate of 1.0 mm/s. Measurements were done on 5 pieces of samples for all the products.

Colour Properties Determination

The colour properties of the vacuum fried products were determined using a Minolta Reflectance Chroma Meter CR 210 (Minolta Corp., Osaka, Japan) by measuring the L*, a* and b* colour values. The L* value range from 0 (Black) and 100 (White), the a* value from -a* (Green) and +a* (Red) while the b* value range from -b* (Blue) and +b (Yellow). The different products were ground in a multi grinder (Sunbeam Corp., Botany, NSW, Australia) and then a 10g sample was placed on a petri dish without cover. Five sets of ground samples were obtained from each trial run and the average of five readings was used. Before each measurement, the instrument was calibrated using a white ceramic tile (L = 98.06, a = -0.23, b = 1.88). Chroma and hue angle of vacuum fried samples were determined using the following equations,

$$\text{Chroma} = \sqrt{a*^2 + b*^2} \quad (3)$$

$$\text{Hue Angle} = [\tan^{-1}(|b*/a*|)][180/\Pi] \quad (4)$$

where: a* and b* = colour values of the vacuum fried product

Π = pi constant (3.14159)

Rehydration Properties Calculations

The rehydration properties of the vacuum fried products were determined by weighing a piece of dried product in a weighing balance with 0.01g accuracy (Mettler Toledo, Greifensee, Switzerland) and then putting the piece of dried product in a thick glass bowl with boiling water. Place a similar glass bowl on top of the dried product so that it will be fully submerged in the hot water. The dried product was left to rehydrate for 3 minutes.

At the end of rehydration, the product was taken out of the water and put on three sets of thick tissue paper to dry out all surface moisture. The rehydrated product were weighed in the same weighing balance. The same procedure was repeated for 5 pieces of dried products. The Percentage Gain, Rehydration Rate and Rehydration Ratio of the individual pieces were calculated as follows,

$$\text{Percentage Gain} = \frac{\text{Initial product weight} - \text{Rehydrated weight}}{\text{Initial product weight}} \times 100 \quad (5)$$

$$\text{Rehydration Rate} = \frac{\text{Percentage Gain}}{\text{3 minutes}} \quad (6)$$

$$\text{Rehydration Ratio} = \frac{\text{Rehydrated weight}}{\text{Initial product weight}} \quad (7)$$

Statistical Analyses

A two-way analysis of variance (ANOVA) using Minitab 15 (Minitab Inc., State College, Pennsylvania, USA) was carried out on the initial and final moisture contents, product yield, fat content, fracturability, colour values (L*, a* and b*) and colour properties (chroma and hue angle) in order to determine the significance of the results. The Tukey's test was used to locate the difference between the means (Walpole et al., 1998).

RESULTS and DISCUSSION

Preliminary Experiments on Vacuum Frying of Frozen Cooked Shrimp

Pan et al. (2015) reported the vacuum frying of breaded shrimp at 80 to 120°C and 12 kPa using frying times of to 10 minutes. Examination of their results showed that the final moisture content of the vacuum fried breaded shrimp were still very high at 80 to 95% dry basis (44.4 to 48.7% wet basis). Longer frying times were used in the study to obtain very dry products that will have good texture qualities and shelf stable. Preliminary studies were carried on frozen cooked shrimp at a temperature of 80°C at a chamber pressure of 0.4 kPa using frying times of 15, 30 and 45 minutes; at a temperature of 90°C at a chamber pressure of 0.4 kPa using frying times of 10, 20 and 30 minutes; and at a temperature of 100°C at a chamber pressure of 0.4 kPa using frying times of 5, 10 and 15 minutes, respectively. It must be noted that the vacuum fryer chamber pressure is very much lower than in the Pan et al. (2015) study. Very low chamber pressures will result in low boiling point of water and therefore speed up the drying of frozen seafood samples to very low moisture levels. Frozen samples were also used in the study in order to enhance the physicochemical properties of the vacuum fried products (Shyu & Hwang, 2011).

Table 1 shows the results of the experiments. The results showed that the vacuum fried cooked shrimp at 80°C and 45 minutes gave a product that was reddish in colour, crunchy texture and very dry with a moisture content of 1.87% wet basis. On the other hand, the vacuum fried cooked shrimp at 90°C and 30 minutes gave a product that was reddish light brown in colour, crunchy texture and very dry with a moisture content of 2.10% wet basis. Lastly, the vacuum fried cooked shrimp at 100°C and 15 minutes gave a product that was reddish light brown in colour, crunchy texture and very dry with a moisture content of 1.76% wet basis.

The vacuum fried cooked shrimp at 80°C gave the most acceptable colour closer to the original sample. The vacuum fried cooked shrimp processed at 90 and 100°C resulted in unacceptable colour of reddish light brown which looked slightly burnt probably due to the high temperatures used. The results suggest that the vacuum frying of frozen cooked shrimp should be limited to a temperature of 80°C at a chamber pressure of 0.4 kPa and frying of about 45 minutes to give a final moisture content of around 2% wet basis and to have acceptable colour and texture properties.

Moisture Contents of Vacuum Fried Cooked Shellfish Products

The initial and final moisture contents of vacuum fried raw and cooked prawns, cooked shrimp, cooked whole and half mussels processed at a frying temperature of 80±1°C, chamber pressure of 0.4±0.1 kPa and different frying times are shown in Table 2. The results show that the cooked shrimp sample had the highest initial moisture content of 89.21% wet basis while the cooked half mussel sample had the lowest of 72.24% wet basis. After processing, the highest final moisture content was obtained for the vacuum fried raw prawn of 2.72% wet basis while the cooked shrimp had the lowest of 1.31% wet basis. The vacuum fried cooked shrimp had the lowest final moisture content in spite that it had the highest initial moisture content due its smaller size which speed up the drying process. In addition, the final moisture content of the different products decreased with increasing frying time. Pan et al. (2015) also reported that vacuum frying of breaded shrimp at 80°C and 12 kPa pressure gave a decreasing moisture content at increasing frying time from 1 to 10 minutes while Andres-Bello et al. (2010) presented a similar trend for vacuum frying sea bream fillets at 90°C and 15 kPa pressure using different frying times from 1 to 10 minutes. Preliminary studies on the vacuum frying of frozen cooked shrimp indicated a final moisture content of around 2% wet basis to give an acceptable product crunchiness. Hence, the target final moisture content of around 2% wet basis for all samples was attained at 50 to 60 minutes of frying time. As a result, the different vacuum fried products processed at 50 minutes were further analyzed for selected properties. It must be noted that when a raw prawn is cooked (steamed), it evaporated some of the moisture resulting in the initial moisture content decrease and when vacuum fried resulted in a lower final moisture content. In addition, when the whole mussel was cut in half, its surface area increased and when subjected to cooking (steaming), there was some moisture evaporation thereby lowering its initial moisture content and when vacuum fried resulted also in a lower final moisture content.

Table 1. Results on the preliminary experiments for vacuum frying of peeled cooked shrimp (Initial MC = 89.21% wet basis) processed using 500g frozen sample for each run at different frying temperatures and times, chamber pressure of 0.4 ± 0.1 kPa with centrifugation of fried samples under the same chamber pressure at 670 rpm for 4 minutes.

Frying temperature/ Frying time	Informal observation of the product		Moisture content (MC) of the product	
	Colour description	Texture description	Description	Value (% wet basis)
80ºC/ 15 minutes	light reddish (similar to original sample)	soft (similar to original sample	slightly wet	not determined
80ºC/ 30 minutes	moderately reddish	rubbery	moderately dry	not determined
80ºC/ 45 minutes	reddish	crunchy (easily broken when pressed)	very dry	1.87
90ºC/ 10 minutes	light reddish (similar to original sample)	soft (similar to original sample	moderately wet	not determined

90ºC/ 20 minutes	moderately reddish	rubbery	slightly dry	not determined
90ºC/ 30 minutes	reddish light brown	crunchy (easily broken when pressed)	very dry	2.10
100ºC/ 5 minutes	light reddish (similar to original sample)	soft (similar to original sample	slightly wet	not determined
100ºC/ 10 minutes	moderately reddish	rubbery	moderately dry	not determined
100ºC/ 15 minutes	reddish light brown	crunchy (easily broken when pressed)	very dry	1.76

Table 2. Initial and final moisture contents (MC) of vacuum fried peeled raw and cooked prawns, peeled cooked shrimp, shelled cooked whole and half mussels processed using 500g frozen sample for each run at a frying temperature of 80 ± 1ºC, chamber pressure of 0.4 ± 0.1 kPa and different frying time with centrifugation of fried samples under the same chamber pressure at 670 rpm for 4 minutes.

Sample	Replication	Initial MC* (% wet basis)	Frying time/ Final MC* (% wet basis) 40 minutes	50 minutes	60 minutes
Raw prawn	R1	86.88^{c}	5.74^{d}	2.83^{c}	1.43^{d}
	R2		5.01^{d}	2.60^{b}	1.50^{d}
Cooked prawn	R1	83.80^{b}	3.13^{b}	1.53^{a}	1.10^{b}
	R2		3.02^{b}	1.41^{a}	1.05^{b}
Cooked shrimp	R1	89.21^{d}	2.61^{a}	1.37^{a}	0.90^{a}
	R2		2.51^{a}	1.24^{a}	0.81^{a}
Whole mussel	R1	74.00^{a}	3.94^{c}	1.87^{b}	1.34^{c}
	R2		4.06^{c}	1.88^{b}	1.20^{c}
Half mussel	R1	72.24^{a}	3.04^{b}	1.57^{a}	1.13^{b}
	R2		3.00^{b}	1.31^{a}	1.02^{b}

* Mean of 3 measurements; means with the same letter are not significantly different from each other at 95% confidence level

Product Yield, Fat Content and Fracturability of Vacuum Fried Shellfish Products

Table 3 presents the product yield, fat content and fracturability of vacuum fried raw and cooked prawns, cooked shrimp, cooked whole and half mussels processed at a frying temperature of 80±1°C, chamber pressure of 0.4±0.1 kPa and 50 minutes frying time. The vacuum fried cooked prawn (25.45%) and cooked whole mussel (27.85%) gave the highest product yields while the cooked shrimp (14.08%) yielded the lowest. When the raw prawn is cooked (steamed) before vacuum frying, it decreased its initial moisture content thereby increasing its solids content resulting in higher product yield. Furthermore, when the whole mussel was cut in half, its surface area increased resulting in some moisture evaporation and therefore increased its solids content and its product yield.

Table 3. Product yield, fat content and fracturability of vacuum fried peeled raw and cooked prawns, peeled cooked shrimp, shelled cooked whole and half mussels processed using 500g frozen sample for each run at a frying temperature of 80 ± 1°C, chamber pressure of 0.4 ± 0.1 kPa and frying time of 50 minutes with centrifugation of fried samples under the same chamber pressure at 670 rpm for 4 minutes.

Sample	Replication	Product yield (%)	Fat content* (% wet basis)	Fracturability**
Raw prawn	R1	18.33	25.41^{b}	1.77^{c}
	R2	18.59	24.86^{b}	1.77^{c}
Cooked prawn	R1	27.06	28.03^{c}	0.95^{b}
	R2	23.83	25.99^{bc}	0.92^{b}
Cooked shrimp	R1	13.26	25.83^{bc}	0.87^{ab}
	R2	14.90	26.48^{c}	0.65^{a}
Whole mussel	R1	27.97	25.42^{b}	0.96^{b}
	R2	27.73	24.76^{b}	0.93^{b}
Half mussel	R1	30.90	21.10^{a}	0.66^{a}
	R2	31.54	22.70^{a}	0.69^{a}

*Mean of 3 measurements; ** mean of 5 measurements; means with the same letter are not significantly different from each other at 95% confidence level

The vacuum fried cooked half mussel (21.90 wet basis) yielded the lowest fat content while both the cooked prawn (27.02% wet basis) and shrimp (26.16% wet basis) gave the highest. Generally, the fat contents of all the vacuum fried products were relatively similar except for the cooked half mussel. Pan et al. (2015) reported that vacuum fried breaded shrimp processed at 80°C and 12 kPa pressure had a fat content of 7.5% wet basis at a moisture content of 9.1% wet basis. The lower fat content observed was due to the breading of the shrimp which restricted fat absorption during frying. When the raw prawn is cooked (steamed) and vacuum fried, its final moisture content decreased resulting in higher fat content. Vacuum fried products with higher moisture content usually will have lower fat content (Diamante, Savage & Vanhanen, 2013; Diamante, Savage, Vanhanen & Ihns, 2012; Tan & Mittal, 2006; Song, Zhang & Mujumdar, 2007; Shyu & Hwang, 2001). As pointed out earlier, when the whole mussel was cut in half, its surface area increased which resulted in the efficient centrifugation of oil resulting in lower fat content of the product.

The vacuum fried cooked half mussel (0.68 kg force) gave the lowest fracturability indicating a crunchier product while the cooked raw prawn (1.77 kg force) yielded the highest value which would be less crunchy. Pan et al. (2015) reported that the hardness of the vacuum fried breaded shrimp at 80°C and 12 kPa pressure was about 0.47 kg force (4.6 N) which was lower than that obtained from this study for the vacuum fried cooked shrimp of 0.76 kg force. The difference observed was due to the removal of the crust from breaded shrimp before texture measurement thereby exposing a high moisture and softer flesh resulting in a lower hardness value. When the raw prawn was cooked and then vacuum fried, it resulted to a product with lower fracturability due to its lower final moisture content. As shown in the previous section, the vacuum fried raw prawn had an average final moisture content of 2.72% wet basis while the cooked prawn had only 1.47% wet basis. Generally, the lower the moisture content of the vacuum fried products the crunchier their texture (Song et al., 2007; Shyu & Hwang, 2001). Furthermore, when the whole mussel was cut in half, its surface area increased resulting in lower final moisture and hence crunchier product.

Colour Properties of Vacuum Fried Shellfish Products

Table 4 shows the L*, a* and b* colour values, chroma and hue angle of vacuum fried raw and cooked prawns, cooked shrimp, cooked whole and half mussels processed at a frying temperature of 80±1°C, chamber pressure of 0.4±0.1 kPa and 50 minutes frying time. The vacuum fried raw prawn had the highest L* (74.72) value indicating a lighter colour while the cooked whole mussel had the lowest L* (35.91) value suggesting a darker colour. While the highest a* value was obtained by the vacuum fried cooked shrimp (20.89) and the lowest a* value was that of the cooked whole mussel (2.21). Lastly, the vacuum fried cooked prawn had the highest b* value (34.55) while the cooked whole mussel had the lowest b* value (13.51). When the raw prawn is cooked (steamed) and vacuum fried, its L* value decreased but its a* and b* values increased due to the effect of heating resulting in the colour change of the flesh. In addition, when the whole mussel was cut in half, it resulted to the increase in the L*, a* and b* values due to the increase in its surface area giving a significant color change of the flesh.

The highest chroma were obtained by the vacuum fried cooked prawn (38.01) and cooked shrimp (35.08) while the lowest chroma was attained by the cooked whole mussel (13.69). The high chroma values was brought about by the higher a* and b* color values as shown in both the cooked prawn and shrimp samples which were more reddish and yellowish in colour. When the raw prawn is cooked (steamed) before vacuum frying, its flesh turned reddish in colour and then when it was vacuum fried it further increased its redness and therefore its chroma. In addition, when the whole mussel was cut in half, its surface area increased thereby increasing to more reddish and yellowish changes and hence its chroma.

The vacuum fried whole mussel (80.73°) and half mussel (77.51°) had the highest hue angle and the cooked shrimp (54.44°) had the lowest value. The high hue angle values were brought about by the higher b* value against the a* value as shown in both the cooked whole and half mussels which were more yellowish in color. When the raw prawn is cooked (steamed) and vacuum fried, their a* and b* colour values increased resulting in the hue angle decrease. Furthermore, when the whole mussel was cut in half, its surface area increased thereby increasing the a* and b* colour values which also decreased the hue angle.

Table 4. L*, a* and b* colour values, chroma and hue angle of vacuum fried peeled raw and cooked prawns, peeled cooked shrimp, shelled cooked whole and half mussels processed using 500g frozen sample for each run at a frying temperature of 80 ± 1^{o}C, chamber pressure of 0.4 ± 0.1 kPa and frying time of 50 minutes with centrifugation of fried samples under the same chamber pressure at 670 rpm for 4 minutes.

Sample	Replication	L* value** (no units)	a* value** (no units)	b* value** (no units)	Chroma** (no units)	Hue angle** (degree)
Raw prawn	R1	74.69^{a}	7.14^{c}	28.15^{b}	29.04^{b}	75.78^{b}
	R2	74.75^{a}	7.79^{c}	26.61^{b}	27.73^{b}	73.69^{b}
Cooked prawn	R1	66.53^{a}	15.91^{a}	34.94^{a}	38.39^{a}	65.52^{c}
	R2	68.93^{a}	15.76^{a}	34.16^{a}	37.62^{a}	65.24^{c}
Cooked shrimp	R1	72.83^{a}	17.98^{a}	28.31^{b}	33.54^{a}	57.58^{d}
	R2	66.47^{a}	23.80^{a}	29.70^{b}	38.06^{a}	51.30^{d}
Whole mussel	R1	35.83^{c}	2.23^{d}	13.29^{d}	13.48^{d}	80.49^{a}
	R2	35.99^{c}	2.18^{d}	13.72^{d}	13.89^{d}	80.97^{a}
Half mussel	R1	40.81^{b}	6.23^{c}	25.53^{c}	26.27^{c}	76.29^{b}
	R2	39.49^{b}	4.78^{c}	24.00^{c}	24.47^{c}	78.73^{b}

** mean of 5 measurements; means with the same letter are not significantly different from each other at 95% confidence level

Rehydration Properties of Vacuum Fried Shellfish Products

A vacuum fried shellfish product can be a ready-to-eat product or it can be incorporated in instant noodles with shellfish flavor. Hence, the rehydration properties such as the rehydration rate and ratio are important properties for the vacuum fried products as a noodle ingredient. The rehydration rate and ratio of vacuum fried raw and cooked prawns, cooked shrimp, cooked whole and half mussels processed at a frying temperature of 80±1°C, chamber pressure of 0.4±0.1 kPa and 50 minutes frying time is shown in Table 5. The vacuum fried cooked shrimp had the highest rehydration rate (32.84%) while the vacuum fried cooked prawn had the lowest value (13.66%). When the raw prawn is cooked (steamed) before vacuum frying, the prawn tissue had already shrunk due to the heating process and then it further shrunk due to the additional heating during the vacuum frying resulting in lower rehydration rate. On the other hand, cutting the whole mussel in half did not affect the rehydration rate.

The highest rehydration ratio was obtained for the vacuum fried cooked shrimp (1.99 kg rehydrated product/kg dried product) while the lowest value was that of vacuum fried cooked prawn (1.41 kg rehydrated product/kg dried product). Again, when the raw prawn is cooked (steamed) before vacuum frying, the prawn tissue had already shrunk due to the heating process and then it further shrunk due to the additional heating during the vacuum frying resulting in lower rehydration ratio. While the cutting of the whole mussel in half did not also affect the rehydration ratio.

Table 5. Rehydration rate and ratio of vacuum fried peeled raw and cooked prawns, peeled cooked shrimp, shelled cooked whole and half mussels processed using 500g frozen sample for each run at a frying temperature of 80 ± 1°C, chamber pressure of 0.4 ± 0.1 kPa and frying time of 50 minutes with centrifugation of fried samples under the same chamber pressure at 670 rpm for 4 minutes.

Sample	Replication	Rehydration rate** (% /min)	Rehydration ratio** (kg rehydrated/kg dried product)
Raw prawn	R1	22.02^{b}	1.66^{b}
	R2	22.50^{b}	1.68^{b}
Cooked prawn	R1	12.05^{e}	1.36^{d}
	R2	15.27^{d}	1.46^{cd}
Cooked shrimp	R1	32.48^{a}	1.97^{a}
	R2	33.20^{a}	2.00^{a}
Whole mussel	R1	16.58^{cd}	1.50^{c}
	R2	16.92^{cd}	1.51^{c}
Half mussel	R1	17.96^{c}	1.54^{c}
	R2	18.09^{c}	1.54^{c}

** Mean of 5 measurements; means with the same letter are not significantly different from each other at 95% confidence level

Implications of the Results

When vacuum frying shellfish samples (prawn, shrimp and mussel), frying temperatures should be limited to 80°C when processing at longer frying times in order to achieve low final moisture for the products and obtain an acceptable crunchiness. The suggested temperature was to avoid the undesirable colour changes on the vacuum fried shellfish products.

Cooking (steaming) the raw prawn before freezing and then vacuum frying obtained a product with low final moisture content, higher product yield and low fracturability value and hence a crunchier product. The average fat content of both vacuum fried products was very close to each other.

However, the rehydration rate and ratio of this vacuum fried product both decreased which means that it is not good a noodle ingredient. The vacuum fried cooked prawn was darker, more reddish but less yellowish in colour.

Cutting in half the frozen cooked whole mussel before vacuum frying also gave a product with low final moisture content, higher product yield and low fracturability value and hence a crunchier product. The average fat content of the vacuum fried cooked half mussel was lower than that for the whole mussel product. But the rehydration rate and ratio of both vacuum fried products were similar and therefore both are not good noodle ingredients. The vacuum fried cooked half mussel was lighter, more reddish but less yellowish in colour.

Vacuum frying of frozen cooked shrimp provided a product with low final moisture content and low fracturability value and hence a crunchier product but a low product yield because of the high initial moisture content of the sample. The average fat content of the vacuum fried product was similar to that of vacuum fried cooked prawn. In addition, the vacuum fried product had high rehydration rate and ratio indicating that it is good a noodle ingredient. The vacuum fried cooked shrimp was similar to cooked prawn in terms of lightness and redness but less yellowish in colour.

CONCLUSION

The vacuum frying of frozen cooked shellfish samples (prawn, shrimp and mussel) can be carried out at 80°C frying temperature, 0.4 kPa of chamber pressure and 50 minutes frying time.

The vacuum fried cooked prawn, shrimp and half mussel had the lowest final moisture contents while the vacuum fried raw prawn had the highest.

The vacuum fried cooked half mussel had the highest product yield while the vacuum fried cooked shrimp had the lowest. The average fat content of the vacuum fried raw and cooked prawns, cooked shrimp, as well as cooked whole mussel were very close to each other but the vacuum fried cooked half mussel had the lowest. The vacuum fried cooked shrimp and half mussel had the lowest fracturability and therefore the crunchiest while the vacuum fried cooked raw prawn had the highest and would be less crunchy.

The vacuum fried cooked whole mussel had lighter colour but the vacuum fried raw prawn had darker colour. The vacuum fried raw prawn had the highest chroma value and hence the most reddish colour while the vacuum fried cooked whole mussel had the lowest. The vacuum fried cooked whole mussel had the highest hue angle value thus the most yellowish colour but the vacuum fried cooked shrimp had the lowest.

The vacuum fried cooked shrimp had the highest rehydration rate and ratio and therefore had the best rehydration abilities while the vacuum fried cooked prawn had the lowest values.

ACKNOWLEDGMENTS

The Project was financed from funds of the New Zealand Ministry of Science and Innovation (PROP-20729-LNNR-LIN). The authors would like to thank the Department of Wine, Food and Molecular Biosciences, Lincoln University for providing the research facilities.

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Natural Plant Pigments and Derivatives in Functional Foods Developments

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Abstract

Food colour contributes to food acceptability. Hitherto, colours for foods are obtained from artificial sources or chemicals. However, there is a gradual shift in sourcing food colouring materials from artificial sources to natural pigments. This was meant to utilize functional properties in natural pigments such as bioactive activities, anticancer potentials, production of vitamin A, and so on in addition to enhancing consumers' acceptability. Some of the functional compounds in natural pigments are polyphenols, antocyanins, chlophyll a & b, carotenoids, betalains, and so on. These compounds possess potent antioxidants, antidiabetics, vasoprotective, anti-inflammatory, anti-cancer, chemoprotective and anti-neoplastic properties. Carotenes serve as precursor of vitamin A. Isolation and utilization of natural pigments will prevent side effects notable in artificial colouring agents in addition to reducing the prevalence of some diseases like diabetics, cancer and cardiovascular disease. The functionalities of these natural compounds in foods promotes health of the consumers.

Keywords: Antioxidants; Carotenoids; food colours; functional properties; natural pigments; vasoprotective.

Review article
Received date: 23 Jaunary 2021
Accepted date:28 April 2021

INTRODUCTION

Food Pigments

Food pigments are substance in food that determine colour of food and or can be used to colour foods. Colour of food influence consumer acceptability of a particular food. First colouring agent was produced from natural source (Petropolous *et al*., 2019), example is the pigments from beet root, but was later abandon due to high cost of production, and variation in resulting colour. However, natural source of colour additives is gaining interest nowadays due to several health deteriorating issues resulting from the use of synthetic colouring agents. Pigments of natural source has been reported to contribute to the overall antioxidant capacity in a dose-dependent and compound-specific manner (McGill, 2009; Agcam *et al* 2017; Petropoulos *et al*., 2019). Important compounds in natural pigments are some polyphenols, carotenoids, chlorophylls and betalains (Shoji, 2007; Aberoumand, 2011).

There are other types of pigment made by modification of plant components such as protein, amino acids, lipids and sugars which result in pigments such as caramel, vegetable carbon and Cu-chlorophyllium (Aberoumand 2011).

Polyphenols are secondary metabolites of plants, which are usually classified into non-flavonoids and flavonoids based on their structure. Non-flavonoids are mostly colourless and do not contribute to the pigment in plant while most flavonoids do. Main flavonoids that contribute to pigment are flavonols, flavones and anthocyanin. Flavonols and flavones are yellow whereas anthocyanins can be orange, red, blue or purple in colour. Flavonols and flavones generally have relatively low solubility in water and this reduces its contribution to food colour as compared to anthocyanins (Shoji, 2007). Xanthones and quinones are flavonoids that also exist in plants as minor pigments.

Anthocyanins pigment provide the red, purple, blue, and pink shade of many fruits, vegetables and flowers and participate in various physiological processes including photosynthesis and pollination by attracting pollinators (Menzies *et al*., 2016; Petropoulos *et al*., 2019). Anthocyanins are glycosides and acylglycosides of anthocyanidins. There are about 250 naturally occurring anthocyanins in form of delphinidin, cyanidin, pelargonidin, marvidin and peonidin and all are o-glycosylated with different sugar substitute. The common anthocyanins are 3- or 3,5-glycosylated (Wang *et al*.,1997; Espin *et al*., 2000). A single anthocyanin can produce various tones and strength of colours in different flowers. Anthocyanins has been detected in appreciable quantity in many fruits and vegetables which include.

Chlorophylls are green pigments found in all higher plants which take part in the photosynthesis. Chlorophyll is a macrocyclic tetrapyrrole with coordinated magnesium in the center. There are two types of chlorophyll (chlorophyll a and b) found in green plant (Ngamwonglumlert *et al*., 2015; Rodriguez-amaya, 2018). Chlorophyll a and b contain methyl group (CH_3) and formyl group (CHO) respectively on carbon C-3 of tetrapyrrole ring of the chlorophyll structure. Chlorophyll a appears blue-green and chlorophyll b yellow-green (Rodriguez Amaya, 2018). Derivatives of chlorophyll a and b due to degradation by heat (Rodriguez-Amaya, 2018) are pheophytin (formed by replacement of magnesium atom by hydrogen), pyrophophytin (formed by replacement of carboxymethoxy group at C-10 by hydrogen), chlorophyllide (formed by removal of phytol at C-7), pheophorbide (removal of phytol and magnesium), pyropheophorbide (removal of phytol, magnesium and carboxymethyl group) (Von Elbe and Schwartz, 1996; Rodriguez-Amaya, 2018). Loss of chlorophyll pigment in thermally processed green vegetables has been reported to be due to formation of pheophytin and pyropheophytin derivatives of chlorophyll giving olive brown colour (Ngamwonglumlert *et al*., 2015; Rodriguez-Amaya, 2018). Chlorophyll b has been reported to be more heat stable than chlorophyll a. (Vonelbe and Steven Schwartz 1996). The stability was attributed to electron-withdrawing effect of its C-3 formyl group (Rodriguez Amaya, 2018).

Carotenoids are also part of photosynthetic process and are nature's most widespread pigments. Carotenoids, mostly identified with yellow-orange colour of many plants (Petropoulos *et al.,* 2019) serves as secondary pigments to harvest light energy in all chlorophyll-containing tissues (Ngamwonglumlert *et al*., 2015; Petropoulos *et al*., 2019). Some carotenoids are bound with chlorophyll which gives rise to a variety of attractive colours in plants, fruits and vegetables (Rao and Rao, 2007). Carotenoids are generally of two types, carotenes containing only carbon and hydrogen, and xanthopyll made up of carbon, hydrogen and oxygen (Mortensen, 2006). Common carotenoids in plants are α-carotene, β-carotene, capsanthin, lutein, lycopene, zeaxanthin. Animals obtained carotenoid from plant, human body cannot synthesis carotenoid but are accumulated in some organs and tissues.

Betalains composed of a nitrogenous central structure, betalmic acid, glucosyl derivatives or aminoacid derivatives. Betalmic acid condenses with imino compounds (like cyclo-L-3,4-dihydroxy-phenylalanine) to form variety of colorful pigments (Qaisar *et al*., 2019). Betalain pigment is an important natural pigment earlier developed from beet roots for use in food industry.

Colouring agents and additives from natural source is gaining more interest food industries due to health benefit to be derived from such. There had been increase in research focusing on natural pigments source, extraction, stability during processing, health benefits and limitation to the use of such pigments in food production. Hence, this study is to review some pigments with special emphasis on their functional properties. The sources of such pigments were also reviewed.

FUNCTIONAL FOODS

Functional foods (nutraceuticals) are whole, fortified or enhanced foods that can provide health benefits beyond the provision of essential nutrients (vitamins and nutrients) when they are consumed. In addition to improving the well-being of consumers, they also reduce risks of diseases (Coisson *et al*., 2005). Functional foods have a potentially positive effect on health beyond basic nutrition (Coisson *et al*., 2005). The functionality of functional foods is as a result of the presence of bioactive compounds in them. Bioactive compounds (also known as nutraceuticals) have been defined as essential and nonessential food compounds (carotenoids, essential oils, antioxidants, flavours, pigments, vitamins, polyphenols) that occur naturally and have an effect on human health (Biesalski *et al*., 2009). A vital benefit of nutraceuticals is that they are associated with a lower risk of chronic diseases (Biesalski *et al*., 2009).

Sources of bioactive compounds include, fruits, vegetables, vegetable oils, essential oils, plant by-products, plants, have been discovered to be rich sources of natural bioactive compounds (Awolu, Ojo, Igbe *et al*., 2017; Ajila *et al*., 2007). Other sources are, peels, stems, seeds, shells, bran and residues remaining after extraction of juice, oil, starch and sugar (Awolu *et al*., 2017; Baiano, 2014).

COMMON PLANT PIGMENTS AND FUNCTIONALITIES

Common colourants, their chemical classifications, source and E numbers are presented in Table 1. They include anthocyanins, betalains, caramel, carotenoids, chlorophyll and curcuminoids.

Anthocyanins pigments

Anthocyanins are recognized as natural alternatives for dyes, though commercial use as dye may not be economically feasible. Anthocyanins are reported to be used as functional compounds for colouring food and as a potent agent against oxidative stress (Downham and Collins, 2000). It prevented lipid peroxidation of cell (Noda *et al*., 2002). Studies have revealed that anthocyanins possess vasoprotective, anti-inflamatory, anti-cancer, chemoprotective, and anti-neoplastic properties (Awika *et al*., 2004) thereby showing a protective effect against coronary diseases. Researchers has established that natural anthocyanin-based pigments extracted from black-chokeberry (623 μg/mg), black-thorn (151 μg/mg), strawberry (54.8 μg/mg) and elderberry (5 μg/mg) serves as colourant and at the same time possess antiradical properties (Espin *et al*., 2000).

Anthocyanins has been reported to be effective in the treatment of diabetic retinopathy, in fibrocystic disease of the breast in human and in treatment of various microcirculation diseases resulting from capillary fragility (Wang *et al*., 1997). Anthocyanin have increased stability at reduced water activity, therefore, they are suitable for colouring dried and intermediate moisture foods.

Table 1. Major colorants from natural sources (or nature identical): chemical classification sources, colours, and regulatory status in the United States and European Union (Sigurdson *et al*., 2017).

Chemical classification	**Additive**	**Source**	**Color**	**Use in the United States**	**E number**
Anthocyanin	Grape skin extract	Grapes (marc from pressing)	Red to purple to blue	Beverage food	E163
	Grape color extract	Concord grapes (*Vitis labrusca*)		Non-beverage	
	Fruit juice	Berries		General	
	Vegetable juice	Carrot, cabbage, and others		General	
Betalain	Beet powder	Beet (*Beta vulgaris*)	Pink to red	General	E162
Caramel	Caramel	Heating of sugars	Brown	General	E150
Carminic acid	Cochineal extract	*Dactylopius coccus*	Orange to red	General	E120
Carotenoid	Annatto	*Bixa orellana* L.	Yellow to orange to red	General	E160b
	Astaxanthin	Synthesized, bacterium (*Paracoccus carotinifaciens*), yeast (*Phaffia rhodozyma*), algae (*Haematococcus pluvialis*)		Fish feed	NA
	β-Carotene	Natural (carrots) or synthesized		General	E160a
	Canthaxanthin	Synthesized		Fish feed and chicken skin	E161g
	Carrot oil	Carrot (*Daucus carota* L.)		General	NA
	Corn endosperm oil	Yellow corn (*Zea mays*)		Chicken feed	NA
	Paprika (oleoresin)	Paprika (*Capsicum annum* L.)		General	E160c
	Paprika			General	E160c
	Saffron	Stigma (*Crocus sativus* L.)		General	NA
	Tagetes	Aztec marigold (*Tagetes erecta* L.)		Chicken feed	NA
	Tomato lycopene	Tomato (*Solanum lycopersicum*)		General	E160d
	β-Apo-8^{r}-carotenal	Synthesized		General	E160a
Chlorophyll	Sodium copper chlorophyllin	Alfalfa (*Medicago sativa*)	Green	Citrus-based dry beverage mixes	E141
Curcuminoid	Turmeric (oleoresin)	Rhizome (*Curcuma longa* L.)	Yellow	General	E100

Carotenoids pigments

Common carotenoids in plants are α-carotene, β-carotene, capsanthin, lutein, lycopene, zeaxanthin with β-carotene as the most common carotenoid in plant tissue and is commonly used as colourant in foods.

Carotenoid serves as precursor of vitamin A with carotenoids containing β rings having greatest provitamin A activity. Provitamin A carotenoids are converted into retinol and other related retinols, which play important roles in the visual cycle and in gene regulation, in mammals (Roridguez *et al*., 2018). High intake of carotenoids has been reported to promote cognitive function, improve immune system and lower the risk of developing cardiovascular and degenerative chronic diseases such as cancer and macular degeneration (Voutilainen *et al*., 2006; Rao and Rao, 2007; Rodriguez-Amaya, 2018). Action of carotenoids against diseases has been attributed to their antioxidants ability to scavenge free radicals that cause oxidative damage which result from such diseases. Carotenoids have also been reported to possess some non-antioxidants mechanisms such as retinoid-dependent signalling, modulation of carcinogen metabolism, regulation of cell growth, modulation of DNA repair mechanisms (Stahl *et al*., 2002; Rao and Rao, 2007; Rodriguez-Amaya 2018).

Chlorophylls pigments

Artificial chlorophyll pigments, chlorophyllin, is commonly used as food-colouring agents in sugar confectionaries, desserts, sauces and condiments, cheese and soft drinks (Inanc, 2011). Chlorophyll from plant source has been reported to have positive effect when used to treat wound and inflammation. Studies have shown that chlorophyll form complexes with certain chemicals that cause cancer and the complex structure formed interfere with gastrointestinal absorption of potential carcinogen, thereby reducing the amounts of carcinogen substances in susceptible tissue (Breinholt *et al.*, 1995; Egner *et al*., 2003; Cing-Yun *et al*., 2008; Inanc 2011). Several researchers (Hoshina *et al*., 1998; Ferruzzi *et al*., 2002) reported that chlorophyll and its derivatives support antioxidants, however, other researchers reported that antioxidant activity of chlorophyll is light dependent as it has pro-oxidants effects in the presence of light (Wanasundara and shahidi 1998; Inanc 2011). Lanfer-Marquez et al. (2005) reported effective protection of chlorophyll derivatives, pheophorbide b and pheophytin b, against linoleic acid using β-carotene bleaching method compare with BHT. However, when compared with Trolox using DPPH assay, all chlorophyll derivatives were reported to have low antioxidants activity (Lanfer-Marquez et al., 2005). Chlorophyll a pigment showed the weakest antioxidant activity among all the greenish pigments the researchers observed. Other healthy importance of chlorophyll include ability to rejuvenate and energize the body, detoxification of the liver, cleaning of the intestine, ability to normalize blood pressure and ability to combat bad odours, bad breath and body odour due to magnesium it contains. (Ferruzzi *et al*., 2007; Inanc, 2011).

Betalains pigments

Betalains pigments are water soluble and have been reported to maintain their colour over a wide range of pH, from 3 to 7, making it suitable for use in colouring food in the pH range (Aberoumand, 2011; Qaisar *et al.*, 2019). Betalains are of different type which are betanin, betacyanin, amaranthine, betacyanin, betaxanthin, vulgasanthin, gomphrenin. Betanin has been used to colour foods such as youghurt, confectionery, ice creams, syrups etc though the usage is limited by the typical earthy flavour.

Betacyanins type of betalains are the main compound associated with the red colour exhibited by flowers, fruits and other plant tissue (Aberoumand, 2011).

Structure of Betacyanin is made up of glycosides of the aglycones betanidin/isobetanidin. This pigment has been said to be nutritionally importance to reduce the risk of coronary disease.

PLANT SOURCES FOR FUNCTIONAL PLANTS PIGMENTS

Pigments in Sweet Potato

Anthocyanin pigment extracted from sweet potato were more than 15 consisting mainly of peonidin, cyanidin and pelargonidin derivatives (Stinizing *et al*, 2002; Petropoulos, 2019). Purple fleshed sweet potatoes contain acylated anthocyanins making it suitable for use as colourants (Esatbeyoglu *et al*., 2017; Petropoulos et al., 2019). Anthocyanin pigments extracted from sweet potato had no negative flavour and may be used in place of red cabbage (Stinizing *et al*, 2002). Antioxidant activity of sweet potato has been reported to be due to the anthocyanin contents of the plant (Kubow *et al*., 2016). Sun *et al*. (2018) reported a prebiotic-like activity of anthocyanins from sweet potatoes through the modulation of microbiota in the intestine. Anthocyanin from purple potato was reported to inhibit tyrosine kinase activity thereby, said to be effective against colon cancer cells (Mazewski *et al*., 2018).

Teow *et al*. (2007-564) evaluated white, yellow, orange and purple skinned sweet potato for antioxidant capacity by ORAC, DPPH and ABTS. The researchers established that purple and orange sweet potato had detectable total anthocyanin with the purple sweet potato having the highest value. Purple sweet potato also possesses highest hydrophilic antioxidant activity with ORAC assay having the highest value and DPPH having the least value; implying that anthocyanin contribute significantly to antioxidant activity of sweet potato (Teow *et al*., 2007).

Orange fleshed sweet potato had the highest β-carotenoid. Orange sweet potato also possess highest lipophilic antioxidant activity which was related to high level of carotenoids contents.

Pigments in Black Sorghum

Sorghum is cereal with significant amount of anthocyanin pigment which compare well to what is found in fruits and vegetables. Significant amount of anthocyanins pigment is concentrated in the bran of black sorghum (Awika, 2004). Anthocyanin contents in the bran was reported to be three to four times the amount in the grains and higher (4.0-9.8mg/g) than anthocyanin level obtained from several commercial sources (usually fruits) except for Elderberry (2.0-10.0 mg/g) which were almost in the same range with sorghum (Awika, 2004).

Several anthocyanin including apigeninidin, apigeninidin-5-glucoside, luteolinidin, luteolinidin-5-glucoside, cyanidin and pelargonidin has been isolated from sorghum. Anthocyanin most commonly found in black sorghum is 3-deoxyanthocynidins and it exist in nature as aglycones (Clifford, 2000) consisting of luteolinidin and apigeninidin. 3-deoxyanthocynidins is said to be stable in acidic solution with improve stability over anthocyanin pigment commonly found in fruits and vegetables due to lack of oxygen at C-3. Awika (2004) recommended extraction of sorghum antioxidant using acidified methanol for high antioxidants activity.

Anthocyanins in black sorghum (3-deoxyanthocynidins) contribute greatly to high antioxidant activities observed in black sorghum and the antioxidant activities were similar to activities of anthocyanins found in fruits and vegetables. Lower quantity of sorghum bran may be required for antioxidant compare with bran of other cereals (wheat, barley, rice) currently use as antioxidant in various food products. The pigment in black sorghum bran due to 3-deoxyanthocyanidin imparts natural dark appealing colour and is more stable than pigment obtained from other source of anthocyanin making resulting colour superior to others (Awika, 2004). Pigment extracted from sorghum bran have been proven to produce acceptable quality colour in bread and cookies (Gordon, 2001; Mitre-Dieste *et al*., 2000)

Pigments in Pomegranate fruit

Major anthocyanin pigments in pomegranate are delphinidin, cyanidin and pelargonidin (Noda *et al*, 2002). The main anthocyanin in the juice is delphinidin-3,5-diglucoside while other anthocyanin such as cyanidin, pelargonidin and even delphinidin 3 and 3,5-glycosylated can be found in the seed coat. Pomegranate extract was reported to show potent radical scavenging activity against superoxide which was reported to be largely due to presence of delphinidin, a major component of pomegranate juice and partly cyanidin which is present in the seed coat (Nodal *et al*, 2002). Nodal et al. (2002) also reported that superoxide radical scavenging activity and H_2O_2-induced lipid peroxidation in rat brain homogenate of anthocyanin were highest in delphinidin, followed by cyanidin and was least in pelargodin. Anthocyanidin was reported not to directly scavenge hydroxyl radical but showed apparent hydroxyl radical scavenging activities which was said to be due to chelating of ferrous ion in the hydroxyl radical of delphinidin and cyanidin (Nodal *et al*.,2002). Anthocynidin in pomegranate extract show superoxide radical scavenging activity value of 16±2 lower scavenging activities when compare to scavenging value of pure dephinidin, cyanidin and pelargonidin which are 1360, 240, and 52 SOD-equivalent units/mg respectively indicating that anthocyanidin in pomegranate did not effectively scavenge nitric oxide (NO) (Nodal et al., 2002)

Pigments in tomatoes

Pigments in tomatoes are chlorophyll, β-carotene and lycopene though the content of this pigment depends on the maturity of tomatoes (Kozukue and Friedman, 2003). Tomato has been reported as the best source of lycopene which is orange in solution. Lycopene has been reported as one of the most effective antioxidants having twice antioxidant activity when compared with β-carotene (Mezzomo and Ferreira, 2016; Clinton S. K., 1998). Boileau et al. (2003) reported that lycopene fed rats show high blood lycopene content with reduced risk of prostate cancer death in rat fed with tomato powder. This may be an indication that consumption of lycopene and tomato products may favourably influence markers of oxidative stress, prostate specific antigen or tissue biomarkers (Chen et al., 2001; Hadly et al., 2003). Studies have also shown that lycopene lower the risk of chronic degenerative disease and cardiovascular disease, as well as, risk of developing prostate, lung and ovarian cancers (Rao, 2002; Cramer et al., 2001). Lycopene pigment mainly from tomato is hardly use commercially as colourant because it is expensive and is more prone to oxidative degradation than β-carotene (Mortensen, 2006).

Pigments in Red Pepper (Capsicum)

Red pepper is known to impact red colour and pungency to food items (Arimboor *et al*., 2014). Pigments found in red pepper include capsanthin, capsorubin, betacarotene, betacryptoxanthin and zeaxanthin with capsanthin and capsorubin been the most dominant pigments in red ripe pepper (Deli *et al*., 2001; Marin *et al*, 2004; Kim *et al*.,2016; Hassan *et al*., 2019). Carotenoids, especially capsanthin, content of red pepper significantly increase with fruit ripening (Hassan *et al*., 2019). Capsanthin accounts for almost 80% of total carotenoids in red pepper and possesses higher bioaccessibility and stability than other carotenoids of red ripe pepper (Pugliese *et al*., 2013; Hassan *et al*., 2019). Carotenoids pigments of red pepper has been reported to possess good antioxidants activity with increasing activity at advance stages of ripening (Cervantes-Paz *et al.*, 2012; Hassan *et al*., 2019).

Capsanthin and capsorubin are the most powerful antioxidant pigments in red pepper. Capsorubin and capsanthin are reported to possess stronger lipid peroxidation than β-carotene (Maoka *et al*., 2002) while capsorubin have relatively higher antioxidant activity than capsanthin (Nishino *et al*., 2015).

High level of the capsanthin and beta-carotene has been reported to possess stronger antioxidant activity in term of DPPH free radicals scavenging and reducing power activities (Bae *et al*., 2012; Hassan *et al*., 2019). Free radical scavenging activity of red pepper is dependent of the ratio and constituent of the carotenoids. Thermal treatment of pepper during postharvest handling and processing also influence antioxidants activity of the carotenoid pigments present (Hassan *et al*., 2019; Cervantes-Paz *et al.*, 2014; Topuz *et al*., 2011; Sayin and Arslan, 2015; Ornelas-Paz et al., 2013). Studies have also shown that carotenoid pigments of red pepper especially capsanthin possess antiadipogenic, antiobesity activities. Jo *et al*., (2017) reported capsanthin of red ripe pepper to have potential insulin sensitizing activity in high fat diet-induced obesity mouse models. Antiadipogenic activity of capsanthin in red pepper was reported to be superior to activity observed in other carotenoids pigments including capsorubin, betacarotene, betacryptoxanthin and zeaxanthin (Jo *et al*., (2017). Commercial natural pigment made from capsanthin and other carotenoid pigments of pepper is called paprika. Paprika pigment is commonly used as flavouring and colouring agents in food industries with little or no side effects (Palma and Robert, 2019). Paprika pigment is cheaper, more stable and produce similar colour shade as lycopene (Mortensen, 2006).

Pigments in palm fruits

Carotenoids and chlorophyll are the pigments found in oil palm fruits depending on the age of maturity (Sundram *et al*.,2003). Red palm oil is extracted from palm fruit with carotenes, lycopene and Xanthophylls as the carotenoid pigments. However, carotenes (β and α carotene) is the major carotenoid pigment in palm oil which contribute about 90% of the total carotenoids (Mortensen, 2006; Yap *et a*l., 1997) making palm oil the richest source of carotene among other vegetable oils. Carotene pigments imparts an orange-red colour to crude palm oil. Provitamin A activity of palm oil is reported to be 15 and 300 times more retinol equivalents than carrot and tomato respectively (Sundram *et al*., 2003). Studies has shown that carotene in palm oil has chemopreventive activities against a number of human cancer cells including breast, pancreatic and gastric cancer cells (Nesaretnam *et al*., 2002; Sundram *et al*., 2003).

Studies also exist on inhibitory effect of palm carotene against liver, lung and skin tumor with α-carotene showing higher activity than β-carotene (Nishino *et al*., 1992). However, these chemopreventive activity of carotene in palm oil could not be attributed to synthetic carotene (Sundram *et al*., 2003). Palm oil carotene has also shown high antioxidant activity with different antioxidant assays in dose dependant manner (001-0.1%).

β and α-carotene has shown effective reducing activity and lipid peroxidation with DPPH, superoxide radical scavenger and Metal chelator while only 0.1% concentration was effective using ABTS assay. 0.1% activity inhibit almost 50% lipid peroxidation in lipophilic medium indicating palm carotene can act as substantial chemoprotective agents and prevent harmful physiological activities (Gupta and Ghosh, 2013).

Pigments in beet roots

Betanin from red beet root is marketed as E-162 in the European Union and is commonly used as red food colourant. Studies revealed that betalains from beet roots, identified as betanin, possessed high antiradical effect and antioxidant activity which is stronger than catechin (Kanner et al., 1994).

Betanin at very low concentration was found to inhibit peroxidation and low-density lipoprotein (LDL) oxidation was found to be better than that of catechin. However, there was a conflicting result on antioxidant activity of betalain from beet root according to Zakharova and Petrova (1998). this was reported by Cai et al., (2003) to be due to different purification procedure for betalains samples and antioxidants activity assessment methods. Betalain is said to be good electron donour, made up of monoglucosylated O-diphenol group and a cyclic amine group in their structure. Increase in the number of hydroxyl groups (-OH) or amine group in the molecular structure was said to lead to higher antioxidant activity. The position of the hydroxyl group and glycosylation of aglycon in the chemical structure is also of great importance in determining the activity level. (Kanner et al., 2001; Cai et al., 2003).

Pigments in Amaranthaceae plants

Plants of Amaranthaceae family has been reported to possess total of 16 red-violet betacyanins and three yellow betaxanthins (Cai *et al*., 2003). Evaluating antioxidant activity of these series of betalain from amaranthaceae family using DPPH showed that all betalains exhibited stronger antioxidative power than ascorbic acid. The study also revealed that red-violet gomphrenin type betacyanins and three betaxanthins from amaranthaceae plant have higher antiradical activity than catechin; and are highly efficient natural antioxidants. DPPH radical scavenging activity of the betalain compounds from amaranthacea family was due to different structural features of betalain compounds with their ability to donate hydrogen ion (Cai et al., 2003).

Betacyanin and betaxanthin (from Amaranthus) were also found to have high potential not only as food colourant but as antioxidant which could be a substitute for betalain from beet root (Kanner et al., 2001; Cai et al., 2003). Sarker et al., (2020) reported that green morphological type of amaranth possesses remarkable amount of several pigments which include carotenoids, chlorophyll a, chlorophyll b, betalain and betacyanin. The pigments were said to have strong antioxidants activity and contribute significantly to the antioxidant activity of green morphological type amaranth (Sarker et al., 2020).

In the same vain, red morphological type amaranth was said to contain abundant pigments as found in green morphological amaranths with the addition of betaxanthin, anthocyanin, and amaranthine (Sarker and Oba, 2019). These pigments were said to detoxify free radicals in human body therefore acts as potent antioxidants (Wagnet et al., 2015; Sarker and Oba, 2019). Protection of carotenoids, betalains, betacyanin and betaxanthins against lung and skin cancers and cardiovascular diseases has also been reported which give room for their use as additive and colouring agents in cosmetic products, drugs and food (Isabeller et al., 2010; Sarker and Oba, 2019).

Sarker and Oba (2019) stated that significant positive correlation exists between all the pigments found in red morphological type amaranths and TAC ($ABTS^{+}$), TFC, TPC, TAC (DPPH) which was an indication of strong antioxidants activity of the pigments.

Tumeric pigments

Turmeric has brilliant yellow pigment called curcuminoids. Curcuminoids consist of curcumin and its derivatives- demethoxycurcumin, 5-methoxycurcumimg and dihydrocurcumin which are found to be natural antioxidants (Prasad and Aggarwal, 2011). Turmeric is commonly used as spice at household level to impart yellow colour. Curcumitable 1n extracted from turmeric can be used as preservative, colouring and flavouring agents in the food industry, as well as, a substitute for synthetic colouring agents. Curcumin from turmeric has been reported to have some pharmacological activities including inhibition of various cancer cells, improving colon health and cardiovascular system (Shalaby and Amin, 2018).

Rauf et al., (2018) in their review established from various studies that curcumin, yellow pigment from turmeric has numerous pharmacological activities including anticancer (Liu Wang et al., 2017), antidiabetic (Wang et al., 2017), cardiovascular protective (Li et al., 2017), antiobesity (Ding et al., 2016), antimicrobial (Sarker et al., 2016), wound healing (Hussain et al., 2017), and nephroprotective (Hashish and Elgami, 2016) activities. Curcumin is reported to have strong antioxidant activity which protect from reproductive and respiratory disorders (Radaeva et al., 2004; Gan et al., 2016).

Pigments due to chemical reaction of plant components

Brown Pigment Formation in honey

Turkmen et al., (2006) reported the effect of heating on honey and established that antioxidant activity and brown pigment formation in honey is time and temperature dependent. Heating at 70^{o}C favours antioxidant activity and brown pigment formation than heating at lower temperatures. The studies also establish strong relationship between antioxidant activity and brown pigment formation at different temperature. Antioxidant activity was linear with increase in time and Brown pigment formation at 50 and 60^{o}C but the increase was logarithmic at 70^{o}C. The behaviour was attributed to the formation of different compounds following different pathway during non-enzymatic browning reactions as a result of composition of the product and processing conditions (Manzocco et al., 2001; Vav Boekel, 2001 Turkmen et al., 2006).

Limitations of natural pigments

The application of anthocyanin as food colourant and functional ingredients may be limited to acid foods due to its sensitivity to oxidation, bleaching by Sulphur dioxide and variation with pH. Anthocyanins are also limited by their low stability and interaction with other ingredients in the food matrix (Qasir *et al*., 2019).

Anthocyanins can be affected by the co-existence of other polyphenols and metal ions. Likewise, betalains and chlorophylls are sensitive to light, heat, pH change and oxygen which in turn had adverse effect on the food in which it added during processing, storage and consumer evaluation after storage. Chlorophylls hydrolyze in the acidic medium to give a brown colouration (Qasir et al., 2019). Degradation of chlorophyll usually occur during ripening of fruits, senescence of green vegetables and thermal processing (Rodriguez Amaya, 2018).

Carotenoids is moderately heat stable and subject to loss of colour by oxidation during processing and storage (Arimboor, 2014). Lower pH, presence of oxygen, catalytic metal, oxidative enzymes and unsaturated lipids in food also cause carotenoid degradation (Boon et al., 2010; Gao and Kispert, 2003).

Curcumin usually extracted from turmeric is also limited by its spicy, curry flavour. Some of these pigments also possess earthy flavour which limited their application to some food and additional processing may also be required to remove the flavour. Betanin form beet root possess earthy flavour, riboflavin have bitter taste and curcumin may need to be debittered to avoid its odour and taste. Generally, Sigurdson et al., (2017) also stated that lower stability, weaker tinctorial strength, interactions with food ingredients and inability to match desired hue are all factors that can affect application of natural pigments. Availability of all these pigment for commercial purpose in food industry and the tone of colour obtained is largely dependent on the availability of the source materials and method of extraction of the pigment to obtain pure substance and pH of the food they are in (Qasir et al., 2019).

However, recent studies have shown that acylation of anthocyanin and copigmentation interactions with colourless molecules in solution with anthocyanin can be used to stabilize and strengthen the colour of anthocyanins thereby improving its application (Sigurdson et al, 2017; Malien-Aubert et al., 2012; Paccheco-Palencia and Talcott, 2010). Bioavailability of carotenoids in food is affected by factors such as food matrix characteristics, properties of co-ingested food, generic profile of the host (Fernandez-Garcia et al., 2012). Carotenoids are resistance to heating, sterilization and freezing (Qasir et al., 2019) and are oil soluble.

CONCLUSIONS

Some of the sources and benefits of natural pigments were highlighted. A vital benefit of natural pigments is that they are readily available locally at cheap costs. Some of the raw materials highlighted were sweet potato, black sorghum, pomegranate fruits, tomatoes, beet roots and palm fruits. They are rich sources of polyphenols (flavonoids, flavonones), antocyanins (glycosides, acylglycosides), chlorophyll a&b, carotenoids (carotenes, xanthophyll) and betalains. These compounds are potent antihypertensive, antidiabetics, anticancer, anti-inflamatory and vasoprotective compounds, valuable in fighting coronary diseases.

Conflict of interest Statement

Authors declare there are no conflict of interest

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Adaptive Neural Network Based Fuzzy Inference System for the Determination of Performance in the Solar Tray Dryer

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Abstract

This study aims to apply the adaptive neural network based fuzzy inference system (ANFIS) were used to modeling the apple solar drying conditions in the solar tray dryer. Apple slices were dried by solar drying techniques as a solar tray dryer, exposure to direct sunlight and in the shade. Drying air temperature, the air humidity, apple slice load, apple slice thickness and solar drying time has been investigated with the prediction of the drying in the solar tray dryer on water loss, drying rate and shrinkage ratio. The model results clearly showed that the use of ANFIS led to more accurate results. The correlation coefficient (R^2) values of the water loss, drying rate and shrinkage ratio were found as 0.9968, 0,9675 and 0,9918, the water loss, drying rate and shrinkage ratio respectively.

Keywords: Anfis, Drying, Solar drying, Solar tray dryer

Research article
Received date: 25 January 2021
Accepted date:28 April 2021

INTRODUCTION

Fresh fruits and vegetables are dried to preserve them for a long time without spoiling due to transportation, lack of storage and supply that may exceed the demand. Drying of foodstuffs at high temperatures negatively affects product quality, samples shrink due to loss of water during the drying process and a decrease in volume is observed. However, while the quality properties of various food products are preserved in the traditional drying process by spreading under the sun, drying of the products takes longer than necessary with this drying technique. If the product is in direct contact with the sun, there is a decrease in the color and nutrient values of the product. The drying method with a solar dryer is a suitable method for the economical drying of food products that are heat sensitive and where color quality and sensory properties are important (Demirhan and Ozbek, 2011; Decareau,1992; Askari et al., 2008; Cui et al., 2008).

Apple is the high drying temperature and high moisture content. It is clear that the use of solar dryers provide benefits in a shorter time to achieve a better quality of dry products. Since Mersin is a leading province in both agriculture and high sunshine potential, a significant contribution can be made to the agriculture-food economy of the country by benefiting from the heating effect of the sun in the drying process in the field of food.

In this study, apple slices with moisture content which should be removed to safe storing were dried in a solar energy dryer with natural convection. Apple slices were dried using 3 methods; a solar tray dryer, exposure to direct sunlight and in the shade. Drying conditions were chosen as the amount of loaded material; slice thickness, drying time and air conditions in the drying cabinet. The effect of these drying conditions on apple solar drying was determined by water loss, drying rate and shrinkage ratio.

The aim of this study was to test the importance and efficiency of neural networks to model and predict the temperature and moisture transfer during air drying of foodstuff. The model validation was made with experimental solar drying data of apple slices. For predicting effects of the parameters such as the apple slice thickness, drying time, the apple slice load, air temperature and air humidity on water loss, shrinkage ratio and drying rate. ANFIS could be the identification of an input-output relationship between the involved solar drying parameters based on the experimental data.

On the other hand, the model structure based on input and output data during the drying process was developed using adaptive network based fuzzy inference system. This system is an artificial neural network developed by Jang (1993). It is an effective method which eliminates the inefficiency of the parameter definition process in membership functions (MF) in complex systems. Recently, it was widely used in many different field areas, such as maximum power point tracking (MPPT) (Abbasov et al., 2011; Kharb et al., 2014; Esen et al, 2009), face recognition (Sharma and Patterh, 2015), and object tracking (Choi, 2015) which transformed algorithm of ANFIS.

In this study, ANFIS is intended to be used to transform into a prediction algorithm for changes in the drying conditions of apple slices during solar powered drying process. Estimation results showed that a good modeling design was made using ANFIS. In addition, the results of error revealed that ANFIS was successfully performed.

MATERIALS and METHOD

Consists of dryer, solar collector, drying chamber and chimney. Steel perforated shelves are placed inside the dryer room, which can be easily removed and installed. The solar collector heats the air in the dryer by holding the sun rays, while the heated air rises, it dehumidifies the product on the shelves and the moist air is thrown out of the dryer and the drying process is performed (Ozturk, 2008).

Starking Delicious apple was used for the experiments. Apples went through a number of preparation stages before experiments. The apples, which were washed and peeled, were removed from the seeds and then cut into two halves and sliced in slice thicknesses determined as half a circular shape. In order to prevent browning caused by the phenolic compounds found in the apple structure (such as peeling, slicing), apple slices were immersed in a 0.3% citric acid solution (Ceylan et al., 2006).

Excess water of the sliced apples taken from the solution was filtered with the help of strainers and placed on the tray in the solar dryer, direct under sunshine and under shadows. Then, apple samples were measured both weight and volume during the drying period. These experiments were replicated five time to obtain a reasonable average.

The water loss, drying rate and shrinkage ratio were calculated according to the following equations (Aboud, 2013; Ochoa-Martínezet al., 2006).

$$\text{Water Loss} = \frac{M_0 - M_t}{Mo} \quad (1)$$

$$\text{Drying Rate} = \frac{M_0 - M_t}{\Delta t} \quad (2)$$

$$\text{Shrinkage Ratio} = \frac{V_0 - V_t}{V_0} \quad (3)$$

M_0 and M_t in Equations 1 and 2 show the weight of the apple slice before drying and the weights (g) of the apple slice after drying. V_0 and V_t in Equation 3, the sample volume before drying and the sample volume at the end of the drying process (ml). Δt indicates the duration of the drying process. Air temperature and humidity in the drying cabinet were monitored by CEM brand DT-802 model air quality measuring device.

To develop an ANFIS model for estimating the water loss, drying rate and shrinkage ratio, the data set was partitioned into a training set and a test set. Out of 120 data set available, 90 were used for training, and the remaining for testing. The performance function was the regression for analyzing. The network was trained for 100 epochs. The computation was performed in MATLAB 7.0 by the Mathworks Inc. environment.

RESULTS and DISCUSSIONS

In this study, ANFIS is intended to be transformed into a prediction algorithm for changes in the drying conditions for solar drying process. Input variables, the apple load (in1), the apple slice thickness (in2), drying time (in3), air temperature (in4) and air humidity (in5); the output variables (f) were selected in order to determine the moisture loss, shrinkage rate and drying rate. According to these design data, the developed ANFIS model structure is given in Figure 1. An ANFIS system has been arranged for each output variable estimation. The neural network in the ANFIS structure conforms to the following rule.

$$if\ (in1\ is\ A_i)\ and\ (in2\ is\ B_i)\ and\ (in3\ is\ C_i)\ and\ (in4\ is\ D_i)\ and\ (in5\ is\ E_i)$$
$$then$$
$$f_i = \rho_i in1 + q_i in2 + x_i in3 + y_i in4 + z_i in5 + r_i$$

where, Ai and Bi fuzzy set are variables of the parameter set. f_i outputs are output variables calculated from FUZZY field. ANFIS structure used for estimation process, It can be arranged in the following form.

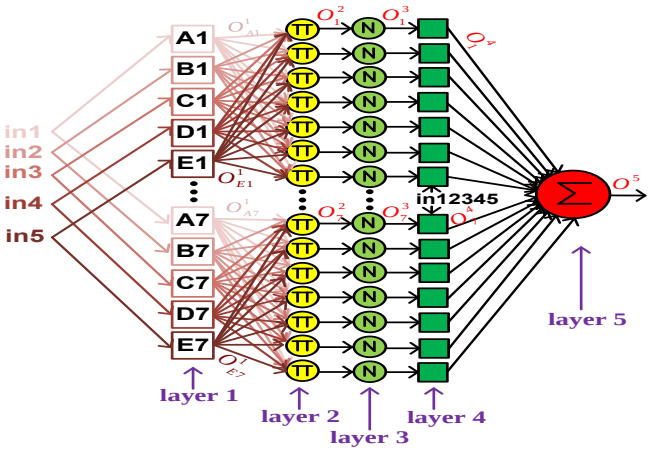


Figure 1. View of the ANFIS model structure developed.

If the layer outputs in the ANFIS system are expressed mathematically,

$$\left.\begin{aligned} O_{A_i}^1 &= \mu_{A_i}(in1) = \frac{1}{1+\left|\frac{in1-c_i}{a_i}\right|^{2b_i}}, O_{B_i}^1 = \mu_{B_i}(in2) = \frac{1}{1+\left|\frac{in2-c_i}{a_i}\right|^{2b_i}} \\ O_{C_i}^1 &= \mu_{C_i}(in3) = \frac{1}{1+\left|\frac{in3-c_i}{a_i}\right|^{2b_i}}, O_{D_i}^1 = \mu_{D_i}(in4) = \frac{1}{1+\left|\frac{in4-c_i}{a_i}\right|^{2b_i}} \\ O_{E_i}^1 &= \mu_{E_i}(in5) = \frac{1}{1+\left|\frac{in5-c_i}{a_i}\right|^{2b_i}} \end{aligned}\right\} \quad (4)$$

$$O_i^2 = \mu_{A_i}(in1)\,\mu_{B_i}(in2)\,\mu_{C_i}(in3)\,\mu_{D_i}(in4)\,\mu_{E_i}(in5)\} \quad (5)$$

$$O_i^3 = \frac{O_i^2}{\sum_i O_i^2}\Bigg\} \quad (6)$$

$$O_i^4 = O_i^3 f_i = O_i^3\left(\rho_i in1 + q_i in2 + x_i in3 + y_i in4 + z_i in5 + r_i\right)\} \quad (7)$$

$$O^5 = f = \sum_i O_i^4\Bigg\} \quad (8)$$

and output function statements,

a_i, b_i, c_i, d_i, e_i : The input is the membership function parameters.

$\rho_i, q_i, x_i, y_i, z_i, r_i$: 1st degree is polynomial parameters.

i : $0 < i < 8$

It is defined in the above format.

In order to measure the efficiency of the designed ANFIS system, 30 actual test data were used. These actual data are given in Table 1, and the estimation results obtained. The predicting of the drying efficiency of the of the water loss, shrinkage ratio and drying rate of the solar drying process is modeled ANFIS.

The model was constructed by using the experimental observations as the input set in order to identify the effects of operating parameters on the water loss, shrinkage ratio and drying rate. The data set was divided into two separate data sets randomly, the training data set and the testing data set. The training data set was used to train the proposed ANFIS model, whereas the testing data set was used to verify effectiveness of the trained ANFIS model for the solar drying process. The adequate functioning of the ANFIS depends on the sizes of the training set and the test set. The data set for the water loss, shrinkage ratio and drying rate of the solar drying process available included 120 data. From these, 90 data patterns were used for training ANFIS, and the remaining 30 patterns were used as the test data set for trained ANFIS model.

Table 1. The actual test data based on measurement

in1 (g)	in2 (mm)	in3 (min)	in4 (^{0}C)	in5
11,91	10	270	38,8	35,1
11,8508	10	300	37,2	40,8
13,0422	10	30	31,6	55,3
12,8938	10	60	44,5	31,3
12,7832	10	90	45,9	28,5
13,1514	10	120	38,9	39,3
12,908	10	150	39,7	39,3
12,8168	10	180	42,1	35,8
12,4308	10	210	42	35,2
12,2506	10	240	41	36
11,4712	10	270	53,7	16
11,2594	10	300	50	14,7
10,8878	8	30	31,6	52,4
10,5300	8	60	43	32,1
10,0402	8	90	46,2	29,6
6,49480	4	30	34,6	53,2
6,1158	4	60	42,5	37,3
5,7936	4	90	47,9	27,5
5,3712	4	120	46,7	28,4
4,952	4	150	49,4	25,5
3,6946	4	270	41,8	37,7
3,4198	4	300	41,1	38,6
6,582533	4	30	33	57,1
6,078867	4	60	40,3	41,7
5,5462	4	90	47,5	31,2
5,17505	4	60	42,7	39,3
4,63415	4	90	46,4	32,8
4,2499	4	120	46,3	30,4
3,8507	4	150	45,5	28,7
3,44555	4	180	44,6	27

In this study, an ANFIS model was developed for the prediction of the water loss, shrinkage ratio and drying rate of the solar drying process. The proposed ANFIS model predicts the water loss, minimum shrinkage ratio and drying rate when the apple slice thickness, drying time, the apple slice load, air temperature and air humidity are given. ANFIS model achieved a mean absolute percentage error of output property of only 0.001. The model is expected to be of use for predicting the water loss, shrinkage ratio and drying rate to a satisfactory degree encouraged by these results. The error values based on the difference between the actual data and the estimation data are shown in Table 2.

Table 2. The output and error data based on predicted

Water Loss			**Shrinkage Ratio**			**Drying Rate**		
Predicted data	**Measured data**	**% error**	**Predicted data**	**Measured data**	**%error**	**Predicted data**	**Measured data**	**%error**
0,169101	0,169095	-0,00374	0,209615	0,209524	-0,04324	0,008553	0,008546	-0,07779
0,174169	0,17416	-0,00509	0,214288	0,214286	-0,00106	0,007892	0,007889	-0,04394
0,033255	0,033254	-0,00281	0,066671	0,066667	-0,00656	0,014202	0,0142	-0,0159
0,045803	0,045802	-0,00215	0,066253	0,066667	0,620337	0,009507	0,009573	0,689835
0,053696	0,053666	-0,05543	0,073208	0,070085	-4,4563	0,008144	0,007611	-7,00247
0,081819	0,081821	0,002323	0,009456	0,009458	0,024611	0,082289	0,082292	0,003931
0,099789	0,099787	-0,00224	0,009174	0,009189	0,165267	0,105	0,105	0,00031
0,105785	0,105794	0,008929	0,008168	0,008164	-0,04567	0,130555	0,130556	0,000677
0,135024	0,135027	0,002095	0,008807	0,008836	0,326047	0,13124	0,13125	0,007576
0,149189	0,149161	-0,01856	0,008493	0,008483	-0,11225	0,144177	0,144167	-0,00713
0,131973	0,131979	0,004399	0,006484	0,00646	-0,36746	0,16025	0,160256	0,003654
0,147986	0,147992	0,003723	0,006534	0,00652	-0,20892	0,167397	0,167399	0,001322
0,056059	0,056059	-0,00048	0,102856	0,102855	-0,00051	0,021527	0,021527	-0,00043
0,087165	0,087166	0,000721	0,118655	0,118648	-0,00609	0,016726	0,016727	0,005397
0,129831	0,129832	0,001023	0,151986	0,151981	-0,00311	0,016383	0,016385	0,011773
0,109689	0,10969	0,000587	0,136902	0,136905	0,002107	0,026566	0,026567	0,002789
0,161846	0,161848	0,001243	0,169039	0,169048	0,005489	0,019751	0,0199	0,749673
0,206264	0,206265	0,000246	0,247023	0,247024	0,000498	0,016648	0,016647	-0,00688
0,264401	0,264402	0,000458	0,29285	0,297619	1,602414	0,015825	0,015642	-1,17121
0,322012	0,322012	0,000127	0,391361	0,391369	0,00194	0,015424	0,015248	-1,15099
0,43595	0,435949	-0,00015	0,527765	0,527778	0,002521	0,009944	0,009942	-0,02494
0,466259	0,466259	-3,10e-5	0,559376	0,559392	0,002858	0,009865	0,009864	-0,01346
0,065776	0,065776	-1,76e-5	0,142855	0,142857	0,0013	0,019267	0,019267	0,002579

0,159608	0,159608	-0,0001	0,149988	0,15	0,008243	0,014332	0,014331	-0,00527
0,24896	0,24896	4,80e-5	0,24026	0,24026	6,02E-06	0,01293	0,012928	-0,01281
0,259821	0,259822	0,000252	0,16666	0,166667	0,004448	0,018926	0,018925	-0,00492
0,336217	0,336217	0,000128	0,324994	0,325	0,001917	0,01697	0,016969	-0,00691
0,403741	0,403742	0,000311	0,350011	0,35	-0,0032	0,015396	0,015394	-0,01116
0,439838	0,439837	-0,00017	0,416653	0,416667	0,003348	0,014897	0,014895	-0,01044
0,507857	0,507858	0,000158	0,50833	0,508333	0,000623	0,014012	0,01401	-0,01687

The ANFIS parameters were optimized using Matlab's Neuro Fuzzy Tool. The input MF function gauss and the output MF function as linear were selected for the optimization process. In addition, the training process was carried out by selecting hybrid. Then, the predictive power of the optimized ANFIS was calculated based on the regression function value.

$$R^2 = 1 - \frac{\sum(y-\hat{y})}{\sum(y-\bar{y})} \Bigg\} \qquad (9)$$

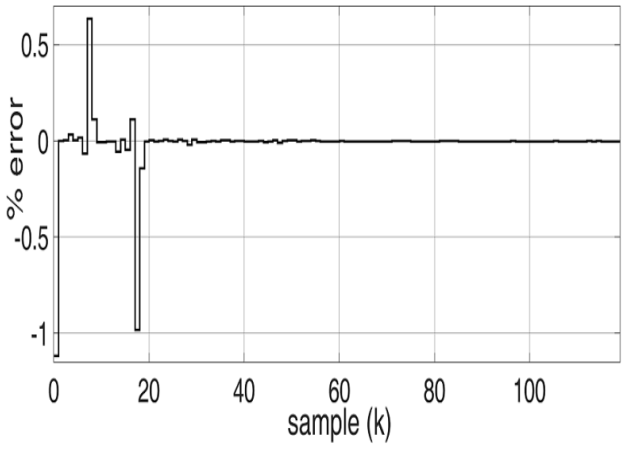
Where,

y: real data

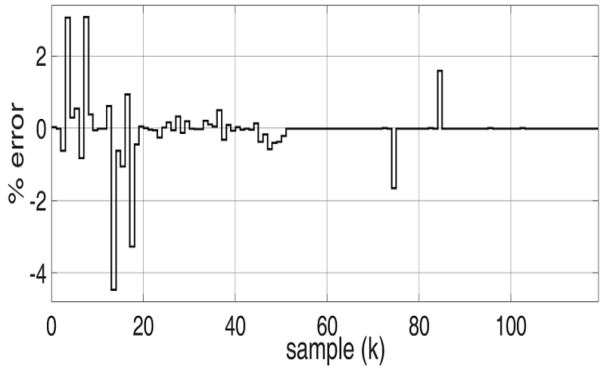
ŷ: estimated data

$\bar{y}$: the average of measured data.

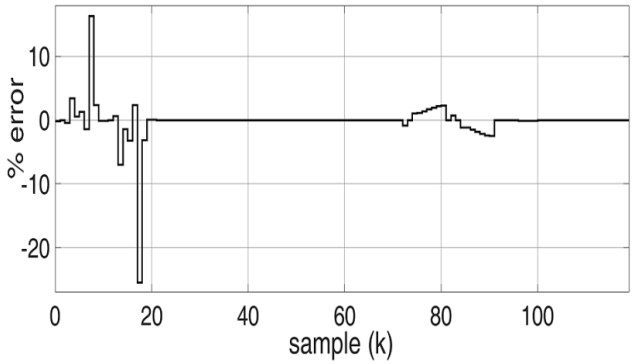
The R^2 values, in the prediction simulation performed were obtained as shown in Figure 2.



$$R^2_{water\ loss} = 0.9968$$



$$R^2_{shrinkage\ ratio} = 0.9675$$



$$R^2_{drying\ rate} = 0.9918$$

Figure 2. The R^2 values based on the water loss, shrinkage ratio and drying rate

CONCLUSIONS

The drying of apple slices with a solar tray dryer has been quicker than the direct sunshine and drying in the shade. Both water loss and shrinkage ratio have increased due to increased air temperature in the dryer and it also reduced slice thickness of the apple whilst increasing drying time and amount of loaded material. This study shows that the adaptive neural network based fuzzy inference system modeling can be used to obtain good solar drying modeling of apple slice. The ANFIS modeling was validated with experimental drying data. The modeling must be related to the fact that it is elaborated without any preliminary assumptions on the underlying mechanisms as simply and rapidly. The applications of ANFIS can be used for the on-line state estimation and control of solar drying processes.

ACKNOWLEDGEMENTS

The study was supported by Mersin University Science Research Project Department (BAP) with 2018-1-TP2-2840 project.

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In vitro Investigation of Deoxyribonucleic Acid Interaction and Anti-Acetylcholinesterase Activity of Turnip (*Brassica Rapa Subsp. Rapa)*

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Abstract

Turnip (*Brassica rapa subsp. Rapa*) is a herbaceous and seasonal plant found in the cruciferous family. It is possible to grow in many regions of Europe and West Asia. Although many studies have been carried out to show that turnip juice obtained from turnip root has high biological activity, there has been no previous study on the interaction of turnip root with DNA or its anti-acetylcholinesterase activity. In this study, the interaction and anti-acetylcholinesterase activity of turnip, which obtained three different extracts by applying Soxhlet extraction, were tried to be determined. The DNA binding properties, DNA protective effects, DNA restorative effects and anti-acetylcholinesterase activity of the obtained water, ethanol and ethyl acetate extracts were calculated and the results obtained were compared with other plants of the same species in the past studies.

Keywords: Turnip, DNA, DNA interaction, acetylcholinesterase, Soxhlet extraction, natural product

Research article
Received Date: 18 April 2021
Accepted Date:4 May 2021

INTRODUCTION

DNA is a nucleic acid that contains the genetic instructions required for the vital activities and biological development of all existing organisms and some viruses as well as these organisms (Nelson et. al., 2013). The coding of genetic information in living things takes place thanks to the sequence of nitrogenous organic bases that run along the backbone of the DNA double helix. The encoded genetic information is read with the help of the genetic code during protein synthesis, and thanks to this reading, the amino acid sequences of the synthesized proteins are determined (Butler, 2001). Damages that occur on the DNA double chain and can occur due to endogenous or exogenous reasons are most frequently encountered by free radicals (Lodish et. al., 2004). These damages are repaired either by destroying the damaged cells or by removing the DNA with their own repair systems (Larsen et. al., 2005).

The non-covalent interactions of DNA with molecules smaller than itself are generally grouped in three groups: Intercalation, electrostatic interactions and binding to grooves (Yıldız et. al. 2015). Intercalation, which can be defined as an intervention in short, is the type of bonding realized by the molecules entering between the double chains in a reversible way (Hasanzadeh and Shadjou, 2016).

In addition to electrostatic interactions that express positive molecules that bind to the negatively charged phosphate-sugar backbone of the DNA chain, molecules can also bind to the grooves of the DNA chain by van der Waals interactions and hydrogen bonds (Rajendiran et. al., 2012).

Acetylcholinesterase, also known as AChE or acetylhydrolase, is an enzyme that is cholinesterase. It is an enzyme that catalyzes the breakdown of acetylcholine and some other choline esters that act as neurotransmitters. AChE is mainly found in cholinergic type chemical synapses and is responsible for terminating synaptic transmission. It belongs to the carboxylesterase enzyme family (Katzung, 2001). Alzheimer's disease is the most common neurodegenerative disease. It can be summarized as the accumulation of extracellular amyloid plaques, shortly acetylcholine. The signal of the acetylcholine molecule is terminated by acetylcholinesterase. Drugs that limit acetylcholine degradation or mimic acetylcholine activity, briefly have anti-acetylcholinesterase activity, are used to increase cholinergic signal transmission in patients with Alzhemier (Nwidu et al., 2017).

Turnip (*Brassica rapa subsp. Rapa*) is a plant in the Brassica genus of the Brassicaceae family (cruciferous or cruciferous) and has a wide cultivation area. The turnip, which loves warm and cool seasons, is resistant to cold despite its structure. Due to the flexible about tolerance and cold temperatures, West Asia and Europe are grown in many regions of Turkey in the overall Adana, Osmaniye, Mersin is grown around. However, it is also possible to grow in colder regions (Padilla et al., 2005).

As stated by the authors, there is no study on the interaction of the turnip root with DNA and its anti-acetylcholinesterase activity. Studies on the biological activity of natural resources have gained importance in recent years, and in this study, the binding, protective and restorative effects and anti-acetylcholinesterase activity of the extracts obtained from turnip by Soxhlet extraction were investigated. The results obtained were compared and interpreted with other plants of the same species.

MATERIAL and METHOD

Source Plant and Extraction Process

To be used in the experiments, the turnip plant was used for this study, purchased from a local market in Küçükçekmece district of Istanbul province. The plant itself can be consumed both in the form of leaves and in the form of a root, and the interaction with DNA and anti-acetylcholinesterase activity were investigated, by the extracts provided by Soxhlet extraction. In order to obtain the extracts, Soxhlet extraction with 100 mL water, ethanol and ethyl acetate solvents for 4 hours each was applied to the 50 gram samples taken from the turnip root. Then, the solvents were removed from the extracts using a rotary evaporator, oven and fume hood respectively. Both the plant and the extracts obtained were stored at +4 °C throughout the study.

Enzyme Activity Assay

Anti-acetylcholinesterase Activity Assay

While studying anti-acetylcholinesterase activity, Ingkaninan et al., (2003)'s method was followed (Ingkaninan et. al., 2003). At the first step, 325 µL Tris-HCl buffer (at 50 mM and pH 8) was added to each 100 µL of extract samples prepared at a concentration of 1 mg/mL. 25 µL of enzyme solution (0.28 U/mL) was added on each of the samples to which buffer was added, and the samples were incubated for 15 minutes at room temperature.

After 15 minutes, 75 µL of acetylcholine iodide (15 mM) and then 475 µL of DTNB (3 mM) solution were added to each sample, respectively. After the addition of DTNB, the absorbance values at 405 nm of the samples, which were incubated for another 30 minutes at room temperature, were read. During the measurements, Tris-HCl buffer was used as a blank solution, and samples containing each sample's own solvent were used as the control solution. While calculating the % anti-acetylcholinesterase activity, the equation where A_0 corresponds to the absorbance of the control solution and A_1 to the absorbance of the samples was applied.

$$\text{Anti-acetylcholinesterase Activity (\%)} = [1 - (A_1 / A_0)] \times 100 \qquad (1)$$

Interaction with DNA

DNA For studies involving DNA binding effect, DNA protective effect and DNA restorative effect assays, a working solution of 3×10^{-2} mM from CT-DNA was prepared using Tris-HCl buffer solution. This prepared working solution and the main stock DNA solution were stored at +4 °C throughout the studies. In all studies on DNA, water extract, ethanol extract and ethyl acetate extract obtained from the turnip root were prepared at a concentration of 1 mg/mL and using Tris-HCl buffer solution.

DNA Binding Effect

Before observing the change in the absorbance values of the samples added to the DNA solution, in the wavelength range of 200-400 nm, absorbance values of 3×10^{-2} mM DNA working solution and water, ethanol and ethyl acetate extracts at a concentration of 1 mg/mL against the Tris-HCl buffer used as a blank solution were measured and recorded separately. Subsequently, the changes in absorbance values were observed by adding 10 µL of each extract up to 100 µL with the addition of 10 µL onto 1 mL and 3×10^{-2} mM DNA working solution. All measurements were repeated at the 24th hour with samples stored at +4 °C in order to observe the time-dependent changes in the DNA binding capacity of water, ethanol and ethyl acetate extracts.

DNA Protective Effect

In studies for DNA protective effect, changes in absorbance values were observed with the help of extracts added to DNA working solutions that were denatured with ethanol and UV light, separately. During the study of denaturation by the addition of ethanol, an equal volume of DNA working solution and ethanol were mixed first and the its absorbance between 200-400 nm against Tris-HCl buffer was measured.

In order to observe the DNA protective effect of the extracts, first, 250 µL of ethanol and then 500 µL of all three extracts each were added to the 250 µL DNA working solution, respectively, and the measurements were taken in the range of 200-400 nm. In order to compare the protective effect more accurately, the absorbance value of pure ethanol was also read in the same wavelength range.

All measurements were repeated at 24th hour with samples stored at +4 °C to observe the time-dependent change of the protective effect of the extracts. However, it was observed that repeated measurements at 24th hour did not give a different result from the first measurements.

During the investigation of denaturation with UV light, the wavelengths of 254 nm and 365 nm were studied, respectively. 500 µL of each extract was added onto 500 µL of DNA working solution and the solutions were exposed to UV light for 30 minutes, separately.

Then, absorbances of the samples were read against the Tris-HCl buffer solution used as a blank solution in the wavelength range of 200-400 nm. For a better interpretation of the results, pure water extract, ethanol extract, ethyl acetate extract and DNA working solution were kept under UV light for 30 minutes and absorbance values were measured in the same wavelength range. In the protective effect tests performed after a second 30-minute UV light denaturation, it was determined that the second period had no effect.

DNA Restorative Effect

In order to determine whether the turnip extracts have a restorative effect, the DNA working solution was first kept under UV light for 30 minutes at 254 and 365 nm, respectively. Then, the absorbance between 200-400 nm was scanned against the Tris-HCl buffer solution. Afterwards, absorbances of the solutions formed by mixing the UV-damaged DNA working solution and the extracts in an equal volume were studied to see if the extracts had a restorative effect. In the experiments carried out with another 30-minute UV light denaturation, it was determined that the second 30-minute period had no effect.

RESULTS and DISCUSSION

Enzyme Activity Assay

Anti-acetylcholinesterase Activity Assay

The inhibition percentages for the anti-acetylcholinesterase activities of the samples obtained from the turnip root were found to be 0.76% for water extract, 1.38% for ethanol extract and 3.70% for ethyl acetate extract.

Interaction with DNA

In order to determine whether the extracts obtained from turnip root have an antimutagenic effect on CT-DNA, the values reached by the measurements should be interpreted through the wavelength changes divided into two as hypsochromic effect and batochromic effect, and absorbance values, which are divided into two as hyperchromic effect and hypochromic effect. In studies on DNA, a purity control should be calculated by proportioning the absorbance of DNA at 260 nm to 280 nm absorbance and A_{260}/A_{280} ratio should be 1.8 or more than value of 1.8. For the CT-DNA used in the study, the value of absorbance ratio calculated as A_{260}/A_{280} = 2,4209/1,3126 = 1,8444.

DNA Binding Effect

Initially, the maximum absorbance of the pure DNA sample was obtained at 258 nm and 2,4378. After the addition of 100 µL water extract, the maximum absorbance value of 2.0194 was obtained at the same wavelength, 258 nm. By adding 100 µL ethanol extract to the pure DNA sample, the maximum absorbance was again at 258 nm, but this time with a value of 2.0014. After the addition of 100 µL of ethyl acetate extract to the pure DNA sample, the maximum absorbance value of the DNA sample was again measured at 258 nm and this time as 1.9735.

Although the wavelength at which the DNA solution gives maximum absorbance does not change with the addition of all three extracts, the absorbance at this wavelength has changed and this decrease is expressed as a hypochromic effect. Based on the numerical data, it can be interpreted that the binding that occurs between all three extracts and DNA is intercalated, separately. Studies based on the binding effect between DNA solution and water, ethanol and ethyl acetate extracts were repeated after 24 hours, and no significant changes were observed in the second measurements.

Maximum wavelength of 100 µL water extract, ethanol extract and ethyl acetate extract interacting with DNA, maximum wavelength change caused by these extracts, the hypochromic effect percentages of the extracts and the binding constants of their binding with DNA are given in Table 1. Equation 2 was used for hypochromic effect values calculated in percentage, and Equation 3 was used for binding constants calculated by absorbance values and DNA concentration. In these equations, A_0 indicates the absorbance value that the DNA sample in free state reaches at the maximum wavelength, while A_1 indicates the absorbance value reached by the DNA sample to which the extract is attached at the maximum wavelength, and C_{DNA} indicates the concentration of the DNA working solution.

$$H\ (\%) = 100 \times [(A_0 - A_1) / A_0] \quad (2)$$

$$K_b = (A_0 - A_1) / (A_1 \times C_{DNA}) \quad (3)$$

DNA Protective Effect

Denaturation with Ethanol

During the examination of the protective effects of the extracts on DNA denatured with ethanol, firstly, the maximum wavelength of DNA and ethanol mixed in equal volume was determined as 259 nm, while the absorbance value at this wavelength was found to be 2.4032. When water extract was added to DNA and ethanol, the maximum wavelength did not change, while the absorbance value was observed as 0.7727. While the maximum absorbance value of the ethanol extract added DNA and ethanol solution was read at 260 nm, the absorbance value at this wavelength was read as 0.9958. The addition of ethyl acetate extract decreased the wavelength at which the maximum absorbance was read to 256 nm, while the absorbance value was measured as 0.8637. The decreasing absorbance value in all three extracts indicates the hypochromic effect. In terms of wavelength change, it can be said that there is a batchromic effect, which means the π-π^* transition in the ethanol extract, and a hypsochromic effect in the ethyl acetate extract, except for the water extract which did not perform any change. In the light of these results, it can be interpreted that all three extracts obtained from the turnip root are intercalated to DNA.

In addition, as a result of repeated measurements of the DNA protective effect at the 24th hour, no significant difference was observed from the values obtained in the first measurements. The results for the effect of preventing the denaturation in DNA with ethanol, studied for water extract, ethanol extract and ethyl acetate extract, were calculated using Equation 1 and Equation 2 and shown in Table 2.

Denaturation with UV Light

During the investigation of the reparative effect of water, ethanol and ethyl acetate extracts against denaturation with UV light on DNA, the maximum wavelength of the DNA sample exposed to UV light and the change in the absorbance value at this wavelength were considered.

When water extract was added to the DNA sample denatured by UV light, the maximum wavelength did not change at both 254 nm and 365 nm, while the maximum absorbance value decreased. Changes of 0.9497 and 0.9735, respectively, can be expressed as a hypochromic effect. In the measurements performed with ethanol extract, the maximum absorbance value decreased while the maximum wavelength increased at both 254 nm and 365 nm. While the wavelength increasing from 258 nm to 260 nm expresses the batchromic effect, the changes in absorbance values of 0.8425 and 0.8636, respectively, can be expressed as hypochromic effect. During the examination of the restorative effect of the ethyl acetate extract, both at 254 nm and 365 nm, the maximum wavelength and maximum absorbance value decreased as a result of the addition of ethyl acetate extract. While the wavelength from 258 nm to 257 nm is described as hypsochromic effect, the changes in absorbance values of 0.9549 and 0.9788, respectively, can be expressed as hypochromic effect. In the second measurements repeated 30 minutes after the first measurements, no serious difference was observed for all three extracts. The values calculated for the DNA protective effect at both 254 nm and 365 nm using Equation 1 and Equation 2 are shown in Table 3 and Table 4, respectively, for two wavelengths.

DNA Restorative Effect

When the effects of the extracts obtained from turnip root on DNA to restore the denaturation caused by UV light are examined, it is seen that the maximum absorbance values decrease with the addition of all extracts. Moreover, the maximum wavelength unchanged with the addition of water extract increased after the addition of ethanol extract and decreased after the addition of ethyl acetate extract. As a result, it can be interpreted that all extracts cause hypochromic effect, additionally there is a batchromic effect in ethanol extract and a hypsochromic effect in ethyl acetate extract. The results obtained in the second measurements performed after a period of 30 minutes do not differ significantly from the first measurement results. The values calculated using Equation 1 and Equation 2 at 254 nm and 365 nm, which are two wavelengths where the DNA restorative effect is studied, are given in Table 5 and Table 6, respectively.

LIST OF TABLES

Table 1. λmax, Δλ, H% and K_b values of water extract, ethanol extract and ethyl acetate extract

Sample	**$\lambda_{maximum}$ (nm)**	**Δλ**	**H%**	**K_b (x 10^5 M^{-1})**
Distilled water extract with DNA	258	0.4184	17.1630	0.0691
Ethanol extract with DNA	258	0.4364	17.9014	0.0727
Ethyl acetate extract with DNA	258	0.4643	19.0459	0.0784

Table 2. λmax, Δλ, H% and K_b values of DNA denaturation of water extract, ethanol extract and ethyl acetate extract with ethanol

Sample	**$\lambda_{maximum}$ (nm)**	**Δλ**	**H%**	**K_b (x 10^5 M^{-1})**
Distilled water extract with DNA and ethanol	259	0.7727	67.8470	0.7034
Ethanol extract with DNA and ethanol	260	0.9958	58.5636	0.4711
Ethyl acetate extract with DNA and ethanol	256	0.8637	64.0604	0.5941

Table 3. λmax, Δλ, H% and K_b values for the restorative effect of extracts against UV light denaturation of 254 nm in DNA

Sample	**$\lambda_{maximum}$ (nm)**	**Δλ**	**H%**	**K_b (x 10^5 M^{-1})**
Distilled water extract with DNA and UV Light	258	0.6971	57.6694	0.4541
Ethanol extract with DNA and UV Light	260	0.8043	51.1234	0.4029
Ethyl acetate extract with DNA and UV Light	257	0.6919	57.9852	0.4566

Table 4. λmax, Δλ, H% and K_b values for the restorative effect of extracts against UV light denaturation of 365 nm in DNA

Sample	**$\lambda_{maximum}$ (nm)**	**Δλ**	**H%**	**K_b (x 10^5 M^{-1})**
Distilled water extract with DNA and UV Light	258	0.7145	57.6718	0.4542
Ethanol extract with DNA and UV Light	260	0.8244	51.1611	0.3492
Ethyl acetate extract with DNA and UV Light	257	0.7092	57.9858	0.4600

Table 5. λmax, Δλ, H% and K_b values for the restorative effect of extracts against UV light denaturation at 254 nm on DNA

Sample	**$\lambda_{maximum}$ (nm)**	**Δλ**	**H%**	**K_b (x 10^5 M^{-1})**
Distilled water extract with DNA and UV Light	258	1.3623	19.1823	0.0736
Ethanol extract with DNA and UV Light	259	1.4368	8.6387	0.0525
Ethyl acetate extract with DNA and UV Light	256	3.4798	8.6387	0.0413

Table 6. λmax, Δλ, H% and K_b values for the restorative effect of extracts against UV light denaturation at 365 nm on DNA

Sample	**$\lambda_{maximum}$ (nm)**	**Δλ**	**H%**	**K_b (x 10^5 M^{-1})**
Distilled water extract with DNA and UV Light	258	1.2770	18.0966	0.0791
Ethanol extract with DNA and UV Light	259	1.4436	13.6175	0.0315
Ethyl acetate extract with DNA and UV Light	256	3.4436	11.0323	0.0315

CONCLUSION

Synthetic antioxidants such as BHA, BHT, Trolox and α-tocopherol have been used for a long time for Today, in daily life, many changes occur in DNA structure due to many endogenous and exogenous factors. These changes, which are important and damaging enough to affect the continuation of life, cause mutations on the DNA molecule and diseases such as cancer that are very difficult to reverse. The fact that some natural sources have antitumor and anticarcinogen effects allows them to have a protective and restorative effect on DNA. In addition, the number of natural resources used for the treatment of many neurodegenerative diseases, especially Alzheimer's, is rapidly increasing.

In the study, turnip (*Brassica Rapa Subsp. Rapa*) was subjected to Soxhlet extraction and three different extracts were obtained with the help of water, ethanol and ethyl acetate solvents which have different polarity. With the help of different parameters, binding, restorative and protective effects and anti-acetylcholinesterase activity on the DNA molecule were investigated.

In addition to many natural sources that have been investigated for enzyme inhibition and interaction with DNA, the fact that turnip has not been included in such a study before has caused some limitations to compare our results with similar studies. Likewise, turnip could not be compared with other plant samples in Brassica, since no study has been conducted on these subjects. Interaction with DNA was not studied in any plant of Brassica species, and only one reference of the same species was found for anti-AChE activity. In a study conducted with red cabbage (Brassica oleracea var. Capitata f. Rubra), the high anti-AChE activity of the plant stands out (Archana et. al., 2018).

In conclusion, the study used a material that had not been evaluated in any similar study before. Comparison of the obtained results with the literature is not easy in this respect. The results obtained in the study show that the interaction of turnip with DNA molecule changes depending on the parameter studied. Similarly, the anti-acetylcholinesterase activity of turnip could not be obtained at high values due to the results compared with other plant in the same family. The importance of the study appears before us in terms of giving an idea for the future studies. In the future, the interaction with DNA and anti-acetylcholinesterase activity of turnip root, the content of which will be completely purified and determined, will be revealed more clearly.

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Proximate and Some Phytochemical Constituents of Three West African Vegetable Spices

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Abstract

The proximate and some phytochemical composition of three African vegetable spices, namely celery, bay, and scent leaves, were investigated in this study. Fresh samples of the spices were obtained from a local market. They were cleaned, sorted, dried, and blended to reduce the particle size to flour. From the results of the analysis, the particle size of the scent, bay, and celery leaves flour ranged from coarse to fine size in that order, respectively. The water absorption capacity of celery leaf flour was greater among others, but bay leaf flour's oil absorption capability was the highest. The moisture content, fat, and crude fibre content of the spices flour ranged between 10.71 –10.84%, 1.81 – 4.5%, and 4.44 – 11.41%, respectively. While the ash, protein, and carbohydrate content were 2.18 – 8.69%, 0.92 – 14.88%, and 61.28 – 71.88%, respectively. The concentration of alkaloids, phenols, tannins, and saponins was higher in scent leaf flour when compared to others. However, flavonoids were more present in celery leaf flour. The large proportion of phytochemicals contents of the spices suggest that they are a good source of minerals. Generally, all the spices investigated are cheap, nutritionally rich, and have potentials for pharmacological applications.

Keywords vegetable spices, minerals, proximate analysis, properties, herbs

Research article
Received Date: 3 February 2021
Accepted Date:28 April 2021

INTRODUCTION

Food spices are part of the human diet, they are aromatic and pungent dried vegetable substances, which add colour, aroma, flavour, and tastes to foods (Gadekar and Yerramilli, 2006). Spices have vast applications such as food seasoning, food preservation, and medicinal uses. According to Altemimi *et al.* (2017), vegetable spices parts such as seeds, fruits, roots, flowers, leaves, and barks have been documented as valuable for several food, medicinal and industrial application.

Bay leaf (*Laurus nobilis*) is an herbal vegetable spices commonly used in cooking especially for their distinctive flavour and fragrance for the special aroma it gives in soups, stews, brines, meat, seafood, vegetable dishes, and sauces. The leaves also flavour many classic continental dishes; although often removed before serving especially because it can be abrasive on the digestive tract if eaten (WebMD, 2021). Its medicinal application as antedote for some stomach related problems have also been documented. Dried bay leaves have a fairly herbal and floral fragrance and the essential oil that makes it useful as an ingredient in perfumery. Scent leaf (*Ocimum gratissimum)*, is a perennial homegrown shrub and an aromatic herb that has been extensively used across tropical and subtropical regions of the world. It was reported by Pharmapproach (2021), that the leaves are mainly used as a spice for cooking delicacies due to its aromatic taste. The Scent leaf has been reported to be rich in plant chemicals and the crushed leaf juice is used in the treatment of convulsion, stomach pain and catarrh. Oil from the leaves has been shown to possess antiseptic, antibacterial, and antifungal activities (Ezekwesili et al, 2004). O. gratissimum has proved to be an effective anti-microbial (Orafidiya et al, 2001) and hypoglycemic and hepatoprotective agent *and also* in trado-medical practice, scent leaf is extensively used throughout West Africa as anti-malarial, mosquito repellent and anti-convulsant. Celery (*Apium graveolens*) is a biennial plant that occurs around the globe, it is a marshland plant in the family *Apiaceae* that has been cultivated as a vegetable since antiquity (Medhat, 2017).

Celery is an umbelliferous, aromatic, herbaceous plant grown for its leaves, seeds, oleoresin and essential oil. its plant is usually 30-60 cm high, erect with conspicuously jointed stems, bearing well-developed leaves on long expanded petioles (Elliot, 1999). The rigid fruit is small, ovoid, 1 to 1.5 mm long, 1 to 2 mm in diameter, contains a small brown seed. Celery has a long fibrous stalk tapering into leaves. Depending on location and cultivar, either its stalks, leaves or hypocotyl are eaten and used in cooking (Ballmer, 2000). The dried ripped fruits (celery fruit) are used as spice, Leaves and stalks are used as salads and in soups which makes the vegetable a popular low-calorie snack, Celery also provide a range of health benefits as the fiber in celery can benefit the digestive and cardiovascular systems, the leaf ontains antioxidants and used in preventing disease. Thekitchn (2021). In Nigeria, celery leaf is commonly known as Seleri (Yoruba, Ibo, and Hausa).

Some works have been carried out on the engineering properties of some African vegetable spices for food such as savory, caraway, dill (Desai *et al.*, 2013), thyme (Mustafa, and Cemalettin, 2014), curry (Kulathooran *et al.*, 2000), and cilantro (Rahman *et al.*, 2017). However, information regarding the proximate and some phytochemical constituents of these three spices namely, Bay (*Laurus nobilis)*, Celery (*Apium graveolens)*, and Scent leaves (*Ocimum gratissimum)*, are rarely obtained in the literature; hence in this work, proximate and some phytochemical constituents of three common vegetable spices found in Nigeria were investigated.

MATERIALS and METHOD

The materials obtained for the study include bay, scent, and celery leaves. They were obtained from a local market in Ile-Ife, Nigeria. It was cleaned and the initial moisture content of each of the samples was determined. Thereafter, it was sun-dried and blended to reduce the size of the material. The physiochemical properties and phytochemical contents of the spices (Figure 1) were determined in this study.

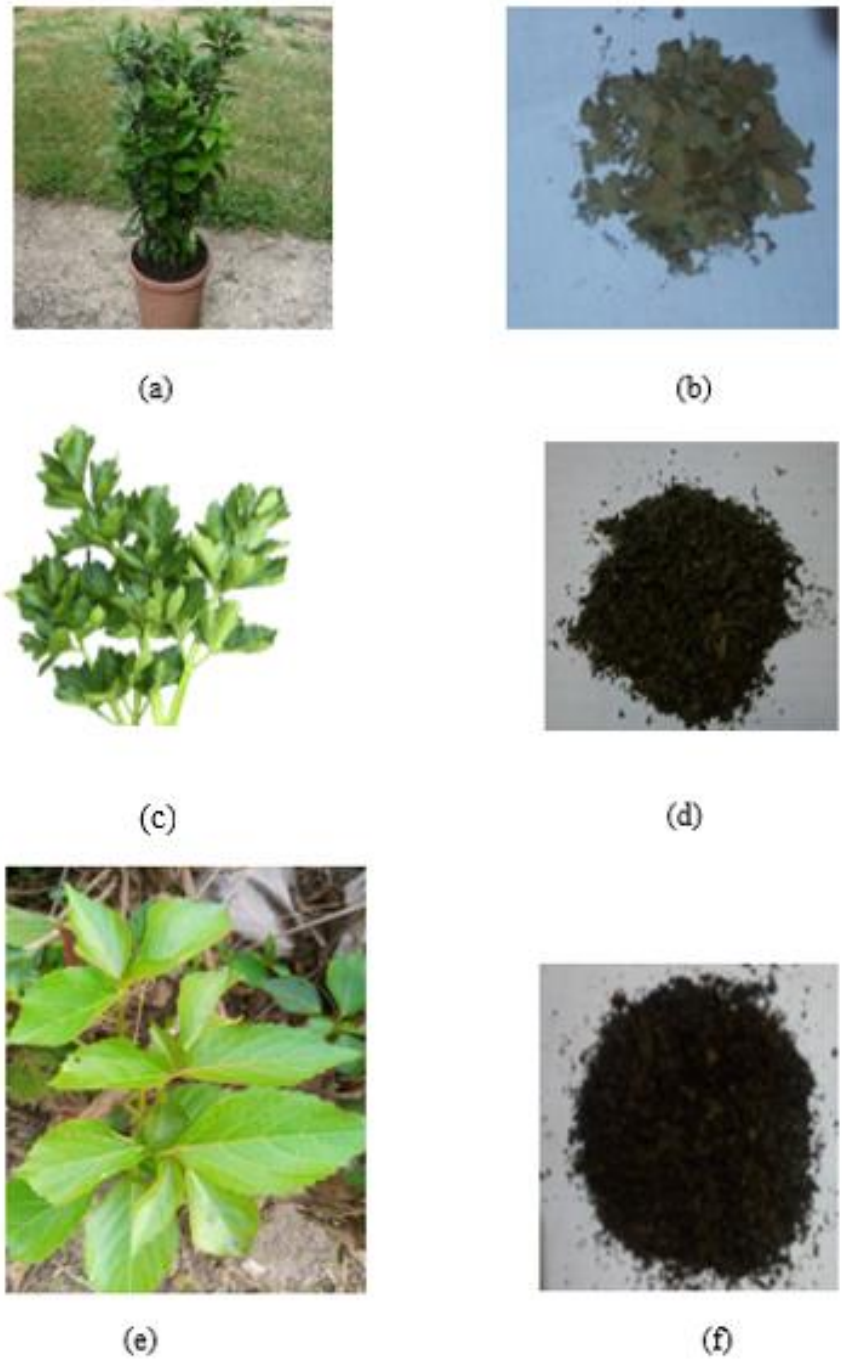

Figure 1. (a) Fresh bay leaf, (b) dried bay leaf (c) fresh celery leaf, (d) dried celery leaf, (e) fresh scent leaf, and (f) dried scent leaf

Particle size distribution analysis

The particle size analysis was carried out by weighing about 30 g of the flour samples and sieved with sieves of mesh sizes 850, 600, 425, and 300 µm using a mechanical shaker operated for 5 mins (Ogunsina *et al*., 2016).

The weight of the sample retained on each mesh was determined and the particle size distribution was calculated in percentages of the powder sample retained on each mesh. This procedure was replicated three times for each flour sample.

$$PSD = \frac{Wsr}{Wf} * 100\% \quad (1)$$

Where: PSD = Particle size distribution, W_{sr} = Weight of sample retained, W_f = Total weight of flour sample

Water and Oil Absorption Capacity

The water and oil absorption capacities were determined following standard procedure (Beuchat, 1977; Okunade *et al*., 2019). A Known weight (1 g) of each sample was mixed with 10 ml of distilled water (or refined groundnut oil for fat absorption capacities) in a 15 ml centrifuge tube. The mixture was centrifuged at 5000 g for 30 min at 27.5^0C, and the supernatant was quantified using a 10mL graduated cylinder. Water and oil absorption capacities were expressed as grams of water or oil bound per 100 g of flour from equation (2) and (3)

$$\text{Water Absorption Capacity} = \frac{Volume\ of\ Water\ Absorbed}{\text{Weight of sample used}} * 100\% \quad (2)$$

$$\text{Oil Absorption Capacity} = \frac{Volume\ of\ Oil\ Absorbed}{\text{Weight of sample used}} * 100\% \quad (3)$$

Proximate analysis

The protein content (Kjeldahl method), fat content (solvent extraction), ash content, crude fibre content was determined using the method described by AOAC (2005). While the carbohydrate content was determined following Olawoye and Gbadamosi (2020) approach. The mineral contents of the samples were determined in triplicates.

Determination of chemical composition

The chemical composition is the phytochemicals which include alkaloids, saponins, phenols, tannins, and flavonoids. The alkaloids content was determined following the methods of Okwu and Emenike (2006). The saponin content of the samples was determined following the method described by Okwu (2005). The tannin content was determined following Van-Burden and Robinson (1981) approach. The total phenolic content (TPC) and flavonoids of each sample were determined using modified Folin–Ciocalteu and aluminium chloride methods (Khodaie, *et al.*, 2012).

RESULTS and DISCUSSION

Proximate composition

Table 3 shows the result of the proximate analysis of bay, scent, and celery leaves. The Bay leaf has the highest fat, ash, and crude fibre content of 5.05%, 9.74%, and 12.79%, respectively. The carbohydrate content for each of the spices are of high values and are higher than each of the other contents.

Celery leaf has the highest carbohydrate content of the three spices with a value of 71.39%. Since ash content is a measure of the mineral content of the plant, it indicates that the bay leaf is a very good source of minerals. Crude fibre is an indication of the number of lipids in vegetable (Ejoh *et al*., 1996), The low crude fibre content in celery indicates its poor sources of lipids, while the high crude fibre in the bay and scent leaf makes them a good source of lipids.

However, excess consumption of fats in humans leads to cardiovascular diseases, ageing, and cancer (Kris-Etherton *et al*., 2002), the low concentration of lipid in celery makes it of more advantage to human health. Carbohydrate is a good source of energy which is essential for the maintenance of life in plant, animals, and human, celery indicating the highest carbohydrate content makes it a good source of energy.

The carbohydrate content of the studied vegetable spices is similar to those reported for alligator pepper, black pepper, clove, and ginger (Okunade *et al*., 2019). While the ash and crude fibre of the leaves are higher than those reported for garlic and African nutmeg (Onimawo *et al*., 2019).

Table 3. Proximate composition of scent leaf, bay leaf, and celery leaf flour

Property	Bay leaf	Celery leaf	Scent leaf
Fat (%)	5.05 ± 0.17^{b}	2.06 ± 0.11^{b}	2.14 ± 0.04^{b}
Fibre (%)	12.79 ± 0.35^{b}	5.04 ± 0.13^{b}	9.98 ± 0.02^{a}
Ash (%)	9.74 ± 0.26^{a}	6.21 ± 0.16^{a}	2.45 ± 0.04^{a}
Protein (%)	1.03 ± 0.03^{c}	5.04 ± 0.03^{c}	16.69 ± 0.01^{c}
Carbohydrate (%)	71.39 ± 0.17^{b}	81.64 ± 0.64^{a}	68.74 ± 0.51^{c}

Values are expressed as mean ± standard deviation of three replicates; Means having the same superscript in a row are not significantly different ($p<0.05$)

Phytochemical contents of scent leaf, bay leaf, and celery leaf

The result of the phytochemical analysis of the spices are shown in Table 4. The phytochemicals analysis of scent, bay, and celery leaf flour samples differed significantly. Scent leaf has significantly high alkaloids (12.17%), tannins (10.25 mg GAE/g), and saponins (9.65 mg/100g) contents when compared to bay leaf, which has alkaloids (2.45%), tannins (5.31 mg GAE/g), and saponins (4.47 mg/100g) and celery leaf, which has alkaloids (4.26%), tannins (6.88 mg GAE/g), and saponins (0.20 mg/100g). The vegetable spices studied have a wide range of pharmacological applications, which includes the production of quinine, ephedrine, galantamine, vincamimine, quinidie, morphine, and many others. This is because scent, bay, and celery leaves have a high percentage of alkaloids, flavonoids, tannins, and saponins, which are present in herbs. However, they are low in phenols. Many plants used in traditional medicines worldwide contain some certain level of the phytochemical contents, which can often account for their therapeutic action (Anupam *et al.*, 2018).

Table 4. Phytochemical composition (Quantitative) composition of Bay, Scent, and Celery flour

Property	Scent	Bay	Celery
Alkaloids (%)	12.17±0.15[a]	2.45±0.04[b]	4.26±0.07[b]
Flavonoids (mg GAE/g)	8.30±0.07[b]	5.72±0.20[c]	13.58±0.31[a]
Phenols (mg GAE/g)	1.08±0.05[a]	0.48±0.05[b]	0.32±0.06[b]
Tannins (mg GAE/100g)	10.25±0.15[a]	5.31±0.09[b]	6.88±0.06[b]
Saponins (mg/100g)	9.65±0.23[a]	4.47±0.23[b]	0.20±0.03[c]

Values are expressed as mean ± standard deviation of three replicates; Means having the same superscript in a row are not significantly different ($p<0.05$)

CONCLUSION

The particle size distribution, physical properties, and chemical composition of scent leaf, bay leaf, and celery leaf have been established in this study. The vegetable spices investigated are rich in carbohydrates, crude fibres, fats, and proteins, which form a large portion of the human diet. Scent leaf produced a coarse particle-sized flour while blended celery leaf has a fine particle flour. The low oil absorption capacity of celery leaf flour enhances its flavour retention capability, which made it a preferred flavouring agent. The large proportion of phytochemicals contents of the spices suggest that they are a good source of minerals. Generally, all the spices investigated are cheap, nutritionally rich, and have potentials for pharmacological applications.

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Determination of Heavy Metals in Some Fruits, Vegetables and Fish by ICP-MS

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Abstract

This study was designed to determine arsenic, lead, mercury and cadmium levels in Spinacia oleracea (spinach), Daucus carota (carrot), Malus pumila (apple), Solanum tuberosum (potato) and canned Thunnus thynnus (tuna fish), Engraulis encrasicolus (anchovy fish), Salmo trutta labrax (sea trout) and Salmo trutta (farm trout). Samples were mixed, blended raw, weighed and placed in labeled sample cups. All samples were analyzed using Inductively Coupled Plasma Mass Spectrometry (ICP-MS). Arsenic, Pb and Hg concentrations were lowest in potato; As, Cd and Pb concentrations were highest in spinach. As and Cd loads were highest in anchovy fish and Pb load was highest in canned tuna. Lead concentrations of all fruits and vegetables exceeded the safe limits. Arsenic concentration in anchovy fish was above the acceptable limit. Some fruits, vegetables and fish species as analyzed in our study may threaten human health because of their heavy metal loads.

Keywords: Contamination, fish, fruit, heavy metal, vegetable.

Research article
Received date: 8 February 2021
Accepted date:28 April 2021

INTRODUCTION

The dissemination of heavy metals into the environment adversely affects the quality of foods. Trace metals such as copper (Cu), zinc (Zn), manganese (Mn), cobalt (Co) are necessary for healthy growth and development of humans and animals. In contrast; arsenic (As), lead (Pb), mercury (Hg) and cadmium (Cd) are indicated as toxic metals for living things and for these toxic metals there is no known homeostasis mechanism in the body. (Morais et al., 2012).

Heavy metals can be spread to the environment in many different ways. Factors leading to the dissemination of heavy metals to the environment include natural degradation of the earth's crust, mining, soil erosion, industrial discharges, effect of industrial and domestic wastes blending in to the seas, urban flow, sewage wastes, harmful agents applied to plants, and air pollution (Morais et al., 2012). Heavy metal pollution in the soil and seas affects the nutritional quality of foods and can reach animals and humans through the food chain. The main sources that cause heavy metals to mix into nature are; mines, wastewater of various metal and paper industries, fertilizers, fossil fuels, pesticides, various chemicals and household waste (Afoakwa, 2008). Lead, As, Cd and Hg are the most common heavy metals that cause soil pollution (Wuana and Okieimen, 2011). Long-term accumulation of heavy metals in soil can lead to a reduction in buffering capacity and pollution of groundwater (Sun et al., 2010). Heavy metals present as particulates or vapor in the atmosphere can be taken into plants, although the uptake is typically less than that of from soil and water (Nagajyoti et al., 2010). For example, vegetables and fruits which have beneficial effects on health with rich vitamin mineral content can affect human health negatively as a result of heavy metal pollution. Same way while some heavy metals such as Cu, Zn, Fe are used by human metabolism in many events, heavy metals such as Hg, Cd and Pb which have no beneficial effect and function, can be transmitted to the water in various ways. At the same time, the presence of these metals in high amounts of water leads to the death of many living creatures, as well as disrupting the natural balance of life (Karayakar et al., 2017).

Heavy metals in food are not found in safe concentrations and the consumption by humans for a long time causes heavy metals to accumulate chronically in the human body. The accumulation of heavy metals in the body can cause many biochemical processes to deteriorate and cause kidney, liver, heart, nerve and bone diseases in humans. Therefore, the international and national regulations on food quality have reduced the permissible maximum levels of toxic metals in foodstuffs as these metals increase the risk of food chain pollution (Sharma et al., 2009).

The aim of this study was to determine the concentrations of As, Pb, Hg and Cd in one edible portion of some fruits, vegetables and some fish species frequently consumed in Turkey.

MATERIAL and METHOD

Sample Selection and Collection

The selection of vegetables and fruits was made in March 2017 and the selection of fish species was made in January 2018. One kg of each fruit and vegetable samples of the products were purchased from local markets in Kartal, Maltepe and Sarıyer districts of Istanbul. Potatoes, spinach and apples were grown at garden conditions, only carrots were grown in greenhouse conditions. For the fish samples; 324 g anchovy, 772 g farm trout, 1980 g sea trout and 80 g canned tuna were purchased from local markets and bazaars in Bakirkoy, Pendik and Uskudar districts of Istanbul. All fish species (except canned tuna fish) were obtained from Marmara Sea.

Sample Preparation

The fruit and vegetable samples were washed under flowing warm water for 3 minutes. Non-renewable parts were removed. The roots of spinach and pale leaves were discarded. Potatoes were peeled and damaged potatoes and the peelings were discarded. Two centimeters of the beginning and end of the carrot, and the skins were discarded. Samples taken from different district markets and bazaars were mixed and blended raw.

For the fish samples; the head, internal organs, bone and tail parts were discarded.Fish taken from different districts were blended homogenously. The samples were weighed with precision kitchen scale and placed in plastic sample cups and labeled. Sixty-three g of spinach, 113 g of potatoes, 130 g of apples, 107 g of carrots, 153 g of anchovy, 164 g of farm trout, 176 g of sea trout and 80g x 3 canned tuna were sent for analysis.

Chemical Analysis

Analysis of the samples was carried out in Yıldız Technical University Central Laboratory. Firstly, samples were digested by microwave digester. A wet ground weight (0.5 g) was digested with 6 mL of nitric acid (HNO_3, 65%) and 2 mL of hydrogen peroxide (H_2O_2, 30%). The digested samples were evaporated to dryness and diluated to 20 mL with deionized water. Concentrations of heavy metals (As, Hg, Pb and Cd) in the digested samples were analyzed with Agilent 7700 ICP-MS. Standard 1: Agilent 8500-6940 2A (10 ppm in 5% HNO_3): As, Cd and Pb, standard 2: Agilent 8500-6940 Hg (10 ppm in 5% HNO_3): Hg, internal satandard: Agilent 5188-6525(100 ppm in 5% HNO_3): Li, Sc, Ge, Rh, In, Tb, Lu, Bi and tune solution: Agilent 5185-5959: Li, Y, Tl, Ce, Co, Mg (1ppb in 2% wt HNO_3) solutions used during analysis.

Calculation

The results were reported as ng/g of fresh food. For this reason, the residual percentages found in the "Food Composition Table" were removed and after the net weight was found, the heavy metal load of one portion was calculated by multiplying the value per gram in the analysis report. The residual ratio of non-edible parts were; 8% for apple, 22% for carrot, 17% for spinach, 19% for potato, 23% for anchovy, 51% for farm trout and 62% for sea trout. There was no residue of non-edible parts for canned tuna.

RESULTS and DISCUSSION

Arsenic, lead and mercury concentrations were lowest in potato. In contrast, spinach had the greatest concentrations of As, Cd and Pb. Mercury levels were found low in all samples (shown in Table 1). Anchovy was found to have the highest levels of As and Cd. Canned tuna and farm trout had the highest concentrations of Pb (shown in Table 2).

According to the comparison of heavy metal levels of all samples with reference codex values; all fruit and vegetable samples had higher Pb concentrations than the values set by both Joint Expert Committee on Food Additives (JECFA) and Turkish Food Codex (TFC) limits (shown in Table 3). In all analyzed fish species, Zn, Cd and Hg values were found below the reference. Lead levels of canned tuna and farm trout were determined as close to the upper limit, and the anchovy's As level exceeded the reference limit (shown in Table 4).

Heavy metals found in food may cause heavy metal accumulation in human body. For this reason, food quality should be monitored within the framework of national and international regulations (Morais et al., 2012). The comparison of the results of heavy metal analysis of fruits and vegetables with the reference codex values is given in Table 3. According to the table, Cadmium level of apple was found to be higher than the reference value of Turkish Food Codex (Turkish Food Codex Regulation on Contaminants, 2011). Moreover, Lead levels of all fruits and vegetables were found to be over the limits of both Joint FAO/WHO Expert Committee on Food Additives (JECFA, 2002) and Turkish Food Codex (Turkish Food Codex Regulation on Contaminants, 2011).

Investigation of heavy metal levels in aquatic organisms that are consumed by humans has great importance for human health. Heavy metals accumulate in the tissues and organs of the fish, and may cause dysfunctions at the cellular and molecular levels when consumed (Kayhan et al, 2010). One of the main causes of water pollution is petroleum. While the upper limit of the amount of petroleum in sea waters should be 10 µg /g, in a study conducted in 2017; it was found to be 540 µg/g in the Black Sea, 148 µg/g in the Marmara Sea, 1100 µg/g in the Bosphorus (İstanbul) and 592 µg/g in the Dardanelles. These results show that the seas are polluted with high amount of petroleum (TUDAV, 2018). In a study, it was stated that meat yields and fat ratios were negatively affected in fish exposed to heavy metal contamination, and the changes occurred differently according to the type and age of fish, and duration of heavy metal exposure (Kayhan et al., 2010).

Arsenic

In a study held in Bangladesh, As values for vegetables was found as 0.25 µg/g and 0.19 µg/g for fruits (Islam et al., 2014). In Osaili and collegues' study, As values found as; 0.31 µg/g in spinach, 0.6 µg/g in carrot and 0.13 µg/g in potato (Osaili et al., 2016). Another study held in 2016, As values were found as; <0.0007 µg/g in potato, <0.00073 µg/g in carrot, 0.01234 µg/g in spinach and <0.00074 µg/g in apple (Shaheen et al., 2016). The values found in this study were lower than the results of previous studies. Shaheen et al. conducted the study in Bangladesh and there may be differences in soil properties and soil characteristics in this region. Osaili and collegues (2016) pointed out that the use of atmospheric pollution and phosphate fertilizers may contribute to the residue of As in agricultural soils. Moreover, Islam et al. (2014) indicate that the As content in the soil was high due to the excessive use of As contaminated irrigation water. In this study, heavy metal loads of one portion of the foods examined were calculated based on the edible amount of nutrients. Arsenic load of 1 portion was found <0.057 µg in carrot, 1.5 µg in spinach, <0.051 µg in potato, <0.068 µg in apple. There is no limit value specified in the JECFA and Turkish Food Codex for arsenic.

In a study conducted in Iran in, the amount of As in tuna fish was analyzed with the atomic absorption spectrometry (AAS) method and the average value was found to be 0.128 µg/g (Khansari et al., 2005). It has been determined that the As level of anchovy was above the maximum consumable level. Moreover, this limit for canned tuna and farm trout were close to the consumable limit (Turkish Food Codex Regulation on Contaminants, 2011; 2002).

In a study conducted in 2013, anchovy were collected from the Black Sea shores of Turkey and Georgia during the hunting season (January, October and November) and analyzed. The As levels of anchovy's edible parts were found between 0.41-0.69 mg/kg. It is stated that the values in edible tissues are not above the values specified in the regulations and do not pose a health risk for consumers (Bat et al., 2014). In our study, in the anchovy sample collected in January, the As value was found as 1.48 µg/g, which is above the Food Codex limits.

Mercury

Hu et al., (2017) determine the Hg value in leafy vegetables as 0.002±0.001 µg/g, in rootstalk vegetables as 0.0003±0.0001 µg/g and in fruits as 0.0003±0.0002 µg/g. In this study, Hg values were found as <0.00032 µg/g in carrot, <0.00031 µg/g in spinach, <0.00031 µg/g in potato and <0.00033 µg/g in apple. Hg values determined in our study for carrot, potato and apple were higher, and Hg value for spinach was lower than the study of Hu and colleagues.. There is no reference limit specified in the Turkish Food Codex for mercury.

According to JECFA, the limits of one portion of the foods examined were 78 µg for carrots, 124.5 µg for spinach, 72.9 µg for potatoes and 92 µg for apples. In our study, these values are as follows: <0.025 µg/per portion in carrot, <0.038 µg/per portion in spinach, <0.023 µg/per portion in potato and <0.031 µg/per portion in apple. The Hg levels found in this study are below the values of JECFA.

Mercury levels were analyzed with the ICP-MS method in the anchovy fish that were caught every two months between October 2012 and April 2013 from the Marmara Sea and the average value was found within acceptable legal limits (0.04±0.00 mg/kg) (Türksönmez & Diler, 2017). In another study, the highest accumulation of heavy metals (lead, cadmium, mercury, copper and chromium) was observed in copper in tilapia fish obtained from Wadi Hanifah, Saudi Arabia in 2010, while the lowest was Hg bioaccumulation. The results were below safe limits for consumption (Abdel-Baki et al., 2011). In Khansari and colleagues' study, Hg level of canned tuna fish was found as 0.117 µg/g which is below the limits set by FAO and WHO (Khansari et al., 2005). In another study, metal levels in edible tissues of fish sold in Konya were analyzed by using inductively coupled plasma optical emission spectrometry (ICP-OES). As a result of the analysis, it has been determined that the Hg value of fish was at a level that will not cause any harm in terms of public health (Günhan and Yalçın, 2015).

Lead

In a study of Islam and colleagues, it was found that the Pb value for vegetables was 0.25 µg/g and 0.19 µg/g for fruits (Islam et al., 2014). Hu et al. (2017) determined the Pb value as 0.022±0,017 µg/g in leafy vegetables. Kim et al. (2012) determined the Pb value as 0.026 µg/g in tuberous vegetables. Osaili and collegues found the Pb value as 0.503 µg/g in spinach, as 1.26 µg/g in carrot and as 1.061 µg/g in potato (Osaili et al., 2016). In Shaheen and colleagues' study, it was found that the Pb value for carrots was 0.029±0.025 µg/g and 0.007±0.006 µg/g for potato (Shaheen et al., 2016). A study held in Serbia, Pb concentration in spinach leaves was found as 3.56 µg/g (Pajević et al., 2018). Adimula et al. (2019) found the Pb value as 0.459±0.03 mg/kg in carrot and 0.74±0.05 mg/kg in apple. In a study in Turkey by using inductively coupled plasma atomic emission spectroscopy (ICP-AES), Pb value was found as 2.21±0.75 µg/g in apple (Hamurcu et al., 2010). In our study, we found Pb values as 0.432 µg/g in carrot, as 0.430 µg/g in spinach, as 0.408 µg/g in potato and as 0.436 µg/g in apple. The Pb values found in our study are lower than what was found in the studies of Osaili et al. (2016), Pajević et al., (2018) and Adimula et al. (2019). However, our results for Pb is higher than the results of the study of Hu and colleagues (Hu et al., 2017). The reason for this result may be only the vegetables grown in the greenhouse were selected in the study of Hu et al. The maximum acceptable limits in Turkish Food Codex for lead and the general limits in JECFA for one portion are as 7.8 µg for carrots, 37.35 µg for spinach, 7.29 µg for potato and 9.2 µg for apple. In our study, the values of Pb were found as 33.6 µg/per portion in carrot, 53.5 µg/per portion in spinach, 29.7 µg/per portion in potato and 40 µg/per portion in apple. The Pb levels detected in our study were higher than permissible level and these results may indicate that some of the fruits and vegetables might cause a potential risk for human health in terms of Pb content. The soil properties and characteristics of the region where fruits and vegetables were grown can be shown among the reasons why the amount of lead is so high. Moreover, it is thought that the soil may have been exposed to lead especially by air.

In Türksönmez and Dilers' study (2017), Pb level of anchovy fish was found as 0.29 ± 0,03 mg/kg which is within the legal limits. For this reason, it has been concluded that the consumption of anchovy fish is not a problem that would threaten human health. Similarly, Khansari et al. (2005) found the Pb level of canned tuna fish within the acceptable limits (0.726 µg/g). In 2005, the Pb content of canned fish of different species from supermarkets in Turkey was analyzed by using AAS method.

The results were found in the range of 0.09-0.40 μg/g and compared with the values in the literature. The results were above the legal limits. As a result of the study, it was stated that metal levels can be reduced by processing raw materials and more frequent analyzes should be made in canned fish in Turkish supermarkets (Tüzen and Soylak, 2007). In our study, Pb level in canned tuna fish was determined as 0.18 μg/g, which is very close to the legal limits set by the Turkish Food Codex, 0.2 mg/kg.

Trout collected in Rize between April and September 2011 were analyzed with ICP-OES and the heavy metal levels of the fish in the muscles, liver, gills and skins were examined. According to the results of the study, it was concluded that the metal levels in trout were lower than the acceptable limit recommended by FAO and WHO and did not threaten human health (Dizman et al., 2017). In our study, heavy metal levels of sea trout were found below Turkish Food Codex limits. However, in farm trout, the Pb load was found to be 0.28 μg/g, which is close to the upper limit of 0.3 mg/kg.

Cadmium

Hu et al., (2017) determine the Cd value of leafy vegetables as 0.013±0.010 μg/g. Osaili and collegues (2016) found the Cd values as 0.129 μg/g in spinach, 0.07 μg/g in carrot and 0.08 μg/g in potato. These values found as 0.023±0.003 μg/g in carrot and 0.013±0.007 μg/g in potato in the study of Shaheen and colleagues (Shaheen et al., 2016). In the study of Pajević et al. the Cd concentrations were found as 0.48 μg/g in spinach leaves and 0.29 μg/g in carrot (Pajević et al., 2018). Adimula et al. (2019) found the Cd values as 0.108±0.02 mg/kg in carrot and 0.139±0.03 mg/kg in apple. In our study, Cd values were found as 0.055 μg/g in carrot, 0.088 μg/g in spinach, 0.069 μg/g in potato and 0.053 μg/g in apple. These values we found in our study are lower than the findings in the studies of Pajević et al. (2018) and Adimula et al. (2019), however, higher than the findings in the studies of Shaheen et al. (2016) and Osaili et al. (2016). This difference can be explained due to the differences in soil properties and soil characteristics in different regions. According to JECFA, the general limits for Cd in one portion were determined as 7.8 μg for carrot, 24.9 μg for spinach and 7.29 μg for potato, and there is no limit set for apple. In our study, Cd values were found as 4.3 μg/per portion in carrot, 10.9 μg/per portion in spinach, 5 μg/per portion in potato and 4.8 μg/per portion in apple. According to our results, the determined Cd levels were lower than the values of JECFA.

In Türksönmez and colleagues' study (2017), Cd level of anchovy fish was determined as 0.07 mg/kg which can be accepted as a safe limit. Similar to this study, Khansari et al. (2005) found the Cd level of canned tuna fish as 0.0223 μg/g and this value is below the acceptable limits of FAO and WHO. In the study of Tuzen and Soylak with different types of canned fish, Cd load was found as 0.25 μg/g and compared with previous studies. The results were found to be above the legal limits (Tüzen and Soylak, 2007). In contrast of this study, in another study held in Turkey, Cd values of edible parts of fish were analyzed by using ICP-OES and the results were found within the safe limits (Günhan and Yalçın, 2015). Similar to the previous study, Cd loads of the fishes in our study were found below the upper reference limits set by Turkish Food Codex.

One of the limitations of this study may be the foods (fruits, vegetables and fish) that were analyzed had a limited variety. On the other hand, one of the strength of this study may include these specific foods were selected as the most common ones that Turkish people consume.

Table 1. Heavy metal levels of fruits and vegetables

Heavy Metals (µg/g)	**Fruits and Vegetables**			
	Carrot *(Daucus carota)*	Potato *(Solanum tuberosum)*	Spinach *(Spinacia oleracea)*	Apple *(Malus pumila)*
Arsenic	<0.00073	<0.0007	0.01234	<0.00074
Cadmium	0.055	0.069	0.088	0.053
Lead	0.432	0.408	0.430	0.436
Mercury	<0.00032	<0.00031	<0.00031	<0.00033

Table 2. Heavy metal levels of fish species

Heavy Metals (µg/g)	**Fish Species**			
	Canned Tuna *(Thunnus thynnus)*	Anchovy *(Engraulis encrasicolus)*	Sea Trout *(Salmo trutta labrax)*	Farm Trout *(Salmo trutta)*
Arsenic	0.55	1.48	0.30	0.24
Cadmium	0.008	0.013	0.004	0.010
Lead	0.18	0.22	0.15	0.28
Mercury	0.126	0.064	0.065	0.065

Table 3. Comparison of the results of heavy metal analysis (µg/per portion) with reference codex values for fruits and vegetables

Fruits & Vegetables	**Arsenic**			**Cadmium**			**Lead**			**Mercury**		
	Analysis	*JECFA*[*]	*TFC*[**]	*Analysis*	*JECFA*	*TFC*	*Analysis*	*JECFA*	*TFC*	*Analysis*	*JECFA*	*TFC*
Carrot *(Daucus carota)*	<0.057	ND[***]	ND	4.3	7.8	7.8	***33.6***	7.8	7.8	<0.025	78	ND
Potato *(Solanum tuberosum)*	<0.051	ND	ND	5	7.29	7.29	***29.7***	7.29	7.29	<0.023	72,9	ND
Spinach *(Spinacia oleracea)*	1.5	ND	ND	10.9	24.9	12.45	***53.5***	37.35	37.35	<0.038	124.5	ND
Apple *(Malus pumila)*	<0.068	ND	ND	***4.8***	ND	4.6	***40***	9.2	9.2	0.031	92	ND

[*] *Joint FAO/WHO Expert Committee on Food Additives;* [**]*Turkish Food Codex;* [***]*Not determined.*

Table 4. Comparison of the results of heavy metal analysis (μg/per portion) with reference codex values for fish species

Fish Species	Arsenic		Cadmium		Lead		Mercury	
	Analysis	*TFC*[*,1,2]	*Analysis*	*TFC*[*,1,2]	*Analysis*	*TFC*[*,1,2]	*Analysis*	*TFC*[*,1,2]
Canned Tuna *(Thunnus thynnus)*	0.55	1	0.008	0.05	***0.18***	0.2	0.126	0.5
Anchovy *(Engraulis encrasicolus)*	***1.48***	1	0.013	0.30	0.22	0.3	0.064	0.5
Sea Trout *(Salmo trutta labrax)*	0.30	1	0.004	0.05	0.15	0.3	0.065	0.5
Farm Trout *(Salmo trutta)*	0.24	1	0.010	0.05	***0.28***	0.3	0.065	0.5

[*]Turkish Food Codex; [1]Turkish Food Codex Regulation on Contaminants, 2002; [2]Turkish Food Codex Regulation on Contaminants, 2011.

CONCLUSION

Lead concentrations in all fruits and vegetables that were analyzed were found to be higher than the reference codex values of both JECFA and TFC. Moreover, cadmium concentration of apple was determined as higher than the acceptable limit of TFC. Arsenic load of anchovy fish was found to be way higher than the safe limit of TFC. Furthermore, lead loads of canned tuna and farm trout were found to be close to the upper limits of TFC. This study conducted in Turkey has not enough data to comment on human health. There is very limited number of studies about the effects of foods with high heavy metal concentrations on human health. More comprehensive studies are needed to achieve the final results.

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In vitro Investigation of Antioxidant Properties of Turnip (*Brassica Rapa Subsp. Rapa)*

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Abstract

Turnip (Brassica rapa subsp. Rapa), which grows in many regions of Western Asia and Europe, is a seasonal and herbaceous plant belonging to the cruciferous family. As a result of the studies, it has been revealed that the turnip juice has antioxidant properties. However, no previous study has been carried out on the antioxidant activity of turnip root as a source. In this study, the activities of turnip, which were subjected to Soxhlet extraction with solvents with different polarities, were compared with standards known to have antioxidant properties and thus, antioxidant activity of the extracts was tried to be determined. The radical scavenging effects, total antioxidant activities, reducing power, metal chelating capacity, β-carotene bleaching power of water, ethanol and ethyl acetate extracts in different concentrations were studied. In addition, proline determination, total phenolic compound amount, total anthocyanin and total carotenoid amounts were measured in all extracts. As a result of the studies, it was found that the antioxidant activity of the turnip plant studied in the form of root was higher than the standard antioxidants in some parameters studied, and the activity values were interpreted by comparing with other plants in the same species.

Keywords: Turnip, antioxidant activity, free radicals, Soxhlet extraction, Brassica, natural product

Research article
Received Date: 18 April 2021
Accepted Date:4 May 2021

INTRODUCTION

Today, it has been revealed by many studies that free radicals and the oxidative degradation they cause play a significant role in a significant part of the illnesses whose mechanism is illuminated (Koca and Karadeniz, 2003; Lushcha, 2014). Structures that act in response to oxidative degradation on biomolecules such as DNA, protein, carbohydrate, and lipid, which play key functions for the continuity of life, are antioxidants. Antioxidants that prevent oxidative stress caused by free radicals over other molecules also significantly reduce the damage capacity of free radicals. By utilizing this radical cleansing activity of antioxidants, cellular damage caused by free radicals is either inhibited or delayed (Lobo et. al., 2010).

The importance of antioxidants with antitumor activity, preventing cardiovascular diseases, producing remarkable results against aging, and resistance to inflammation are increasing day by day (Podsędek, 2007). The activities and antioxidant effects of antioxidants, which can be taken into the body with many different consumable foods, increase with the consumption of foods with high polyphenol composition (Belguith-Hadriche et. al., 2013).

Turnip radish (Brassica rapa subsp. rapa) whose adaptation ability is very high, is a plant of the Brassicaceae family (cruciferous or cabbage) in the genus Brassica and have a large growing area. Turnip, which loves warm and cool seasons, is also resistant to cold despite this structure. Due to be flexible in the tolerance to cold and warm temperatures, can be grown in many parts of western Asia and Europe but in Turkey, Adana, Osmaniye, Mersin is especially where it is cultivated. However, it is also possible to grow in colder regions (Padilla et. al., 2005).

To the authors' knowledge, there are no published reports on the antioxidant activity of the turnip root. The studies on the antioxidant activity of natural resources have gained speed in recent years, and in this study, antioxidant activity of the extracts of turnip root, which has not been used as a direct material in a similar study, with the help of solvents with different polarity; radical scavenging effects, total antioxidant activities, reduction power, metal chelating capacity, β-carotene bleaching power were studied and proline determination in all extracts, total phenolic compound amount, total anthocyanin and total carotenoid amount were also measured. Antioxidant activity results obtained and compared with standard antioxidants BHA, BHT, Trolox, ascorbic acid, α-tocopherol, catechin and epicatechin.

MATERIAL and METHOD

Source Plant and Extraction Process

In this study, the root part of the turnip plant, which is obtained from a local street market in Küçükçekmece district of Istanbul province and can be used both in leaves and root, was preferred for research purposes. In order to obtain the extracts, 50 grams samples taken from the turnip root were subjected to Soxhlet extraction for 4 hours with water, ethanol and ethyl acetate solvents each of which is 100 mL. Then, solvents were removed from the extracts using an oven, fume hood and the rotary evaporator. Extracts which obtained from turnip and the turnip itself was stored at +4 °C throughout the study for determination of its antioxidant activity.

Measurement of Antioxidant Activity

Lambda 25 UV / VIS Spectrophotometer (Perkin Elmer) was used to measure the antioxidant activity and percent inhibition values. PB-11 pH meter (Sartorius) was used for pH control of the prepared solutions. In the centrifuged solutions, 3K30 model (Sigma) centrifuge device was used. A 1086 model (GFL) shaking incubator was used for the incubation procedures. For distilled water used in solutions, LA620 (ELGA) was used.

DPPH• Radical-Scavenging Assay

The Brand-Williams method was used to determine this parameter (Min and Boff, 1998). During the application of this method, 2.5 mg DPPH• was dissolved in 125 mL ethanol. 0.75 mL taken from BHA, α-tocopherol and catechin samples prepared as standard with plant extracts diluted in different concentrations as 100 µg/mL, 150 µg/mL, 200 µg/mL and 250 µg/mL from stock added over 1.5 mL of DPPH• and the samples were mixed thoroughly using vortex. The absorbance values of the samples kept in the dark for 30 minutes at room temperature against ethanol at 517 nm were measured.

The control solution used during the study was chosen as the DPPH• radical with added water. For the % scavenging activity of the DPPH• radical, the following equation was used where A_0 corresponds to the absorbance of the control solution and A_1 as standard or samples.

$$\text{DPPH}^{\bullet} \text{ radical scavenging effect (\%): } [(A_0 - A_1) / A_0 \times 100] \quad (1)$$

Reducing Power

The basis of this parameter, which is based on Oyaizu method, is the measurement of absorbance values at 700 nm of colored complexes formed by $K_3Fe(CN)_6$, TCA and $FeCl_3$ compounds with molecules that are antioxidant sources (Oyaizu, 1986). In the application of the method, water, ethanol, ethyl acetate extracts and BHA, catechin and α- tocopherol standards were prepared and diluted to different concentrations between 100-250 µg/mL.. 2.5 mL of phosphate buffer (0.2 M and 6.6 pH) and 2.5 mL of 1% (w/v) $K_3Fe(CN)_6$ were added on 1 mL of each sample, respectively. The well mixed samples were incubated for 30 minutes at 50 °C. Then, 2.5 mL of 10% (w/v) TCA solution was added to each sample solution and the samples were centrifuged at 3000 rpm for 10 minutes. After centrifugation, 2.5 mL distilled water and 0.5 mL 0.1% (w/v) $FeCI_3$ solution were added to the 2.5 mL samples taken from the filtrates. The absorbances of the colored solutions formed at 700 nm were read. The green color present at the beginning of the reaction turned blue at the end of the application, indicating the reducing power of the standards and samples.

Metal Chelating Activity

Decker and Welch (1990) method was used to determine the metal chelating activity (Gülçin, 2012). In the implementation of the experiment, 3.7 mL of distilled water and 0.1 mL of $FeCl_2$ (2 mM) were added to 1 mL each of the samples diluted to different concentrations between 100-250 µg/mL. The mixed samples were left in the dark for 10 minutes at room temperature. In this method, in which EDTA, Trolox, BHA and α-tocopherol are used as standard, the absorbance at 562 nm of the colored solutions formed as a result of the complexes was measured. Using the absorbance values of the resulting colored solutions and the equation given below, the percentage of inhibition of the complex formed between Ferrozin and Fe^{2+} was calculated. In this equation, A_0 corresponds to the absorbance of EDTA, which is the control solution, while A_1 refers to the absorbance of samples prepared from extracts and standard antioxidants.

$$\text{Metal chelating activity (\%): } [(A_0 - A_1) / A_0] \times 100 \quad (2)$$

Proline Assay

For proline analysis, a method derived from very little change was used based on the application developed by Bates (Bates et. al., 1973). First of all, the proline sample to be used as standard was prepared and diluted at concentrations of 5 µg/mL, 10 µg/mL, 15 µg/mL, 20 µg/mL and 25 µg/mL. Then, 1 mL each of the acid ninhydrin solution prepared with 1.25 g of ninhydrin, 30 mL of glacial acetic acid and 20 mL (6 M) of phosphoric acid was added onto 1 mL of proline samples. To the resulting solution, 1 mL of glacial acetic acid was added and the mixed solution was incubated at 100 °C for 1 hour. To the samples cooled in an ice bath after incubation, 2 mL of toluene was added to the samples and proline standard samples were obtained. The resulting colored toluene phase was separated from the underlying water phase and the absorbance of the separated phase at 520 nm against toluene was measured. The same procedure was followed for the samples prepared from the extracts. 15 mg samples taken from plant extracts were dissolved in 3 mL and 3% sulfosalicylic acid solution. Samples diluted to concentrations between 100-250 µg/mL were centrifuged.

After centrifugation, 1 mL each was taken from the filtered samples and firstly, 1 mL of acid ninhydrin solution and then 1 mL of glacial acetic acid were added to them. The resulting solutions were left to incubate at 100 °C for 1 hour. After the incubation, 2 mL of toluene was added to the samples cooled in an ice bath, and the absorbance of the colored phases of the samples against toluene at 520 nm was measured. The proline content of the extracts prepared from the turnip root was calculated in terms of proline by using the standard curve drawn.

Determination of Total Flavonoid

In the determination of the total flavonoid content, the colorimetric method was developed by Zhishen et al. (1999) preferred (Zhishen et. al., 1999). While the concentration of (+)-catechin used as standard varies between 20-100 µg/mL, the concentrations of solutions prepared from turnip root extracts were chosen between 100-250 µg/mL. 1.25 mL distilled water was added to each 0.25 mL taken from the samples. Firstly, 75 µL and 5% (w/v) sodium nitrite was added to each sample and waited for 6 minutes. Then, 150 µL and 10% (w/v) aluminum chloride was added to each sample, and another 5 minutes was waited. At the end of the specified time, 0.5 mL and 1 M NaOH were added to each test tube. Then, each sample was completed to a volume of 2.5 mL with the addition of 275 µL of distilled water. The absorbance of the samples, which were mixed thoroughly, against ethanol at 510 nm was measured. The antioxidant activities of the samples were expressed in terms of (+)-catechin used as standard in the method.

Total Antioxidant Activity Assay

Thiocyanate method was used for the determination of total antioxidant activity. In the thiocyanate method, the regulated version of the method developed by Osawa and Namiki in 1981, was used (Osawa and Namiki, 1980). Samples were prepared from each turnip root extract and BHA and α-tocopherol standard solutions in concentrations ranging from 100-250 µg/mL, and the samples were diluted by adding phosphate buffer solution (0.04 M and pH 7.0) to a total volume of 2.5 mL. On the solutions in the tubes and the stock solutions containing only distilled water instead of sample, 2.5 mL of linoleic acid emulsion obtained by combining 1750 µg Tween 20, 1550 µL linoleic acid and 496.7 mL of phosphate buffer solution was added and at 37 °C left for incubation in the dark. By taking 0.1 mL each of the samples at 24 hour intervals, 4.7 mL of 75% ethanol, 0.1 mL of 30% (w/v) ammonium thiocyanate solution and 0.1 mL of 0.02 M $FeCl_2$ solution prepared using 3.5% HCl were added. The % inhibition values of the samples whose absorbance was measured against ethanol at 500 nm by waiting for 3 minutes were calculated according to the following equation, where A_0 corresponds to the absorbance values of the control solution and A_1 corresponds to the absorbance extracts and standards:

$$\text{Inhibition (\%)} = [(A_0 - A_1) / A0] \times 100 \qquad (3)$$

β-Carotene Bleaching Test

In the method, first, 5 mg of trans-β-carotene was dissolved in 5 mL of chloroform. 0.2 mL of trans-β-carotene solution was added to the solution consisting of 20 mg linoleic acid and 200 mg Tween 40. After the chloroform in the mixture was evaporated in fume hood, 50 mL of distilled water was added to the emulsion to reach its final form. While studying this parameter in which the samples were prepared in a single concentration, 0.2 mL of the plant extracts prepared at a concentration of 2 mg/2 mL and the BHA solution used as a standard were taken, and 5 mL of the prepared emulsion was added to each of them. Then, samples were incubated for one hour at 50 °C.

The absorbance values of the samples taken at 0th, 60th and 120th minutes were read at 470 nm. Distilled water was used as a blank solution. In the β-carotene bleaching method, two different control solutions were used: BHA solution was the positive control and distilled water was the negative control. By considering the absorbance values of BHA accepted as 1, the Relative Antioxidant Activity (RAA) calculation determined for bleaching power tests is given in the equation below (Peksel et. al., 2013).

$$RAA = \text{Absorbance of the sample} / \text{Absorbance of the BHA solution} \quad (4)$$

$ABTS^{\bullet+}$ Assay

To determine $ABTS^{\bullet+}$ radical scavenging activity, the method developed by Arnao et al. (2001) was applied (Arnao et. al., 2001). First, 7.4 mM ABTS solution and 2.6 mM $K_2S_2O_8$ solution were prepared and mixed in equal amounts of volume and kept in the dark at room temperature for 12 hours to obtain the $ABTS^{\bullet+}$ radical solution. 20 mL of methanol was added on 1 mL of $ABTS^{\bullet+}$ solution, and dilution with methanol was continued until the absorbance of this radical solution reached 1.1 ± 0.02 at 734 nm. A solution of 2850 µL $ABTS^{\bullet+}$ was added to 150 µL of extract solutions and Trolox, BHA, epicatechin and α-tocopherol standard solutions which have different concentrations as 100 µg/mL, 150 µg/mL, 200 µg/mL and 250 µg/mL. The resulting solutions were kept in the dark for 2 hours. At the end of the determined period, the absorbance values of the prepared samples were measured against distilled water at 734 nm. The antioxidant activities of each extract and BHA, epicatechin and α-tocopherol standards were expressed in Trolox equivalent, which is another standard used in the method.

$DMPD^{\bullet+}$ Radical Scavenging Assay

For determination of $DMPD^{\bullet+}$ radical scavenging activity, method was developed by Fogliano et. al. was applied (Fogliano et. al., 1999). To obtain the $DMPD^{\bullet+}$ radical, 100 mL acetate buffer solution (at 0.1 M and 5.3 pH) and 0.2 mL $FeCl_3$ (0.05 M) solution were added onto 1 mL of DMPD solution (100 mM). 1 mL each of the prepared $DMPD^{\bullet+}$ radical solution was taken and extracts with BHA and α-tocopherol standard solutions which diluted to concentrations between 100-250 µg/mL were added on the $DMPD^{\bullet+}$ radical solution. After waiting for 10 minutes due to the formation steps of $DMPD^{\bullet+}$ radicals, measurement was taken at 505 nm in spectrophotometer. As the control solution, samples containing only distilled water were used instead of extract or standard solution. The following equation was used to calculate the $DMPD^{\bullet+}$ radical scavenging activity in the parameter using buffer solution as blank solution. In equation, A_0 is the absorbance value of the control solution and A_1 is the absorbance values of the standard solution or the extracts.

$$DMPD^{\bullet+} \text{ radical scavenging activity (\%)} = [(A_0 - A_1) / A_0] \times 100 \quad (5)$$

Determination of Total Chlorophyll and Total Carotenoid

The total chlorophyll and carotenoid content determined according to the method developed by Kocaçalışkan and Kadıoğlu in 1990 is based on measuring the absorbance values at 450, 645 and 663 nm of the solutions obtained by dissolving 2.5 mg plant extracts in 2.5 mL distilled water (Kocaçalışkan and Kadıoğlu, 1990). The following equations were used to determine the total chlorophyll and carotenoid content in the plant extracts with the absorbance values obtained, respectively:

$$\text{Chlorophyll a} = 12.7A_{663} - 2.69A_{645} \quad (6)$$

$$\text{Chlorophyll b} = 22.9A_{645} - 4.68A_{663} \quad (7)$$

$$\text{Total Chlorophyll} = 20.2A_{645} + 8.02A_{663} \quad (8)$$

$$\text{Total Carotenoid} = 4.07A_{450} - [(0.0435 \times \text{Chlorophyll a}) + (0.367 \times \text{Chlorophyll b})] \quad (9)$$

Nitric Oxide Radical Scavenging Assay

For nitric oxide radical scavenging activity, Marcocci et al.'s work was taken as reference (Marcocci et. al., 1994). 2 mL each of extract solutions prepared in four different concentrations, 100 µg/mL, 150 µg/mL, 200 µg/mL and 250 µg/mL, were taken and 0.5 mL of sodium nitroprussiate solution prepared in phosphate salt buffer (pH 7,4) was added onto it. The mixed solutions were incubated for 3 hours in a 37 °C water bath. After the incubation process, 0.5 mL of the prepared solutions was taken and 0.5 mL of Griess reagent containing 1% sulfanilamide prepared in 5% phosphoric acid and 0.1% naphthylethylene diamine dihydrochloride was added to them. The absorbance values of the solutions obtained were read at 570 nm and against the phosphate salt buffer. In this experiment, $NaNO_2$ solution at concentrations 2.5 µg/mL, 5 µg/mL, 7.5 µg/mL and 10 µg/mL was used as a standard. As a result of the absorbance values read, the $NO^{\bullet}$ radical scavenging activity of the extracts was expressed in terms of nitrite concentration.

Anthocyanin Assay

On the basis of this parameter, where the plant itself is used instead of the plant extracts, Padmavati et al.'s work is included (Padmavati et. al., 1997). According to the method, 250 mg samples were taken from both the colored and colorless part of the turnip root. The samples taken were treated with methanol acidified with 1% HCl and kept at +4 °C and in the dark. At the end of the 24-hour period, the solutions were stirred at +4 °C for 1 hour and in the dark. The solutions obtained were first filtered with filter paper, then centrifuged at 1000 rpm for 15 minutes. To calculate the anthocyanin content, the absorbance values of the filtrate were read at 530 and 657 nm. In order to reach the anthocyanin content from the absorbance values obtained, an extinction coefficient of 31.6 $M^{-1}cm^{-1}$ was used and with the help of the equation below, the anthocyanin content in the plant sample was calculated.

$$\text{Concentration of anthocyanin (µmol/g)} = [(A_{530} - 0{,}33 \times A_{657}) / 31{,}6] \times [V(mL) / m(g)] \quad (10)$$

Total Phenolic Material Amount Assay

Slinkard-Singleton method was used to determine the total phenolic content of the turnip root (Slinkard and Singleton, 1977). Folin-Ciocalteau reagent which diluted with 22.5 mL distilled water to obtain 1:2 ratio was added to the pyrocatechol standard and extracts prepared at a concentration of 1 mg/mL. After the addition, the well mixed samples were kept for 3 minutes and 1.5 mL of 2% (w/v) Na_2CO_3 was added to each. The prepared solutions were left to incubate for two hours at room temperature with intermittent shaking. After two hours of incubation, the absorbance values of the extracts and the pyrocatchol standard at 760 nm were measured. Distilled water was used as the blank solution during the measurement. The total phenolic substance amount of the extracts obtained from the turnip root was stated as the equivalent of the sample in µg in terms of mg of pyrocatechole.

RESULTS and DISCUSSION

DPPH• Radical-Scavenging Assay

The graphic of DPPH• radical scavenging activities of standard solutions and extracts is given in Figure 1 and Table 1. The best activity among the extracts was in ethanol extract, with a value of 23.04%. While the activity in the water extract was 11.60%, the activity value in the ethyl acetate extract was observed as maximum 5.88% at most.

While the activity of BHA from standard solutions increased to 86.85%, this value was 84.35% for catechin and 86.22% for α-tocopherol.

Reducing Power

As a result of the application of this Oyaizu method, the Fe^{3+} reduction capacity of the turnip root extracts obtained from the turnip plant with the help of different solvents was determined by comparing with the BHA, Catechin and α-tocopherol standards. The results obtained are shown in the graphic in Figure 2 and Table 2. The increase in the absorbance values of each sample indicates the amount of Fe^{2+} released and this amount is directly proportional to the antioxidant activity. The reducing power of water extract and ethanol extract at 100 μg/mL sample concentration was obtained as close to the α-tocopherol standard. However, when the absorbance values of all extracts are compared with the BHA, catechin and α-tocopherol standards, it was seen that the reducing power of the extracts is not concentration dependent.

Metal Chelating Activity

In this parameter, metal chelating capacity of EDTA, standard antioxidants and extracts is summarized with the graphic in Figure 3 and Table 3. According to this graph, it was observed that the metal chelating activity of water, ethanol and ethyl acetate extracts remained at 40% values, whereas the formation of Ferrozin-Fe^{2+} complex reached almost 100% values on EDTA. While the metal chelating activity of all three extracts was higher than the BHA, Trolox and α-tocopherol standards, it was observed that the water extract had the lowest metal chelating activity among the extracts with 39.10%, and the ethanol extract had the highest metal chelating activity with 41.39%.

Proline Assay

Proline, one of the twenty essential amino acids, is responsible for stimulating the pentose phosphate pathway in plants. Synthesis of cytosolic proline helps regulate the pentose phosphate pathway by oxidizing NADPH. According to this embodiment, with increasing proline concentration, the pentose phosphate pathway is induced, thus inducing the synthesis of phenolic compounds. Plants with high proline content also have high amounts of phenolic compounds. This high amount of phenolic compound also expresses the antioxidant capacity of the plants that can be consumed (Peksel et. al., 2013). The proline content of the extracts obtained from the turnip root with the help of water, ethanol and ethyl acetate solvents is given in Table 4 as μg proline/mg extract.

Determination of Total Flavonoid

Flavonoids, which are the most important group of polyphenol compounds in plants, are known as the source of radical scavenging effect with high antioxidant activity. The total flavonoid content in the plants provides high levels of antioxidant activity. The total flavonoid content of the extracts obtained from the turnip root, obtained as a result of the experimental studies of this parameter, is given in Table 5 in terms of μg catechin/mg extract equivalent.

According to the values gathered as a result of the studies, the highest value was found in the ethyl acetate extract and the lowest flavonoid content was reached in the water extract.

Total Antioxidant Activity Assay

Thiocyanate method was used to determine the total antioxidant activity. During the application of the method, the amount of peroxide released in the first steps of lipid oxidation in linoleic acid emulsion was measured for 3 days at 24-hour intervals. The first measurements were carried out before the prepared solutions were left to incubation in the water bath and the values given in the graphic in Figure 4 were obtained.

The second measurements were carried out after 24 hours and the values obtained are shown in Figure 5. The parameter was terminated as a result of the measurements made on the third day, as the gradually decreasing % activity value of the extracts was completely finished at the 48th hour for water extract and ethanol extract and shown in the graphic in Figure 6. The values of each measurement are summarized in Table 6.

The highest activity % as a result of the experiments carried out in the parameter; 40.52% for water extract, 39.51% for ethanol extract and 37.31% for ethyl acetate extract. As a result of the measurements at the 24th hour and repeated once again at the 48th hour, the activities of the extracts decreased at the end point and therefore the highest values for the extracts were obtained and recorded at the 0th hour. The activities of BHA and α-tocopherol standard solutions decreased gradually as from the 24th hour, as in the same extracts. Despite the activity still available at the 48th hour, the highest activity of the standards was determined at the 0th hour as 58.39% and 79.48%, respectively.

β-Carotene Bleaching Test

The results obtained by applying β-carotene bleaching method with the values shown in Table 7 are given. As a result of this parameter, the absorbance values of the extracts and therefore the antioxidant activities determined with these absorbance values were calculated by accepting the BHA standard used as a positive control in the method, and as a result of this parameter, the relative antioxidant activity values of all extracts were obtained very close to the BHA standard solution with the elapsed time.

$ABTS^{\bullet+}$ Assay

The inhibition % achieved in this method is given in Trolox equivalent, which is used as a standard and is shown in the graph in Figure 7 and Table 8 with μg Trolox values per mg extract. According to this method, in which all three extracts have an average activity according to Trolox standart, higher than Epicatechin standard and lower activity than α-tocopherol and BHA standard. Ethyl acetate extract was the most able to remove $ABTS^{\bullet+}$ radical from the environment. In the method, ethanol extract had the least activity in removing the $ABTS^{\bullet+}$ radical from the environment, as can be seen from the values given in the graph. In addition, it has been observed that increasing the concentration does not have much effect on the % activity value.

$DMPD^{\bullet+}$ Radical-Scavenging Assay

The highest activity for the extracts was seen at the concentration value of 250 mg/μL, and the highest activity value was found in the ethyl acetate extract at this concentration with 27.94%. The activity of BHA showed a concentration-dependent increase and was obtained as 53.32% in 250 mg/μL. It can be said that % radical scavenging activity values obtained in this parameter are more affected by concentration than other methods.

Determination of Total Chlorophyll and Total Carotenoid

It has been proven by previous experimental studies that chlorophyll a, chlorophyll b and carotenoid compounds, which are not polyphenolic and have an important place in the pigments class, have antioxidant activity. Chlorophyll a, chlorophyll b, total chlorophyll and total carotenoid contents obtained from three different extracts obtained from turnip root are shown in Table 10, and it was observed that all values appeared differently in different extracts.

Nitric Oxide Radical Scavenging Assay

Nitric oxide, which emerged during the incubation process performed during the application of the method, was released by sodium nitroprusside and enabled the radical scavenging activity of the extracts to be determined. According to the results, the nitric oxide radical scavenging activity, which was obtained very high regardless of the concentration, is shown in the graph given in Figure 9 and Table 11, corresponding to the nitrite concentration that enables the determination of the antioxidant activity of the extracts during the reaction.

Anthocyanin Assay

Anthocyanins, a very common type of flavonoids with antioxidant properties, are the structures that give colour to many plants. Anthocyanins taken into the body by the foods that can be consumed both protect the body against oxidative damage and minimize the risks of diseases caused by oxidative stress. As a result of the experiments repeated separately for the purple and white colored parts of the turnip root, the anthocyanin content of this plant was calculated as 0.5479 μmol/g plant for the coloured part and 0.0048 μmol/g plant for the white part.

Total Phenolic Material Amount Assay

The total phenolic material amounts of the extracts in terms of μg pyrocatechol/mg extract calculated as a result of the values obtained after the experiments are given in Table 5. Accordingly, the highest phenolic material composition was found in the ethanol extract, the lowest phenolic material composition was found in the water extract. As the results show, the total phenolic content of the turnip root is quite low compared to the pyrocatecol standard.

LIST OF TABLES

Table 1. DPPH• radical scavenging activity (%) of extracts and standards

Sample \ Concentration	100 µg/mL	150 µg/mL	200 µg/mL	250 µg/mL
Distilled water extract	5.32%	7.66%	10.34%	11.60%
Ethanol extract	9.95%	14.80%	17.27%	23.04%
Ethyl acetate extract	4.96%	5.43%	5.53%	5.88%
BHA	83.92%	84.86%	85.52%	86.85%
Catechin	82.41%	83.81%	84.32%	84.35%
α-tocopherol	85.52%	85.63%	85.86%	86.22%

Table 2. Reducing power of the extracts and standards (as an absorbance)

Sample \ Concentration	100 µg/mL	150 µg/mL	200 µg/mL	250 µg/mL
Distilled water extract	0.0336	0.0289	0.0273	0.0240
Ethanol extract	0.0272	0.0233	0.0222	0.0173
Ethyl acetate extract	0.0101	0.0110	0.0126	0.0174
BHA	0.1296	0.1505	0.1513	0.1630
Catechin	0.0499	0.0624	0.0657	0.0717
α-tocopherol	0.0305	0.0346	0.0547	0.0823

Table 3. Fe^{2+} chelating capacity of the extracts and standards (%)

Sample \ Concentration	100 µg/mL	150 µg/mL	200 µg/mL	250 µg/mL
Distilled water extract	30.72%	32.69%	36.71%	39.10%
Ethanol extract	36.31%	38.05%	40.51%	41.39%
Ethyl acetate extract	35.06%	36.47%	39.49%	41.15%
EDTA	92.86%	97.09%	98.55%	99.29%
BHA	0.68%	1.07%	1.36%	2.97%
Trolox	2.11%	5.29%	7.78%	8.96%
α-tocopherol	0.54%	1.15%	4.03%	5.39%

Table 4. Absorbance values and proline amounts of extracts

Extract	Absorbance	Concentration (µg proline/mg extract)
Distilled water extract	0.4248	4.7411
Ethanol extract	0.2093	2.3359
Ethyl acetate extract	0.1112	1.2411

Table 5. Absorbance values and Catechin amounts of extracts

Extract	Absorbance	Concentration (µg catechin equivalent/mg extract
Distilled water extract	0.93525	259.7325
Ethanol extract	1.406025	390.5
Ethyl acetate extract	1.8373	510.2975

Table 6. Total antioxidant activities (%) of the extracts and standards measured in three trials

Sample (0th Hour| 24th Hour | 48th Hour) \ Concentration	100 µg/mL	150 µg/mL	200 µg/mL	250 µg/mL
Distilled water extract	32.95 - 12.88 - 0	35.63 - 16.48 - 0	39.47 - 17.68 - 0	40.52 - 20.3 - 0
Ethanol extract	37.72 – 11.53 - 0	38.18 – 15.31 - 0	38.65 – 17.4 - 0	39.51 – 17.88 - 0
Ethyl acetate extract	33.1 – 15.53 - 0	33.96 – 22.22 – 2.31	36.19 – 24.88 – 6.84	37.31 – 25.7 – 11.21
BHA	53.47 – 41.22 – 36.71	55.24 – 43.05 – 37.03	57.05 – 43.35 – 41.19	58.39 – 46.12 – 42.44
α-tocopherol	72.75 – 42.67 – 27.62	75.55 – 46.34 – 33.20	78.51 – 50.42 – 35.82	79.48 – 53.65 – 36.45

Table 7. Antioxidant activity in terms of RAA value

Sample	RAA value (60th minute)	RAA value (120th minute)
Distilled water extract	0.82	0.75
Ethanol extract	0.81	0.74
Ethyl acetate extract	0.80	0.73
BHA	1	1
Control solution	0.76	0.64

Table 8. $ABTS^{\bullet+}$ radical scavenging activity of extracts and standards in µg Trolox per mg extract

Sample \ Concentration	100 µg/mL	150 µg/mL	200 µg/mL	250 µg/mL
Distilled water extract	137	142.25	143.73	151.42
Ethanol extract	100.17	114.83	124.81	137.6
Ethyl acetate extract	139.94	148.81	155.94	158.65
BHA	195.67	226.17	228.96	234.08
Epicatechin	35.52	47.46	61.31	83.33
α-tocopherol	234.44	234.67	234.77	234.90

Table 9. $DMPD^{\bullet+}$ radical scavenging activity (%) of extracts and standards

Sample \ Concentration	100 µg/mL	150 µg/mL	200 µg/mL	250 µg/mL
Distilled water extract	8.95%	10.89%	15.91%	22.06%
Ethanol extract	18.08%	21.34%	24.23%	26.76%
Ethyl acetate extract	21.01%	22.43%	26.23%	27.94%
BHA	35.51%	41.68%	47.33%	53.32%
α-tocopherol	2.73%	6.23%	10.81%	12.83%

Table 10. Chlorophyll a, chlorophyll b, total chlorophyll and total carotenoid values of the extracts

Sample	Chlorophyll a	Chlorophyll b	Total Chlorophyll	Total Carotenoid
Distilled water extract	0.0241	0.0634	0.0875	0.0931
Ethanol extract	0.0139	0.0058	0.1972	0.1536
Ethyl acetate extract	0.0844	0.1508	0.2350	0.0476

Table 11. The value of the nitric oxide radical scavenging activity of the extracts in terms of nitrite concentration (%)

Sample \ Concentration	100 µg/mL	150 µg/mL	200 µg/mL	250 µg/mL
Distilled water extract	96.80	96.46	96.22	95.16
Ethanol extract	94.49	94.09	93.35	92.84
Ethyl acetate extract	96.29	96.01	95.64	95.09

Table 12. Absorbance values and total amount of phenolic material in terms of pyrocatechol

Sample	Absorbance	Concentration (µg pyrocatechol equivalent/mg extract)
Distilled water extract	0.0104	2.4186
Ethanol extract	0.0178	4.1395
Ethyl acetate extract	0.0143	3.3256

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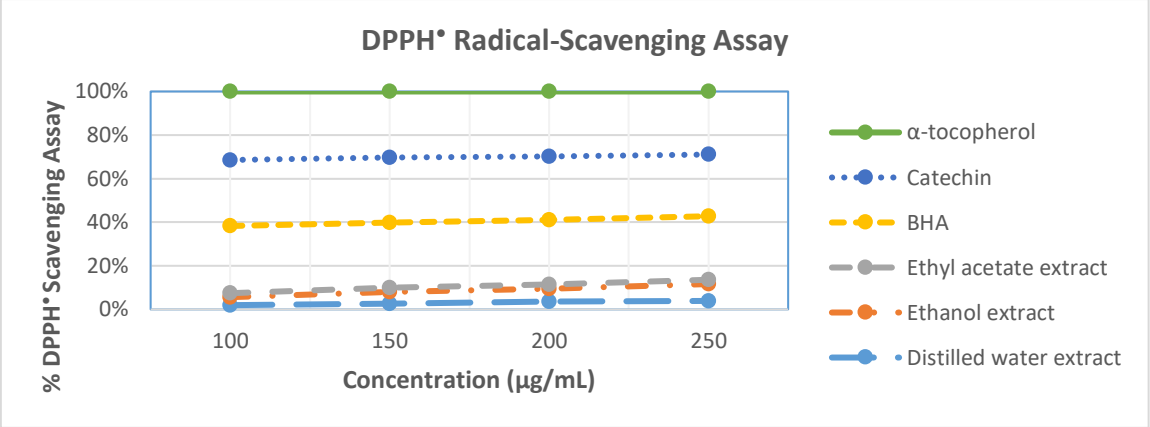

Figure 1. DPPH• radical scavenging activity of extracts and standards

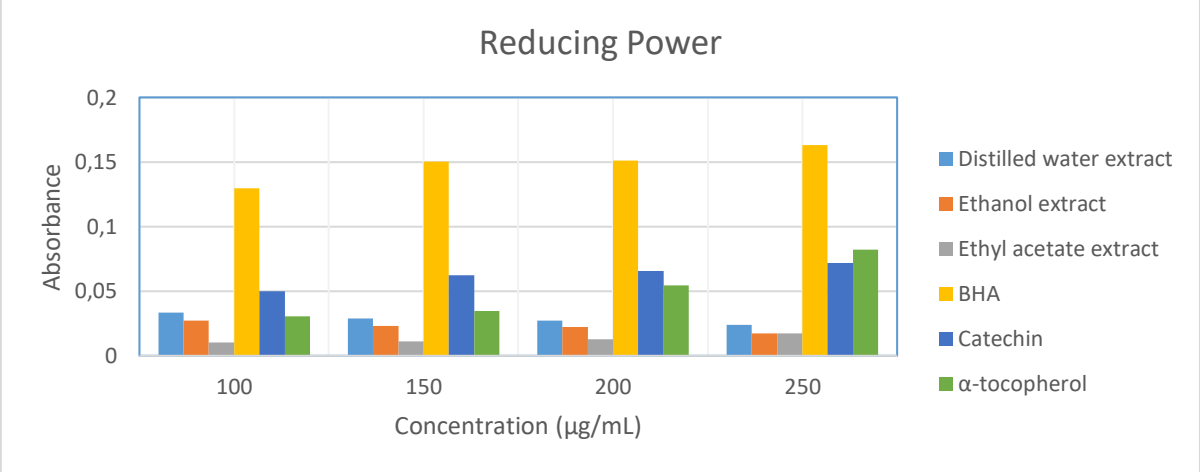

Figure 2. Reducing power of extracts and standards

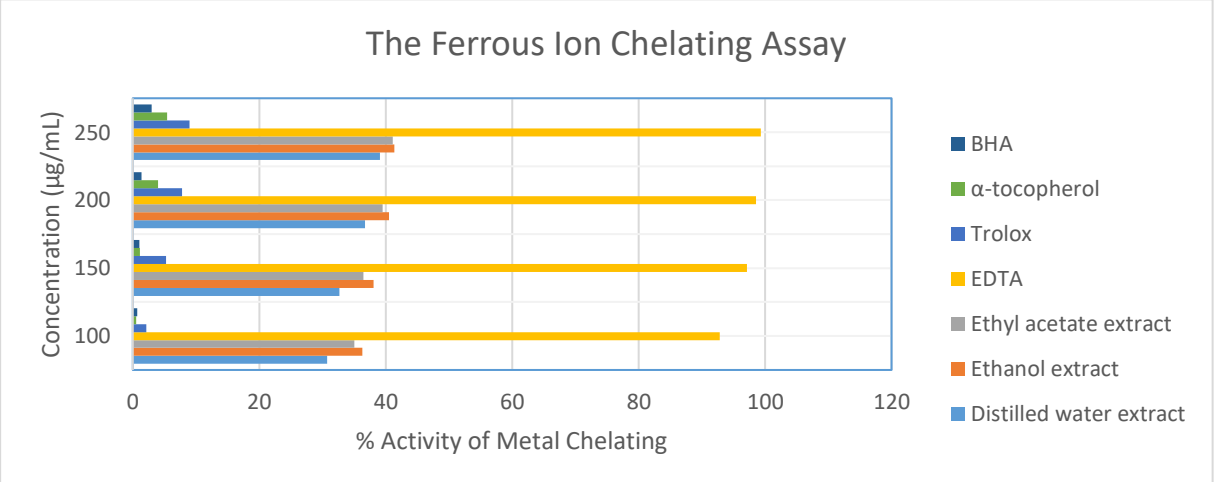

Figure 3. Metal chelating activity of extracts and standards

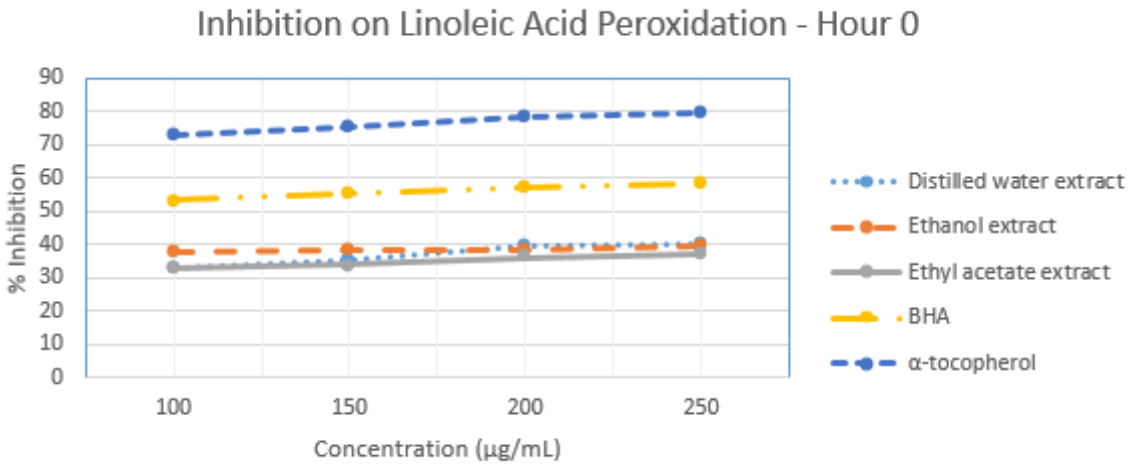

Figure 4. Total antioxidant activities of extracts and standards at 0th hour

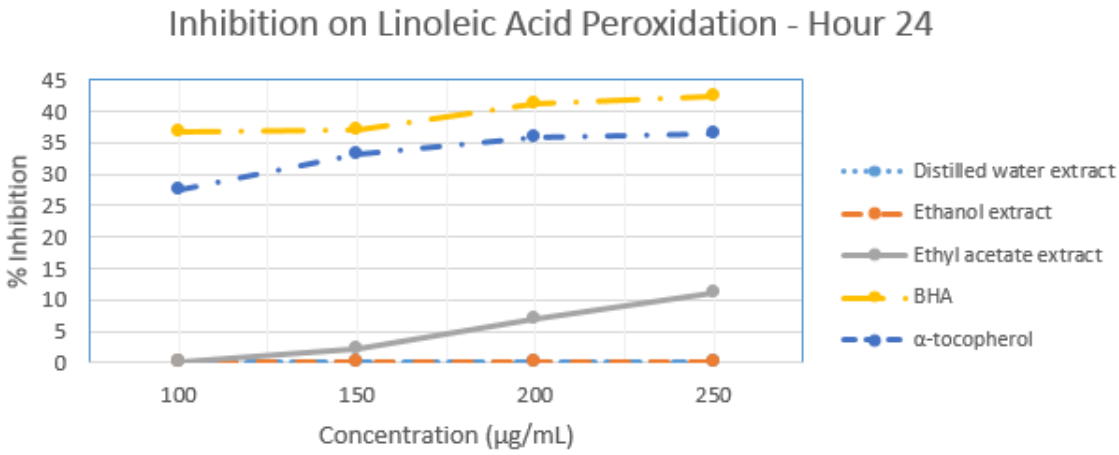

Figure 5. Total antioxidant activities of extracts and standards at 24th hour

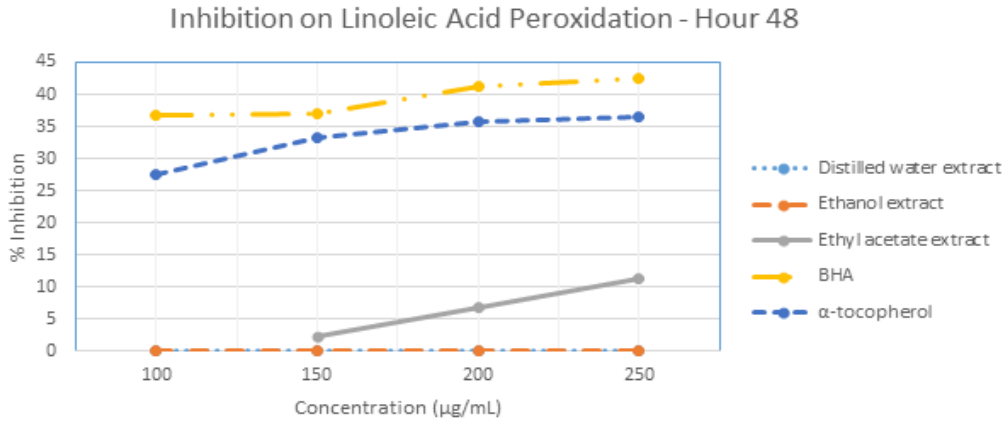

Figure 6. Total antioxidant activities of extracts and standards at 48th hour

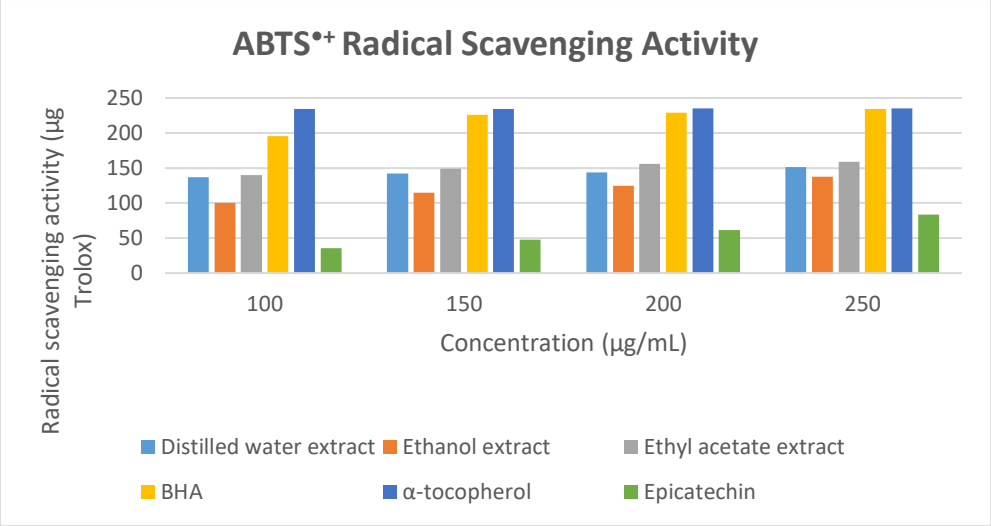

Figure 7. ABTS$^{\bullet+}$ radical scavenging activity of extracts and standards in µg Trolox per mg extract

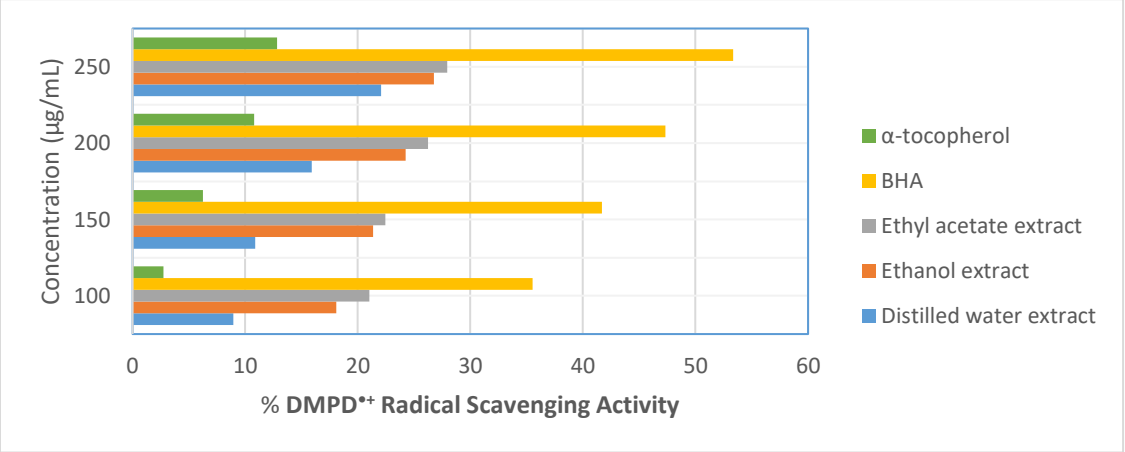

Figure 8. DMPD$^{\bullet+}$ radical scavenging activity of extracts and standards

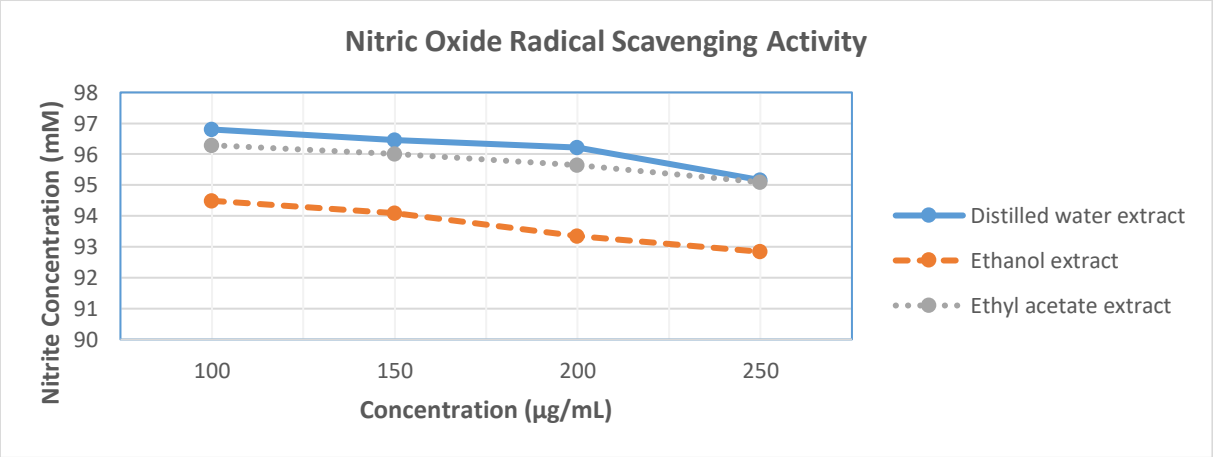

Figure 9. Values of nitric oxide radical scavenging activity of extracts in nitrite concentration

CONCLUSION

Synthetic antioxidants such as BHA, BHT, Trolox and α-tocopherol have been used for a long time for the purposes of extending the shelf life of foods, ensuring that they can be stored for a longer time, and increasing their durability. However, the possibility that synthetic antioxidants have toxic properties and cause carcinogenic effects, it is necessary to use natural antioxidants. In this study, the antioxidant activity of turnip root (*Brassica rapa subsp. Rapa*) was compared with the standard antioxidants.

Regarding the antioxidant activity, many natural and synthetic sources are already used as antioxidant molecules and provide ease of interpretation by providing comparison with the literature. However, since turnip, whose antioxidant activity we examined, had not been included in such a study before as a root form, the results were interpreted by comparing them with other plant species in its family. For $DPPH^{\bullet}$ radical scavenging activity, which is the most studied activity in Brassica species, cauliflower (Brassica oleracea var. Botrytis) with an activity of 23% and cabbage (Brassica oleracea L var. Capitata) with an activity of 9% stand out (Gülçin and Köksal, 2008; Singh et. al., 2006). The highest value in this parameter belongs to rapeseed with 73% scavenging activity (Soengas et. al., 2018). The scavenging activity of cabbage, cauliflower and pyramid cauliflower plants (Brassica oleracea var. Romanesco) compared for $ABTS^{\bullet+}$ radical scavenging activity varies between 1.91 and 3.10 in μg Trolox equivalent (Volden et. al., 2008). In a study using cauliflower, Brussels sprouts, and broccoli, total phenolic contents varied between 746.1 μg and 2306.6 μg in μg of catechin, while $DMPD^{\bullet+}$ radical scavenging activity was obtained as 70% for cauliflower (Florkiewicz et. al., 2018; Nawaz et. al., 2018)

The results obtained in similar experiments show that turnip root has higher antioxidant activity in some parameters.

In conclusion, we subjected a material whose antioxidant activity had not been studied before, to some experiments for this purpose. Although the results we reached were promising for some parameters, they were not obtained as high as expected for some parameters. The fact that it is a plant that grows under the ground is the only reason turnip root lacks some of biochemical components. In future studies, the components of turnip root able to be analyzed as every biomolecule level and their effects on each parameter can be examined in more detail.

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Nano-emulsions: A Novel Approach in Seafood Preservation

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Abstract

Seafood industry has become progressively growing worldwide with fish being the most demanded as it is a rich source of many nutrients including proteins, vitamins and carbohydrates. Fish usually undergo spoilage due to microbial, oxidative, enzymatic and chemical activities. Fish is highly perishable and if not handled properly, its sensory attributes, quality and freshness will be affected. Preservation of fish is mandatory so to assure quality and to extend shelf life. Besides traditional preservation methods including chilling, freezing, drying, salting, smoking, some new methods have also been introduced for preservation that includes, modified atmospheric packaging, irradiation, ozone technology and nano-emulsion. Nano-emulsion is the latest innovation that is being used for preservation to improve food properties such as taste, odour, texture and quality. The advancement in the research showed the several benefits of nano-emulsions for the usage of bioactive and antimicrobial substances due to its minute size ranges and volume to the surface ratio that can increase the ability of digestion and durability of these emulsions. Therefore, it was objected to discuss the application of nano-emulsion as a novel preservation technology in seafood.

Keywords: Seafood, nano-emulsions, preservation techniques, spoilage, emulsifiers

Review article
Received date: 25 January 2021
Accepted date: 28 April 2021

INTRODUCTION

Seafood plays an important role in a person's diet as the reliable source of protein. Moreover, seafood is the rich source of other nutritious compounds as omega-3 polyunsaturated fatty acids, vitamin, and minerals. There is a clear relation of health and life probability in association with the use of seafood. For example, fish is a rich source of protein and it requires a vigilant handling (Eyo, 2004). Seafood freshness is generally termed as the time period for which fish upholds its shelf life, which usually defined by various factors, as the initial presence of different bacteria, their specificity, temperature, pH and surrounding environment.

The concept of quality of fish is very complicated (Bremner, 2000). It is often narrated as nutritional, microbiological, biochemical and physiochemical properties but it must also include perception of quality sensory and acceptability of consumer. Sensory evaluation can either be objective or subjective. In objective sensory evaluation, trained team is used to define freshness. In subjective sensory evaluation, satisfaction and acceptability of consumer and trends of market (Singham et al., 2015).

Spoilage of fresh fish is quite rapid after it is caught. The key to provide high quality of fish need attention throughout the process including catching, handling, transportation and storage. At high temperature, it took 12h after fish catch to begin spoilage process (also known as rigor mortis) (Berkel et al., 2004). In rigor mortis process, after few hours of fish death, it tends to lose its flexibility due to mussels stiffens (Adebowale et al., 2008). Fish is evidently unusable that has become spoiled or addled (Gopakumar, 2000). If the fish is badly handled, it may not be clearly spoiled, but due to off-odour, off-flavours, spongy texture, or substandard colour, it loses its desirability (Burt, 2003). Degradation of fish occur due to digestive enzymes activity, microbe's activity, and oxidation (AMEC, 2003). When spoilage of fish occurs, many components break and new compounds are formed. These newly formed compounds in fish meat result in change of physical attributes like odour, flavour, texture, color of gills and softness of muscles (Baird-Parker, 2000).

Due to the high temperature, bacterial activity, enzymes, chemical changes and fat oxidation in the fish accelerates. Generally, numerous bacteria are present on the skin and gill of fish. When a fish is caught, a series of bacterial, enzymatic and chemical changes occur that results in spoilage of fish (Burt, 2003). Warm climate usually accelerates the rate of spoilage. Stomach of the fish contains enzymes which helps a living fish in digestion (Lima Dos Santos et al., 2011). Enzymes start digesting the stomach itself once the fish is dead. Ultimately the enzymes enter into the fish meat and digest it too. This is the reason why fish becomes mushy and the odour of the fish becomes more prominent. Chemical changes cause spoilage results in enzymatic digestion and bacterial decomposition (Putro, 2005).

Shelf life of seafood is short as it is highly perishable and may cause aerobic spoilage of fish in the presence of air and oxygen. Therefore, it is important to preserve freshness and quality of seafood. Seafood is preserved in order to lessen or inhibit the metabolic changes that cause deterioration of fish quality. By controlling temperature, water content, microbial activity and oxygen availability, shelf life of seafoods can be extended. The purpose of preservation of fish is to reduce or stop the enzymatic, chemical and bacterial deterioration, so that fish meat remains fresh (Bate and Bendall, 2010). Besides traditional techniques, there some minimal processing technologies like high-pressure processing (Ucak et al., 2019; Ucak et al., 2018; Ucak and Gokoglu 2020), irradiation technology, ozone technology, advanced packaging technology such as edible films and coatings (Ucak et al., 2020; Ucak et al., 2019; Ucak 2019), and nano-emulsion applications.

Chilling is the simplest method to preserve fish. Although cool and uncool fish can be spoiled in a matter of few hours but comparatively cool fish remains fresh for a longer period (Tawari and Abowei, 2011). It is essential for fish preservation to store it at 0°C after it is caught as at that time spoilage can be very fast (FAO, 1973). Cooling of fish can be done by covering the fish with layers of ice. But this method of icing is successful only for a short duration such as for transportation purpose. Berkel et al. (2004) stated that fish can either be stored at cooling temperature +1°C to +4°C, that hinders the microbial growth or by freezing at -18°C to -30°C that stops the growth of microorganisms. Fish preservation can be done by adding salt on fish. However, there is usually no choice in remote places other than to use salt in any way to preserve fish. Another way to preserve fish is to keep it in brine solution. The ratio to make brine solution is four parts of water and one part of salt (4:1). If the salt grain size is large, it should be grinded first (Tys and Peters, 2009).

By using a stirrer, it is then dissolved. Modified atmospheric packaging is a type of packaging in which air is eliminated from the pack replacing it with a single gas or mixture of gases. To meet consumer demand, MAP has been progressively accepted preservation technique to fulfil seafood distribution.

A lot of applications in food products are being implemented using MAP techniques that include raw and cooked meats, poultry, sea foods etc (Church, 1998). MAP along with refrigeration is proficient of prolonging the shelf life of sea foods. To preserve fish for a long duration and to improve its quality, irradiation technology is used in which electrons or electromagnetic rays are exposed to food products (Lacroix and Ouattara, 2000). For ionizing radiation, gamma rays, X-rays and electron beams are used to preserve food. Most important material is cobalt 60 that is being used for food irradiation (Clucas and Ward, 1996). Ozone technique is a latest method of food preservation that is being used to examine quality of fish and extending its shelf life (Kim et al., 2000; Campos et al., 2006). Ozonation is a successful technique which is being used to destroy microbes that includes virus, bacteria etc. (Hobbs, 1991; Campos et al., 2006). Ozone technique is promising as it lessens the apparent contamination of fish and reduces formation of volatile nitrogen which ultimately improves sensory attributes and quality of fish for many days (Dondo et al., 1992). Besides all these techniques, the application of natural extracts to fish products are commonly used (Ucak and Zahid 2020; Ucak 2020a; Ucak 2020b).

Nano-emulsions

The food sector has made significant advancements in nanotechnologies known as nano-liposomes, nano-emulsions, nano-fibers, and nano-capsules (Raj et al., 2013). Nanotechnology is an advanced approach that deals with controlling and preventing diseases, extending the shelf-life of food products (Wang et al., 2014). Nano-emulsions are colloidal systems of particular interest because they can be made from elements mainly food graded used in the food sector such as mixing, heat treatment and homogenization (Rao and McClements, 2011). The technologies in food industries are used to transform numerous food products (McClements, 2010). The improvement of appearance, texture or taste is considered as the main benefit for applying nano-emulsions in the food industry by the careful selection process and use of products wisely (McClements, 2015). On the other hand, emulsions can have improved stability against the combination of droplets (Mc-Clements and Rao, 2011). Moreover, droplets are clear when dispersed, so they are appropriate for the accumulation of food without altering the characteristics (Mason et al., 2006). Nanoparticles are being used in advance food processing technology to enhance attributes like flavour, texture, colour and other antimicrobial properties (Chaudhry et al., 2008).

McClements (2012), explained that nano-emulsion is a thermodynamically uneven exhibit dispersal comprising of two immiscible points, particle combinations and gravitation phase separation because of their small droplet sizes as related to other methods. For instance, phases comprised of discrete particles around r<100 nm and minute droplets showing dispersal in a translucent manner this resultant. It can also comprise of oil-in-water emulsions or water-in-oil. Moreover, nano-emulsions can be made up of essential oils, acylglycerols and free fatty acids (Shah et al., 2010). On a wider basis, nano-emulsions have been prepared to condensed sustenance with a lipophilic property because these components may not be well dispersed in an aqueous phase. However, with rheological behaviour such as color, appearance, texture, and stability, it is probable to manufacture water in oil type nano-emulsions in food applications (Jafari et al., 2008).

The free energy of the emulsion is larger than the free energy of the discrete stages (oil and water). Instability in thermodynamics happens by sedimentation, coalescence, occultation, molecular diffusion and Ostwald ripening (Gharib Zahedi et al., 2012; Gupta et al., 2016). This mainly happens when a huge droplet increases its size, incorporating a minute droplet by molecular diffusion (Ahmed et al., 2012).

Nano-emulsions are widely being used in different industries including food, pharmaceutical, medical and cosmetic in order to encapsulate, protect, and release bioactive lipids (McClements et al., 2007). It is vital to know about size of droplet in emulsion as ultimately it will affect emulsion properties including texture, stability of shelf life, rheology (Wolf et al., 2009).

Preparation of nano-emulsion can be done by high-pressure homogenization, ultrasonic homogenization, micro fluidization, phase inversion temperature, solvent displacement, emulsion, and phase inversion (Lovelyn and Attama, 2011; Bradley et al., 2005; Kentish et al., 2008; Shah et al., 2010; Solans et al., 2005; Tadros et al., 2004; Ganachaud and Katz, 2005; Trimaille et al., 2001; Solè et al., 2006; Uson et al., 2004; Shinoda and Saito, 1969; Ahari, 2017). Moreover, these bioactive complexes comprised of ketone, aldehyde, ester bonds and they are beneficial for oxidative deterioration, on the other hand, micro capsulation inhibits light without manipulating the taste and texture of the food products. They can also enhance the stability, solvability in the final by-product (Barani et al., 2018). However, nano-emulsion can be comprised of four components: (a) oil stage (b) water stage (c) surfactant and (d) energy needed to generate.

Nano-emulsions ingredients

Oil phase

The oil phase is comprised of bioactive compounds such as fish oil, essential oils, oil flavors, and Vitamins which are dissolved in carrier oil. Moreover, several oils are associated with the production of nano-emulsions due to their low cost, nontoxicity, and abundance of their raw sources, containing acyl glycerol, essential oils, free fatty acid, organic oils, and waxes.

Water phase

The production of the nano-emulsion water phase is one of the unique ingredients in the creation of nano-emulsion. It can be described that the percentage of water to the oil phase is a significant factor in the formation of nano-emulsion. The other materials are polysaccharides, cosurfactants, proteins, salts, and nutritious materials which are included in the water phase these could alter the pH, polarity, surface tension and ionic structure. Moreover, liquid phase elements can be a definite factor in defining the physicochemical features of the nano-emulsion. While, compounds like proteins, agar resin, alginate could aid the consistency of nano-emulsion

Stabilizers

As described before, nano-emulsions are thermodynamically unstable; hence, their formation requires stabilizers. Emulsifiers are one kind of stabilizer, which has application information of tiny droplets during homogenization; meanwhile, they prevent the aggregation of droplets either during or after homogenization. Another kind of stabilizers used as a texture modifier prohibits gravity separation and Oswald ripening in nano-emulsion (McClements and Rao, 2011).

Emulsifier

Emulsifiers are those materials that are active on the surface and have amplification properties. Lipophilic and hydrophilic compartments are present in the structure emulsifiers. Hence, one part is dependent on the nonpolar (oil) phase, while the other part on the polar (water) phase (Qian and McClements, 2011).

Texture modifier

To change the structural characteristics of nano-emulsion fabrication; a compound used during the continuous step of nano-emulsion fabrication; named as texture modifier. Texture modifier can also be used as a thickening object (McClements and Jafari, 2018)

Weighting agents

These compounds are added to the dispersed phase to change the density of the droplets with the density of the continuous phase. The objective is to lessen the impulsion force of gravity and delaying the creaming or sedimentation (Salvia et al., 2017).

Methods used to form nano-emulsions

Understanding the formation of nano-emulsions; it is necessary to produce a small droplet size; these dispersions are representatively formed in a different procedure in which a macro emulsion is developed and then transformed into nanodroplets (Gupta et al., 2016). Nano-emulsions are created using a series of species methods, and they can be grouped based on the energy input, i.e., low energy and high energy. However, low energy is used for making nano-emulsion elements and the particles size ranges (Ostertag et al., 2012)

Low-energy methods

Low interfacial tension is reached due to differences in the conditions of the suspension and a state of phase inversion is obtained by nano-emulsion (Gupta et al., 2016). The most commonly used methods that are used in low energy methods are phase inversion composition and phase inversion temperature (Jin et al., 2016).

High-energy methods

High-energy methods are made using numerous types of different mechanical apparatus, such as ultrasonic homo-genizers, high pressure homogenizers or micro fluidizer, that produce sufficient levels of concentrated energy to oil and water, producing minute droplets (Jin et al., 2016).

Materials used in the development of nano-emulsions

Nano-emulsion application in the food industry used to encapsulate components with possible biological activity comprised of fatty acids, liposoluble flavors, vitamins, etc. (Rao and McClements, 2011). Emulsifiers aids in the making of emulsions during homogenization by adsorbing at the water-oil interface during homogenization to decrease surface tension (Yan et al., 2013).

The emulsifier must have following features: It must rapidly lessen the facial tension in water and oil interface, once adsorbed it must be strongly bound to the interface, in destabilization it should be protective.

Based on their usage it may differentiate as emulsifiers, foaming agents, wetting agents and dispersants. Moreover, according to physical features their stability for water and oil will be most commonly used. The replacement of synthetic emulsifiers with natural emulsifiers is a rising propensity as numerous synthetic emulsifiers are not allowed for use in different areas (Rao and McClements, 2011). Because of this situation, naturally occurring emulsifiers are an improved option for the development of nano-emulsions. Proteins, polysaccharides, and gums are known as natural emulsifiers. Proteins have the ability to absorb oil and water by having amphiphilic characteristics (Silva et al.,2015). Due to the controlled release of emulsion, there is an improvement for the presence of bioactive compounds (that are extracted mainly from fruits) in terms of antimicrobial process (Ranjan et al., 2017; Angel Robles-Garcia et al., 2016; Lu et. al., 2016; Oehlke et al., 2014; Hernandez-Fuentes et al., 2015; Pimentel-Gonzalez et al., 2015).

CONCLUSION

The usage of nano-emulsions is as useful as the encapsulation system. It has several advantages in the food industry including minute droplet size range, transparency, and high stability. Customer response should be attained by investigations showing that these substances cannot be gathered in the human body because of the low toxic level. In contrast, there must be a need for establishing some laws in the food industry for use of nanotechnology that provides functionality and expand features linked to human health. The advancement in the research showed the several benefits of nano-emulsions for the usage of bioactive and antimicrobial substances due to its minute size ranges and volume to the surface ratio that can increase the ability of digestion and durability of these emulsions.

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