# Comparative Quality Assessments of Five Local Fresh Fish in Sulaimani City Markets

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**Doctor of Philosophy (Ph.D)**

**In quality control and food hygiene**


**By**

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## Supervisor certification

I certify that this thesis was prepared by the candidate **Zaid Khalaf Khidhir** under my supervision at the Department of Microbiology, College of Veterinary Medicine, University of Sulaimani as a partial fulfillment of the requirements for the degree of Doctor of Philosophy in **quality control and food hygiene**. Accordingly, I hereby recommend this thesis to be debated by an examining committee.

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## Resolution of the Examining Committee

We, the chairman and members of the examining committee, have read this thesis and examined the candidate " **Zaid Kh. Khidhir** " in its contents on / / 2011. Accordingly, we found this thesis is accepted as a partial fulfillment of the requirements for the degree of Doctor of Philosophy in **quality control and food hygiene**.

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# List of abbreviation

| | |
|---|---|
| Ah | *Aeromonas hydrophila* |
| AOAC | Association of Official Analytical Chemists |
| Bas | Biogenic amines |
| BPD | Butterfield's phosphate diluent |
| CAC | Codex Alimentarius Commission |
| Cd | Cadmium |
| CFU | Colony forming units |
| DMA | Dimethylamine |
| EPA or USEPA | U.S. Environmental Protection Agency |
| ERV | Extract Release volume |
| FAO | Food and Agriculture Organization |
| FDA or USFDA | Food and Drug Administration |
| FFA | Free fatty acids |
| HPLC | High Performance Liquid Chromatography |
| Hx | Hypoxanthine |
| ICMSF | International Commission on Microbiological specifications for Food |
| IQS | Iraqi standard |
| M1 | Licensed markets |
| M2 | Unlicensed markets |
| MAFF | Ministry of Agriculture, Fisheries and Food |
| MeHg | Methyl mercury |
| Meq | Milli equilibrium |
| $NH_3$ | Ammonia |
| Pb | Lead |
| PV | Peroxide Value |
| QIM | Quality index method |

| | |
|---|---|
| SSO | Specific spoilage bacteria |
| TBA | Thiobarbituric Acid |
| TCA | Trichloroacetic acid |
| TCBS | Thiosulphate Bile Salt Sucrose agar |
| TMA | Trimethylamine |
| TMAO | Trimethylamine oxide |
| TPC | Total plate counts |
| TVB-N | Total volatile basic nitrogen |
| T.V.N | Total volatile nitrogen |
| USDA/FSIS | United States Department of Agriculture/Food Safety and Inspection Service |
| WHC | Water-Holding Capacity |
| WHO | World Health Organization |

**Summary**

The aim of this study to assess the quality of five types of local, most heavily traded and widespread in Sulaymani, fresh fish including Grass carp (*Ctenopharyngodon idella*), Silver carp (*Hypophthalmichthys molitrix)*, Common carp (*Cyprinus carpio)*, Bizz (*Barbus esocinus*) and Shabbout (*Barbas grypus*), different standard inspection tests were applied.

All types of scored acceptable quality index limits valued 6.67 for Bizz to 10.67 for Silver carp with significant differences among them ($p<0.05$). Proximate composition, including moisture, protein, lipid and ash contents, revealed percentages for all types, moisture valued 69.19% for Silver carp to 74.69% for Grass carp, protein valued 16.82% for Silver carp to 21.69 % for Bizz, lipid valued 2.58% to Bizz 11.73% for Silver carp and ash valued 0.99% for Grass carp to 1.95% for Silver carp with significant differences among all. The lipid content of the three farmable types Grass carp, Silver carp and Common carp was low in wild inhabitants and was significantly varied as compared to the higher lipid content farmed counterparts, while moisture was vice versa. The essential amino acids content revealed significant differences among all fish types tested.

Physical evaluation: the extract release volume was 11.15 ml for Bizz to 16.27 ml for silver carp, water holding capacity was15.92% for Silver carp to 29.08 % for Bizz, Cooking loss was 28.83% for Bizz to 42.99% for Silver carp, density was 1.006 for Silver carp to 1.019 for Bizz and pH valued 6.605 for Silver carp to 6.70 for Shabbout. Wild inhabitants graded significantly better in physical properties than farmed amongst farmable types.

Chemical evaluation revealed acceptable results with significant differences in examined fish, total volatile basic nitrogen (TVN) was12.23 for Bizz to 16.41mg N/100gm for Silver carp, trimethylamine (TMA) was 2.35 for Bizz to 5.58 mg N/100gm for Silver carp and Dimethylamine (DMA) valued 0.142 for Bizz to 0.365mg N/100gm for Silver carp. Wild inhabitant graded significantly better chemical properties than farmed amongst farmable types. The licensed markets sold five types which graded chemical properties significantly better than those unlicensed markets sold counterparts. Except Silver carp which contained too low of ammonia concentration (mean value 0.135 mg N/100gm) the remaining four types were ammonia free.

Lipid oxidation evaluation: except Silver carp which revealed unacceptable limits of thiobarbituric acid (TBA) (mean value 5.2mg malonaldehyde/ kg), the remaining four types were within acceptable limits which was 0.87 for Bizz to 3.48mg malonaldehyde/ kg for Common carp, values varied significantly. The peroxide value was within the acceptable limits for all types and valued 3.40 for Bizz to 8.86meq oxygen /kg for Silver carp and varied significantly. Free fatty acids was within the acceptable limits for all types valued 0.77 for Bizz to 1.36% for Silver carp and varied significantly, wild inhabitant showed significantly stability than farmed inhabitants among farmable types. The licensed markets sold five types with lipid stability significantly better than unlicensed markets.

Biogenic amines: histamine, as quality index, was within the acceptable limits for the five fish types which valued 7.26 for Grass carp to 26.28 ppm for Common carp, where here Common carp showed significant difference with the other four types while putrescine, cadaverine, spermine and spermidine were within the ranges reported in literatures for good quality fish. The biogenic amines index (BAI) was within the acceptable limits for the five fish types which ranged between 1.175 for Bizz to 8.657 for Silver carp but Silver carp was significantly different from the others where it was closer to the upper undesirable limit.

Hypoxanthine (Hx) was detected only in Silver and common carps with acceptable limits (0.003 For Silver carp and 0.002ppm for common carp).

Microbiology evaluation: total plate count (TPC), Psychrophilic bacterial count and Psychrotrophic counts were within the acceptable limits for all types (TPC valued 8.37 For Bizz to $25.80\times10^5$ Cfu/g for Silver carp, Psychrophils valued 6.83 for Bizz to $63.91\times10^5$ Cfu/g for silver carp and Psychrotrophs valued 7.7 for Bizz to $20.70\times10^5$ Cfu/g for Silver

carp) but they varied in count. Wild inhabitant showed significantly better microbiology evaluation results than farmed among farmable types. Bizz and Shabbout have been sold from licensed markets had better significantly than those unlicensed markets.

*Pseudomonas* spp. were detected in all five types in a range of 45.75 for Shabbout to 59.16 $\times 10^2$ Cfu/g for silver carp with insignificant differences. Randomly selected *Pseudomonas* colonies revealed isolation of *P.aeruginosa* and *P.putida*. *Vibrio* spp. were detected in Silver carp (0.658×10Cfu/g), Common carp (0.908×10 Cfu/g) and Bizz (1.942×10 Cfu/g), randomly selected *Vibrio* colonies revealed isolation of *V.metschnikovii* and *V. alginolyticus*. *Aeromonas* spp. was detected in Silver carp (3.325×10 Cfu/g), Common carp (2.842×10 Cfu/g) and Shabbout (0.450×10 Cfu/g), randomly selected *Aeromonas* colonies revealed isolation of *A. hydrophila* and *A. caviae.*

The sensory evaluation: All fish types scored acceptable sensory properties which valued 4.00 for Bizz to 2.75 for Silver carp. Wild inhabitant showed significantly better sensory properties than farmed among farmable types. The licensed markets sold five types of graded sensory property which significantly better than those unlicensed markets which sold counterparts.

Heavy metal residues: The five fish types recorded acceptable limits of mercury (as methylmercury) that valued 0.083 in Shabbout to 0.407ppm in Common carp. Cadmium was 0.451 in Grass carp and 0.475ppm in Common carp. Lead was 0.306 in Grass carp and 0.364ppm in Shabbout. Farmed Silver and Common carps contained lower concentrations of mercury and cadmium in comparison to wild counterpart while Grass carp showed insignificant differences for both metals.

There was a relationship between biogenic amines and TPC and between TPC and TMA. Inverse relationship was shown between moisture and lipids.

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# Introduction

Food quality refers to the sensory, chemical, physical and microbiological characteristics, as well as nutritional value, safety and other characteristics of a food product. With respect to fish, quality involves nutritional, microbiological, biochemical and physicochemical aspects (Bonilla, 2004).

One of the most highly perishable food products is fish; during handling and storage, quality deterioration of fresh fish rapidly occurs and limits the shelf life of the product (IFST, 1993). The quality of fish could be degraded through a complex process, in which the physical, chemical and microbiological forms of deterioration are involved; enzymatic and chemical reactions are usually responsible for the initial loss of freshness whereas microbial activity is responsible for the obvious spoilage and thereby establishes product shelf life (Gram & Huss, 1996). Several factors lead to the perishability of fish meat, which are: more rapid autolysis by fish enzymes, less acid reaction of fish meat which favors microbial growth, many of fish oils seem to be more susceptible to oxidative deterioration, softness of the fish flesh and high water content (Huss, 1994). Since fish is a very perishable commodity, it has drawn special attention. Like most raw materials, fish consists of a large number of species of widely differing appearances and flavors so that customers are often unsure if a particular species is suitable and safe to eat. The public also become more demanding in respect of freshness, microbiological safety, free from pollutants, protection from damage, and convenience (Leo & Fidel Toldra, 2010). The main problem with aquatic animals is the fact that from the moment that they are caught or harvested, a change in properties starts, which continues until a state of spoilage is reached. After catch and harvest, not only is spoilage and freshness parameters are changing due to metabolic (autolytic) and microbiological processes but also the microbial flora is changing. The number of bacterial gastroenteritis associated to seafood products has been increased considerably during the last decades by the rapid globalization of the food market ,the increase of personal and food transportation, and profound changes

in the food consumption habits (Mead *et al*.,1999). Aquatic animals from some areas of the world can carry microorganisms such as *Vibrio* spp. that are harmful to human health and must be destroyed or removed before selling the products (Lee *et al.,* 2008).

The analytical methods used for seafood analysis can be divided into objective methods and subjective methods. The objective methods are chemical/ biochemical methods, physical methods, and microbiological methods. The chemical/biochemical methods are mostly traditional methods that were developed earlier than the physical (instrumental) methods and have been mostly applied as methods for freshness/ spoilage determinations. Methods that are still being used are, amongst others, hypoxanthine value, which is based on ATP breakdown products, analysis of TMA, DMA, ammonia, TMAO, and total volatile basic nitrogen (TVB-N) (Malle & Poumeyrol, 1989); determination of TBA and formaldehyde; and analysis of biogenic amines as histamine or cadaverine ( Leo & Fidel Toldra,2010). The sensory methods can also be divided into two principal methodologies: methods based on outer inspection of the sample (without cooking) and methods based on assessing the cooked sample. Outer inspection is carried out by the European Union quality-grading scheme (ECTS grading scale) and by the quality index method (QIM) (Parisi *et al.*, 2002).

Fish and fish products on local markets have not been investigated by analytical methods at all. Some is doubted to be exotic, enter local markets in large quantities or as single fish specimen and are not thoroughly investigated for their microbiological status, their spoilage characteristics and shelf life, sensory characteristics, and contents of all the beneficial components. This is a vast area needed to be considered.

Thus, this study was aimed to assess the quality characteristics of local fresh fish by means of standard methodologies including:

1. Use of quality index method (QIM) for outer inspection of the sample.

2. Evaluation of the physical, chemical, microbial and sensory properties as well as the nutritional value.
3. Detection the specific spoilage and pathogenic organisms.
4. Detection the biogenic amines and hypoxanthine value.
5. Detection of the heavy metals residues.
6. Assessment of the quality characteristics according to valid international and local regulations.

# Chapter 1.

# Literature reviews

## 1.1 Food quality control, a historical background

As Laztity *et al.* (2001) state in the Encyclopedia of Life Support Systems (EOLSS), food laws can be traced back to times of the earliest societies. Veteran food regulations are referred to in Egyptian, Chinese, Hindu, Greek, and Roman literature. In the Middle Ages, the trade guilds exerted a powerful influence on the regulation of food trade and the prevention of falsification of food products. Later, the initiative in food control was taken on by the state, municipal, or other local authorities. The big changes in food production and distribution because of the industrialization and rapid growth of urban population, together with public health problems, resulted in the production of many food laws in industrialized nations during the latter part of the nineteenth century. Following examples of the introduction of early food control measures by developed nations, some of the larger, more established and non-industrialized societies also took steps in order to set up measures of control. During the latter nineteenth and early twentieth centuries, a general consolidation of earlier rules took place, but more crucially, this period saw the creation of a new set of laws relating to food. Most of the national standards organizations were established in Europe in the 1920s. The need for improved health and food control and the rapidly expanding international food trade stimulated cooperation on an international level. After World War II, activity in international standardization started intensively in the framework of ISO. A Joint FAO/WHO Food Standards Program was established in 1962, and a joint subsidiary body was also created called the Codex Alimentarius Commission (CAC). The trend in the field of food regulation is characterized by the growing efforts for harmonization at an international level.

## 1.2 Fish quality

The most important indicators of fish flesh quality are: safety, fat content and distribution, color and texture (Gill, 1990), however, nutritional factors such

as n-3 (omega-3) fatty acids and mineral content (essential and heavy metals), also play important roles in quality attributes. With the introduction of farmed fish into the market, a variety of differences in their composition and quality have been observed when compared with their wild counterparts.

Fish freshness is fundamental to fish quality. The state of freshness can be described by a variety of definite properties of the fish which can be assessed by various indicators (Bremner and Sakaguchi, 2000).These properties, and thus the freshness and quality of the end product, are dependent on different biological and processing factors that influence the degree of various physical, chemical, biochemical and microbiological changes occurring post mortem in fish (Botta, 1995). Rapid, inexpensive and accurate instrumental and sensory methods have been developed, that can be correlated with time after catch or attributes related to fish freshness (Connell, 1995; Olafsdottir *et al.*, 1997). An estimate of freshness can be obtained by the defining criteria of the changes in the sensory attributes like appearance, odor, color and texture, which can be measured and quantified by sensory or instrumental methods.

**1.3 Nutritional value of Fish**

Fish is considered one of the natural sources which human body needs to build tissue and activity, as it fish contains high percentage of protein (18.5%) in comparison with cattle meat (16.8%), egg (13.6%) and milk(3.5%) also some species of fatty fish contain 25% fat as well as fish meat considered a rich source of minerals, calcium, phosphorus and lodin; especially marine fish (Ali,1980) .

Hassan *et al*. (1981) mentioned that some studies found that the human body needs to approximate 18kg fish per year in addition to other types of meat.

Approximately 1,000 fish and shellfish species are consumed in the world (Fraser and Sumar, 1998) varying in nutritional and sensorial characteristics

The most common difference between wild and farmed fish is the fillet lipid content (Rasmussen, 2001).

Fish fat is high in omega 3 fatty acids, which are heart-friendly, the regular diet of fish is highly recommended by nutritionists. Nutritionists recommend that fish should be eaten at least 2-3 times a week (Jimenez-Colmenero *et al.*, 2001).

Fish is a good source of protein, amino acids, minerals and n-3 fatty acids; more specifically, fish fat (higher amounts of n-3 fatty acids delivered) are valuable food products recommended to help lower triglycerides and prevent heart attacks and strokes in humans (American Heart Association, 2002).

Fish is a good source of iron, zinc, phosphates and cobalt (Miller, 1996). However their surrounding environment influences in the final mineral content. For instance, fish from acid waters have higher contents of manganese, zinc and heavy metals like mercury. Minerals not only play an important role in the nutritive value of fish, but also could impact sensorial properties such as flavor (Haard, 1992a).

Mineral contents in fish is roughly estimated by ash determination, where minerals are in the form of oxides, sulfates, phosphates, nitrates and chlorides. Minerals are deposited in fish flesh from the aquatic environment and nutritional sources (Miller, 1996; DeMan, 1999).

### 1.4 Quality index method (QIM)

Sensory evaluation of food, according to Huss (1995) and Meilgaard *et al.* (1999) is defined as the scientific means of quantifying and interpreting the variations in food characteristics (odor, taste, appearance) through the use of human senses of sight, smell, taste and touch. Studies have shown that assessment of food freshness and characteristics using sensory methods are capable of giving objective and reliable results when assessments are done under controlled conditions. Generally, trained and experienced taste panel is essential to obtain accurate and reproducible result (Connell, 2001). Sensory methods are divided into two groups; discriminative and descriptive tests. However, the most commonly used is the descriptive test which measures the difference or absolute

value thereby indicating the different quantitative levels (Meilgaard *et al*., 1999). There are several grading methods used to assess freshness in fish and fish products, for example the EU scheme and the Torry system (Huss, 1994).

New sensory schemes exist like the quality index method (QIM), originally developed in Tasmania (Bremner, 1985). QIM is a tool, for the estimation of the quality attributes in a more objective way, based on the significant parameters for raw fish (Frederiksen, 2002); with a score system ranging from 0-1; 0-2; 0-3; 0-4 or more, demerit points (Jonsdottir,1992). The main advantages of the QIM method is that it is specie specific and confusion about attributes is minimized. Each fish species has its own characteristic sensory attributes (flavor, appearance, odor, and texture) which changes with time and temperature, after harvest (Martinsdóttir, 2002). QIM schemes have been developed for species such as European cuttlefish (*Sepia officinalis*), Arctic charr (*Salvelinus alpines*) and fresh cod (*Gadus morhua*) fillets (Martinsdóttir, 2002). Sensory methods in general are known to be irrationally expensive due to the high training requirement of the panel; the cost of running and the need for individual scheme for individual fish species given the different spoilage patterns and physiological and psychological limitations of the analyst (Connell, 2001).

QIM gives scores close to zero for very fresh fish whilest it increases the scores as the fish deteriorates (Martinsdóttir *et al*., 2001). One of the unique advantages of QIM is that it can be used to estimate storage time and remaining shelf life of the studied fish species. QIM is based on a scheme originally developed by the Tasmanian Food Research Unit (Bremner, 1985).

To date the QIM system incorporates fresh herring (*Clupea harengus*), cod (*Gadus morhua*) (Jonsdo´ttir, 1992; Larsen *et al*., 1992), Atlantic mackerel (*Scomber scombrus*), horse mackerel (*Trachurus trachurus*) and European sardine (*Sardina pilchardus*) (Andrade *et al*., 1997), red fish (*Sebastes mentella/ marinus*), brill (*Rhombus laevis*), dab (*Limanda limanda*), haddock (*Melanogrammus aeglefinus*), pollock (*Pollachius virens*), sole (*Solea vulgaris*),

turbot (*Scophtalmus maximus*) and shrimp (*Pandalus borealis*) (Martinsdo´ttir *et al*., 2001). It also incorportes gilthead seabream (*Sparus aurata*) (Huidobroa and Tejada, 2000) and farmed salmon (*Salmo salar*) (Sveinsdo´ttir *et al*. 2002). Warm *et al*. (1998) describes the development of QIM for frozen cod.

QIM has several advantages, including estimation of past and remaining storage time in ice (Luten and Martinsdo´ttir, 1997). The method is based on characteristic changes that occur in raw fish. QIM is based on significant and well defined characteristic changes of outer appearance attributes (eyes, skin, gills, smell) for raw fish, and a score system from 0 to 3 demerit (index) points.

### 1.5 Proximate composition of fish

Proximate composition involves the determination of moisture, lipid, protein and ash content. The proximate composition of fish is affected by a diversity of factors such as: species, age, sex, sexual maturation, season, salinity, exercise, ration, time and feeding frequency, starvation, migration, type and amount of dietary ingredients (Shearer, 1994).

Protein and ash contents do not vary as often as lipid, since it is not impacted by diet, but mainly is determined by the species, genetic characteristics and size (Haard, 1992a; Morris, 2001).

Lipid content of fish flesh is directly related to the nutrition of the fish. When comparing wild and farmed fish yellow perch, higher lipid contents are found in farmed fish mostly due to the accessible and well formulated diets (Jankowska *et al*., 2003; Orban *et al*., 2003). The lipid content of wild fish, however, cannot be manipulated by the fisherman, amongst other factors (Haard, 1992b).

The chemical composition of fish is relatively similar to that of other animals, the main compositions are: water 66- 84%, protein 15-25 %, fat 0.1-24%, minerals 0.8- 2% and glycogen 0.3%, there is also water soluble vitamins and fat soluble vitamins (Salh, 2009). The composition of fish meat is different due to the physical factors like mating season, puberty level also in many cases

it had been noted in various cases that the female flesh contains more protein than the male flesh for the same species will the opposite was found in the salmon cod species (Hassan, 2001).

Friestly (1790), Biot (1802), Morin (1822) and Atwtare (1880) were considered the first researchers to analyze chemical composition of many types of fish as indicator for nutrient value (Hanne ,1987).

There are many studies in the Arabic area about the chemical composition of fishes meat such as, Idlerand Bitners, (1959;1960) on red salmon (*Oncorhynohus nekka* ) and the study of Karrick and Thorston (1965) on silver salmon. Foda *et al.* (1969) also in a study on 3 types of Egyptian shore sardine, that there were significance differences in chemical composition according to the fat percent, they also found a reverse relationship between the fat level and moisture of the edible part of Egyptian sardine; here the moisture levels were 71.63-75.65%, protein percent of 14.4-15.17% according to the dry matter weight and the fat percent being 1.83-5.23% for wet weight base, while the ash percent were 9.98-13.17 % for dry weight base during the study period.

McComish *et al*. (1974); Das *et al*. (1976) studied the chemical composition of fish in the Arabian gulf, also Denton and Yousef (1976); Niimi,(1976a); Raouf *et al*.(1976) who all found in their studies on fish in the Arab gulf that the moisture percent was 76.3% in sardine families (Clupeidae) while the largest percent was in Synodontideae which was 78.7% while the protein percent was 19.4% in Syndontideae and the higher percent were in Sparideae family. Niimi (1976b) analyzed the chemical composition of *Micropetrus saimoide* and *Lipomis macrochirus* fish.

A study by Dennis and Robert (1977) 14 types of fishes on the shore of the pacific ocean were chemical analyzed, and chemical composition for 4 species of Albayah type in Arab gulf (Mugilidae) were studied( Marais andErassmus,1977), Cyprinideae (Dabrowski *et al*., 1978), while Kamal and Allam (1979) studied the chemical composition of fishes in Kuwait and they found that they contain 57.9-80.99% moisture, 17.69-23.6% protein, 0.4-20.11%

fat and 0.76-2.53% ash. Joadder and Islam (2005) clarified that there are significant changes in chemical composition due to seasonal changes for Gobi fishes in Padma River in Bangladesh.

Zuraini *et al.* (2006) studied the amino acids composition and fatty acids for 3 species of local fishes in Malaysia. Kandemir and Polat (2007) noticed the seasonal changes in total fat and total fatty acids through their studied on Rainbow trout which rose in Derbent Dam Lake in Turkey.

Hong-gang *et al.*(2009) studied the chemical composition and fatty acids in big yellow croaker fishes in two different ages, Crude protein, fat and ash levels showed a tendency to increase with age.

Boran *et al*. (2008) suggested that the proximate composition of fish species greatly varies during the catching season. This might be due to physiological reasons and changes in environmental conditions, i.e., spawning, migration, and starvation or heavy feeding. Species-specific physiological characteristics might greatly affect the proximate composition.

The proximate composition and amino acid profile of farmed and wild silver carp and grass carp was determined in Pakistan, The proximate composition of fish flesh of wild silver and grass carp showed significantly higher moisture contents and low protein contents than their farmed counterparts while lipid was significantly higher in wild grass carp and lower in silver carp (Ashraf *et al.*, 2011).

There were many different studies In Iraq about the chemical composition and nutritional value of fishes like the study of Al-Habbib *et al.* (1980) were the chemical composition of 10 different Iraqi fish species in Tigris, were studied of Al-Habbib (1983) studied the chemical composition of *Barbus sharpeyi and Barbus xanthopterus,* Al-Habbib and Al-Aswad (1985) studied the chemical composition for Iraqi fish *Barbus xanthopterus*.

Both of Al-Badri *et al*. (1991); Al-Habbib *et al*. (1991) studied the chemical composition for *Siliurus triostegus*, Jassim (1996) studied the chemical composition of AlShekh fish.

Al-Habbib (2001) studied the chemical composition of *Carassius carassius* and their presence in Iraqi inland rivers. Al-Shatty (2006) studied the chemical composition of 4 species of Iraqi fish, and in (2008) studied the fat quality properties of *Nematalosa nasu*. Another Study was carried out on five species fresh water fish species which speared in Dukan lake that are Gattan (*Barbus xanathupterus*), Bizz (*Barbus esocinus)*, Carseen (*Carassius carassius*), Tawnni (*Barbus belayewi*) and Shabbout (*Barbus grypus*) (Salh, 2009).

## 1.6 Physical indicators

### 1.6.1 pH for flesh fish

The glycogen levels of fish muscles are lower than those of mammalian muscles, mainly due to the stress of fishing. As a result, the pH of fish muscle remains high after death (>6.0) favoring microbial growth and enzymatic activity (Church, 1998). There were many studies involving the pH of fishes meat, such as Orban *et al.* (2000) study which reported that the pH of Bream flesh was 6.3-6.4 also Taşkaya *et al.* (2003) also demonstrated the pH of fresh flesh was 6.83-7.03 but there was change in this value through storage period to reach 5.67-5.82. Kilinc and Cakli (2004) noted in their study on fresh Sardine fishes that the pH was 6.72, while Patir *and* Duman (2006) noted that the pH on common carp reached 5.6-6.95.

Al-Shatty (2008) reported that the pH of *Nematalosa nasu* reaches 6.5-6.6. Ibrahim *et al. (*2008) noted that pH of Tilapia about 6.70-7.21.

The initial pH values of red mullet and gold band goatfish stored in ice were 7.06 and 7.03 respectively (Ozyurt *et al.,* 2009).

### 1.6.2 Water-Holding Capacity

Water holding capacity is defined as the ability of meat to retain its water during application of external forces such as cutting, heating, grinding, or pressing, many physical properties of raw meat (including color, texture, and firmness of raw meat, and tenderness, juiciness of cooked meat) are partially

dependent on water holding capacity (Hedrick *et al*., 1994). The water-holding capacity of meat is closely related to tenderness and juiciness, an increase in water-holding capacity is associated with a loosening of the network of the protein gel which results in an increase in tenderness (Lawrie, 1985). Increased shortening of muscle fibers is accompanied by a decrease in water-holding capacity as was observed during the freezing and thawing (Behnke *et al*., 1973). Cross and Overby (1988) stated that the considerable decrease of water-holding capacity during the heating of meat, which resulted in the release of juice (as cooking drip), was due to the tightening of the myofibrillar network by heat-denaturation of proteins, and changes in water holding capacity during heating were closely connected with alterations in tenderness and rigidity of tissue. Most of the decrease in water-holding capacity occurs between 30°C and 50°C (Ning, 1998).

### 1.6.3 Extract Release volume (ERV)

The measurement of ERV is a simple objective method for assessing freshness of refrigerator-stored meat and fish. It was introduced in 1964 (Jay, 1964). Seafood products (Shelef and Jay, 1971). Vacuum packaged meat (Patterson and Gibbs, 1977).

## 1.7 Fish spoilage

Essentially, fish spoilage changes, which take place in a sequential process occur as soon as the fish dies, and they. These processes have been categorized into: autolytic, microbiological and chemical changes. According to Huss (1995) spoilage of iced Cod has been stratified into four phases; in orded to indicate the deterioration of the eating quality of fish during storage in which autolytic spoilage changes predominate in the initial storage period and is accelerated by changes due to bacterial activity; thus, apart from spoilage changes due to chemical reactions of the fish lipids, autolytic and bacterial activity constitute to the main spoilage changes in fish during storage.

The contemporary consumer demand for fresh food products and the strict food safety regulations in general, requires appropriate assessment and management of safety and quality of food products, by using methods that can quantitatively relate microbial growth to the characteristics of the products during storage, to avoid unnecessary economic losses (McMeekin and Ross, 1996; Connell, 2001).

The various methods commonly used for assessment of fish spoilage have been classified into two categories: sensory methods and instrumental (microbiological, biochemical, and physical) methods (Huss, 1995). However, the most commonly used of these methods for evaluation of wet fish freshness in the fish industry is sensory method, due to its reliability that has been found to coincide with the need to assess the freshness of wet fish within the limited short time in light of its perishability (Connell, 1990).

### 1.7.1 Autolytic spoilage changes

The initial quality loss in fish is basically due to autolytic changes and is not related to microbiological activity (Gram and Huss, 1996). The autolytic spoilage changes precede the other changes responsible for the loss of fish quality during storage. Enzymes and other related chemical reactions do not immediately cease their activities in the fish muscle after fish death (Howgate 1982).

Most importantly in the autolytic spoilage changes is the degradation of fish nucleotides, Adenosine triphosphate (ATP) degrades to adenosine diphosphate (ADP), adenosine monophosphate (AMP), inosine monophosphate (IMP), Inosine (Ino) and hypoxanthine (Hx), associated with bitter fish flavors (Huss, 1995). Generally, the biochemical changes due to enzymatic activity related to freshness deterioration in fish change in the flavor and color and may vary from fish to fish (Haard, 2002).

Unlike in the degradation of other nucleotides, which are known to be principally as a result of enzymatic activity; the ultimate degradation of ATP

intermediate nucleotides, (IMP and inosine) to hypoxanthine (Hx) is attributed to bacterial activity (Haard, 2002). The presence of Hx is usually characterized by undesirable, bitter flavor, an indication of spoilt fish product under chilled storage (Huss, 1988).

### 1.7.2 Chemical spoilage

The chemical spoilage associated with fish during storage is mainly due to fish lipid degradation (auto-oxidation). In general, fish have high degree of unsaturated lipids than other food commodities (Huss, 1995). During fish storage, fish lipids are known to susceptible to oxidative rancidity.

According to Haard (2002) fish lipids are subjected to two main changes, lipolysis and auto-oxidation. The main reactants in these processes involves atmospheric oxygen and fish unsaturated lipids, leading to the formation of hydroperoxides, associated with tasteless, flavor and accompanied by brown yellow discoloration of the fish tissue (Huss, 1994).

Upon further degradation of hydroperoxides is the formation of strong rancid flavors e.g. aldehydes and ketones, usually associated with spoilt fatty fish species (Ashton, 2002). However, these reactions are initiated and accelerated by heat, light, especially the UV-light and several inorganic and organic substances such as copper, iron ions and several antioxidants with the opposite effect, such as alpha- toceopherol, ascorbic acid, citric acid and carotenoids (Huss, 1994).

### 1.7.3 Chemical methods for fish spoilage detection

Chemical methods of food evaluation are normally used to indirectly predict the level of a sensory attribute, which allows for immediate determination of freshness (Huss, 1995).

With regard to evaluation of fish quality using chemical methods, the total volatile basic amines (TVB) constitute to the commonly measured chemical indicators. TVB is a general phrase used to include volatile amines such as,

trimethylamine (TMA), ammonia ($NH_3$) produced by spoilage bacteria and dimethylamine (DMA) produced by autolytic enzymes during fish storage by freezing (Huss, 1988). The concentration of these chemicals in fish tissues can be determined by steam distillation methods (Malle and Poumeyrol, 1989).

Conversely the measurement of the amount of hypoxanthine (Hx) in fish is one of the chemical methods of determining fish freshness (Huss, 1995). Other methods such as Peroxide Value (PV) and Thiobarbituric Acid (TBA) also constitute to the chemical methods that are used to measure rancidity in fish and fish products generally. There are a number of limitations associated with traditional methods of food/fish evaluation (Chebet, 2010).

**1.7.3.1 Amines - Total Volatile Basic Amines**

Total Volatile Bases Nitrogen (TVB-N) is one of the most widely used methods today to estimate the degree of decomposition in fish. It includes the measurement of trimethylamine (produced by spoilage bacteria), dimethylamine (produced by autolytic enzymes during frozen storage), ammonia (produced by the deamination of amino-acids and nucleotide catabolizes) and other volatile nitrogenous compounds associated with seafood spoilage (Malle and Poumeyrol, 1989).

The level of TVBN for white fish is generally considered to be fresh if the TVB is less than 20mg N/100g sample. If the TVB reaches 30mg N/100 g most authorities consider the fish to be stale, whilst at 40mg N/100g the fish is regarded as unfit for consumption. The Codex Alimentarius Committee proposed in 1968 the TVB assay by steam Distillation (Egan *et al*., 1981). The growth of specific spoilage bacteria and the accumulation of their metabolic – by-products constitute to the major spoilage changes in fish during storage. Essentially, freshly caught fish are usually characterized sensorally by fresh fish flavors (sweet, sea weedy); during storage, a period is reached where the odors and flavors are described as neutral or non-specific, (the first indications of off-odors and flavors) which progressively become more pronounced and ultimately renders the fish unacceptable for consumption (Gram and Huss, 1996).

### 1.7.3.2 Trimethylamine (TMA)

Trimethylamine (TMA) is formed in ice-stored fish from the reduction of trimethylamine oxide (TMAO) by bacteria predominately of the genus *Shewanella*. In fish harvested from temperate waters spoilage is predominately due to bacteria of the genus *Pseudomonas* and ammonia rather than TMA is produced. Neither TMA nor TVB are effective indices of spoilage in either commercial quality control, or in official regulatory control (Howgate, 2010).

According to Huss (1988) the source of TMAO in fish is known to be from the biosynthesis of certain species of zooplanktons. These organisms possess an enzyme, mono-oxygenase, which oxidizes TMA commonly found in marine plants as are many other methylated amines (mono-methylamine and dimethylamine) to TMAO, thus, the plankton eating fish obtain their TMAO from these zooplanktons. However, the reduction of TMAO to TMA does not independently the function the TMAO-reducing bacteria, as other systems have also been found to participate in its reduction.

Though it has been known from the mid-19th century that trimethylamine (TMA) was present in Herring (*Clupea harengus*) pickle (Winkles, 1855), it was not until several decades later that Poller and Linneweh (1926) reported the presence of TMAO in fresh Herring. Previous to that Suwa (1909) had identified TMAO in dogfish muscle and many reports since have shown its presence in tissues of both invertebrate and vertebrate aquatic animals from various habitats. The literature on TMAO contents of fishery products has been reviewed in Reay and Shewan (1949); Shewan (1951); Ruiter (1971) and Hebard *et al.* (1982), but there does not seem to be a more recent review. The data presented in these reviews show a wide range of TMAO concentration, including undetected TMAO, in aquatic animals, although some generalizations are possible (Carr *et al*., 1996).

Gadoids fish generally have high TMAO concentrations, in the range of 70 to more than100 mgN /100g, flatfish have moderate levels of around 40mgN /100g, and pelagic, dark fleshed, species contain low levels of around 10 mg N

(100g), although concentrations show high variance within species including seasonal effects (Hebard *et al.*, 1982).

The early measurements of freshwater species found that TMAO was absent, or in very low concentrations, and this was believed to be case for all freshwater species (Dyer, 1952), however further surveys have demonstrated exceptions to this generalization (Anthoni *et al*., 1990). There is evidence that TMAO in the muscle of teleost vertebrate fish is derived from the diet (Hebard *et al.,* 1982) and this could account for patterns of variations between species and for variations within species. The role of TMAO in the biochemistry of vertebrate and invertebrate fishery products is not clear; it may be that it has more than one role depending on the species and environment, or perhaps it has no role at all ( Seibel and Walsh, 2002).

The total volatile bases developed during the storage of unfrozen fish consist primarily of ammonia and trimethylamine (TMA). Depending on the species (ground fish, pelagic species, and shellfish), it has been observed to be a useful measure of freshness quality (particularly flavor and odor aspects) of a variety of seafood. but this usefulness depends upon the time of the year and/or the location of catching, stage of spoilage, type of processing, and/or storage, and method of analysis (Malle and Poumeyrol, 1989).

Trimethylamine provides an accurate indication of bacterial spoilage in some species, even TMA is believed to be generated by the action of spoilage bacteria (Malle and Poumeyrol, 1989).

### 1.7.3.3 Dimethylamine (DMA)

TMAO dimethylase (TMAO-ase) which converts TMAO into equimolar quantities of DMA and formaldehyde(FA). It is noted that certain types of fish contain this enzyme, DMA is produced along with FA in frozen storage with the accompanying FA-induced toughening of the proteins (Masette, 1999). The amount of protein denaturation is roughly proportional to the amount of

FA/DMA produced, but it is most common to monitor the quality of frozen-stored gadoid fish by measuring DMA.

Dimethylamine is produced autolytically during frozen storage. For gadoid fish such as hake, it has been found to be a reliable indicator of FA-induced toughening (Gill *et al*., 1979). Because it is associated with membranes in the muscle, its production is enhanced with rough handling and with temperature fluctuations in the cold storage facility. Dimethylamine has little or no effect on the flavor or texture of the fish per se, but is an indirect indicator of protein denaturation which is often traceable to improper handling before and/or during frozen storage (Rey-Mansilla *et al*., 1999).

Early reports of DMA in chill-stored fish associated its production with bacterial spoilage(Shewan, 1937; Beatty and Collins, 1940) but Amano and Yamada (1965) presented evidence to show that is was formed by splitting of TMAO into DMA and formaldehyde by enzymatic action. Subsequently the property of this TMAOase enzyme system has been studied most often in the context of frozen-stored fish, but there is little doubt that the same reaction is responsible for DMA production during chill storage (Rey-Mansilla *et al*., 1999).

The enzyme is not active in all species of fish; Mackie and Thomson, (1974) measured DMA concentrations during ice storage of three gadoids, Atlantic cod, haddock, saithe (*Pollachius virens*), and two flatfish, plaice (*Pleuronectes platessa*) and lemon sole (*Microstomus kitt*) the study found DMA was formed in the gadoids, but not in the flatfish. Where it is formed the rate of formation during iced storage is low, typically 2-3 mg DMA N (100g) in 14 day, and is approximately linearly related to storage time without any dwell (Oehlenschläger, 2002).

### 1.7.3.4 Ammonia

The bacterial degradation/ deamination of proteins, peptides and amino-acids leads to the forming of Ammonia (Oehlenschläger, 1997b). It is also

produced in the autolytic breakdown of adenosine monophosphate in chilled seafood products. Although ammonia has been identified as a volatile component in a variety of spoiling fish, few studies have actually reported the quantification of this compound since it was impossible to determine its relative contribution to the overall increase in total volatile bases (Howgate, 2006).

Ammonia has been found to be an excellent indicator (LeBlanc and Gill, 1984) and comprised a major proportion of the TVB value for chilled short-finned squid. However, ammonia would appear to be a much better predictor of the latter changes in quality insofar as finfish is concerned.

LeBlanc (1987) found that for iced cod, the ammonia levels did not increase substantially until the sixteenth day of storage. It would appear that at least for herring, the ammonia levels increase far more quickly than trimethylamine (TMA) levels which have traditionally been used to reflect the growth of spoilage bacteria on lean demersal fish species. Thus, ammonia has potential as an objective quality indicator for fish which degrades autolytically rather than primarily through bacterial spoilage.

The ammonia present in immediate post-mortem flesh derives from the deamination of adenine nucleotides to inosine monophosphate, a process which occurs rapidly during the harvesting and death of the fish or within a few hours of subsequent storage in ice (Jones and Murray, 1961; Fraser *et al*., 1966). Howgate (2006) in a review of the kinetics of loss of inosine monophosphate in iced fish found that the median value of the extrapolated initial adenine nucleotide concentration over the species reviewed was 6.8 micromole gm. equivalent to the ultimate formation of 9.5mg ammonia nitrogen /100g, a value similar to the mean concentration of 10.0 mg ammonia nitrogen /100g observed by Oehlenschläger (1997a). In cod a dwell of 14 days before the ammonia concentration started to rise was reported by Oehlenschläger (1997b), 13 days in haddock (Shewan, 1937), and 10 days in farmed catfish (*Silurus glanis*) (Manthey *et al*., 1988).

Vallé *et al*. (1996) measured ammonia contents in three species of fish during its storage in ice.

### 1.7.3.5 Lipid oxidation in fish

Lipid oxidation involves the reaction of oxygen with free radicals from unsaturated fatty acids, which leads to the production of off-flavors and off-aromas (Gorga and Ronsivalli, 1988).

There are different types of lipid oxidation: photo oxidation, thermal oxidation and enzymatic oxidation, but autoxidation of unsaturated fatty acids is the most common. Lipid oxidation is influenced by different factors: fatty acid profile, oxygen, light, metals, temperature, and water activity, among others(Labuza, 1975). The main steps of autoxidation are: initiation, propagation and termination. Hydroperoxides are the first oxidation products (no flavor) which will subsequently form compounds such as aldehydes, alkanols, alkanes, alkenes that produce an off-flavor (Nawar, 1996; DeMan, 1999).

Volatile aldehydes are responsible for the rancid flavor in catfish fillets, most specifically (Freeman and Hearnsberg, 1993).

Rancidity is a major problem in frozen fish, whilst in fresh fish the main quality problem is microbiological spoilage (Ackman and Ratnayake, 1992). The high content of PUFAs in fish increases the rate of oxidation, since the increased double bonds accelerates oxidation (Flick *et al*., 1992). Lipid oxidation is one of the main factors limiting the quality and acceptability of meats and other muscle foods (Morrissey *et al*., 1998: Zamora and Hidago, 2001).

Post-slaughter period, during handling, processing, storage and cooking. This process leads to discoloration, drip losses, off-odor and off-flavor development, texture defects and the production of potentially toxic compounds (Richards *et al*., 2002). Lipid oxidation is a chain reaction that consists of initiation, propagation, and termination reactions, and involves the production of free radicals (Nawar, 1996; Renerre, 2000).

A three-step simplified free-radical scheme has been postulated as follows (Figure 1.1):

1-Initiation

Initiator $\longrightarrow$ free radicals (R°, ROO°)

2. Propagation

R°+ O2$\longrightarrow$ ROO°; ROO° + RH $\longrightarrow$ROOH + R°

3. Termination

R° + R° $\longrightarrow$ R-R

R° + ROO° $\longrightarrow$ ROOR

ROO°+ ROO° $\longrightarrow$ ROOR + O2

Figure 1.1: Mechanism of lipid oxidation (Nawar, 1996).

This phenomenon can be influenced by both intrinsic and extrinsic factors such as the concentration of pro oxidants, endogenous ferrous iron, myoglobin, enzymes, pH, temperature, ionic strength, oxygen consumption reaction and the fatty acid composition of the meat (Andreo *et al*., 2003; Undeland, 2001). Meats such as fish and poultry contain a high concentration of polyunsaturated fatty acids and are therefore more susceptible to oxidation (Pacheco-Aguilar *et al*., 2000). Lynch *et al*. (2001) demonstrated that lipid oxidation occurred progressively in stored ground beef at 4°C and produced a variety of aldehydes.

Fatty fish such as sardine underwent rapid lipid oxidation during ice storage due to the high content of polyunsaturated fatty acids (Pacheco-Aguilar *et al*., 2000). Chaijan *et al*. (2004) reported that lipid and myoglobin contents were higher in dark muscle than in ordinary muscle of both sardine and mackerel. Saturation of red color in meat was directly related to myoglobin concentration (Faustman *et al*., 1992). Other constituents of meat including enzymatic and non-enzymatic reducing systems can accelerate oxidation by converting iron from the inactive ferric form to the active ferrous state (Foegeding *et al*., 1996). As with most chemical reactions, lipid oxidation rates increase with increasing temperature and time (Hultin, 1992).

Saeed and Howell (2002) reported that the rate of lipid oxidation in frozen Atlantic mackerel increased with increasing storage time and storage temperature. Furthermore, freezing can facilitate lipid oxidation, partly because of concentration effects (Foegeding *et al*., 1996).

Oxidative spoilage: Lipid oxidation is a major cause of deterioration and spoilage for the pelagic fish species such as mackerel and herring with high oil/fat content stored fat in their flesh (Fraser and Sumar, 1998).

In fish, lipid oxidation can occur enzymatically or non-enzymatically. The enzymatic hydrolysis of fats by lipases is termed lipolysis (fat deterioration). During this process, lipases split the glycerides forming free fatty acids which are responsible for:

(a) Common off flavor, commenly referred to as rancidity.

(b) Reducing the oil quality (Huis in't Veld, 1996; FAO, 1986).

The fatty acids formed during hydrolysis of fish lipids interact with sarcoplasmic and myofibrillar proteins causing denaturation (Anderson and Ravesi, 1969).

Modern instrumental methods allow analysis of better defined oxidation products (specific hydroperoxides, actual content of malonaldehyde), but for general quality estimation, methods that determine a broader range of oxidation products (such as PV and TBA-RS) are to be preferred.

Hydrolysis of ester bonds in lipids by enzymatic action or heating in the presence of water liberates free fatty acids (FFA). In animal tissues, once sacrificed, FFA can be liberated by enzymatic action. Lipolysis of triacylglycerol and phospholipid fractions has been shown to occur during frozen storage of non-heated food systems. Moreover, fish enzymes may be active even at temperatures below -20 ºC (Erickson and Hung, 1997).

FFA content in hake and anchovies has been used to establish the grade of deterioration (De Koning andMol, 1991).

Triacylglyceride hydrolysis has been suggested to lead to increased oxidation, whereas phospholipid hydrolysis produces the opposite effect (Shewfelt, 1981).

Accumulation of these lipids causes disagreeable flavors in foodstuffs. The flavor impairment caused by lipolysis is usually described as “rancidness” or “soapiness”. In some case, the quantification of FFA serves to establish the limits by which the food is not organoleptically acceptable. Studies on frozen fish have shown that lipid hydrolysis plays a key role in sensory deterioration (Refsgaard *et al*., 2000). In addition, the rancid odor detection showed good correlation values with some biochemical lipid damage indices (FFA, PV and TBA-i) (Aubourg *et al*., 2004; Rodríguez *et al*., 2009)

Peroxide value provides a measure of the degree of lipid oxidation and indicates the amount of oxidized substances (Yanishlieva and Marinova, 2001; Barthet *et al.*, 2008; Aberoumand, 2010).

#### 1.7.3.5.1 Thiobarbituric acid(TBA)

The most common method to determine lipid oxidation in fish is the measurement of thiobarbituric reactive substances (TBARS) (Freeman and Hearnsberger, 1993). The 3-carbon compound malonaldehyde (MDA) is a major carbonyl decomposition product of autoxidized, polyunsaturated lipid materials (Pegg *et al*., 1992). Spectrophotometric detection of the malonaldehyde-thiobarbutiric acid (TBA) complex has been widely used for measuring lipid oxidation in food and biological tissues (Esterbauer and Cheseeman, 1990). The basic principle of the method is the reaction of 1 molecule malonaldehyde and 2 molecules TBA to form a pink pigment malonaldehyde-TBA complex, which can be quantitated spectrophotometrically (Gutteridge, 1981).

TBA Consumption limits are from 7-8mg malonaldehyde/kg (Schormüller, 1969) Nonetheless, Tarladgis *et al.* (1960) reported that rancidity was occurred when TBA value exceeded to 4mg malonaldehyde/kg.

### 1.7.3.6 Biogenic amines

Biogenic amines (BAs) are mainly formed in foods by microbial decarboxylation of amino acids and transamination of aldehyde and ketones (Silla-Santos, 1996). BAs are of importance due to risk of food intoxication and serve as chemical indicators of fish spoilage (Alberto *et al*., 2002).

However, food intoxication may occur if the amine-metabolizing capacity is over-saturated and/or the metabolic activity is impaired by specific inhibitors (Taylor *et al*., 1978). Putrescine and cadaverine can enhance histamine toxicity through interfering with histamine detoxification system. Moreover, biogenic polyamines, such as putrescine, cadaverine, spermidine, spermine and agmatine, are potential carcinogens to be converted to nitrosamine when exposed to nitrite (Bills *et al*., 1973). Common symptoms of BA intoxication are migraine, brain haemorrhage, heart failure, hypertension, urticaria, headache, flushing, abdominal cramps and hypotension (Rice *et al*., 1976). An upper limit of histamine for human consumption has been suggested to be 100mg/kg food, and 2mg/l alcoholic beverage and 100–800 mg/kg of tyramine and 30mg/kg of phenylethylamine these have been reported to be toxic doses in foods, respectively (Brink *et al*., 1990). Total BA levels of 1000 ppm in food are also considered dangerous for human health (Taylor, 1985).

Histamine production in fish is related to the histidine content of the fish, the presence of bacterial histidine decarboxylase (HD), and environmental conditions (Ienistea, 1973; Love, 1980). During spoilage, certain bacterias produce decarboxylase enzymes, which act on free histidine and other amino acids in the fish muscle to form histamine and other biogenic amines. Chemically, histamine (from histidine), putrescine (from ornithine), cadaverine (from lysine), and spermidine and spermine (from arginine), which are produced post-mortem in fish muscle, are low-molecular-weight, aliphatic, alicyclic or heterocyclic organic bases (Rawles *et al.*, 1996).

Fish species belonging to the families of Scombridae (e.g. tuna and mackerel) and Scomberescoidae (e.g. saury) are most commonly associated with

HFP, but non scombroid fish, such as mahi-mahi, sardines, pilchards, anchovies, herring, marlin and bluefish can also be involved (Taylor, 1986). These fish species are characterized by having relatively high levels of histidine in their flesh (Institute of Medicine, 1991). Histidine levels vary from 1g/kg in herring to as much as 15g/kg in tuna (Ijomah *et al.,* 1992). Frank *et al.* (1981) found that fresh (skipjack) tuna (*Katsuwonus pelamis*) contained negligible quantities of histamine, usually <0.1 mg/100 g.

After investigating HD production by *Morganella morganii* in mackerel, Eitenmiller *et al.* (1982) concluded that the ready availability of free histidine in the muscle to act as both inducer and substrate makes scombroid fish muscle an ideal environment for histamine formation.

The main bacteria responsible for histidine decarboxylation and HFP are members of the family Enterobacteriaceae (Frank *et al.,* 1985; Taylor and Sumner, 1986). Endogenous production of decarboxylase enzymes is insignificant when compared with the exogenous (bacterial) pathway (Rawles *et al.,* 1996). Spoilage, ammonia production and biogenic amine production are enhanced at elevated storage temperatures, with histamine production being optimal at around 30°C (Arnold *et al.,* 1980). Once a large bacterial population has been established, residual enzyme activity continues slowly at refrigeration temperatures (0–5°C), even though bacterial growth ceases (Institute of Medicine 1991). Histamine is also produced, but to a lesser extent, by bacteria that can grow at refrigeration temperatures (Okuzumi *et al.,* 1981). Only free histidine can be decarboxylated (Arnold and Brown, 1978).

The assessment of the biogenic amine content in foods has received much attention for many years due to the possibility of using amine concentrations as an index of food quality (Mietz and Karmas 1977; Edwards *et al.,* 1987).

### 1.7.3.7 Hypoxanthine (Hx)

Shortly after the death of the fish, Hypoxanthine (Hx) begins to accumulate. However, changes in TVB-N and TMA-N are related to the

microbiological activity and they do not increase at the beginning of storage. Thus, nucleotide and Hx measurements have some advantages over TMA and TVB analyses. Spinelli (1967) reported that the total volatile amine and trimethylamine tests measure the various stages of spoilage caused by bacteria, but the assay of nucleotides and Hx reflects enzymatic spoilage.

The detection of xanthine during advanced spoilage indicates that hypoxanthine is lost through bacterial oxidation (Kassemsarn *et al*., 1963).

Adenosine triphosphate (ATP) is degraded at the postmortem stage by endogenous enzymes in the fish flesh.

ATP →ADP → A MP→ IMP →HxR→ Hx (Vilhelmsson, 1997).

Any quality control parameter should accumulate or disappear quickly during spoilage. In addition, it should be absent or present in constant amounts in fresh fish (Jahns *et al*., 1976).

### 1.7.4 Microbiological spoilage

Microorganisms exist on the skin/slime, gills and the gut of live and newly caught fish. According to Huss (1995) the proportion of microorganisms commensal the fish on the surface and gills/ guts of fish is 102-107 colony forming units (cfu) /cm2 and between 103 and 109 cfu/g respectively.

Spoilage of fish under aerobic conditions becomes apparent when specific spoilage bacteria (SSO) reaches the values of $10^8$-$10^9$/g flesh (Gram and Huss, 1996). Normally, this occurrence is after a lag phase, the time-span of which mainly depends on the temperature before the bacteria enter into exponential growth particularly in tropical fish species stored under low temperatures (0˚C ) (Gram *et al*., 1990).

According to Huss (1995) microbial spoilage of fish may take diverse forms which manifest itself as changes in the sensory characteristic such as; off-odors, flavor, taste, formation of slime, visible pigmented and non-pigmented colonies; discoloration and gas production; that finally cause sensory rejection. Dalgaard *et al.* (1993) suggested that the utilization of TMAO by these

organisms lead to the formation of trimethylamine (TMA), one of the most important compounds responsible for off-odors, "fishy" odors at later stages of spoilage in many marine fish. According to Huss (1988) and Gram and Huss (1996) these compounds are characterized by volatile foul-smelling, pungent off-odors, which can be detected organoleptically even in very low concentrations. Whereas, *Pseudomonas* spp, Enterobacteriaceae and anaerobic spoilers lead to the production of fruity, sweet-smelling esters, ketones, aldehydes, and putrid sulphur compounds, non-$H_2S$ sulphides (Huis in't Veld, 1996).

### 1.7.5 Microbiological methods for fish spoilage detection

The major changes in fish freshness such as unattractive change in food characteristics such as, flavors and odors and color are largely due to bacterial growth and activity (Connell, 1990). Microbiological methods are used to estimate bacterial numbers, in order to determine fish freshness, hygiene and or evaluate the possible presence of bacteria or organisms of public health importance (Huss, 1994) Microbiological prediction/estimation of bacterial numbers therefore, in order to serve the purpose of food safety and shelf life determination, is expected to relate quantitatively to the characteristics of the food during storage (Dalgaard, 2000).

#### 1.7.5.1 Total plate counts (TPC)

This parameter is synonymous with Total Aerobic Count (TAC) and Standard Plate Count (SPC). The total count represents, if carried out by traditional methods, the total number of bacteria that are capable of forming visible colonies on a culture media at a given temperature. The total count was $10^9$ cfu/g for days before the fish was rejected (Gram *et al*., 1989).

The number and types of bacteria which lived on the skin of a live animal depends on the quality and the water contamination level, fresh water like river flakes contain several types of microorganism which has more variety than sea and Oceans waters which contain a specific rate of soluble salt which determine the type and number of living bacteria, the psychrophilic and

mesophilic type of microorganisms had been isolated from fish body surface, these group found of the body surface of fresh water and salt water fish ,the genus *Pseudomonas* spp. Formed 40-50% from the total contamination (Aldulaimi,1978).

The increase in total microorganisms count in fresh fish is due to the contamination after fishing or from boats, fishing tools also by the invasion of meat microbes (Frazier andWesthoff, 1988).The limited of total bacteria count is $10^5$-$10^7$ cfu/g for fresh fish (ICMSF,1986).

The average of total bacterial count on skin, Gills and intestine of different types of Microorganism were $10^2$-$10^7$, $10^3$-$10^5$ and $10^4$-$10^8$ cfu /$cm^2$, gm and ml respectively (Liston *et al*., 1976 ; Huang andLeung, 1993).

After rigor mortis the bacteria found on the surface of the body and slime, gills and intestine will rapidly invade the fish muscles (Frazier and Westhoff, 1988).

The psychrophilic bacteria in the chilled stored fishes leading to spoilage due to Proteolysis and lipolysis (Haard ,1992a), Determining the microbial load of fishes and their products considered as the most important tests to determine the flesh quality and storage period (Al-Basuni,1993) .

Fish is considered as a very perishable (fast spoiled) food due to its high moisture contain and free amino acids, low connective tissues and neutral pH and because they move a lot at fishing so most of glycogen will convert to Pyruvic acid (aerobic ally) or lactic acids(an aerobically), and because these acids will be removed physiologically so the pH will remain neutral after hunting which lead to the growth of microorganism which are on the surface (Al-Taaee, 2001).

Sikorski and Kalodziejska (2002) found that the pathological bacterial contamination were very low which could be happen due to unsanitary conditions and using improper preparation, bad handling or air contaminations at packaging.

### 1.7.5.2 Spoilage bacteria

The total number of bacteria on fish rarely indicates sensory quality or expected storage characteristics (Huss *et al*., 1974). However, it is recognized that certain bacterias are the main cause of spoilage. Ambient spoilage is often caused by members of *Vibrionaceae* and *Pseudomonas* spp. which spoils some tropical and freshwater fish (Gram *et al*., 1987).

#### 1.7.5.2.1 *Pseudomonas* spp

The *Pseudomonadaceae* family represents a large and poorly defined group of Microorganisms. They are generally characterized as Gram-negative rods, motile with polar flagella, oxidase-positive, catalase-positive, obligate respiratory bacteria. The spoilage compounds associated with the growth of psychrotrophic *Pseudomonas* spp (Gram and Dalgaard, 2002), on fish are diverse and in many cases species-specific. *Pseudomonas* spp. mediated spoilage is characterized by ‘fruity’, ‘oniony’ and ‘fecal’ odors from the production of ketones, aldehydes, esters and non-hydrogen sulphide, sulphur-containing compounds such as methyl sulphide, Members of the genus are able to produce pigments and proteolytic and lipolytic enzymes that may affect the quality of fresh and, more especially, processed (e.g. frozen) fish products. The spoilage of freshwater fish is generally ascribed to the growth of *Pseudomonas* spp. (Gram and Huss, 1996).

#### 1.7.5.2.2 *Vibrio* spp.

*Vibrio* spp. is Gram-negative, facultative anaerobic motile curved rod bacteria with a single polar flagellum. The genus contains twelve species that can cause foodborne illness, although most of this is caused by *V. cholera*e, *V. parahaemolyticus* or *V. vulnificus* (Oliver and Kaper, 1997; Dalsgaard, 1998). Some species are primarily associated with gastrointestinal illness *(V. cholerae* and *V. parahaemolyticu*s) while others can cause non-intestinal illness, such as septicaemia *(V. vulnificu*s).

Pathogenic *Vibrios* can also be recovered from freshwater reaches of estuaries (Desmarchelier, 1997). The occurrence of these bacteria does not correlate with numbers of fecal coli forms and the depuration of shellfish may not reduce their numbers. Based on data from the United States, there is a positive correlation between water temperature and both the number of human pathogenic *Vibrios* isolated and the number of reported infections, a correlation particularly marked by *V. parahaemolyticus* and *V. vulnificus.* In Japan (Ministry of Health, Labour and Welfare, Japan, 2000) and eastern Asian countries, *V. parahaemolyticus* has been recognized as a major cause of foodborne gastroenteritis.

In most countries outside of Asia, the reported incidence is low, perhaps reflecting a different mode of seafood consumption, Gastroenteritis caused by this organism is almost exclusively associated with consumed seafood which is either raw, inadequately cooked, or contaminated after cooking. In the United States prior to 1997 the illness was most commonly associated with crabs, oysters, shrimps and lobsters (Oliver and Kaper, 1997).

*V. vulnificus* has been associated with primary septicaemia in individuals with chronic pre-existing conditions, following consumption of raw bivalves. This is a serious, often fatal, disease. To date, *V. vulnificus* disease has almost exclusively been associated with oysters (Oliver, 1989). Recently, *V. vulnificus* infections have been associated with a variety of raw seafood products in Korea and Japan.

Wong *et al.* (1999) recovered *V. parahaemolyticus* from 315 (45.9%) seafood samples from Asian countries. The incidence of *V. parahaemolyticus* in shrimp, crab, snail, lobster, sand crab, fish and crawfish was 75.8%, 73.3%, 44.3%, 44.1%, 32.5%, 29.3% and 21.1%, respectively; recovery from products from Hong Kong and Thailand was markedly higher than those from Indonesia and Vietnam.

**1.7.5.2.3 *Aeromonas***

Species of Aeromonas are Gram-negative, non-spore-forming, rod-shaped, facultative anaerobic bacteria .Although historically the Aeromonas genus has been placed in the *Vibrionaceae* family (Popoff, 1984); the current edition of *Bergey's* lists three genera in the Aeromonadaceae family, including *Aeromonas*, *Oceanimonas*, and *Tolumonas* (Carnahan and Joseph, 2005). Although members of the genus have classically been divided into three biochemically differentiated groups (typified by *A. hydrophila*, *A. caviae*, and *A. sobria*), these contain a number of geno species, to which new species have been added (Carnahan and Altwegg, 1996).

*Aeromonas hydrophila* (Ah) is an emerging foodborne disease agent, widely distributed in the environment. Recently, the genus *Aeromonas* has been classified within the family *Aeromonadaceae* and consists of 14 different confirmed species, one of which is Ah (Joseph and Carnahan, 2000). It is well known that the microorganism is the cause of several diseases in cold (fish, reptiles, amphibians) and warm (mammals and birds) blooded animals as well as of a zoonotic disease (Daskalov, 2006). Ah is widely spread in waters, water habitants and many food products (seafood, shellfish, raw foods of animal origin like poultry, ground meat, raw milk, and raw vegetables) (Fricker andTompsett, 1989; Gobat and Jemmi, 1993). With regard to foods of animal origin, according to Kumar *et al*. (2000) and Neyts *et al*. (2000), seafood products are more frequently contaminated by Ah in consequence of the wide diffusion in the aquatic environment and the ability to grow at cold temperatures. Furthermore, since Ah is an important agent of several freshwater (Aoki, 1999) and marine (Balebona *et al*., 1998; Zorrilla *et al*., 2003) reared fish diseases, its spread in aquaculture environment could be a significant public health concern (Daskalov, 2006). However, the above food safety implications are strictly related to the pathogenicity and virulence of the strain as well as to the bacterial concentration which Ah is able to reach during the storage. In the case of fish, the main Ah growth during the storage, like for several other microorganisms, occurs on the

skin and the gills which are considered the most important source of spoilage bacteria and pathogen bacteria (Kumar *et al*., 2000).

#### 1.7.5.2.4 *Shewanella putrefaciens*

The genus *Shewanella* was first described by MacDonell and Colwell (1985). The definition of *Shewanella* was based almost entirely on rRNA structure and described it as straight or curved rods, Gram-negative, non-pigmented, motile by polar-flagella, chemo-organotrophic, oxidase positive generally associated with aquatic or marine habitats (Lee *et al*., 1977; Jensen *et al*., 1980)

*Shewanella putrefaciens* has been studied since its first description as *Achromobacter putrefaciens* by Derby and Hammer (1931), because of its special interest in the areas of applied and environmental microbiology.

*Shewanella putrefaciens*, first isolated as *Achromobacter putrefaciens* from rancid butter, was classified in 1960 as *Pseudomonas putrefaciens* by Shewan *et al*. (1960) and, later, as *Alteromonas putrefaciens* by Lee *et al*., (1977). This micro-organism has been found to be associated with the spoilage of proteinaceous foods (Shewan, 1977). *Shewanella* species are widely distributed and have been isolated from diverse sources such as aquatic environments (Nealson *et al*., 1991), sediments (Myers and Nealson, 1988), oilfield fluids (Semple and Westlake, 1987) and, as mentioned above, spoilage of proteinaceous foods (Stenstrom and Molin, 1990), and are considered opportunistic pathogens of humans (Brink *et al*., 1995) and aquatic animals (Aguirre *et al*., 1994).

## 1.8 Sensory evaluation for fish (Organoleptic Taste)

The sensory evaluation defined as the art that depends on measuring and analyzing the translation of the impression of food quality, also by observation, tasting, touching and hearing. Most of the sensory evaluation can be made by the human being himself although the development in equipment field helped in measuring the quality changes for each property (Lawless and Heymann, 1999)

Sensorial attributes of food products are perceived by consumers in a specific order (Meilgaard *et al*., 1999):

1. Appearance: color, size, shape, clarity, surface

2. Odor (Aroma): "Aroma is the odor of a food product". Volatile compounds of products are affected by temperature and biochemistry of the compounds

3. Consistency and texture: viscosity, consistency and texture. Texture (hardness/ firmness, cohesiveness, dryness, moistness): sensory perception of mechanical or physical properties of the food product by the sense of touch (hand, tongue, lips, jaw).

4. Flavor: sensorial perceptions of chemical compounds of the food product by tasting in the mouth. Flavor is a complex concept that integrates the aroma (volatile substances), tastes (salty, sweet, sour and bitter) in the mouth and chemical factors (astringency, heat, metallic flavor, umami taste).

Sensory analysis, as mentioned before, is of vital importance to the fish industry, for its assessment of products freshness. Fish consumers expect a product that is safe and has good appearance, odor, taste and texture (Parisi *et al*., 2002) and their decision to purchase a fish product is based first on appearance, followed by flavor and then texture.

Also the sensory evaluation considered as one of the important tests used for general acceptance of meat, consumer desire; this includes flavor ,tenderness, Juiciness and general acceptance. Although the meat have great nutritional value so the consumption can be increased by improving the palatability through flavor, smell, color, appearance, Juiciness and tenderness(Al-Aswad, 2000).

Tenderness is considered as the most important quality for the consumer because it's the first thing that the consumer feels at chewing (Al-Taaee, 1987).

Juiciness is considered important for general acceptance for meat; both of muscle fat and water is responsible for this. Flavor and meat odor help in saliva

and gastric juice stimulation so they help to increase appetite and acceptance of the meat (Tahir, 1983)

Al-Habbib (1983) found there were insignificant difference in general acceptances for frozen meat of *Barbus sharpeyi and Barbus xanthopterus* while the flavor properties showed significant difference at storage period. Bilgin *et al*. (2008) studied the quality properties for chilled and smoked Sea Bream which has removing gills, the consistency has the 4.43 point from 5, odor has 4.57 from 5 and flavor has 4.43 from 5.

## 1.9 Heavy metal residue

The term 'heavy metal' has been used extensively to describe metals that are environmental pollutants (Walker *et al*., 2001). According to Francis (1994), even though some metals are essential when taken up by organisms, their excessive presence will reverse the effect so that its benefits become toxicity. Heavy metals can be critically important to the life processes of marine organisms. Aluminium, arsenic, chromium, cobalt, copper, iron, manganese, molybdenum, nickel, selenium, tin, vanadium, and zinc are essential heavy metals for one or more organisms. Usually, they are present in living organisms in trace amount not exceeding 1μg/g. Copper and zinc are necessary in trace amounts for the functioning of biological systems (Markert, 1994). The non-essential heavy metals include cadmium, gold, lead, mercury, silver, and metals (including radionuclides) of higher atomic weight (Rainbow and Furness, 1990). Lead and cadmium are known to interfere with the functioning of the biological systems (Villareal-Trevino *et al*., 1986). Due to the fact that even trace amounts of some heavy metals can generally exhibit high toxicity to marine biota and human, there is an increasing interest in studying these metals in the marine environments (Sadiq, 1992).

Mercury levels in fish have gained recent media attention after the FDA published a consumer advisory (2001) where recommendations were made to pregnant women and young children to restrict consumption of shark, swordfish,

king mackerel and tilefish because of a probable risk of high methyl mercury concentrations.

Many nations have considerable concern over the presence of high levels of toxic elements in fishes. For example, over 1970mg Hg/g liver was reported for two of the 26 liver samples of whale meat examined in Japan (Endo *et al*., 2002). This is nearly 5000 times the Japanese government's limit of 0.4 mg/g for mercury. The WHO has adopted the US EPA levels for mercury and recommends that food with mercury concentrations of 0.5 mg/kg or more should not be sold for human consumption. In Japan, because of the high consumption of fish, the government has recommended that fish with mercury level of 0.3 mg/kg (wet weight) or over should not be sold (Dickman and Leung, 1998).

Industrial and agricultural processes have resulted in an increased concentration of heavy metals in air, water and soil, subsequently; these metals are taken by plants and animals and take their way into the food chain. Man is exposed to uptake of heavy metals from air, food and water (Sohair and Mahmoud, 1992). Fish intake is the major source of exposure to mercury, mainly in the form methyl mercury, which it accumulates from the surrounding, waters (McKinney and Rogers, 1992).

There is a very narrow range in which the heavy metal is considered essential or toxic, these metals includes: Lead, cadmium and mercury which is a cause for concern due to the variety of their uses which increases their level in the environment (Highom and Tomkins, 1993).

A study reported mercury levels in the 12 most commonly consumed fish in the Seychelles Islands, the analysis showed 5-6 samples of each fish, and found that several of the larger predatory species exceeded the FDA action limit of 1.0 ppm, the European Commission's Decision's maximum mercury level of 1.0 ppm for trophic fish (Storelli *et al*., 2002).

In 1993-94 a survey was conducted on the total mercury and methyl mercury content in edible fish and invertebrates of the coast of the Azores Islands in the Atlantic Ocean (Andersen and Depledge, 1997). Methylmercury

was, on average, 80% or more of the total mercury in the samples. All levels of total mercury reported for the fish and shellfish were within the European Union's safety limit. Romeo *et al*.(1999) evaluating Hg levels in muscle, gills and liver tissues in different fish species from the Mauritania coast, observed that Hg concentrations in the gills and muscle of the pelagic species are very low, whereas higher concentrations were found in the liver of benthic species .

Total and MeHg concentrations in fish differed between species and ranged from 0.073µg/ g (*Liza subviridis*) to 3.923 µg/g (*Epinephelus coiodes*) (AL-Majidand Preston, 2000).

Storelli *et al.* (2005) measuring concentrations of total Hg and organic-Hg in the edible fish tissue from the Ionian and Adriatic seas, detected higher concentrations in striped mullet (*Mullus barbatus*), a benthic species, than in hake (*Merluccius merlucciu-s*), a pelagic species.

# Chapter 2:

# Material and methods

Table 2.1: instruments used in the study

| **Instrument** | Company | Country |
|---|---|---|
| Oven | Lab tech | Korea |
| Hot plate stirrer | Lab tech | Korea |
| Colony counter | W.T.W .BZG30 | Korea |
| Muffle furnace | Carbolite | England |
| kejldahal (distillation unit) | Local production | Iraq |
| Autoclave | Lab tech | Korea |
| Shaking incubator | Lab tech | Korea |
| Automatic water still (distilater) | Lab tech | Korea |
| Cold incubator | Electro mag .M7040R | Germany |
| Water bath | GFL | Germany |
| Magnetic stirrer hot plate | Sturt | England |
| sensitive balance | Mettler Toledo | Switzerland |
| Centrifuge | Hettich | Germany |
| pH meter | WTW 2f40-11420 D | Germany |
| Blender and mixer | Cook Works | England |
| Incubator | Memmert | Germany |
| Hood chamber | Local production | Iraq |
| Meat thermometer | Metaltex | France |
| Spectrophotometer | Shimdzu | Japan |
| Balance | Sartorius | Germany |
| Microkejldahal(digestion unit) | Buchi .K424 | Switzerland |
| Microkejldahal(distillation unit) | Buchi .K350 | Switzerland |
| HPLC. | shimdzu | Japan |
| Cold vapor atomic absorption spectrophotometer | shimadzu 6800 | Japan |
| Homogenizer | Lab tech | Korea |
| VITEK® 2 SYSTEM | BIOMÉRIEUX | Germany |
| atomic absorption spectrophotometer | Perkin Elmer | US A |

Table 2-2: Media and chemical used in the study

| | | |
|---|---|---|
| Starch ampicillin agar | Biolife | Italy |
| MacConkey agar | Hi media | India |
| Kligler Iron agar | Lab | England |
| Iron agar | Lab | England |
| Plate count agar | Biolife | Italy |
| TCBS cholera medium | Lab | England |
| Triple sugar iron agar | Merck | Germany |
| Nutrient agar | Biolife | Italy |
| Bacteriological pep ton | Lab | England |
| Tryptic soy agar | Lab | England |
| Citermide agar | Biolife | Italy |
| Tryptic soy broth | Lab | England |
| N-N-N-N-Tetra methyl Phenylendiamine dihydro-chloride | BDH | England |
| Ethanol | BDH | England |
| Hydrochloric acid | Merck | Germany |
| Sulfuric acid | Merck | Germany |
| Glacial acetic acid | BDH | England |
| Methanol | Gcc | England |
| Chloroform | Bio solve | Netherlands |
| Formic acid | Merck | Germany |
| Methyl red | Georage.T.Gurr Ltd | England |
| Sodium hydroxide | Merck | Germany |
| Copper sulfate-s-hydrate | Riedel-de-haen ag seelze | Germany |
| Magnesium oxide | BDH | England |
| Potassium sulfate | Merck | Germany |
| Potassium dihydrogen ortho-phosphate | Thomas baker | India |

| | | |
|---|---|---|
| Sodium thio sulfate 5-hydrate | Merck | Germany |
| Potassium hydroxide | Thomas baker | India |
| Phenolphthalein | BDH | England |
| Potassium dihydrogen phosphate | Analar R | England |
| Trichloro acetic acid | Thomas baker | India |
| Sodium sulfate anhydrous | Merck | Germany |
| Boric acid | Fluka AG ,Buchs SG | Switzerland |
| Cristal iodine | JDB | England |
| Nitric acid | BDH | England |
| Aceton | BDH | England |
| Thiobarbaturic acid | BDH | England |
| Phenol red | haen ag seelze | Germany |
| Bromocresol green | BDH | England |
| Formaldehyde | Analar R | England |
| acid bromide | BDH | England |
| dithizone | Fluka AG | Switzerland |
| amino acids standard | Sigma | USA |
| Hypoxanthine standard | Sigma | USA |
| Biogenic amines standard | Sigma | USA |
| Ammonia standard | Sigma | USA |
| Dimethylamine standard | Sigma | USA |
| Heavy metals standard | Alpha | USA |
| Dithylether | BDH | England |
| trichloroethylene | BDH | England |
| sodium bicarbonate | BDH | England |
| acetonitrile | Analar R | England |

Culture media: The Media were prepared according to the directions of manufacturers then sterilized by Autoclave at 121 ºC and 15 Ibs /inch$^2$ for 15 min, except if it was not subjected to autoclaving.

## 2.1 Sampling

This study was conducted in the laboratories of the Agriculture science Faculty/ University of Sulaimani/Kurdistan Region/ Iraq, for the period from 1st February 2010 to 1st October 2010. Five types of fish most commercialy traded and widespread in Sulaimani governorate, which were grass carp, silver carp, common carp, Bizz and Shabbout (table, 2.3), were chosen randomly from two fish retail outlet, licensed (M1) and unlicensed (M2) markets. Analyses were achieved on a sample of twelve fishes for each type, their weight ranged 2-3Kg. Grass, Silver, and Common carps, were of two sources (farmed and wild), while Bizz and Shabbout were wild only. Both licensed and unlicensed retail outlet exhibit commodity cooled by ice. Grass, Silver and common carps were identified according to FAO (2004) while Bizz and Shabbout were identified according to Al-Daham (1982) (figures, 2.1, 2.2, 2.3, 2.4, 2.5). Fishes were transported in cool box within 2-3 hr to the laboratory to determine the Quality index. From each fish, five flesh specimens, 100 gram for each from different parts of the fish were obtained; scales were removed with skin by a sterile scalpel, flesh were picked out from five parts, cut into smaller pieces, mixed together and put in a sterile plastic bag. Sizes needed for microbiological analyses were taken and analyzed directly, the remaining lot was stored in a deep freezer (-40ºC) for further analysis. The flowcharts of tests achieved are shown in figure (2.6).

Table 2.3: Species of fish, scientific and Kurdish nomenclature

| species | Local name | vernacular name |
|---|---|---|
| ***Ctenopharyngodon idella*** | **Grass carp** | **قاشنئ** |
| ***Hypophthalmichthys molitrix*** | **Silver carp** | **سیلفر** |
| ***Cyprinus carpio*** | **Common carp** | **کارب** |
| ***Barbus esocinus*** | **Bizz** | **لوتھ** |
| ***Barbas grypus*** | **Shabbout** | **سووره** |

Figure 2.1: Grass carp *(Ctenopharyngodon idella)*

A Grass carp: large, elongated, laterally compressed, and can grow to a length of 1.6m and weight of 37kg. The head is slightly flattened, with moderately small eyes centered on the side of the head. The body is covered with large cycloid scales. Coloration of the body varies from blackish or olive-brown, grading to brassy or silvery-white on the sides and belly. Scale pockets on the back and sides are outlined by dusky pigment, giving a crosshatched effect. Enter to Iraq in the eighties of the twentieth century (FAO, 2004).

Figure 2.2: Silver carp (*Hypophthalmichthys molitrix)*

A Silver carp: large, deep-bodied, and can grow to a lengths of 1m and weights of 27kg. It has a moderately large and broad head encompassing just less than 1/3 of its body size, a toothless upturned lower jaw, and eyes located below the axis of the body. Coloration of the body is generally silver on the sides with a slate grey head and dorsal surface, and the belly is white. Enter to Iraq in the eighties of the twentieth century (FAO, 2004).

Figure 2-3: Common carp (*Cyprinus carpio)*

A Common carp: Body elongated and somewhat compressed. Lips thick. Two pairs of barbels at angle of mouth, shorter ones on the upper lip. Dorsal fin base long with 17-22 branched rays and a strong, toothed spine in front; dorsal fin outline concave anteriorly. Anal fin with 6-7 soft rays; posterior edge of 3rd dorsal and anal fin spines with sharp spinules. Lateral line with 32 to 38 scales. Pharyngeal teeth 5:5, teeth with flattened crowns. Color variable, are brownish-green on the back and upper sides, shading to golden yellow ventrally. The fins are dusky, ventrally with a reddish tinge. Golden carp are bred for ornamental purposes. Enter the to Iraq in the fifties of the twentieth century (FAO, 2004)

Figure 2.4: Bizz (*Barbus esocinus)*

**A Bizz**: Body elongated and smooth, the head is relatively large, snout prominent jaws equal length, mouth front with the lips thick and wide and has a couple of feelers oral small scales small, eyes small, the color of the dorsal olive to gray and the abdomen are lighter in color container on a lot of points scattered black, a maximum length of about 1.5 m and weighing approximately 100 kg. fish which comes first prize in Iraq and neighboring countries, one of the largest freshwater fish in the basin of the Tigris and Euphrates (Encyclopedia of Iraqi fish, 2011).

Figure 2.5: Shabbout (*Barbas grypus*)

A **Shabbout:** body elongated and compressed from both sides, the total length equal to 6 times the length of the head, the head is small, the length of the head is greater than the depth of the body, mouth average basement contains a couple of tentacles, lips thick, eyes small, scales relatively small, body color is olive-brown in the dorsal and dorsal, ventral aspects and the implications of silver, has a maximum length of about 100 cm and weight 10 kg (Encyclopedia of Iraqi fish, 2011).

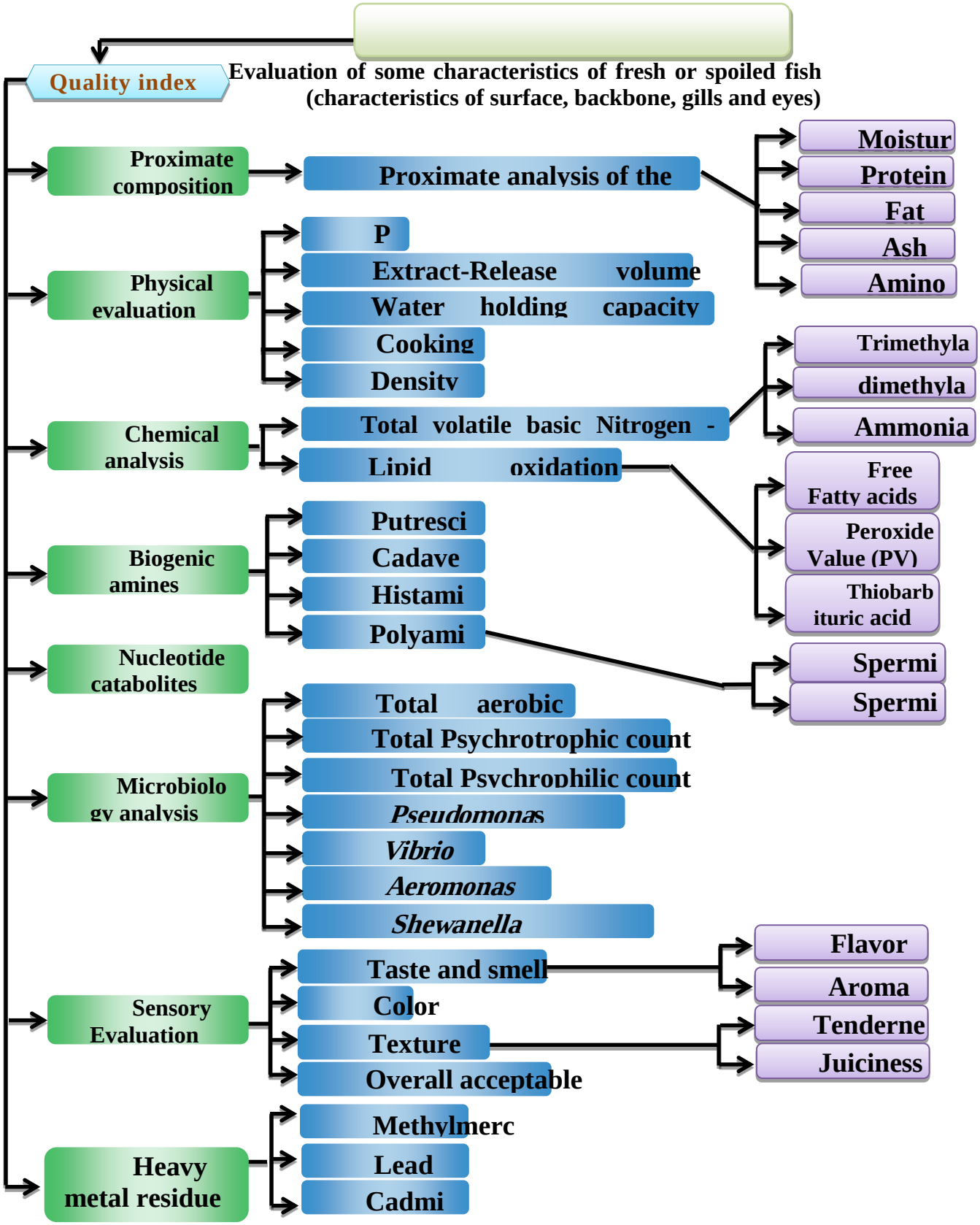


Figure 2.6: a flowchart describes all tests achieved for all samples according to standard regulations.

**2.2 Quality index method** (Botta, 1995)

Sensory analyses were performed by a panel of five experienced assessors. Raw fish were evaluated using the quality index method (QIM) shown in table 2.4. This structured category scale is based on the freshness quality grading system. The QIM involves specifying the characteristics of appropriate sensory attributes of the raw fish. Once the characteristic of a sensory attribute is determined, it is assigned a demerit score ranging from 0 to 3. The scores for all characteristics are then summed to give an overall sensory score. The scale gives zero score for absolutely fresh fish, while increasingly larger totals result as the fish deteriorates.

Table 2.4: Quality index method (Martinsdóttir *et al*., 2001).

| Quality parameter | | Description | Score |
|---|---|---|---|
| Appearance | Skin | Very shiny | 0 |
| | | Shiny | 1 |
| | | dull | 2 |
| | Blood on Gill cover | None | 0 |
| | | Very little (10-30%) | 1 |
| | | Some (30-50%) | 2 |
| | | Much (50-100%) | 3 |
| | Stiffness | Hard | 0 |
| | | Firm | 1 |
| | | Yielding | 2 |
| | | Soft | 3 |
| | Belly | Firm | 0 |
| | | Soft | 1 |
| | | burst | 2 |
| | Odor | Fresh odor | 0 |
| | | neutral | 1 |
| | | Slightly secondary odor | 2 |
| | | Strong secondary odor | 3 |
| Eyes | Clarity | Bright | 0 |
| | | Somewhat luster less | 1 |
| | Shape | Convex | 0 |
| | | Flat | 1 |
| | | Sunken | 2 |
| Gills | Color | Characteristic red | 0 |
| | | Somewhat pale, non-glossy, | 1 |
| | Odor | Fresh, seaweed, metallic | 0 |
| | | Neutral | 1 |
| | | Some secondary odor | 2 |
| | | Strong secondary odor | 3 |
| Quality index | | | 20 |

## 2.3 Proximate composition

### 2.3.1 Moisture content

Moisture content was determined as weight loss after samples were dried in the convection oven at 105°C until weight was stabilized (AOAC, 2000).

### 2.3.2 Protein content

Protein content determined according to method of AOAC (2000) by using microkejldahal and was calculated as follows:

**Protein% =nitrogen ×6.25**

### 2.3.3 Crude lipids content

The crude lipid of ground fish meat was determined according to Folch *et al*. (1957). The lipid was extracted by homogenizing 3 grams of meat with 30 ml chloroform–methanol (2:1); the lipid residue was washed twice with 15ml of chloroform–methanol and the homogenate with washing solvent was filtered through whatman no.1 filter paper. The filtrate was made up to 60ml by passing additional chloroform–methanol through the filter and then washed with 12ml of distilled water by magnetic starrier, and then centrifuged at 1000×g for 20min. the lower lipid containing layer was removed through evaporation of the upper layer solvent. Percentage of lipid was determined according to the following formula:

Lipid % = (weigh of extract / weigh of sample) × 100

### 2.3.4 Ash content

Ash content was determined according to the method of AOAC (2000) by taking a specimen of a known weight of flesh and placed in a muffle furnace at 550 ºC for 16 hr, and then the ash percent was determined as the follows:

Ash % = (W1/W2) x 100

W1 = weight of ash, and W2 = initial weight

**2.3.5 Amino acid analyses** (Schuster, 1988**).**

**2.3.5.1 Sample Preparation**

Twenty five gram of flesh were taken and homogenized with 10 to 20 ml of distilled water by a laboratory mixer to obtain a homogenate that digesed with 6N-Hcl at 110 ºC (AOAC, 2000).

The digest was filtered through a Buchner filter (0.45 µm micropore seized filter) and metallic vacuum trap. Much of the 10-20 ml of the distilled water was added and the mixture was filtered again. This procedure was repeated several times until 100ml was collected. A 10ml of aliquot of the extract and 2ml of trichloroethylene were added to a centrifuge tube, stirred and centrifuged at 3000rpm for 15min. The organic phase was discarded; the aqueous was used to chromatography analysis.

**2.3.5.2 High Performance Liquid Chromatography**

To detect the amino acids it is necessary to derivatize with dansyl chloride (DNS). The pH of the supernatant was adjusted to 7.50 to 8.0 by adding appropriate amounts of sodium bicarbonate (5%). A small aliquot sample (0.50ml) was transferred to a test tube and 10µl of DNS was added, then the mixture was shaken in the dark at 40ºC for 3hr in a shaking incubator. The excess of DNS was removed by extracting with diethylether. The remaining aqueous fraction was acidified by 50µl of 6M HCL and DNS-amino acid was extracted with diethylether until the ether no longer became colored. The ether was evaporated and the residue was taken up in 0.50ml of methanol and injected into the HPLC apparatus.

All runs were made at the ambient temperature 25ºC using a solvent flow rate of 1.2ml/ min. The ultraviolet detector was set at 254nm. The mobile phase was prepared with 25% acetonitrile and 75% glacial acetic acid (1% w/v).

The calibration curve was set by using different aliquots of standard solutions of amino acids needs to assay by subjected to pre-chromatography derivation and processed as the samples. The concentrations used were ranged

between 14.45 to 144.50mg /100g of 25g of meat samples. The samples spiked with a known amount of standard solution were processed using the same conditions mentioned above. It was necessary to use the values obtained in calibration curve, considering the peak, where the retention time agrees with standard solution retention time of 9.2 min.

Concentration = area of peak /area of standard peak × conc. of standard ×dilution.

The quantity of each of the eight amino acids (Lysine, Methionine, Tryptophan, Phenylalanine, Leucine, Isoleucine, Threonine and Valine) was obtained as concentration (% of protein).

**2.4 Physical evaluation:**

**2.4.1 Measurement of pH**

The pH of the meat specimens were measured according to Naveena & Mendiratta (2001). 10grams were homogenized with 50ml of distillated water then filtered through whatman no.1 filter paper. pH of the filtrate was measured using digital pH meter.

**2.4.2 Extract release volume:**

The extract release volume (ERV) was measured according to the Pearson (1976). 15grams of minced sample were weighted into a 100ml beaker, 60 ml extraction reagent (50ml 0.2M $KH_2PO_4$ and 3.72ml 0.2M NaOH diluted to 200ml with water, check pH is 5.8) were added, blended for 2min, the mixture was poured into a filter paper (whatman No.1) and the volume collected within 15 min was measured as ERV.

**2.4.3 Water holding capacity (WHC)** (Wardlaw *et al*., 1973).

A meat sample of 8 g and 12ml of 0.6M NaCl solution were put into a 25ml test tube. The tubes were placed into a water bath at 5°C for 15min. Then, the tubes were cooled centrifuged at 4100rpm at 5°C for 15min. The supernatant

was then poured into a volumetric cylinder. The WHC was calculated as the volume of separated fluid (ml).

$$WHC = \frac{\text{final volume – initial volume}}{\text{Sample weight}} \times 100$$

**2.4.4 Cooking loss** (Cyril *et al*., 1996)

Twenty gram of flesh specimens were taken and placed in closed aluminum boxes and cooked for 15 min in an oven pre-heated at 200ºC, after cooking, the specimens were dried with paper towel. Total cooking loss was estimated on each specimen (cooled for 30 min to 15°C) as percentage ratio between cooked and raw weight.

**2.4.5 Density**

This test was performed according to Rattry *et al*. (1973) to measure the density of fish according to following formula:

Density=Wt. of fish in water/Wt. on water-Wt. on air

**2.5 Chemical indicators**

**2.5.1 Total volatile nitrogen (T.V.N.)** analysis (Malle & Poumeyrol, 1989).

One hundred grams of fish specimens were minced by ceramic mortar and mixed for 1min with 200ml of 7.5% trichloroacetic acid (TCA) in a blender, the mixture was filtered, 25ml of the filtrate were transferred to micro-kjeldahl distillation apparatus of 250ml capacity, then 5ml of 10% NaOH solution were added to the distillation apparatus, then distillation was a carried out, the distillate was collected in 15ml of 4% boric acid. The distillate was titrated against 0.05N $H_2$ $So_4$ using methyl red– bromocresol green (1:5 v/v) as indicator, the T.V.N. value was estimated as following:

**T.V.N. (mg N/100gm)** = $\frac{V \times 14 \times (200 + M/100 \times 100)}{25 \times 100}$

* V= ml of $H_2 So_4$

* M= moisture content (section, 2.3.1)

**2.5.2 Trimethylamine (TMA)** analysis (Malle & Poumeyrol, 1989).

Using the method mentioned above (section, 2.5.1) with adding 20 ml of 35% of formaldehyde to the filtrate in addition to the NaOH 10% (formaldehyde was added to the distillation flask to block the primary and secondary amines), and then distillated, The distillate was then titrated against 0.05 N $H_2 So_4$ using methyl red –bromocresol green (1:5 v/v) as indicator, the TMA value was estimated as following:

**TMA (mg N/100gm)** = $\frac{V \times 14 \times (200 + M/100 \times 100)}{25 \times 100}$

* V=ml of $H_2 So_4$

* M=moisture content (section, 2.3.1)

**2.5.3 Dimethyl amine and ammonia analysis** (Malle & Poumeyrol, 1989)

Thirty ml of 8% trichloroacetic acid were added to 20gm of fish meat and deionized water was added to make 100ml solution, this was then blended for 5 min, Homogenized, Centrifuged at 6000rpm for 10min, TCA was removed with 50ml ether, the supernatant was filtered within 0.45 µm micropore filter, 20µl of the filtered was injected on HPLC system under the optimum separation condition. The separation was set out as follows:

High performance liquid chromatography (system model 2010 HPLC System; shimadzu, Kyoto, Japan). Column: shim-pack ISC-o7/s1504 Na (100×4.6mm H.D), the mobile phase: A: 0.6N sodium citrate, 0.1N boric acid

pH 10, 5% ethanol. Flow rate of 0.4 ml/min. Column temperature: 65ºC. Detection: the florescent detector was set at 360nm emission 465 nm.

All chemicals, solvents, and biogenic amines standards were of HPLC grade under the above condition (Shimadzu, Kyoto, Japan).

Concentration of sample was calculated as the following:

**Conc.of compound in sample (mg N/100gm meat)** = area of peak /area of standard peak × conc. of standard × dilution.

**2.5.4 Thiobarbituric acid (TBA) value analysis (**Tarladgis *et al*., 1960 as adopted by Witte *et al*., 1970)

Twenty grams of fish meat were full speed blended for 1-5 min in a blender with 50ml of the extraction solution containing 20% trichloroacetic acid in 2M phosphoric acid, the resulting slurry was transferred quantitatively to the 100ml volumetric flask with 40ml distilled water , the sample was diluted to 100ml with distilled water and homogenized by shaking by hand ,a 50ml portion was filtered through whatman No.1 filter paper, 5ml of filtrate was transferred to a test tube followed by adding 5ml thiobarbituric acid (0.005M in distilled water), the tube was then placed in a boiling water bath for 1 hour. The resulting color was measured using spectrophotometer at absorption spectrum 530 nm.

TBA values as mg malonaldehyde/ kg were calculated by multiplying absorbance value of sample by 5.2.

**2.5.5 Free fatty acids (FFA) and peroxide value (PV) analysis (Egan *et al*., 1981)**

Weights of 80-150 gram of sample were placed in a mechanical blender and about 250ml of chloroform was added, the mixture was blended for 2-3 min and filtered immediately through a large filter paper. This was then re-filtered through a paper containing a small amount of anhydrous sodium sulfate.

Portions of the filtrate were used for the determination of free fatty acids and peroxide value as follows:

### 2.5.5.1 Weight of fat in the solution

Ten ml of filtrate were pipetted into a weighed dish; the solvent was removed, dried at 100°C, cooled in a desiccator and weighed. This weight was used for the below calculation:

### 2.5.5.2 Free fatty acids (FFA) analysis

Twenty five ml of 95% ethanol were neutralized with drops of 0.1N NaOH after adding phenolphthalein, this solution was then added to 25ml of the filtrate above and the mixture was tittered against 0.1N NaOH until the pink color persisted for 15 seconds. The FFA as percent oleic acid in the sample was calculated by the following formula:

$$\text{Free fatty acids (FFA.)\%} = \frac{\text{ml of 0.1 NaOH} \times 0.0282 \times \text{dilution factor}}{\text{Weight of sample}} \times 100$$

### 2.5.5.3 Peroxide value (PV) analysis

Twenty five ml of the filtrate above were pipetted into a volumetric flask. 37ml of glacial acetic acid and 1ml of freshly prepared saturated potassium iodide solution were added. The solution was allowed to stand with swirling for exactly 1min, and then 50ml of distillated water was added and titrated against 0.001 N sodium thiosulphate using starch as indicator. The peroxide value (meq oxygen/kg fat) was calculated as follows:

$$\textbf{Peroxide value (P.V.)} = \frac{\text{ml of 0.001 N sodium thiosulphate} \times N \times 1000}{\text{Weight of sample}}$$

*N = normality of sodium thiosulphate.

* (mequiv =milli equivalent of peroxide oxygen /kg fat).

**2.6 Biogenic amines analysis (**Gingerich *et al*., 1999).

Thirty ml of 8% trichloroacetic acid were added to 20gm of fish meat and deionized water was added to make a 100ml solution, Centrifuged at 6000 rpm for 10 min, TCA was removed with 50ml ether, supernatant was filtered within 0.45µm micropore filter, Pre–column orthopthaldehyde(OPA) was derivitized by reaction of 20µl samples with 20µl OPA for 60 sec, 20µl were injected on HPLC system under the optimum separation condition. The separation was set out as follows:

High performance liquid chromatography (system model 2010 HPLC System; shimadzu, Kyoto, Japan), Column: shim-pack ISC-o7/s1504 Na (100×4.6mm H.D), the mobile phase: A: 0.6N sodium citrate, 0.1N boric acid pH 10,B: acetonitrile under gradient, flow rate of 0.8 ml/min, Column temperature: 65ºC, detection: The florescent detector was set at 340nm emission 445nm. All chemicals, solvents, and biogenic amines standards were of HPLC grade under the above condition (Shimadzu, Kyoto, Japan).

Concentration of sample was calculated as follows:

**Conc.of compound in sample** = area of peak/ area of standard peak × conc. of standard × dilution.

Each of the five compounds per fish sample in ppm was submitted to the formula (Bunčič, 1993):

$$\text{Index} = \frac{\text{ppm histamine + ppm putrescine + ppm cadaverine}}{1 + \text{ppm spermine + ppm spermidine}}$$

Nominal cut-off values were established for the index scores to establish Class 1, Class 2 or Class 3 fish. The decomposition index followed an exponential value. The values were Class 1, 0–1; Class 2, 1–10; and Class 3, >10. These nominal cut-off values were found to be effective in discriminating between the classes of fish, and appeared not to be dependent on species of fish or packing media.

**2.7 Hypoxanthine (Hx) analysis (**Ryder, 1985)

Fish extracts used for the analysis were prepared by homogenization of 5g of flesh with 25ml of chilled 0.6M perchloric acid in a laboratory homogenizer at 0°C for 1min. The homogenate was centrifuged at 6000 rpm for 10 min; the supernatant was then decanted and immediately neutralized to pH 6.5–6.8 with 1M potassium hydroxide solution. After standing at 2 °C for 30 min, the precipitated potassium perchlorate was removed by filtration through Whatman No.1 filter paper. The filtrate was diluted to 20 ml prior to storage at −80°C until analyzed. Twenty microliter aliquots of the sample extracts were injected in duplicate into the HPLC. The separation was set out as follows:

High performance liquid chromatography (system model 2010 HPLC System, shimadzu, Kyoto, Japan).The florescent detector was set at 360 nm emission 465 nm. Column: shim-pack ISC-o7/s1504 Na (100×4.6mm H.D). Mobile phase: 0.6N sodium citrate, 0.1N boric acid pH 10:5% ethanol. Flow rate of 0.8 ml/min. Column temperature: 65ºC. The peaks obtained from fish muscle extracts were identified by comparing against the standard solutions.

All chemicals, solvents, and nucleotide standards were of HPLC grade under the above condition (Shimadzu, Kyoto, Japan).

Concentration of sample was calculated as follows:

**Conc.of compound in sample** = area of peak /area of standard peak ×conc. of standard × dilution.

**2.8 Microbiological tests:-**

**2.8.1 Sample preparation**

To 25gram flesh, 225ml of Butterfield's phosphate diluent were added and homogenized, this is the first $10^{-1}$ dilution, series of dilutions were prepared in 9ml Butterfield's phosphate diluent untill $10^{-7}$.

One ml was removed from each dilution and put in 3 petri-dishes, Plate Count Agar (Biolife, Italy) was cooled in a water bath to 45 ± 1°C and poured into the petri-dishes, mixed gently by swirling or tilting each plate. Incubated for periods and temperatures according to the bacteria detected.

**2.8.2 Total plate count** (USDA/FSIS, 1998)

Plates were Incubated at a 35 ± 1°C for 48 h. colonies on the duplicate plates from dilutions gave 30-300 colonies per plate were counted using a colony counter. Colony forming units (cfu)/ml were obtained by multiplying the colony number with the dilution.

**2.8.3 Psychrophilic count** (Swanson *et al*., 1992).

Plates were Incubated at a 5± 1°C for 48 h. colonies on the duplicate plates from dilutions gave 30-300 colonies per plate were counted using a colony counter. Colony forming units (cfu)/ml were obtained by multiplying the colony number with the dilution.

**2.8.4 Psychrotrophic count (**Swanson *et al*., 1992**).**

Plates were Incubated at a 15 ± 1°C for 48 h. colonies on the duplicate plates from dilutions gave 30-300 colonies per plate were counted using a colony counter. Colony forming units (cfu)/ml were obtained by multiplying the colony number with the dilution.

**2.8.5 *Pseudomonas* spp**. (APHA, 1984)

Plates (ceterimide agar) were Incubated in a 20 ± 1°C for 48 h. colonies on the duplicate plates from dilutions gave 30-300 colonies per plate were counted using a colony counter. Colony forming units (cfu)/ml were obtained by multiplying the colony number with the dilution. Those Colonies on citermide agar have (typically producing a small, rough colony) further analyzed biochemically then fully identified by VITEK® 2 SYSTEM.

**2.8.6 *Vibrio* spp.** (Elliott *et al*., 1995)

*Vibrio* population was enumerated by adopting spread plate method. 50gm of flesh specimens were added to 450ml of alkaline peptone water, Serial dilutions in alkaline peptone water were done, duplicate of 0.1ml of each dilution spread on duplicate of petriplates containing the selective medium thiosulphate Bile Salt Sucrose agar (TCBS) and incubated at 37 ºC for 24h. *Vibrio cholera* appears on TCBS agar as small or medium sized, yellow or greenish yellow, 2-3mm in diameter colonies. *Vibrio parahaemolyticus* appears as punctuate, <2 mm in diameter, green or bluish green colonies. Colonies were selected depending cultural morphology and purified again on TCBS agar. A number of colonies for each suspicious species were transferred to nutrient agar slants for biochemical testing and identification by VITEK® 2 SYSTEM.

**2.8.7 *Aeromonas* spp.** (USDA/FSIS, 1998).

Twenty five grams of flesh were blended in 225 ml of Tryptic soy broth enriched with 10 µg/ml ampicillin (TSBA) with a blender for 2 minutes, and then incubated at 28$^{o}$C for 24 h. After incubation of enrichment cultures, serial dilutions were prepared in Butterfield's phosphate diluent (BPD). Aliquots of 0.1 ml from the dilutions $10^{-4}$ to $10^{-6}$ were transferred in duplicates onto the surface of starch ampicillin agar (SA) plates and spread on. The plates were incubated at 28$^{o}$C for 18 to 24h. Three typical colonies per samples were picked out from the SA agar plates and transferred to TSI agar and nutrient agar slants there were incubated an overnight at 28$^{o}$C. *Aeromonas* colonies are typically 3 to 5 mm in diameter and appear yellow to honey-colored on SA agar. A number of colonies for each suspicious species were transferred to nutrient agar slants for biochemical testing and identification by VITEK® 2 SYSTEM.

**2.8.8 *Shewanella putrefaciens***

Twenty five grams of flesh were blended in 225 ml of Tryptic soy broth in

a blender for 2 minutes, and then incubated at 28$^{o}$C for 24 h, after incubation of cultures; serial dilutions were prepared in BPD. Aliquots of 0.1 ml from the dilutions $10^{-4}$ to $10^{-6}$ were transferred in duplicates onto the surface of iron agar plates and spread on. The plates were incubated at 28$^{o}$C for 2 days. A number of Black colonies for each suspicious species were transferred onto nutrient agar slants for biochemical testing and identification by VITEK® 2 SYSTEM.

### 2.8.9 Gram stains procedure

Smears of bacteria on microscopic slides were heat-fixed, flooded with crystal violet for 1 minute, all cells turned purple. Iodine solution added for 3 minutes, all cells remain purple. Smears were decolorized with 95% ethanol for 20 seconds, Gram-positive was purple and Gram- negative was colorless, smears were then counter stained by flooding with safranin for 1 to 2 minutes. Gram-positive returned purple and gram negative remained red when seen microscopically under 1000x magnification.

### 2.8.10 Biochemical test:

#### 2.8.10.1 Oxidase test

A suspected colony from nutrient agar slants was scraped with a sterile wooden applicator stick and was rubbed across a wet filter paper with 1% oxidase reagent. A reaction was considered positive when the color of growth turned to dark purple within 10 sec.

#### 2.8.10.2 Triple Sugar Iron agar (TSI)

A colony was picked out from nutrient agar slants with a sterile needle and streaked onto the TSI slant and then stabbed butt of agar, incubated for 24 h at 35°C. The *Vibrio* spp. Produced a purple (alkaline) or acid slant and yellow (acidic) bottom with no gas or hydrogen sulfide production.

#### 2.8.10.3 Kliglar Iron agar (KIA)

A colony was picked out from nutrient agar slants with a sterile needle and streaked onto the KIA slant then stabbed butt of agar, incubated for 24 h at 35°C. The *Vibrio* spp. Produced a purple (alkaline) or acid slant and yellow (acidic) bottom with no gas or hydrogen sulfide production.

### 2.8.11 MICROBIAL IDENTIFICATION USING THE BIOMÉRIEUX VITEK® 2 SYSTEM:

Identification at the species level was confirmed by using BIOMÉRIEUX VITEK® 2 SYSTEM performed in the Bacteriological lab, Central lab, Ministry of Health, Erbil, Kurdistan Regional Government / Iraq.

#### 2.8.11.1 Suspension Preparation

A sterile swab or applicator stick was used to transfer a sufficient number of colonies of a pure culture and to suspend the microorganism in 3.0 ml of sterile saline (aqueous 0.45% to 0.50% NaCl, pH 4.5 to 7.0) in a 12 x 75 mm clear plastic (polystyrene) test tube. The turbidity was adjusted accordingly and measured using a turbidity meter called the DensiChekTM.

#### 2.8.11.2 Inoculation

Identification cards were inoculated with microorganism suspensions using an integrated vacuum apparatus. A test tube containing the microorganism suspension was placed into a special rack (cassette) and the identification card was placed in the neighboring slot while inserting the transfer tube into the corresponding suspension tube. The cassette can accommodate up to 10 tests (VITEK 2 Compact). The filled cassette was placed manually (VITEK 2 compact) into a vacuum chamber station. After the vacuum was applied and air was re-introduced into the station, the organism suspension was forced through the transfer tube into micro-channels that fill all the test wells.

#### 2.8.11.3 Card Sealing and Incubation

Inoculated cards were passed by a mechanism, which cuts off the transfer tube and seals the card prior to loading into the carousel incubator. All card types were incubated on-line at 35.5 + 1.0ºC. Data were collected at 15-minute intervals during the entire incubation period.

**2.8.11.4 Test Reactions**

Calculations were performed on raw data and compared to thresholds to determine reactions for each test. On the VITEK 2 Compact, test reaction results appeared as "+", "–", "(–)" or "(+)". Reactions that appeared in parentheses are indicative of weak reactions that are too close to the test threshold.

**2.8.11.5 Database Development**

The databases of the VITEK 2 identification products were constructed with large strain sets of well-characterized microorganisms tested under various culture conditions. These strains are derived from a variety of clinical and industrial sources as well as from public (e.g., ATCC) and university culture collections. There are two identification cards, each with substrates for either gram negative (GN) (table, 2.5) or gram positive (GP) bacteria

**2.8.11.6 Analytical Techniques**

Test data from an unknown organism are compared to the respective database to determine a quantitative value for proximity to each of the database taxa. Each of the composite values is compared with the others to determine if the data is sufficiently unique or close to one or more of the other database taxa. If a unique identification pattern is not recognized, a list of possible organisms is given, or the strain is determined to be outside the scope of the database.

Figure 2.7: VITEK® 2 SYSTEM

Table 2.5: Test Substrates on GN Card**.**

| Well | Test | Mnemonic | Amount/Well |
|---|---|---|---|
| 2 | Ala-Phe-Pro-ARYLAMIDASE | APPA | 0.0384 mg |
| 3 | ADONITOL | ADO | 0.1875 mg |
| 4 | L-Pyrrolydonyl-ARYLAMIDASE | PyrA | 0.018 mg |
| 5 | L-ARABITOL | IARL | 0.3 mg |
| 7 | D-CELLOBIOSE | dCEL | 0.3 mg |
| 9 | BETA-GALACTOSIDASE | BGAL | 0.036 mg |
| 10 | H2S PRODUCTION | H2S | 0.0024 mg |
| 11 | BETA-N-ACETYL-GLUCOSAMINIDASE | BNAG | 0.0408 mg |
| 1 2 | Glutamyl Arylamidase pNA | AGLTp | 0.0324 mg |
| 1 3 | D-GLUCOSE | dGLU | 0.3 mg |
| 14 | GAMMA-GLUTAMYL-TRANSFERASE | GGT | 0.0228 mg |
| 15 | FERMENTATION/ GLUCOSE | OFF | 0.45 mg |
| 1 7 | BETA-GLUCOSIDASE | BGLU | 0.036 mg |
| 18 | D-MALTOSE | dMAL | 0.3 mg |
| 19 | D-MANNITOL | dMAN | 0.1875 mg |
| 20 | D-MANNOSE | dMNE | 0.3 mg |
| 21 | BETA-XYLOSIDASE | BXYL | 0.0324 mg |
| 22 | BETA-Alanine arylamidase pNA | BAlap | 0.01 74 mg |
| 23 | L-Proline ARYLAMIDASE | ProA | 0.0234 mg |
| 26 | LIPASE | LIP | 0.0192 mg |
| 27 | PALATINOSE | PLE | 0.3 mg |
| 29 | Tyrosine ARYLAM1DASE | TyrA | 0.0276 mg |
| 31 | UREASE | URE | 0.15 mg |
| 32 | D-SORBITOL | dSOR | 0.1875 mg |
| 33 | SACCHAROSE/SUCROSE | SAC | 0.3 mg |
| 34 | D-TAGATOSE | dTAG | 0.3 mg |
| 35 | D-TREHALOSE | dTRE | 0.3 mg |
| 36 | CITRATE (SODIUM) | CIT | 0.054 mg |
| 37 | MALONATE | MNT | 0.15 mg |
| 39 | 5-KETO-D-GLUCONATE | 5 KG | 0.3 mg |
| 40 | L-LACTATE alkalinisation | ILATk | 0.15 mg |
| 41 | ALPHA-GLUCOSIDASE | AGLU | 0.036 mg |
| 42 | SUCCINATE alkalinisation | SUCT | 0.15 mg |
| 43 | Beta-N-ACETYL-GALACTOSAM 1 Nl DASE | NAGA | 0.0306 mg |
| 44 | ALPHA-GALACTOSI DASE | AGAL | 0.036 mg |
| 45 | PHOSPHATASE | PHOS | 0.0504 mg |
| 46 | Glycine ARYLAMIDASE | GlyA | 0.01 2 mg |
| 47 | ORNITHINE DECARBOXYLASE | ODC | 0.3 mg |
| 48 | LYSINE DECARBOXYLASE | LDC | 0.15 mg |
| 52 | DECARBOXYLASE BASE | ODEC | N/A |
| 53 | L-HISTIDINE assimilation | IHISa | 0.087 mg |
| 56 | COUMARATE | CMT | 0.1 26 mg |
| 57 | BETA-GLUCORONIDASE | BGUR | 0.0378 mg |
| 58 | 0/1 29 RESISTANCE (comp.vibrio.) | 01 29R | 0.0105 mg |
| 59 | Glu-Gly-Arg-ARYLAM 1 DASE | GGAA | 0.0576 mg |
| 61 | L-MALATE assimilation | IMLTa | 0.042 mg |
| 62 | ELLMAN | ELLM | 0.03 mg |
| 64 | L-LACTATE assimilation | ILATa | 0.186 mg |

**2.9 Organoleptic evaluation** (Castellini *et al*., 2002)

The fish fillets specimens were placed in open aluminum boxes and cooked for 15 min in a pre-heated oven at 200°C, after cooking nine teaching staff of the Department Animal Production- Agricultural Science faculty/University of Sulaimani were randomly determined as a sensory evaluation panel. Each member of the panel has filled a sensory evaluation chart, as shown in table (2.6):

Table 2.6: Sensory evaluation form

| Type of fish | Tenderness | Juiciness | Color | Flavor and aroma | Overall acceptability |
|---|---|---|---|---|---|
| Grass carp | | | | | |
| Silver carp | | | | | |
| Common carp | | | | | |
| Bizz | | | | | |
| Shabbout | | | | | |
| | 5 =extremely like<br>4 = like<br>3 = neither like nor dislike<br>2 = dislike<br>1 = extremely dislike | | | | |

## 2.10 Heavy metals analysis

**2.10.1 Mercury** (Ministry of Health and Welfare, 1991).

Fish specimens were homogenized by electrical blender, 2grams of fish (wet weight) were weighed and placed into 40ml Pyrex conical centrifuge tubes fitted with poly– seal screw cap. The sample was shaken vigorously by hand with 25ml aliquots acetone. The mixture was centrifuged for 3 minutes at 1500rpm, and the acetone was removed by aspiration. A 5ml of acid bromide and 10ml ($HNO_{3+}$ 4N $H_2So_4$) solution was then added to the residue, and the mixture was shaken gently by hand. Next 10ml of toluene was added and the tube shaken by hand for 1min, and then centrifuged for 10minutes at 1500 rpm .the solution was prepared for analysis in an atomic absorption spectrophotometer by preparing 0.5ml of toluene extract and 0.5ml of 10% dithizone into glass stoppered tube on vortex mixture.

A standard was prepared in the same manner using 0.05-0.4ppm methyl mercury in the dithizone– toluene solution; a 20ml of the solution was injected into the furnace for each determination.

The peak height was measured and used to calculate the Hg concentration by the method of standard addition.

Methyl mercury chloride (95%) was used to prepare 100mg/ml as (Hg) stock solution in toluene.

All chemicals reagents used were of analytical grade.

**2.10.2 Cadmium and lead** (AOAC, 2000)

Chemical elements were estimated in the samples using Atomic Absorption after incineration of samples at 600 ºC by muffle furnace.

Certain weight of ash was taken and put in a 100ml beaker, nitric acid (15-20ml) was added to form a 1:1(V/W) suspension, covered by a watch glass, the mixture heated on a hotplate until ash was solubilized, and then left to cool at room temperature, this was then transferred quantitatively to a volumetric

flask (100ml volume) and completed with distilled water, and thus became a model ripe for estimating the elements by an atomic absorption spectrometer.

Standard solutions: cadmium and lead intermediate standards were diluted with 1% nitric acid in a volumetric flask, stored in plastic bottles. Typical standard solutions for lead analysis were 3, 5, 10 and 20 µg/L. Typical standard solutions for cadmium analysis were 0.3, 0.5, 1.0, 2.0 µg/L.

Standard blank: 1% nitric acid. The separation condition were set out as follows:

| **Lead** | **Cadmium** |
|---|---|
| Injection temperature: 100 °C | Injection temperature: 100 °C |
| Wavelength: 283.3 nm | Wavelength: 228.8 nm |
| Slit width: 0.7 nm | Slit width: 0.7 nm |
| Sample Volume: 20 µL | Sample Volume: 20 µL |

The blanks and calibration standard solutions were also analyzed as the sample solutions and calibration curves constructed

## 2.11 Statistical analysis

All data were subject to one-way analysis of variance (ANOVA) using SPSS 18.0 and XL Stat program for Windows. Differences Between the means were tested by Duncan's multiple range tests. The level of significance was chosen at $P<0.05$ and the results are presented as mean (Steel *et al.*, 1996).

# Chapter 3

# Results and Discussion

## 3.1 Quality Index evaluation

As shown in table 3.1, there were significant differences ($P< 0.05$) among the sum of scores for the five types. Both Bizz and Grass carp scored low sums of 6.67 and 7.00 respectively, which reflects good quality freshness, whilst the other three types (Shabbout, Common carp and Silver carp ) scored higher sums (8.17, 9.17 and 10.67 respectively). There were insignificant differences both between Bizz and Grass carp and between Shabbout and Common carp ($p< 0.05$). There were no standard score limits for the fish types under the present study with respect to Quality Index Methods (QIM) in standard quality regulation. Thus, the obtained data may be adopted as standard quality index for the examined fish.

Sveinsdo´ttir *et al*. (2002) have mentioned that high scores, near 20, indicate fish deterioration and storage for long times, whereas low scores, near zero, indicate more freshness. It was suggested that, when the sum of scores for the batch of fish reach demerit points of 10, the remaining storage time in ice maybe estimated to be about five days (Huss, 1995; Kyrana, 2001).The application of QIM is an excellent indication in the first part of the storage period for fish stored in ice, as during first stage storage other instrumental method results are inaccurate or unreliable (Nielsen *et al.*, 1992; Mhongole, 2009). This could be similar to the conditions at which fish were sampled stored during the present investigation.

Table 3.1: Quality Index scores for five types of local fresh fish.

| Type of fish | score | | | | | | | | | Sum score* |
|---|---|---|---|---|---|---|---|---|---|---|
| | General appearance | | | | | Eye | | Gills | | |
| | skin | Blood spot | stiffness | belly | odor | clarity | shape | color | odor | |
| Grass carp | 0.833 b | 0.833 b | 1.250 b | 1.00 a | 0.917 ab | 0.00 a | 0.750 b | 0.250 c | 1.167 c | 7.00 c |
| Silver carp | 1.333 a | 1.667 a | 2.250 a | 1.00 a | 1.00 a | 0.00 a | 1.167 a | 0.333 bc | 1.917 a | 10.67 a |
| Common carp | 1.00 ab | 1.333 ab | 1.583 b | 0.917 ab | 1.00 a | 0.167 a | 0.917 ab | 0.667 ab | 1.583 ab | 9.17 b |
| Bizz | 0.750 b | 0.833 b | 1.583 b | 0.583 b | 0.750 b | 0.083 a | 0.167 c | 0.833 a | 1.083 c | 6.67 c |
| Shabbout | 1.00 ab | 1.417 ab | 1.333 b | 0.750 ab | 1.00 a | 0.083 a | 0.833 ab | 0.500 abc | 1.250 bc | 8.17 b |

Mean having different letters in the same column are significantly different.
The differences at $p<0.05$.
The means average of 12 replicates.
*sum scores: summation of total scores.

### 3.1.1 Quality Index evaluation for farmed and wild fish

The quality index evaluations of the three farmable types of fish (Grass, Silver and Common carps); for farmed and their wild counterpart are shown in table 3.2, here, the total sum scores showed insignificant differences between the farmed breed and wild for each type where they scored the sums of 7.00; 7.00, 10.83; 10.50 and 9.50; 8.83 for Grass, Silver and Common carps respectively. All score were within acceptable scores according to Sveinsdo´ttir *et al*. (2002 ) even if they scored near the mid value between zero and 20 values of Sveinsdo´ttir *et al*. (2002) which intern reveals the residual storage life on ice

can be estimated to be around five days as suggested by Huss(1995) and Kyrana ( 2001).

Table 3.2: Quality Index scores for wild and farmed sources of three types of local fresh fish.

| Type of fish | Breed | Score | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | General appearance | | | | | Eye | | Gills | | Sum score* |
| | | skin | Blood spot | stiffness | belly | odor | clarity | shape | color | odor | |
| Grass carp | farmed | **0.667 a** | **1.667 a** | **1.0 a** | **1.0 a** | **0.833 a** | **0.0 a** | **0.833 a** | **0.333 a** | **1.167 a** | **7.00 a** |
| | wild | **1.0 a** | **0.5 b** | **1.5 a** | **1.0 a** | **1.0 a** | **0.0 a** | **0.667 a** | **0.167 a** | **1.167 a** | **7.00 a** |
| Silver carp | farmed | **1.5 a** | **1.667 a** | **2.333 a** | **1.0 a** | **1.0 a** | **0.0 a** | **1.167 a** | **0.167 a** | **2.00 a** | **10.83 a** |
| | wild | **1.167 a** | **1.667 a** | **2.167 a** | **1.0 a** | **1.0 a** | **0.0 a** | **1.167 a** | **0.5 a** | **1.833 a** | **10.50 a** |
| Common carp | farmed | **1.0 a** | **0.5 b** | **1.333 a** | **1.167 a** | **1.0 a** | **0.167 a** | **1.50 a** | **0.833 a** | **2.00 a** | **9.50 a** |
| | wild | **1.0 a** | **2.167 a** | **1.833 a** | **0.667 a** | **1.00 a** | **0.167 a** | **0.333 b** | **0.500 a** | **1.167 b** | **8.83 a** |

Means having different letters in the same column are significantly different within same types
The differences at $p<0.05$.
The means average of 6 replicates.
*sum scores: summation of total scores.

### 3.1.2 Quality Index evaluation in licensed and unlicensed markets

The quality index was used to compared the five fish types in the licensed markets (M1) and unlicensed markets (M2), as indicated in table, 3.3, itisappeared to be insignificant differences between the two markets for all types

where they scored sum of 6.66; 7.33, 6.17; 7.16, 8.00; 8.33, 9.00; 9.33 and 10.5; 10.83 for Grass carp, Bizz, Shabbout, Common carp and Silver carp respectively. Although there were no previous studies to compare between licensed and unlicensed markets in this respect, it is clearly appeared that the style of fishing and handling procedures including the time elapsed is the same for both M1 and M2 marketing. This may due to the sale mode of fresh fish in Iraq, including Kurdistan Region, that followed same manner, as a habit, depended on the consumer preferences, so long as no alternative modern style of fresh fish sale is applied.

Table 3.3: Quality Index scores for five types of local fresh fish from licensed (M1) and unlicensed (M2) markets.

| Type of fish | Market | Score | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | General appearance | | | | | Eye | | Gills | | Sum score * |
| | | skin | Blood spot | stiffness | belly | odor | clarity | shape | color | odor | |
| **Grass carp** | M1 | 0.667 a | 1.00 a | 1.167 a | 1.167 a | 0.833 a | 0.0 a | 0.667 a | 0.167 a | 1.00 a | 6.66 a |
| | M2 | 1.00 a | 0.667 a | 1.333 a | 0.833 a | 1.00 a | 0.0 a | 0.833 a | 0.333 a | 1.333 a | 7.33 a |
| **Silver carp** | M1 | 1.00 b | 1.50 a | 2.167 a | 1.00 a | 1.00 a | 0.0 a | 1.333 a | 0.500 a | 2.000 a | 10.50 a |
| | M2 | 1.667 a | 1.833 a | 2.333 a | 1.00 a | 1.00 a | 0.0 a | 1.00 a | 0.167 a | 1.833 a | 10.83 a |
| **Common carp** | M1 | 0.667 b | 1.500 a | 2.00 a | 0.833 a | 1.00 a | 0.167 a | 0.667 a | 0.833 a | 1.333 a | 9.00 a |
| | M2 | 1.333 a | 1.167 a | 1.167 a | 1.00 a | 1.00 a | 0.167 a | 1.167 a | 0.500 a | 1.833 a | 9.33 a |
| **Bizz** | M1 | 0.50 b | 0.667 a | 1.500 a | 0.667 a | 0.500 b | 0.167 a | 0.167 a | 1.0 a | 1.0 a | 6.17 a |
| | M2 | 1.0 a | 1.0 a | 1.667 a | 0.500 a | 1.00 a | 0.0 a | 0.167 a | 0.667 a | 1.167 a | 7.16 a |
| **Shabbout** | M1 | 1.00 a | 1.50 a | 1.33 a | 0.833 a | 1.0 a | 0.0 a | 0.833 a | 0.167 b | 1.167 a | 8.00 a |
| | M2 | 1.0 a | 1.33 a | 1.33 a | 0.667 a | 1.00 a | 0.0 a | 0.833 a | 0.833 a | 1.33 a | 8.33 a |

Means having different letters in the same column are significantly different within same types
The differences at $p<0.05$.
The means average of 6 replicates.
*sum scores: summation of total scores.

### 3.2 Proximate composition

As shown in table 3.4, there were significant differences ($p<0.05$) in the chemical composition (moisture, protein, lipid and ash) amongst the five types of fish examined. Grass carp, Bizz and Shabbout contained high percentage of **moisture** (74.69, 74.56 and 74.15 % respectively) while Silver and Common carps contained low percentages (69.19 and 71.20% respectively). Silver and Common carps contained high percentage of lipid (11.73 and 9.28% respectively) while Bizz, Grass carp and Shabbout contained low percentages (2.58, 2.88 and 3.80% respectively). It was appeared that there was an inverse relationship between lipid and moisture contents in the five species of fish as inversely correlated (appendix 2). This result is in agreement previous investigation that stated an inverse relationship between lipid and content of fish (Hassan, 2001). The results of Bizz and Shabbout were lower that of Salh (2009) who found that the moisture contents were 79.66 and 79.93% and lipid contents were 1.48 and 1.47% in the Bizz and Shabbout flesh respectively, Other studies reported for Rainbow trout and Tilapia muscles (Tozer, 2001) which found the moisture contents were 72.2 and 76.7% and lipid contents were 5.4, 3.2% respectively. Ali *et al*. (1986) found moisture and lipid contents in Shabbout were 74.81 and 4.93% respectively and Buyukcapar and Kamalak (2007) reported the range of lipid was between9.5-13.3% in Common carp.

High **protein** contents were shown in Bizz and Grass carp (21.69 and 20.77% respectively) while lower contents were in Silver and Common carps (16.82 and 17.43% respectively). Shabbout contained a moderate percentage (19.77%) of protein in comparison to the four types presented in table 3.4. The protein contents for all the five types occurred within the normal range values of protein in fish (15-25%) (Huss,1995). Other studies reported similar protein contents in Shabbout, Bizz, Carsean, Tawni and Gattan fish meat in Iraq /Sulaimani (Salh ,2009) and in Jufout in Basrah (Al-Habbib *et al*. ,2008; Al-Shatty, 2008); Ali *et al*. (1986) found that protein content in Shabbout was

17.29%. Buyukcapar and Kamalak (2007) recorded the range of protein was between12.1-13% in Common carp.

Data of ash contents are shown in table 3.4, the five types ranged ash contained between 0.99 and 1.95 %. Other studies have mentioned values of 1.05 - 1.29 % which are within the ranges found in the present study; in Shabbout, Bizz, Carsean, Tawni and Gattan (Salh, 2009) and in Rainbow trout and Tilapia muscles (Tozer, 2001), Ali *et al*. (1986) found that ash content in Shabbout was 1.24 % respectively. Results converged to the results of Buyukcapar and Kamalak (2007) that recorded the range of ash being 1.7-2.3% in Common carp. In any case, the proximate compositions of the fish that have been inspected in this study are healthy.

The proximate composition is species specific (Shearer, 1994), so it is logic to see differences amongst all examined fish. However all values were within the normal ranges of proximate composition (water 66-84%, protein 15-25 %, fat 0.1 -24 %) (Huss, 1995). Variation within the individuals in proximate compositions may occur; this may be related to feed intake, migratory swimming or sexual changes in connection with spawning (Oduor-Odote and Kazungu, 2008).

With respect to their importance to human satisfaction and health, Protein, lipid and water of fish meat are crucial to consumers, therefore scientists and manufacturers have considered many aspects including nutritional value and considerations regarding processing to satisfaction the consumers (Murray and Burt, 2001). Furthermore, some nutritional components of fish meat have functional effects on human health; lipid of fish is one of the most important natural sources of polyunsaturated fatty acids which has proven to have effects on human health (Saoud *et al*., 2008).

Table 3.4: Proximate analysis of five types of local fresh fish.

| Type of fish | Chemical composition (%) | | | |
|---|---|---|---|---|
| | Moisture | Protein N×6.25 | Lipid | Ash |
| Grass carp | 74.69±0.04 a | 20.77± 0.02 b | 2.88 ± 0.03 d | 1.20 ± 0.01 c |
| Silver carp | 69.19±0.11 d | 16.82 ±0.01 e | 11.73± 0.04 a | 1.95 ± 0.06 a |
| Common carp | 71.20 ±0.10 c | 17.43± 0.08 d | 9.28 ± 0.06 b | 1.61 ± 0.03 b |
| Bizz | 74.56±0.07 a | 21.69±0.04 a | 2.58±0.03 e | 0.99 ± 0.01 d |
| Shabbout | 74.15±0.01 b | 19.77±0.03 c | 3.80± 0.03 c | 1.29 ± 0.02 c |

Mean having different letters in the same column are significantly different.
The differences at $p<0.05$.
The means average of 12 replicates.

### 3.2.1 Proximate composition for farmed and wild fish

The **proximate composition** of the three farmable types (Grass, Silver and Common carps) for farmed and their wild counterpart are shown in table 3.5. There were significant differences ($P<0.05$) in proximate composition (moisture, protein and lipid) in Grass carp (wild and farmed sources), Wild Grass carp contained high percentage of moisture and protein (74.81 and 20.85% respectively) and low percentage of lipid and ash (2.79% and 1.19% respectively) while the moisture, protein, lipid and ash of the farmed Grass carp were 74.57, 20.42 and 2.98% and 1.21% respectively. There were insignificant differences have been shown between wild and farmed Silver carp for moisture ,protein and ash, while significant differences was demonstrated for lipid percentage between wild and farmed Silver carp (11.60 and 11.87% respectively).

The results thereby corroborate the finding of others when comparing between wild and farmed fish (Grigorakis *et al*., 2003; Orban *et al*., 2003).

Table 3.5 shows significant differences in the content of moisture and protein between wild and farmed Common carp, values were 71.49 and 17.64 % respectively in wild, while being 70.91 and 17.22 % respectively in farmed Common carp. Insignificant differences in lipid and ash content between farmed and wild Common carp ($P \geq 0.05$); Blanchet *et al.* (2005) found no difference in fat content in farmed and wild Atlantic salmon.

The results showed that moisture and protein content are higher in wild grass carp in comparison to farmed while, in contrast, lipid content is greater in farmed. The lipid content appeared to be higher in farmed Silver carp than in wild type. Other studies reached the same results, and revealed that the high lipid content of farmed fish flesh is related to the dietary lipid level; they opined that the feed offered to farmed specimen had direct impact on its body fat increments (Boujard *et al*., 2004; Gonzáles *et al*., 2006; Jankowska *et al*., 2007; Bendiksen *et al*., 2011). Oduor-Odote and Kazungu (2008) found that lipids in fish vary greatly and this variation is related to feed intake, migratory swimming or sexual changes in connection with spawning, lipids vary in different parts of fish body and also they show enormous variation in different seasons of the year (Ashraf *et al*., 2011).

Flesh protein content is less influenced by external feeding since it is mainly dependent on intrinsic factors such as the fish species, variety and size (Børresen, 1992; Shearer, 1994). However the differences in protein content between wild and farmed Grass and Common carps may belong to the tendency of these farmable types to stores lipids as fuel for energy which theoretically high in farmed because of the regular feeding and small space for movement. As amino acids cannot be stored in major amounts their concentration in blood and tissue rises while relatively little is known about the extent to which amino acids can serve directly as sources of energy in the various tissues (Krebs, 1972), so that speaking on differences between protein contents showed to be not related

to storage fuels such as that of lipids while the elevated protein in wild grass and Common carps may relate to the intrinsic factors or environment.

Table 3.5: Proximate analysis for wild and farmed sources of three types of local fresh fish.

| Type of fish | Breed | Chemical composition (%) | | | |
|---|---|---|---|---|---|
| | | Moisture | Protein N×6.25 | Lipid | Ash |
| Grass carp | farmed | 74.57 b | 20.42 a | 2.98 a | 1.21 a |
| | wild | 74.81 a | 20.85 b | 2.79 b | 1.19 a |
| Silver carp | farmed | 69.18 a | 16.79 a | 11.87 a | 2.08 a |
| | wild | 69.21 a | 16.85 a | 11.60 b | 1.83 a |
| Common carp | farmed | 70.91 b | 17.22 b | 9.30 a | 1.65 a |
| | wild | 71.49 a | 17.64 a | 9.21 a | 1.57 a |

Means having different letters in the same column are significantly different within same types
The differences at $p<0.05$.
The means average of 6 replicates.

### 3.3 Amino-acids composition:

The essential amino acids composition of the protein of the five species is shown in table 3.6. Significant differences in amino acids were shown among the five types of fish. Shabbout and Silver carp fish appeared to have higher contents of both lysine (8.65 and 8.07%) and methionine (4.95and 4.95%) respectively. Grass carp, Common carp and Bizz have lower content of lysine (8.02, 7.82 and 7.65%) and methionine (4.27, 4.47 and 4.62 %) respectively.

Bizz and Grass carp flesh contained a high level of tryptophan (2.70 and 2.12 % respectively) and phenylalanine (3.95 and 3.90 % respectively) while

Silver carp, Common carp and Shabbout fish had lower content of tryptophan (1.92, 1.47 and 1.75% respectively) and phenylalanine (3.27, 2.57 and 3.45 % respectively).

Common and Silver carps contained high Leucine (9.37 and 9.17% respectively) and Isoleucine (6.47 and 5.60%) respectively, while Shabbout, Bizz and Grass carp have low contents for both of leucine (7.00, 8.00 and 8.82 % respectively) and Isoleucine (5.55, 5.55 and 5.57% respectively).

Bizz have high contents of threonine (6.00%) followed by Silver carp (5.85%) and Grass carp (5.17%); Shabbout fish contains less content of threonine (4.40%) followed by Common carp (4.45%). Bizz and Shabbout fish flesh contain of high level of valine (7.28 and 7.25% respectively) followed by Grass carp (6.95%). Common and Silver carps had less content of valine (6.60 and 6.78% respectively).

So, the studied fish appeared to have high contents of lysine, leucine and valine and low contents of tryptophan and phenylalanine, the results were similar to that reported by Alipour *et al.* (2010) who recorded lower contents of tryptophan in Persian sturgeon fillets. Huss (1995) mentioned that the content of arginine, tryptophan, tyrosine, methionine and phenylalanine are lower during the breeding season which means the critical value of fish is lower in this season, it is noted that the breeding season was within the sampling period of this study which makes comparison of those fish hunted during breeding season and after impossible.

The concentrations of phenylalanine and valine were more prominent in Grass carp .These remarkable differences in the levels of amino acids in Grass carp entailed more balanced diets due to its limited feeding scope (Ashraf *et al*., 2011), it is herbivorous. Availability of essential Amino acids in fish meat was a good indicators of higher protein feed value (Yang *et al*., 2010).

Table 3.6: Amino-acids composition for five types of local fresh fish.

| Type of fish | protein% | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Lysine | Methionine | Tryptophan | Phenylalanine | Leucine | Isoleucine | Threonine | Valine |
| Grass carp | 8.02 b | 4.27 b | 2.12 b | 3.90 a | 8.82 a | 5.57 b | 5.17 b | 6.95 abc |
| Silver carp | 8.07 b | 4.95 a | 1.92 bc | 3.27 b | 9.17 a | 5.60 b | 5.80 b | 6.77 bc |
| Common carp | 7.82 bc | 4.47 ab | 1.47 c | 3.57 ab | 9.37 a | 6.47 a | 4.45 c | 6.60 c |
| Bizz | 7.65 c | 4.62 ab | 2.70 a | 3.95 a | 8.00 ab | 5.55 b | 6.00 a | 7.27 a |
| Shabbout | 8.65 a | 4.95 a | 1.75 bc | 3.45 ab | 7.00 b | 5.55 b | 4.40 c | 7.25 ab |

Mean having different letters in the same column are significantly different.
The differences at $p<0.05$.
The means average of 12 replicates.

### 3.4 Physical evaluation

As shown in table 3.7, significant differences ($P<0.05$) in pH values, Extract release volume (ERV), water holding capacity and cooking loss were found amongst all five types. Meat of all types were weak acidic, near to neutral pH 7, the pH ranged between 6.605 for Common carp and 6.98 for Bizz. The pH is the only measurement which has been commonly used as physical method for quality assessment of fish meat (Mhongole, 2009). Mhongole (2009) found pH values range was 6.4 and 7.0. A previous study in Sulaimani (Salh, 2009) had reported a pH 6.85 for Bizz, and a weak alkaline pH value (7.13) for Shabbout!. The pH value of local fresh fish meat in Egyptian-Yemen Gahsh (*Lethrinus elongatus*) was reported to be 5.7 (Gamal El-Deen and Shamery, 2010).

Both Silver and Common carps resulted significantly and higher $ERV_{(s)}$ (16.27 and 15.49 ml) and significantly and low WHC (15.92, 18.25% respectively) in comparison to Bizz, Grass carp and Shabbout which showed low ERV (11.15, 13.21 and 14.05 ml respectively) and high WHC percentages (29.08, 22.83 and 21.08% respectively). However the ERV all five types ranged between 11.15-16.27 ml and WHC was ranged between15.92-29.08%.

Bizz, Grass carp and Shabbout showed significant lower values of ERV whilst both showed increased WHC. It is thought that high pH has effects on ERV and WHC; it is thought to alter the functional properties and denature the myofibril proteins and also ERV and WHC may be related to higher protein content (Mackie, 1984). Water holding capacity (WHC) value of local fresh fish meats in Egyptian fresh Yemen Gahsh (*Lethrinus elongatus*) was 5.5% (Gamal El-Deen and Shamery, 2010) which may belong to the effect of species.

Bizz, Grass and Shabbout showed significant ($p<0.05$) low cooking loss percentages (28.83, 31.66 and 37.73% respectively). Silver carp and Common carp showed significant high cooking loss percentages (42.99 and 40.35% respectively). The low cooking loss may be due to increased moisture content, water holding capacity which leads to an increased ability of meat tissue to retain water and reducing loss during cooking and depended on protein percentage, lowering pH value also affects cooking loss by changes in ionic balancees of proteins on acidic pH (pH was 6.612 and 6.605 for Silver and Common carp respectively).

The density values for the five fish types are shown in table 3.7. A significant difference in density between both Bizz and Common carp and between Grass carp and Shabbout was observed. The density values recorded for Bizz, Common, Shabbout, Grass carp and Silver carp were 1.019, 1.017, 1.011, 1.008 and 1.006 respectively.

The determination of the WHC and cooking loss allows conclusions to be drawn about the degree of denaturation of the proteins and therefore the quality of the fish (Skipnes *et al*., 2007).

Table 3.7**:** Physical evaluation of five types of local fresh fish.

| Type of fish | Test | | | | |
|---|---|---|---|---|---|
| | Extract release volume ( ml) | Water holding capacity (%) | Cooking loss (%) | Density (floating) | pH |
| Grass carp | 13.21 d | 22.83 b | 31.69 d | 1.008 bc | 6.76 b |
| Silver carp | 16.27 a | 15.92 e | 42.99 a | 1.006 c | 6.605 e |
| Common carp | 15.49 b | 18.25 d | 40.35 b | 1.017 a | 6.612 d |
| Bizz | 11.15 e | 29.08 a | 28.83 e | 1.019 a | 6.98 a |
| Shabbout | 14.05 c | 21.08 c | 37.73 c | 1.011 b | 6.70 c |

Mean having different letters in the same column are significantly different.
The differences at $p<0.05$.
The means average of 12 replicates.

### 3.4.1 Physical evaluation of farmed and wild fish

The physical evaluation of three farmable types (Grass, Silver and Common carps) for farmed and their wild counterpart are shown in table 3.8. There were significant differences ($p<0.05$) in the Extract release volume (ERV), water holding capacity (WHC), cooking loss, density and pH values between the farmed and wild fish for the three examined types. The values of ERV, WHC, cooking loss and pH for wild Grass carp were 13.07ml, 24.33%, 30.88% and 6.79 respectively, While for farmed counterpart the values were 13.35ml, 21.33%, 32.43% and 6.73 respectively table 3.8.

For wild Common carp, ERV, WHC, cooking loss and pH were 15.33ml, 18.67%, 39.68% and 6.64 respectively while in their farmed counterpart were 15.65ml, 17.83%, 41.02% and 6.59 respectively. For the same attributes, wild Silver carp resulted values of 16.17ml, 17.33%, 42.37% and 6.51 respectively,

while farmed counterpart showed (16.38ml, 14.50%, 43.62% and 6.50 respectively).

The wild sources of the three types had low ERV, high WHC value, low cooking loss and high pH when compared to their farmed counterparts. These results may be related to highly protein content, moisture content in fish meat of these types and to farming systems and feed on concentrated diet on farmed types compared to wild counterpart of the same (Orban *et al*., 2003).

A difference between farmed and wild fish in pH has previously been observed in other fish species (Olsson *et al*., 2007; Fuentes *et al*., 2010). The difference in post-rigor pH may be due to the difference in the access of feed in the two groups. In intensive farming the fish has access to unlimited amounts of feed, which leads to a high muscle glycogen level, and subsequently a low ultimate muscle pH post-rigor due to anaerobic degradation of glycogen after slaughtering (Ofstad *et al*., 1996).

Table 3.8**:** Physical evaluation for wild and farmed sources of three types of local fresh fish.

| Type of fish | Breed | Test | | | | |
|---|---|---|---|---|---|---|
| | | Extract release volume ( ml) | Water holding capacity % | Cooking loss % | Density | pH |
| Grass carp | farmed | 13.35 a | 21.33 b | 32.43 a | 1.011 a | 6.73 b |
| | wild | 13.07 b | 24.33 a | 30.88 b | 1.006 b | 6.79 a |
| Silver carp | farmed | 16.38 a | 14.50 b | 43.62 a | 1.008 a | 6.50 b |
| | wild | 16.17 b | 17.33 a | 42.37 b | 1.003 b | 6.51 a |
| Common carp | farmed | 15.65 a | 17.83 b | 41.02 a | 1.018 a | 6.59 a |
| | wild | 15.33 b | 18.67 a | 39.68 b | 1.015 b | 6.64 a |

Means having different letters in the same column are significantly different within same types
The differences at $p<0.05$.The means average of 6 replicates.

### 3.5 Chemical quality evaluation

### 3.5.1 Total volatile basic amines (TVBA)

#### 3.5.1.1 Total volatile nitrogen (T.V.N.)

The **T.V.N**. values for the five types of fresh fish were 12.89, 16.41, 15.31, 12.23 and 13.61 mg N/ 100gram for Grass carp, Silver carp, Common carp, Bizz and Shabbout respectively (figure 3.1). Silver carp was of the highest value while Bizz showed the lowest. Significant differences were shown among all five types ($P<0.05$), all showed submaximal acceptable levels; If the T.V.N. reaches 30mg N/100g most authorities would consider the fish to be stale, whilst at 40 mg N/100 g the fish is regarded as unfit for consumption. The level of TVBN for white fish is generally considered to be fresh if the T.V.N. is less than 20mg N/100 g sample according to the Codex Alimentarius Committee proposed in 1968 the TVB assay by steam distillation (Egan *et al.*, 1981). However, levels exceeding 28 mg N TVB-N/100g meat has been reported "unacceptable" according to the Turkish Manual of Seafood Quality Control Limits (Anonymous, 2008) in high of Turkey being neighbor country. Sikorski *et al*. (1990) suggest that fish and fish products is unfit for human consumption when exceeding the value (T.V.N) 30mg N/100g meat. In any case, there are no standard limits for T.V.N. values in Iraqi food quality regulations.

These relatively high values of Silver and Common carps may be related to the effect of microbial enzymes due to higher bacterial growth as Chytiri *et al*. (2004) suggested. So, the T.V.N values for both species were submaximal limit; good fish quality, as suggested by Connell (1995) and Dalgaard *et al*. (1993), necessitates that T.V.N should be lower than a maximal acceptable limit of 35.40 mg N/100g. The T.V.N. value of Common carp here is less than that of Common carp reported by Ježek and Buchtová (2010) which was 18.06 mg N/100g. T.V.N. value of the grass carp was lower than that reported by Scherer *et al.* (2006) for the same type which was 30 mg N/100 g.

The results of the present study converged with results of T.V.N in fresh Suboor, Jaffout, Biyah and Thelah which were 14.2, 13.8, 13.2 and 14.6mg N/100 g respectively (Al-Shatty ,2006).

The results of all fish types here converged that for other types obtained by Kyrana and Lougovois (2002); Chytiri *et al*. (2004); Castro *et al*. (2006) who showed that T.V.N. values in Rainbow fish, European bass and fresh Mediterranean fish (≤ 25 mg N / 100g), but they did not converge T.V.N. value for whale Nile perch which was 6.8mg N /100g (Mhongole, 2009). However, it was recorded T.V.N. values between 25 - 35 mg N /100g (Connell, 1995)

It is worth mentioning that estimation of TVB.N is an important and a very helpful test in assessing fish spoilage, but not freshness, even if it is influenced by several factors including the type, catching season, region, age and sex (El- Marrackchi *et al*., 1990; Oehlenschläger, 1997b).

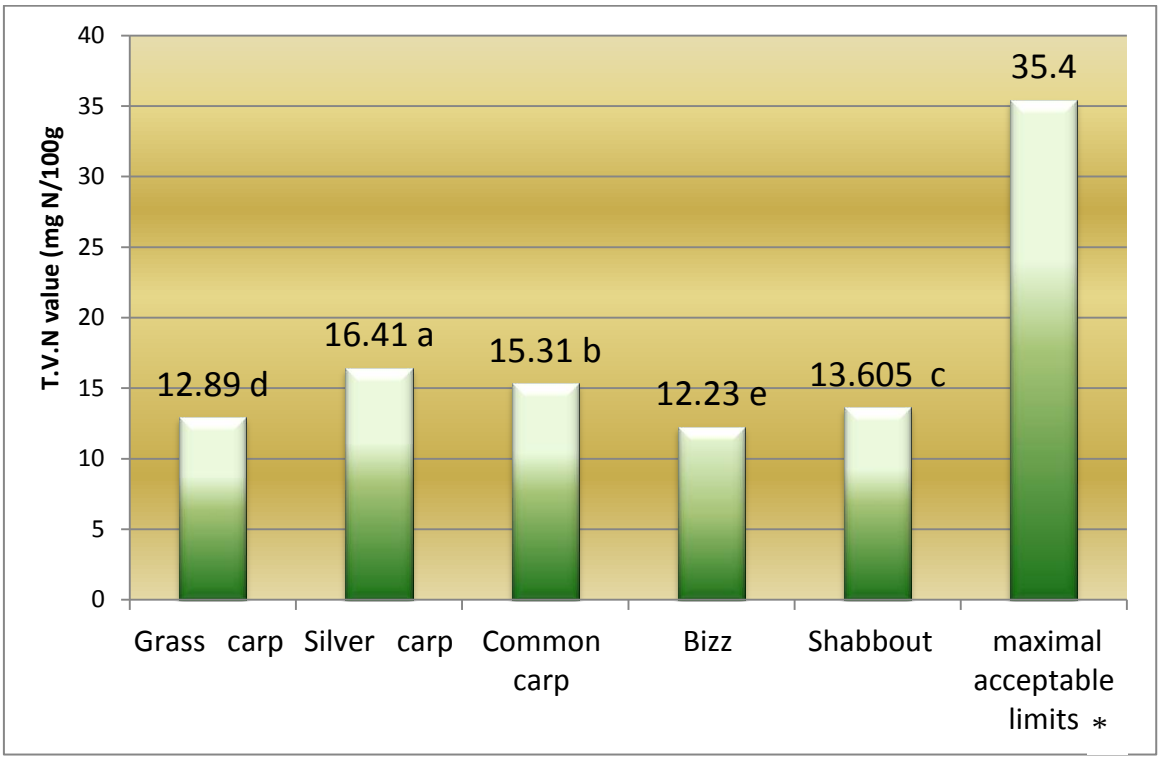


Figure 3.1: Total volatile nitrogen value for five types of local fresh fish. (mg N/ 100g ).

Mean having different letters are significantly different.
The differences at P<0.05.
The means average of 12 replicates.
* Egan et al., 1981

### 3.5.1.2 Trimethylamine

As shown in figure 3.2, Bizz, Shabbout and Grass carp showed rather low values of Trimethylamine (TMA) (2.35, 3.20 and 3.43 mg N/ 100g meat respectively) while Silver and Common carp showed higher TMA values (5.58 and 4.52 mg N/100g meat respectively). Significant differences appeared among all five types ($P<0.05$). It has been mentioned that fresh fish with less than 1.5mg TMA-N 100g is considered as good quality while 10-15mg TMA-N /100g is regarded within the acceptable limits (Huss, 1988; Connell, 1995). It should also be mentioned that there are no standard limits for TMA values in Iraqi food quality regulations.

However, TMA is an indicator for fish spoilage and when TMA value increases to more than 10mg N/ 100g meat, the fish meat is considered stale (Dalgaard *et al*., 1993), but when it is increased upper than that it could be an indicator to high bacterial count; may be higher than $10^8$-$10^9$ Cfu/gram meat (appendix 3) (Chytiri *et al*., 2004). The TMA value here converge those reported for Iraqi aquatic fresh fish including Suboor, Jaffout, Biyah and Thelah which were 4.00, 4.92, 5.20 and 0.27 mg N/100g meat respectively (Al-Shatty, 2006). The fresh Yemen Gahsh type (*Lethrinus elongatus*) in Egypt, however, has been recorded a higher value (13.907 mg N/100gm) (Gamal El-Deen and Shamery, 2010).

Trimethylamine oxide (TMA.O.), which Common in fresh fish, is a flavorless non– protein nitrogen compound which has an osmoregulation function and its content varies with the fish species. TMA could be resulted from decomposition of TMA.O by bacterial activity and intrinsic enzymes and so is often used as an index of freshness of fish (Ozogul and Ozogul, 2006). The current results confirmed that the final value of TMA is species specific that there were significant differences among all and in view of this all scored less than 10mg N/100g meat, which excludes the reason of bacterial activity and remains only the probable of the differences intrinsically in TMAO.

Furthermore, the variations in TMA values may rely on the overall condition of the fish and the reduction of TMAO to TMA is pH dependent (Fraser and Sumar, 1998).TMA mightn't be accessed in fresh water fish.

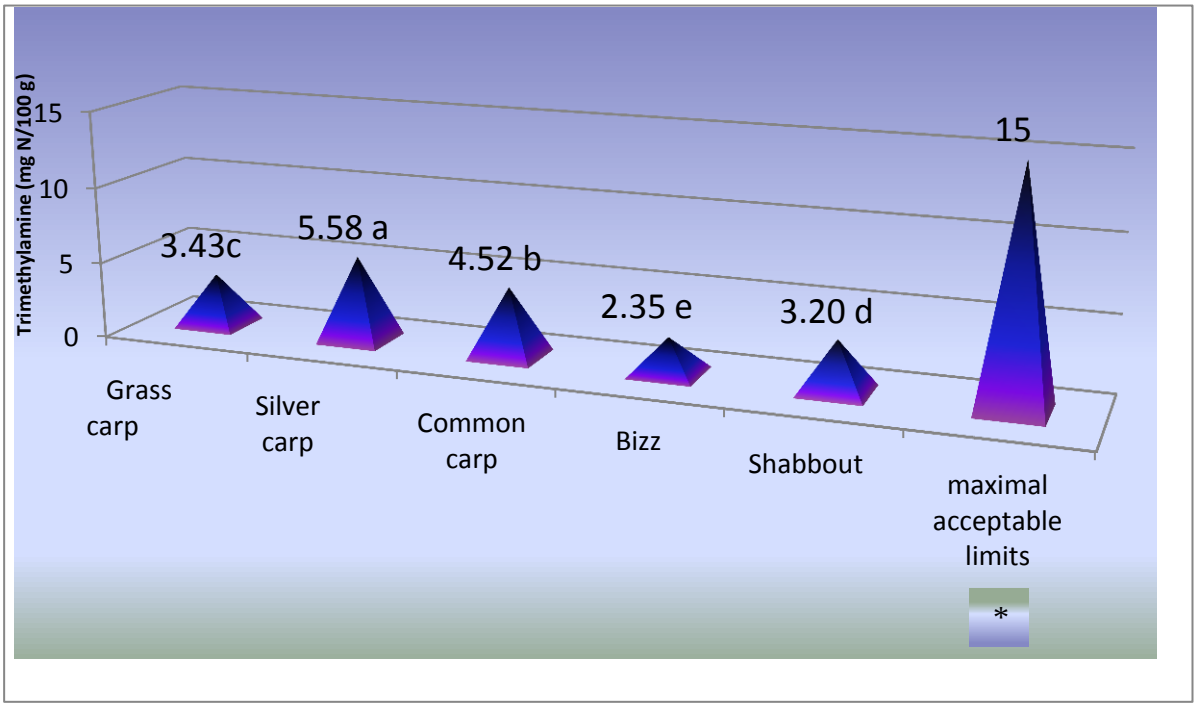


Figure 3.2: Trimethylamine value for five types of local fresh fish. (mg N/ 100g ).

Mean having different letters are significantly different.
The differences at P<0.05
The means average of 12 replicates.
*Connell, 1995

### 3.5.1.3 Dimethylamine

The results of Dimethylamine (DMA) as shown in figure 3.3 for Grass carp, Bizz, Shabbout, Common carp and Silver carp were 0.142, 0.144, 0.161 and 0.305 and 0.365mg N/ 100g meat respectively. In freshly caught fish, DMA is present at a very low concentration of about 0.2mg N/ 100g, with a variation ranging between 0.1 and 0.4mg N/ 100g (Oehlenschläger, 1997a) this means the acceptable values of the current results, even if there are no standard limits for DMA values in Iraqi food quality regulations. There were no significant differences in DMA values among Grass carp, Bizz and Shabbout (P≥0.05) which inturn significantly differ from the two other types Silver and Common carp (P<0.05) that did not differ in between (P≥0.05). Chemical tests for DMA

are most valuable in the early stages of spoilage and could be used as indirect indicators of denaturized protein which is often traceable to improper handling before or during frozen storage (Huss, 1995). DMA is often tested for storage fish; only small amounts of it are formed in fish meat during spoilage and not in all fish species, so that its measurement is not considered useful in monitoring spoilage of fish (Howgate, 2010). DMA mightn't be accessed in fresh water fish.

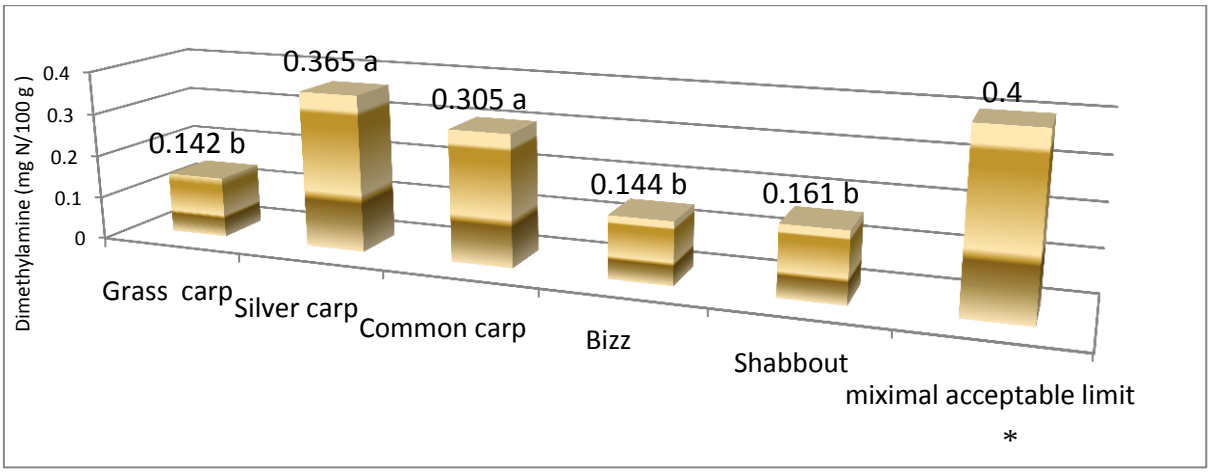


Figure 3.3: Dimethylamine value for five types of local fresh fish. (mg N/ 100 g).

Means having different letters are significantly different.
The differences at P<0.05.
The means average of 12 replicates.
*Oehlenschläger, 1997a

### 3.5.1.4 Ammonia

Except that the Silver carp which recorded 0.135mg N/100g, the other four types showed no ammonia (figure 3.4). Silver carp in turn did not reach the standard maximal limit which is 10mg N/100g meat (Bojanic *et al*., 2009). However, there are no standard limits for ammonia values in Iraqi food quality regulations.

Ammonia is present in freshly caught fish during chilled storage; it is formed by bacterial enzymes which lead to break down of the proteins through proteolysis and decomposition, and considered as a poor indicator of freshness of fish and so cannot be considered as an effective marker of fish spoilage (Etienne *et al*., 2005; Gamal El-Deen, 2007). In general, ammonia increases

with increasing storage time (Bojanic *et al*., 2009). The absence or low Ammonia nitrogen (A.N) values of the current results were incomparable with, for example, the value recorded for fresh Yemen (Gahsh; *Lethrinus elongatus*) fish meats in Egypt which was 25.033 mg N/100gm (Gamal El-Deen and Shamery, 2010). The other investigations confirmed the positive relationship between the storage time and increased ammonia in fish that this study monitored fresh fish only (Bojanic *et al*., 2009).

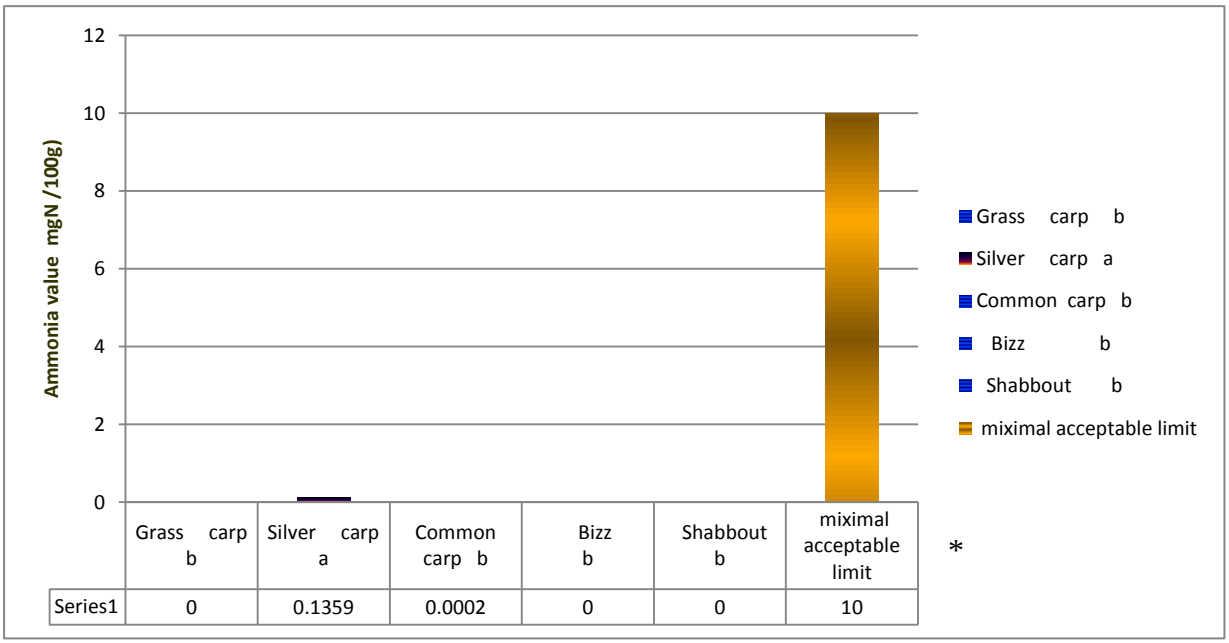


Figure 3.4: Ammonia value for five types of local fresh fish. (mg N/ 100 g).

Means having different letters are significantly different.
The differences at P<0.05.
The means average of 12 replicates.
*Bojanic *et al*., 2009

### 3.5.2 Total volatile basic amines for farmed and wild fish

There were significant differences in T.V.N and TMA of the three farmable types (Grass, Silver and Common carp), between farmed fish and their wild counterpart ($p<0.05$) while ammonia was absent in both. Dimethylamine (DMA) and ammonia appeared not to differed significantly between farmed and wild Silver carp, DMA differed significantly between farmed and wild Common

carp, while ammonia showed no significant difference between farmed and wild Common carp meat (table 3.9).

Wild Grass carp had lower T.V.N, TMA, DMA (12.81, 3.38 and 0.58 mg N/100 gm. meat respectively) and absence of ammonia, than its farmed counterpart which recorded 12.98, 3.48 and 0.67 mg N/100gm meat respectively with no ammonia. T.V.N, TMA and DMA for wild Common carp meat were 15.23, 4.48 and 0.34 mg N/100gm meat respectively with no ammonia, while their farmed counterpart were 15.38, 4.56 and 0.43mg N/100gm meat respectively with no ammonia either.

T.V.N, TMA, DMA and ammonia for farmed Silver carp meat were 16.50, 5.62, 0.41 and 1.92 mg N/100gm meat respectively, while in wild Silver carp meat they were 16.33, 5.53, 0.36 and 0.62 mg N/100 gm. meat respectively.

Results of three species of fish meat indicates that the wild Grass and Common carps had lower T.V.N, TMA, DMA and absence of ammonia; this indicates higher freshness and acceptable quality in comparison to their farmed counterpart and the wild and farmed Silver carp. The high values of DMA in both farmed Silver carp may be related to the high rate of protein decomposition that Chytiri *et al*. (2004) have mentioned such that increasing DMA in meat protein is related to action of bacteria and the presences of free amines.

Table 3.9: Chemical evaluation for wild and farmed sources of three types of local fresh fish.

| Type of fish | Breed | Test | | | |
|---|---|---|---|---|---|
| | | Total volatile nitrogen mg N /100 g | Trimethylamine mg N / 100 g | Dimethylamine mg N / 100 g | Ammonia mg N / 100 g |
| Grass carp | farmed | 12.98 a | 3.48 a | 0.67 a | 0.0 a |
| | wild | 12.81 b | 3.38 b | 0.58 a | 0.0 a |
| Silver carp | farmed | 16.50 a | 5.62 a | 0.41 a | 1.92 a |
| | wild | 16.33 b | 5.53 b | 0.36 a | 0.61 a |
| Common carp | farmed | 15.38 a | 4.56 a | 0.43 a | 0.0 a |
| | wild | 15.23 b | 4.48 b | 0.34 b | 0.0 a |

Means having different letters in the same column are significantly different within same types.
The differences at $p<0.05$.
The means average of 6 replicates.

### 3.5.3 Total volatile basic amines in licensed and unlicensed markets

As shown in table 3.10, there are no significant differences in T.V.N and TMA was appeared between the two markets, licensed (M1) which were 12.84 and 3.40mg N / 100 g meat respectively, and unlicensed (M2) which were 12.95 and 3.46mg N / 100 g meat respectively for Grass carp, DMA mean while showed significant differences ($P<0.05$) between them (0.55mgN/100g in M1 and 0.66mg N/100 g in M2), with absence of ammonia in both markets for this fish type. Silver and Common carp recorded insignificant differences between M1and M2 for all attributes. Significant differences in T.V.N., TMA and DMA for Bizz and Shabbout were demonstrated(for Bizz were 12.19, 2.28 and 0.11mgN/100 g respectively in M1 and 12.27, 2.37 and 0.71mgN/100 g respectively in M2, for Shabbout were13.56, 3.25 and 0.12 mg N/ 100 g

respectively in M1 and 13.65, 3.34 and 0.2mgN/100 g respectively in M2). Ammonia was absent for both Bizz and Shabbout in both markets.

It is observed that T.V.N and TMA values for Bizz of M1 were low, while for Silver carp of M2 they were high (16.43 and 5.59 mg N/ 100 g respectively). The results indicated that the five species of fish from market 1 were characterized with high freshness than fish from market 2 and this may be due to various factors such as handling and storage at inadequate temperature (Fraser and Sumar, 1998) and light; as unlicensed shops are exposed to sun light.

Table 3.10: Chemical evaluation of five types of local fresh fish from licensed (M1) and unlicensed (M2) markets.

| Type of fish | Market | Test | | | |
|---|---|---|---|---|---|
| | | Total volatile nitrogen mg N / 100g | Tri methyl amine mg N/ 100g | Di methylamine mg N / 100g | Ammonia mg N / 100g |
| Grass carp | M1 | 12.84 a | 3.40 a | 0.55 b | 0.0 a |
| | M2 | 12.95 a | 3.46 a | 0.66 a | 0.0 a |
| Silver carp | M1 | 16.40 a | 5.57 a | 0.36 a | 0.70 a |
| | M2 | 16.43 a | 5.59 a | 0.41 a | 1.86 a |
| Common carp | M1 | 15.31 a | 4.51 a | 0.358 a | 0.002 a |
| | M2 | 15.31 a | 4.53 a | 0.415 a | 0.002 a |
| Bizz | M1 | 12.19 b | 2.28 b | 0.11 b | 0.0 a |
| | M2 | 12.27 a | 2.37 a | 0.17 a | 0.0 a |
| Shabbout | M1 | 13.56 b | 3.25 b | 0.12 b | 0.0 a |
| | M2 | 13.65 a | 3.34 a | 0.2 a | 0.0 a |

Means having different letters in the same column are significantly different within same types
The differences at $p<0.05$.
The means average of 6 replicates.

### 3.5.4 Lipid oxidation evaluation

#### 3.5.4.1 Thiobarbituric acid (TBA) values

TBA values for Silver carp, Common carp, Shabbout, Grass carp and Bizz were 5.20, 3.48, 2.11, 1.23 and 0.87mg Malonaldehyde/ kg meat respectively (figure 3.5). Significant differences are found between all species ($P<0.05$). TBA values in Silver and Common carps had higher TBA limit, indicates the higher quality product (3.00 mg malonaldehyde/ kg) while in good quality product should not be more than 5mg malonaldehyde/kg (Günşen *et al*., 2011). Connell (1995) indicated that rancidity appears in fish when TBA become greater than 1-2mg malonaldehyde/kg, this means the Silver carp, Common carp and Shabbout showed higher TBA values. There are no standard limits for TBA values in Iraqi food quality regulations.

The higher values of TBA in Silver and Common carps are probably due to the increase of lipid content than the lipid oxidations resulting from action of lipolytic enzymes (lipases and phospholipases) that fish phospholipids undergo degradation to produce hydroperoxides, Aldehydes and ketones which are responsible for the development of oxidative rancidity (Raharjo *et al*., 1992). It was reported that TBA values of some local fish types in Basrah /Iraq included fresh Suboor, Jaffout, Biyah and Thelah were 3.201, 1.294, 0.551 and 1.170mg malonaldehyde/kg respectively (Al-Shatty, 2006) this is similar to the results of the current study. For other types, however, results may be varied and may be due to species specific depending on the lot of lipid content; the local Egyptian fresh Yemen Gahsh type (*Lethrinus elongatus*) recorded TBA value as 0.163mg N/100gm (Gamal El-Deen and Shamery, 2010). It should be kept in mind that TBA value is a widely used as fish quality indicator and for oxidative rancidity (Pereira – de Abreu *et al*., 2010).

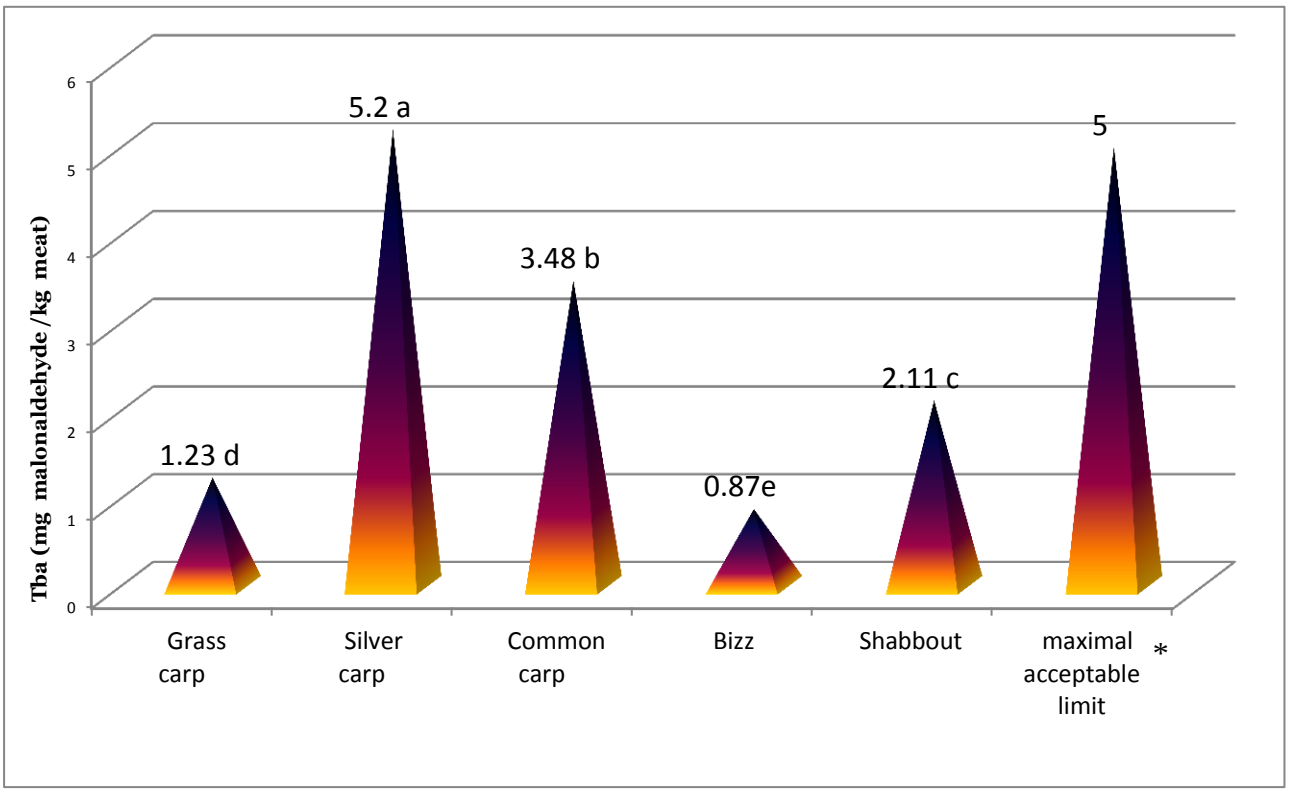


Figure 3.5: TBA means value for five types of local fresh fish.(mg malonaldehyde /kg meat).

Mean having difference letters are significantly different.
The differences at ($p<0.05$).
The means average of 12 replicates.
* Günşen *et al*., 2011

### 3.5.4.2 Peroxide values (PV)

Peroxide values (PV) for Bizz, Grass carp, Shabbout, Common carp and Silver carp were 3.40, 4.47, 5.29, 7.25 and 8.86meq oxygen/ kg fat respectively (figure 3.6). All types varied significantly ($P<0.05$). According to Connell (1995), when the peroxide value exceeded 10meq oxygen/ kg fat of fish meat, the fish meat is then considered unfit for human consumption or refused, all types were within the acceptable limits. Egan *et al*. (1997) suggested that the rancidity flavor occur when peroxide values reach to 20-40meq oxygen/ kg fat. So, the present investigation found that all five tested types haven't showed rancidity flavor. According to Egan *et al*.(1997) suggestion even if the TBA values indicate rancidity for at least three types (section, 3.5.4.1) they appeared to be without affecting flavor attributes (section, 3.7). However, there are no

standard limits for **PV.** values in Iraqi food quality regulations to be compared with. Thus, the PV values obtained in this investigation can be adopted as quality criteria for the Iraqi fish.

Other fresh fish in Africa (albacore and *Claries angullaris)* recorded Peroxide values of 7.23 and 4.00 meq oxygen /kg fat of meat respectively (Ben– gigirey *et al*., 1999 ; Adoga *et al*., 2010), while peroxide values for some Iraqi local fresh marine fish, Suboor, Jaffout, Biyah and Thelah, were 9.2, 17.69, 14.67 and 18.08 meq oxygen /kg fat meat respectively (Al-Shatty, 2006), these were higher than the current values which may be due to species specificity or the condition of fish or fishing.

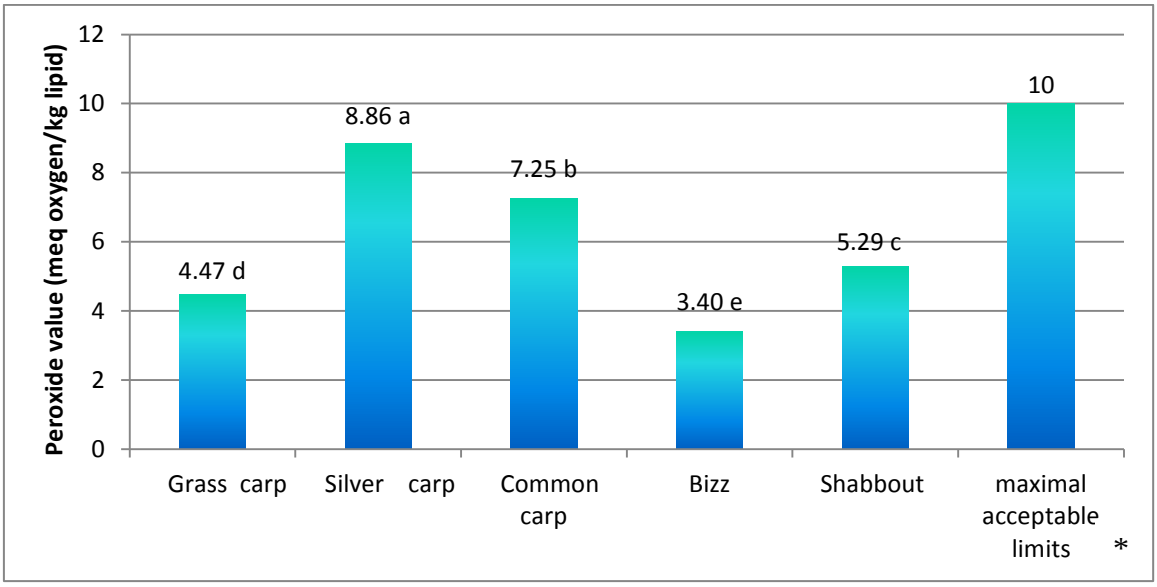


Figure 3.6: Peroxide means value for five types of local fresh fish. (Meq oxygen/kg lipid).

Means having different letters are significantly different.
The differences at $p<0.05$.
The means average of 12 replicates.
*Connell,1995

### 3.5.4.3 Free fatty acids (FFA)

Free fatty acids (FFA) for all species of fish are shown in figure 3.7. The Bizz, Grass carp, Shabbout, Common carp and Silver carp recorded 0.77, 0.84, 0.93, 1.24 and 1.36% FFA respectively, significant differences are found among all species ($p<0.05$). Egan *et al*. (1997) suggested that the acidity could be felt

when FFA calculated, as oleic acid, reach to 0.5-1.5%; all five types were within this range and were within acceptable flavor scores (section, 3.7). There are no standard limits for FFA values in Iraqi food quality regulations. These recorded values may be adopted for Iraqi specifications.

Accumulation of FFA does not in itself affect quality attributes of the product but have been shown to interrelate with lipid oxidation and have been proposed to have a pro-oxidant effect on lipids (Rodriguez *et al*., 2006). Therefore, the production of free fatty acids (FFA) is measured to study the progress of lipid hydrolysis and can be used to determine the degree of deterioration of food products (Barthet *et al*., 2008). The higher value of FFA is possibly due to the action of lipolytic enzymes on lipid from higher bacterial count leading to increase in the release of free fatty acids, which contribute positively to the generation of undesirable aroma and flavor (Al-Sherick, 2005). El– Nady (1997) scored the Egyptian coast's fresh fish free fatty acids, the proportion of which valued to 1.26% is within the same range of the present work, while a paradoxical result had been reported for at least one marine Iraqi type (Biyah) which scored 4.85 % FFA for fresh product in Basrah city (Al-Shatty, 2006). However, various literatures have mentioned that more Iraqi aquatic types have been shown to be closer to the current FFA values. 1.06% in *Silurus triostegus* (AL-Habbib *et al*., 2008) and 0.200, 0.150, 0.140, 0.130 and 0.470% for Shabbout, Bizz, Carsean, Tawni and Gattan fish meat respectively(Salh, 2009).

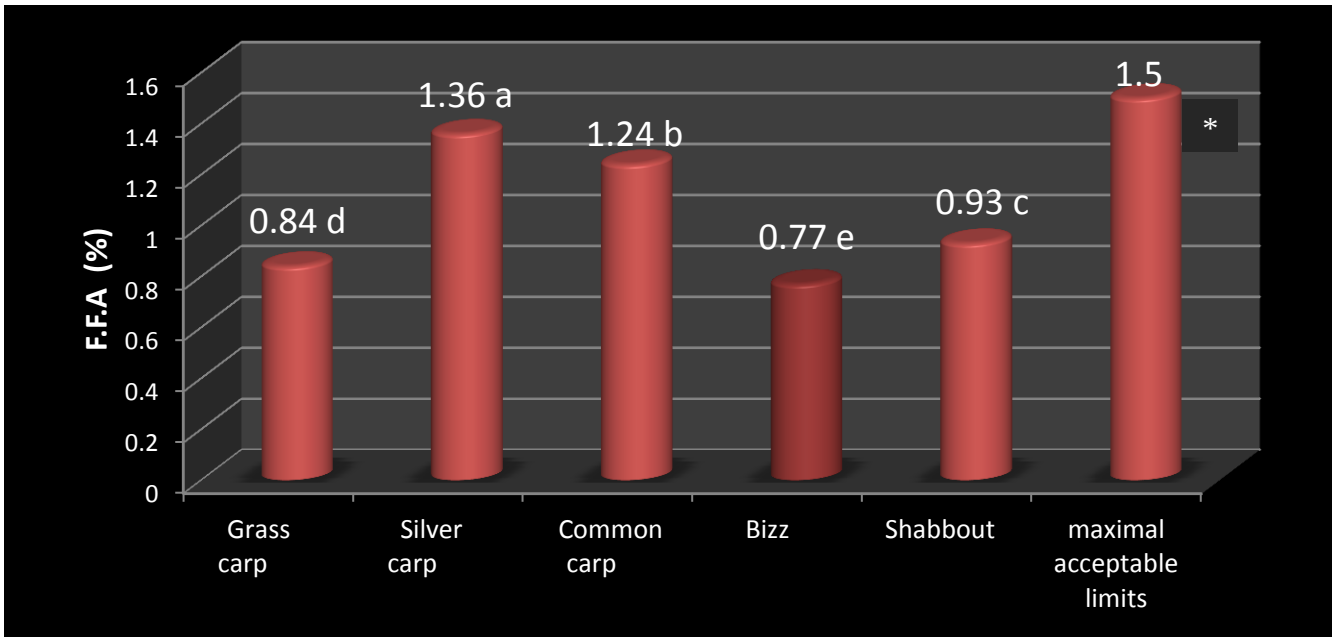


Figure 3.7: FFA mean indicator for 5 species of fish five types of local fresh fish (% lipid).

Means having different letters are significantly different.
The differences at $p<0.05$.
The means average of 12 replicates.
*Egan et al., 1997

### 3.5.5 Lipid oxidation evaluation for farmed and wild sources

The **lipid oxidation** evaluation of the three farmable types (Grass, Silver and Common carps) for farmed and their wild counterpart are shown in table 3.11. where differences were shown in TBA, peroxide and free fatty acid values between wild and farmed types of three types of fish ($P<0.05$). Wild Grass carp recorded lower values of TBA, peroxide and free fatty acids which were 1.14mg malonaldehyde /kg fish, 4.41meq oxygen /kg lipid and 0.82 % respectively while farmed Grass carp recorded 1.32 mg malonaldehyde /kg fish, 4.54meq oxygen /kg lipid and 0.85% respectively.

Wild Common carp had lower TBA, peroxide and free fatty acids percentage which were 3.37 malonaldehyde /kg fish,7.21 meq oxygen /kg lipid and 1.22% respectively; while their farmed counterpart recorded 3.59 malonaldehyde /kg fish, 7.29 meq oxygen /kg lipid and 1.26% respectively. Farmed Silver carp recorded higher values of oxidation lipids indices which

were 5.29 malonaldehyde /kg fish, 8.93meq oxygen /kg lipid and 1.40% respectively than its wild counterparts which were 5.11 malonaldehyde /kg fish, 8.80meq oxygen /kg lipid and 1.32 % respectively.

The high values of lipids oxidation evaluation for the farmed samples of the three farmable types in comparison to their wild counterparts may be related to farming conditions including nutrition and the use of concentrated diet which may accelerate lipid oxidation (Khayat and Schwall, 1983).

Table 3.11: Lipid oxidation evaluation for wild and farmed sources of three types of local fresh fish.

| Type of fish | Breed | test | | |
|---|---|---|---|---|
| | | Free fatty acids ( %) | Peroxide value (meq oxygen/kg lipid) | Thiobarbituric acid (mg malonldehyde /kg lipid) |
| Grass carp | farmed | 0.85 a | 4.54 a | 1.32 a |
| | wild | 0.82 b | 4.41 b | 1.14 b |
| Silver carp | farmed | 1.40 a | 8.93 a | 5.29 a |
| | wild | 1.32 b | 8.80 b | 5.11 b |
| Common carp | farmed | 1.26 a | 7.29 a | 3.59 a |
| | wild | 1.22 b | 7.21 b | 3.37 b |

Means having different letters in the same column are significantly different within same types
The differences at $p<0.05$.
The means average of 6 replicates.

### 3.5.6 Lipid oxidation evaluation in licensed and unlicensed markets

When the lipid oxidation evaluation of the five fish types were compared between to the licensed markets (M1) and unlicensed markets (M2) (table 3.12), insignificant differences between M1 and M2 for Grass carp, Silver carp and Common carp meat were appeared, while, in contrast, significant differences between both markets for Bizz and Shabbout were shown. Bizz of M1 has had

0.75%, 3.23 meq oxygen /kg lipid and 0.83 mg malonaldehyde /kg lipid respectively while its M2 counterpart had 0.79%, 3.50meq oxygen /kg lipid and 0.92 mg malonaldehyde /kg respectively. Shabbout of M1 had 0.92%, 5.21 meq oxygen /kg lipid and 2.04 mg malonaldehyde/ kg while that of M2 showed lipid oxidation indicators of 0.94%, 5.38meq oxygen /kg lipid and 2.19 mg malonaldehyde/kg respectively .

The results of lipid oxidation indices indicate, without doubt, that unlicensed fish selling shops are critical for selling Bizz and Shabbout types in the least.

Table 3.12: Lipid oxidation evaluation of five types of local fresh fish from licensed (M1) and unlicensed (M2) markets.

| Type of fish | Market | Test | | |
|---|---|---|---|---|
| | | Free fatty acids ( %) | Peroxide value (meq oxygen /kg lipid) | Thiobarbituric acid (mg malonaledhyde /kg lipid) |
| Grass carp | M1 | 0.83 a | 4.44 a | 1.21 a |
| | M2 | 0.85 a | 4.50 a | 1.23 a |
| Silver carp | M1 | 1.35 a | 8.84 a | 5.20 a |
| | M2 | 1.37 a | 8.88 a | 5.20 a |
| Common carp | M1 | 1.23 a | 7.26 a | 3.44 a |
| | M2 | 1.25 a | 7.25 a | 3.52 a |
| Bizz | M1 | 0.75 b | 3.30 b | 0.83 b |
| | M2 | 0.79 a | 3.50 a | 0.92 a |
| Shabbout | M1 | 0.92 b | 5.21 b | 2.04 b |
| | M2 | 0.94 a | 5.38 a | 2.19 a |

Means having different letters in the same column are significantly different within same types
The differences at $p<0.05$.
The means average of 6 replicates.

### 3.5.7 Biogenic amines and hypoxanthine ($H_x$)

As shown in table 3.13, there were significant differences among Shabbout, Grass carp and Common carp, with respect to putrescine concentrations ($p<0.05$) which were 13.90, 8.03 and 8.01ppm respectively, while these types were significantly varied with both Bizz and Silver carp in which putrescine were 4.31 and 5.73 ppm respectively.

Bizz showed a higher cadaverine (11.50 ppm) than the other four fish types while Grass carp showed a lower concentration (0.00 ppm) with significant differences ($p<0.05$) amongst all. Common carp had a higher concentration of histamine (26.28 ppm) which varied significantly from the other four types. Spermine concentrations were high in Bizz and Common carp (15.24 and 15.24 ppm respectively) with no significant difference between them, while they varied significantly with the other three types (Grass carp, Silver carp and Shabbout) which in turn showed no significant differences in both between Silver and Grass carps and between Grass and Shabbout, moreover, significant differences was shown between Shabbout and Silver carp.

Spermidine was high in Shabbout, Bizz and Grass carp (3.99, 3.91 and 3.62 ppm respectively) with insignificant differences between them but they were varied significantly with both Silver and Common carps which in turn had insignificant differences between them, furthermore spermidine was absent in Common carp and nearly absent in Silver carp (0.0 and 0.87ppm respectively).

The Biogenic amines indexes (BAI) as shown in table 3.13, were 1.175, 1.673 and 2.117 for Bizz, Grass carp, Shabbout respectively, and were rather higher in Silver and Common Carps (8.657 and 4.612 respectively).

Hypoxanthine ($H_x$) values for the Silver carp and Common carp were 0.003 and 0.002ppm respectively, while the other three fish types were hypoxanthine free (table 3.13).

The current results revealed that all tested types were safe with respect to all biogenic amines content that have been monitored. The maximal acceptable limit of histamine in fish must not exceed 100 ppm (Brink *et al*., 1990; Codex

Alimentarius, 2004); in spite of that, FDA (2008) stated "the level of histamine in a composite sample of fish or fish products, other than crustaceans or molluscs, must not exceed 200 ppm".

The same histamine maximal limit of FDA is the same as Australian legal limit while in South Africa the limit is 100 mg/kg (Auerswald *et al*., 2006), unacceptable limits are those which exceed 50 mg/kg and in Turkey. It is 100 mg/kg, as reported by FDA and Turkish Manual of Seafood Quality Control Limits respectively (FDA, 2001; Anonymous, 2008). There are no standard limits for biogenic amines concentration in Iraqi food quality regulations. Thus, values obtained in this study can be adopted as Iraqi standard specification.

The data obtained for putrescine, cadaverine and histamine were lower when compared to Zaman *et al*. (2009) who reported 1257, 1429 and 1220 ppm respectively in Malaysia.

Histamine was reported for fresh carp in Basrah as 52 ppm (AL-Shatty *et al*., 1999). Another study, Křĩzek *et al*.(2002) revealed critical concentrations of putrescine and cadaverine in Common carp flesh to be around 20 mg/kg and 45mg/kg respectively. In Turkey, the putrescine, cadaverine, spermidine, spermine and histamine values in Common carp were recorded as 1.1, 0.37, 10.7, 10.2 and 0.07 ppm respectively (Křĩzek *et al*., 2004). The average histamine level in fresh marine anchovy (*Engraulis encrasicholus*) samples was 9.18 mg/kg on day zero (Günşen *et al*., 2011).

Estimation of biogenic amines is important not only from the point of view of their toxicity, but also because they can be used as indicators of the degree of freshness or spoilage of fish (Joosten, 1988; Bardócz, 1995 ; Leo and Fidel Toldra, 2010), through their correlation with the bacterial flora count, that biogenic amines increase whenever bacterial growth occurs and increase with storage time (appendix 4)(Silla-Santos, 1996) regarding that biogenic amines production is only as a result of bio activities; microbial action (Institute of Medicine, 1991). It is very good that the fresh fish sold locally are with a good freshness that the current samples were within the allowable limits of biogenic

amines which means low spoilage. This reflects the high awareness of local consumers to obligate fresh fish shops to exhibit the good quality fish to abide by their satisfaction.

According to that biogenic amines index for fish; near 1 is graded as better quality whereas those above 10 indicated the least graded quality (Bunčič, 1993), here, only Silver carp here is approaching to be with least quality (8.657-table 3.16).

Hypoxanthine values revealed freshness for all fish types tested here, while Jahns *et al*. (1976) showed traces or no $H_x$ have mentioned, the very low value of $H_x$ indicated fish freshness. A study on the fresh marine trout fish revealed that it contained 2.63 µg / g hypoxanthine (Metün *et al*., 2002). However, there are no standard limits for hypoxanthine ($H_x$) concentration in Iraqi food quality regulations.

Table 3.13: Biogenic amines means and Hypoxanthine (Hx) for five types of local fresh fish.

| Type of fish | Type of test (ppm) | | | | | | |
|---|---|---|---|---|---|---|---|
| | Putrescine | Cadaverine | Histamine | Spermine | Spermidine | Index | H x |
| Grass carp | 8.03 b | 0.0 e | 7.26 b | 6.67 bc | 3.62 a | 1.673 b | 0.0 c |
| Silver carp | 5.73 c | 4.44 d | 11.68 b | 3.7 c | 0.87 b | 8.657 a | 0.003 a |
| Common carp | 8.01 b | 4.63 c | 26.28 a | 15.24 a | 0.0 b | 4.612 ab | 0.002 b |
| Bizz | 4.31 c | 11.5 a | 7.81 b | 15.24 a | 3.91 a | 1.175 b | 0.0 c |
| Shabbout | 13.9 a | 5.03 b | 8.58 b | 8.22 b | 3.99 a | 2.177 b | 0.0 c |

Means having different letter in the same column are significantly different.
The differences at P<0.05.
The means average of 3 replicates.

### 3.6 Microbiological evaluation

Total plate count (TPC), for Psychrophilic count (PPC) and Psychrotrophic (PTC) are shown in table 3.14. TPC revealed that Bizz have significant differences to Silver and Common carp ($P<0.05$) while there were insignificantly differences to Grass carp and Shabbout. In turn, Common and Silver carps have no differences between them; also there were insignificant differences between Grass carp and Shabbout. Although some of them were with no significant differences. Also there were no differences appeared among Common carp, Silver carp, Grass carp and Shabbout. Bizz, Grass carp and Shabbout had low TPC (8.37, 15.75 and 15.49 $\times 10^5$ cfu/gram respectively). Silver and Common carps had high TPC. (25.80 and 21.71 $\times 10^5$ cfu /gram respectively) (Table 3.14).

The results of TPC for all five types were within the standard range ($5 \times 10^5$ -$10^7$ cfu /gram) for fresh fish according to ICMSF (1986) and even lower than results mentioned by other researches of different countries (Huang and leung, 1993). The maximal limit of TPC of the Iraqi standards for fresh fish is $10^7$ (IQS, 2006) so, all types were safe in this respect.

Other local reports have recorded similar evaluations of total plate count; Al-Sheriffi *et al*. (2002), have shown 56 and 65 $\times 10^5$ cfu /gram in carp and Sabour respectively, Salh (2009) found 270, 220, 231, 289 and 264 $\times 10^4$ TPC in Shabbout, Carsean, Gattan, Tawnni and Bizz respectively. The result for other fish also revealed similar results; Koussemon *et al*. (2008) found a range of $2.2 \times 10^4$ - $4.5 \times 10^5$ cfu/ g in *Cyprinus caprio* and *Cybium tritor*. The standard ranging bacterial count of the current and local and non- local studies demonstrated an acceptable quality. The high total plate count, which may exceed the standard maximal limits, may be related to bad handling and mechanical damages during fishing as well as the time of exposing fish to air (Fraser and Sumar, 1998). Again, consumers experienced on choosing a good quality fresh fish and can differentiate good from spoiled one which lead retailer

to avoid exhibiting spoiled fish, as spoilage is almost a result of high bacterial count. Microorganisms are important reason for spoilage because they break down the food into a form they can utilize. Therefore, food quality decreases and spoilage starts at this stage and estimation of the quality of food products relies on the quantification of total numbers of microorganisms (Brownsell *et al*., 1989). TPC. is an indicator of quality, effectiveness of handling procedures, storage conditions and long storage time at chilled temperature. The factors that influence the microbial contamination and growth include fish species, size, method of catch, handling on-board, fishing vessel sanitation, processing, and storage condition (Chen and Chai, 1982; Ward and Baj, 1988).

With respect to Psychrophilic bacterial count (table, 3-14), all types contained Psychrophils of submaximal standard count ($10^6$ cfu /gram fish meat) as mentioned by Suhendan *et al*. (2007), Bizz, Grass carp, Shabbout and Common carp showed low Psychrophilic count (6.83, 12.73, 14.2 and 15.57 × $10^5$ cfu /gram respectively) while high count was seen in Silver carp (63.91 × $10^5$ cfu/gram). Except Silver carp, the other four types have insignificant differences. Similar findings were reported in local and abroad for fish, Salh (2009) recorded that Psychrophilic count in Shabbout, Carsean, Gattan, Tawnni and Bizz were 218, 196, 288, 245 and 223 × $10^4$ cfu /gram flesh respectively, Al-Sheriffi *et al*. (2002) reported values of 8.8 × $10^4$ and 27 × $10^5$ cfu for Psychrophils/gram in carp and Shabbout respectively. Koussemon *et al*. (2008), study on *Cyprinus caprio* and *Cybium Tritor*, found that Psychrophils ranged between 2.2 ×$10^4$ - 4.5× $10^5$ cfu/gram, and Scherer *et al*. (2006) counted (3 × $10^5$ cfu Psychrophils/gram) in Grass carp. Psychrophils on fresh fish are mostly from water source, in contrast TPC which increases due to contamination by the environment, theoretically Psychrophils become decreased after fishing and extraction of fish from water, but the high psychrophilic count may indicate a pollutant water source.

As shown in table 3.14, all five types of fish under study were lower than the maximal standard limits of Psychrotrophic bacterial count which is $10^9$

cfu/gram meats (Gram *et al*., 1989). The mean values of Psychrotrophic count for Bizz, Grass carp and Shabbout were low (7.7, 9.1 and 10.18 × $10^5$ cfu/gram respectively) while they were higher in Silver and Common carps (20.30 and 15.05× $10^5$ cfu/gram respectively). No significant differences among all species were found. Bojanic *et al*. (2009) have shown that the Psychrotrophic bacterial count may be related to the level of bacterial contamination of fish which depended on the environment and bacterial quality of water where fish was caught. Psychrotrophs in water, from which fish were caught, and environment source which include mesophilis that can tolerate and grow in low temperatures. Psychrophils grow in isolation at the temperature of psychrotrophs (20ºC). So that psychrotrophs, which are an expression for low temperature tolerating mesophiles and some of psychrophils, are within the acceptable levels of quality. On this basis psychrotrophes are of important value in assessing food quality with respect to microbial contamination which is almost of environmental source. The count of Psychrotrophic bacteria increased after prolonged storage time of the fish on ice (Gram *et al*., 1989) and so they are a good indicator for storage time.

As shown in table 3.14 *Pseudomonas*, which is responsible for the development of meat spoilage (Olafsdottir *et al*., 2006), counted, as mean value, 49, 59.16, 55.16, 55.33 and 45.75 × $10^2$ cfu/gram meat for Grass carp, Silver carp, Common carp, Bizz and Shabbout respectively. There were no standard limits for *Pseudomonas* either locally or at the international level compare with. However, no significant differences in pseudomonas bacteria count were shown among the five types. In a study it was shown the count of *Pseudomonas* was 3.9 $\log_{10}$ CFu/gram in fish meat (Bojanic *et al*., 2009). *Pseudomonas* increase with the increase of storage time; as Papsdopoulos *et al*. (2003) observed within 15 days of storage, the number of *Pseudomonas* reached to $10^7$cfu/gram fish meat. Colonies picked out randomly from the selective plates for identification of *Pseudomonas* (Citermide agar) to species level, it appeared that all colonies picked from plates of Grass carp, Silver carp, Bizz and Shabbout were

*Pseudomonas aeruginosa* while 8 colonies picked out from Common carp were *P. putida* and one was *P. aeruginosa* (table, 3-14). *Pseudomonas aeruginosa* is found in many natural and domestic environments including plants, soil and surface water, especially warm moist environments containing organic material or contaminated by human or animal waste (Vasconcelos and Swartz, 1976). *Pseudomonas aeruginosa* were found to cause disease for fish; Kumaran *et al.* (2010), isolated *Pseudomonas* spp from internal organs of sea fish, while Vasconcelos and Swartz (1976) mentioned previously that *P. aeruginosa* does not survive in marine environment because of high salinity. However, isolation of *P. aeruginosa* from fish means that water polluted by the neighbor terrestrial environment. In samples collected from the local markets, the highest count of *Pseudomonas* spp. was found in Sarpunti fish ($4.80\times10^7$ cfu/g) while the lowest count was in Kachki fish ($1.31\times10^4$ cfu/g) Among the samples of super shop, the highest count was found in Tilapia ($8.68\times10^5$ cfu/g) and lowest counts of *Pseudomonas* found in both Rui and Ayr fish ($1.35\times10^3$ cfu/g) (Begum *et al*., 2010).

*Pseudomonas* spp. is frequently associated with fish and have been isolated from skin, gills and intestine. Their load is explained by the population density in water. In an aquaculture, especially *P. aeruginosa* and *P. fluorescens* have been considered opportunistic pathogenic species (Altinok *et al*., 2006).

Table 3.14: Microbiological assessment of five types of local fresh fish**.**

| Type of fish | Total plate count $\times 10^5$ | Psychrophilic count $\times 10^5$ | Psychrotrophic count $\times 10^5$ | *Pseudomonas* count | | |
|---|---|---|---|---|---|---|
| | | | | Total count $\times 10^2$ | Identification of some isolates selected | |
| | | | | | *P. aeruginosa* | *P. putida* |
| Grass carp | 15.75 ab | 12.73 b | 9.1 a | 49 a | 12 | - |
| Silver carp | 25.80 a | 63.91 a | 20.70 a | 59.16 a | 12 | - |
| Common carp | 21.71 a | 15.57 b | 15.05 a | 55.16 a | 1 | 8 |
| Bizz | 8.37 b | 6.83 b | 7.7 a | 55.33 a | 12 | - |
| Shabbout | 15.49 ab | 14.2 b | 10.18 a | 45.75 a | 12 | - |

Mean having different letters in the same column are significantly different.
The differences at P<0.05.
The means average of 12 replicates.

### 3.6.1 Microbiological evaluation in farmed and wild sources of fish

The microbiological evaluation of the three farmable types (Grass, Silver and Common carps) for farmed and their wild counterpart are shown in table 3.15. Significant differences (P<0.05) were found in total plate count (TPC), Psychrophilic, Psychrotrophic and *Pseudomonas* count between wild and farmed Grass carp. In wild Grass carp they were 10.75, 9.80, 5.35 $\times 10^5$and 29.66 $\times 10^2$ cfu/g respectively while in their farmed counterparts they were 20.75, 15.66, 12.96$\times$ $10^5$ and 68.33$\times 10^2$ cfu/g respectively.

Wild Silver carp counted 15.51, 17 $\times 10^5$ and 44.66$\times 10^2$ cfu/g for TPC, Psychrophilic and *Pseudomonas* count respectively which are significantly different to their farmed counterparts that counted 36.10, 110.83$\times$ $10^5$ and 73.66

$\times 10^2$ cfu/g meat respectively. There were significant difference in Psychrotrophic bacteria count between farmed ($40.5 \times 10^5$ cfu/g) and wild ($0.9 \times 10^5$ cfu/g) Silver carp were found.

In Common carp there were significant differences ($p<0.05$) in TPC, Psychrophilic and Psychrotrophic count between wild Common carp (11.76, 5.93 and $0.9 \times 10^5$ cfu/g respectively) and the farmed type (31.66, 25.21 and $40.5\times 10^5$ cfu/g respectively). Insignificant differences were seemed for *Pseudomonas* counts between farmed and wild Common carp.

Results with respect to TPC, Psychrophilic, Psychrotrophic and *Pseudomonas* counts for the three types of carps of both farmed and wild sources, revealed good quality as they were within the standard limits (ICMSF, 1986; Suhendan *et al*., 2007; Gram *et al*., 1989).The wild source of three types showed a better quality than farmed type. Indeed, the TPC is a good indicator for fish quality (Gram and Dalgaard, 2002). The Psychrotrophs count were low in wild sources of three types which indicate better quality than their farmed counterparts because Psychrotrophs are responsible for deterioration of fresh fish during storage in cooling and freezing (Fraser and Sumar, 1998). These results revealed that fish farms exposed to contamination more than natural running water which may be due to feeding processes or farming conditions.

Table 3.15: Microbiological assessment of wild and farmed sources of three types of local fresh fish.

| Type of fish | | Test | | | |
|---|---|---|---|---|---|
| | | Total plate count $\times 10^5$ | Psychrophilic count $\times 10^5$ | Psychrotrophic count $\times 10^5$ | *Pseudomonas* count $\times 10^2$ |
| Grass carp | farmed | 20.75 a | 15.66 a | 12.96 a | 68.33 a |
| | wild | 10.75 b | 9.8 b | 5.35 b | 29.66 b |
| Silver carp | farmed | 36.10 a | 110.83 a | 40.5 a | 73.66 a |
| | wild | 15.51 b | 17.00 b | 0.9 b | 44.66 b |
| Common carp | farmed | 31.66 a | 25.21 a | 29.5 a | 59.16 a |
| | wild | 11.76 b | 5.93 b | 0.59 a | 51.16 a |

Means having different letters in the same column are significantly different within same types
The differences at $P<0.05$.
The means average of 6 replicates.

### 3.6.2 Microbiological indicators in licensed and unlicensed markets

Table, 3.16, showed the microbiological assessment of five fish type in M1 and M2, there were significant differences ($P<0.05$) in TPC, Psychrophilic and Psychrotrophic counts between two markets for Bizz and Shabbout. No significant differences were recorded for Grass, Silver and Common carps between M1 and M2. No significant differences were found for Grass carp, Silver carp, Bizz and Shabbout for *Pseudomonas* count between M1 and M2.

TPC, Psychrophilic and Psychrotrophic counts (as mean values) for Bizz in M1 were 4.38, 1.10 and 5.60 $\times 10^5$ cfu/gm respectively while in M2 the counts

were 12.36, 12.56 and $9.83\times10^5$ cfu/gm respectively. For Shabbout in M1 the counts were 5.06, 6.98 and $7.5\times10^5$ cfu/gm respectively while in M2 they were 25.91, 21.56 and $12.81\times10^5$ cfu/gm respectively. There was a significant difference between *Pseudomonas* counts ($P<0.05$) in Common carp between both M1 ($32.66\times10^2$ cfu/gm) and M2 ($77.66\times10^2$ cfu/gm).

Bacterial counts of Bizz and Shabbout from M1 were freshest due to lower total bacterial count even if M2 was also has not exceeded the maximal acceptable limits $10^7$ cfu/gm (ICMSF, 1986). The result indicated that both Bizz and Shabbout from M1 showed better freshness, due to low bacterial count, than Bizz and Shabbout from M2 which also not exceed the maximum limits $10^7$ cfu/gm (Fraser and Sumar, 1998).

Table 3.16: Microbiological assessment of five types of local fresh fish from licensed (M1) and unlicensed (M2) markets.

| Type of fish | | Type of test | | | |
|---|---|---|---|---|---|
| | | Total plate count $\times10^5$ | Psychrophilic count $\times10^5$ | Psychrotrophic count $\times10^5$ | *Pseudomonas* count $\times10^2$ |
| Grass carp | M1 | 14.95 a | 11.05 a | 7.65 a | 36.66 a |
| | M2 | 16.55 a | 14.41 a | 10.66 a | 61.33 a |
| Silver carp | M1 | 22.73 a | 20.61 a | 1.55 a | 49.50 a |
| | M2 | 28.88 a | 107.21 a | 39.85 a | 68.49 a |
| Common carp | M1 | 20.15 a | 14.36 a | 5.14 a | 32.66 b |
| | M2 | 23.28 a | 16.78 a | 24.96 a | 77.66 a |
| Bizz | M1 | 4.38 b | 1.10 b | 5.60 b | 50.66 a |
| | M2 | 12.36 a | 12.56 a | 9.83 a | 60.00 a |
| Shabbout | M1 | 5.06 a | 6.98 b | 7.5 b | 41.16 a |
| | M2 | 25.91 a | 21.56 a | 12. 81 a | 50.33 a |

Means having different letters in the same column are significantly different within same types
The differences at $P<0.05$.
The means average of 6 replicates.

### 3.6.3 Miscellaneous microbiological evaluation

The results of two miscellaneous bacterial indicators are shown in table 3.17. *Vibrio* species were detected in three types of fish (Silver carp, Common carp and Bizz) with a total number of 0.658, 0.908, 1.942×10 cfu/gm respectively, while it was not detected in both Grass carp and Shabbout. Some colonies were picked up randomly from the selective plates for *Vibrio* (TCBS agar) and fully identified at species level, the results indicated that *V.metschnikovii* isolated from Silver carp and Bizz , while *V. alginolyticus* was isolated from Common carp (table 3.17). Bizz differs significantly from both Grass carp and Shabbout while insignificant difference was found between the latter two. In turn Common and Silver carps have no differences between them; there were also no significant differences between Grass carp and Shabbout; although *Vibrio* was not isolated from Grass carp. There are no authentic standards for *Vibrio* species in fish but surveillances take place on fresh water to monitor the presence of pathogenic *vibrio* such as *V. cholera* and *V. parahaemolyticus* (Begum *et al.*, 2010) and to evaluate the non-pathogenic *Vibrios* in sea or aquatic foods that may cause spoilage. Iraqi standard concentrated only on *V. parahaemolyticus* as a pathogenic species in fresh fish (IQS, 2006) and so the samples with these respects were safe.

*Vibrio* exists in aquatic environment (Uchiyama, 2000). The members of the family *Vibrionaceae* also contribute 60% of the total bacterial population of the coastal ecosystems (Simidu and Tsukamoto, 1985). Since *Vibrio* species are isolated from water, sediment, invertebrates, and fishes, so, it can be considered as autochthonous marine and estuarine microflora (Grimens *et al.*, 1986). *Vibrio* Spp. are predominant spoilage species which has been isolated from chilled fresh fish under aerobic conditions (Hozbor *et al*., 2006). Koussemon *et al*. (2008) found that *Vibrio* spp was not isolated from the aquatic *Cyprinus caprio, Arius* spp and *Cybium tritor*. *Vibrio* species which cause spoilage, produce high amount of volatile compounds (trimethylamine, volatile sulfur compounds, aldehydes, ketones, esters, hypoxanthine as well as other low molecular weight

compounds) (Gramand Huss, 1996). Basti *et al.* (2006) isolated *Vibrio parahaemolyticus* from Alosa kessler, Silver carp, *Liza Aurata* collected from Gaylan markets in Iran. Hadin *et al*. (2004) detected the presence of eight potentially pathogenic Vibrio species, with overall incidence in the samples as 4.6% for *V. cholerae*, 4.7% for *V. parahaemolyticus*, 6.0% for *V. vulnificus*, 11% for *V. alginolyticus*, 9.9% for *V. metschnikovii*, 1.3% for *V. mimicus*, 13% for *V. damsela*, 7.6% for *V. fluvialis* and 52% for a combined population of all of the above from fish samples.

*Aeromoans species* were isolated from Silver carp, Common carp and Shabbout with a total count of 3.325, 2.842 and 0.450× 10 cfu/gm respectively, while not isolated from Grass carp and Bizz (table 3.17). Silver and Common carps differ significantly with the other three types while there was no significant difference in between. The total number in Shabbout was too low where this was no significant difference with both Grass carp and Bizz which failed to isolate *Aeromonas.* Some colonies were picked up randomly from the selective plates for *Aeromonas* (starch ampicillin agar) and fully identified at species level, it was appeared that *A. hydrophila* was isolated from Silver carp and Shabbout, while three of the seven colonies picked up from Common carp were *A. hydrophila* and the remaining four were *A. caviae* (table 3.17). There were no international or local standard of *Aeromonas* in aquatic fresh fish.

*Aeromonads* are Common inhabitants of most types of food, regardless of geographic origin. Palumbo *et al*. (1985) found *Aeromonas* isolates universally present in all foods tested, including sea foods, raw milk, chicken, and meats such as lamb, veal, pork, and ground beef. Initial counts in these foods ranged from $10^2$ to $10^5$ cfu/g at 5°C, after seven-day period at refrigeration temperatures. *Aeromonas* have the largest numbers recorded for fish (Neyts *et al.*, 2000; McMahon and Wilson, 2001).

The microflora consisting of *Aeromonas Spp., pseudomonas, Acinetobacter, Moraxella and Vibrionacea* were reported in newly caught fish of tropical Indian water (Huss, 1995). The off-odors produced by fish are

attributed to the activity of *Aeromonas* spp. through the reducing of trimethylamine- oxide (TMAO) to trimethylamine (TMA) and producing hydrogen Sulphide ($H_2S$) from amino acids (Gram *et al*., 1990).

*Shawanella putrefaciens,* which is a standard indicator of sea food spoilage, is advised to be inspected in aquatic fish also but with minimum importance (Huss, 1995) it has been inspected here but all sample failed to isolate it. This indicate reservoir of this species is saline water and found in little numbers in fresh water especially those waters far from the estuaries. In Iraq, the only study related with isolation of *Shawanella* was an M.Sc thesis conducted on clinical cases in Basrah city which including estuary water (Ataia, 2006).

Table 3.17**:** Miscellaneous microbiological evaluation of five types of local fresh fish.

| Type of fish | *Vibrio* spp. | | | *Aeromonas spp* | | |
|---|---|---|---|---|---|---|
| | Total count ×10 | Identification of some selected isolates | | Total count ×10 | Identification of some selected isolates | |
| | | *V. metschnikovii* | *V. alginolyticus* | | *A. caviae* | *A. hydrophila* |
| Grass carp | 0.0 b | - | - | 0.00 b | - | - |
| Silver carp | 0.658 ab | 2 | - | 3.325 a | - | 6 |
| Common carp | 0.908 ab | - | 4 | 2.842 a | 4 | 3 |
| Bizz | 1.942 a | 4 | - | 0.0 b | - | - |
| Shabbout | 0.0 b | - | - | 0.450 b | - | 2 |

Mean having different letter in the same column are significantly different.
The differences at P<0.05.
The means average of 12 replicates.

### 3.7 Sensory evaluation

The results in table 3.18, show significant differences ($P<0.05$) in the sensory properties (color, flavor, tenderness, juiciness, and overall acceptance) among the five species of fish. Bizz obtained the highest scores (3.33, 3.67, 4.17, 3.83 and 4.00 for the attributed above respectively) followed by the Grass carp which had insignificant difference with Bizz (3.33, 3.25, 3.92, 3.58 and 3.58), and then lower scores were shared by the remaining three species (Shabbout: 3.00, 3.33, 3.42, 3.33 and 3.25, Common carp: 2.83, 3.00, 3.50, 3.08 and 3.00, Silver carp: 2.50, 2.42, 2.92, 2.75 and 2.75). It was clearly appeared that Silver carp has awarded the lowest scores. Even if sardine is a marine type of fish, Kilinc and Cakli (2004) found that it scored evaluations were close to the aquatic types. In their study where they scored 4.43, 4.57, 4.43 and 4.43 for flavor, smell, texture and overall acceptance respectively using the same grading used here.

Meat and fish spoilage means a sequence of changes that are readily detectable by the human senses (sight touch, smile and taste). Sensory evaluation is commonly utilized in determining the shelf–life of muscle food products simultaneously with both analytical and affective tests that allow the best determination of how long the product will have acceptable quality, expiration and freshness assurance (Leo and Fidel Toldra, 2010). Sensory evaluation of fish is the most popular way of assessing the freshness of fish; it is fast, simple and provides immediate quality information. The sensory features are clearly able to be seen to the consumer and are essential for consumer satisfaction (Reineccius, 1990). So that it is logic that, according to their satisfaction, consumers affect the fresh fish quality, and then the acceptable scores here, that fish consumers expect a product that is safe and has good appearance, odor, taste and texture (Warm *et al*., 2000; Parisi *et al*., 2002) otherwise the fresh fish are not displayed in markets, whether licensed or unlicensed.

The lipid content in fish meat can impact the product quality and auto–oxidative deterioration of unsaturated fatty acid, resulting in product deterioration and production of undesirable aroma and flavor (Mottram, 1998). It is observed that the lower scores of the sensory attributes may be related to the oxidative rancidity of fish (Cheng and Ockerman, 1998) which is caused by the higher rate of lipid oxidation that consequently affects the flavor and overall acceptability (Ikeme, 1993).

Table 3.18: Sensory evaluation of five types of local fresh fish.

| Type of fish | Score | | | | |
|---|---|---|---|---|---|
| | tenderness | juiciness | color | flavor | overall acceptability |
| Grass carp | 3.92* ab | 3.58 ab | 3.33 a | 3.25 ab | 3.58 ab |
| Silver carp | 2.92 d | 2.75 d | 2.50 b | 2.42 c | 2.75 c |
| Common carp | 3.50 bc | 3.083 ab | 2.833 b | 3.00 b | 3.00 bc |
| Bizz | 4.17 a | 3.83 a | 3.33 a | 3.67 a | 4.00 a |
| Shabbout | 3.42 c | 3.33 ab | 3.00 ab | 3.33 ab | 3.25 bc |

*From 5 degree grading
Mean having different letters in the same column are significantly different.
The differences at P<0.05.
The means average of 12 replicates.

### 3.7.1 Sensory evaluation for farmed and wild sources

The sensory evaluation of the three farmable types (Grass, Silver and Common carps) for farmed and their wild counterpart are shown in table 3.19. Wild Grass carp scored 4.33, 4.00, 3.83, 3.50 and 4.17 for tenderness, juiciness, color, flavor and overall acceptability respectively, while scored 3.50, 3.17, 2.83, 3.00 and 3.00 in the farmed fish. Wild Silver carp scored 3.17, 3.00, 2.83, 2.83 and 3.17 while scored 2.66, 2.50, 2.17, 2.00 and 2.33in farmed fish. Wild Common carp scored 3.83, 3.33, 3.33, 2.50 and 3.67 while in farmed fish scored

3.17, 2.83, 2.33, 2.50 and 2.33. So, with respect to sensory properties, there were significant differences between farmed and wild fish for all the three types ($p<0.05$) with the exception for one of tenderness in Silver carp (table 3.19). It was clearly appeared that, even if the two sources of carps scored acceptable results, the wild source scored the most acceptable than the farmed source. The difference between wild and farmed fish source evaluation may relate to the feeding pattern of both and then the high percent of the proximate composition of farmed fish (Jankowska *et al*., 2003). It was reported that farmed fish are less firm than wild fish, this is possibly attributed to a higher fat content in farmed fish (Lie, 2001). Diet is also known to influence the organoleptic quality of fish (Spinelli *et al*., 1979).

Table 3.19: Sensory evaluation for wild and farmed sources of three types of local fresh fish.

| Type of fish | Breed | Sensory evaluation | | | | |
|---|---|---|---|---|---|---|
| | | tenderness | juiciness | color | flavor | overall acceptability |
| Grass carp | farmed | 3.50 b | 3.17 b | 2.83 b | 3.00 b | 3.00 b |
| | wild | 4.33 a | 4.00 a | 3.83 a | 3.50 a | 4.17 a |
| Silver carp | farmed | 2.66 a | 2.50 b | 2.17 b | 2.00 b | 2.33 b |
| | wild | 3.17 a | 3.00 a | 2.83 a | 2.83 a | 3.17 a |
| Common carp | farmed | 3.17 b | 2.83 a | 2.33 b | 2.50 b | 2.33 b |
| | wild | 3.83 a | 3.33 a | 3.33 a | 2.50 a | 3.67 a |

*From 5 degree scoring
Means having different letters in the same column are significantly different within same types
The differences at $P<0.05$.
The means average of 6 replicates.

### 3.7.2 Sensory evaluation in licensed and unlicensed markets

When the sensory evaluations of the five fish types were compared in accordance to the license markets (M1) and unlicensed markets (M2) (table

3.20). Common carp showed no significant difference between two markets for all sensory attributes. Silver carp showed the only a difference for juiciness. Grass and Shabbout showed differences for two attributes (tenderness and flavor for grass carp and tenderness and color for Shabbout). Bizz has shown the apparent significant differences ($p<0.05$) in three attributes (tenderness, juiciness and overall acceptable) out of five. The most probable interpretation for the relatively unsuitability of unlicensed markets for the unfarmed Bizz type may be high deterioration rate in comparison to other types which may be avoided in licensed markets.

Table 3.20: Sensory evaluation of five types of local fresh fish from licensed (M1) and unlicensed (M2) markets.

| Type of fish | Market | Sensory evaluation | | | | |
|---|---|---|---|---|---|---|
| | | tenderness | juiciness | color | flavor | overall acceptability |
| Grass carp | M1 | 4.33* a | 3.67 a | 3.50 a | 3.50 a | 3.67 a |
| | M2 | 3.50 b | 3.50 a | 3.17 a | 3.00 b | 3.50 a |
| Silver carp | M1 | 3.00 a | 3.00 a | 3.00 a | 2.50 a | 2.50 a |
| | M2 | 2.83 a | 2.50 b | 2.50 a | 2.50 a | 2.33 a |
| Common carp | M1 | 3.50 a | 3.33 a | 3.17 a | 3.17 a | 3.33 a |
| | M2 | 3.50 a | 2.83 a | 2.50 a | 2.83 a | 2.67 a |
| Bizz | M1 | 4.50 a | 4.33 a | 3.83 a | 3.83 a | 4.50 a |
| | M2 | 3.83 b | 3.33 b | 2.83 a | 3.50 a | 3.50 b |
| Shabbout | M1 | 3.83 a | 3.50 a | 3.50 a | 3.50 a | 3.67 a |
| | M2 | 3.00 b | 3.17 a | 2.50 b | 3.17 a | 2.83 a |

*From 5 degree scoring
Means having different letters in the same column are significantly different within same types
The differences at $p<0.05$.
The means average of 6 replicates.

### 3.8 Heavy metals analysis

#### 3.8.1 Mercury (as methylmercury)

The results in figure 3.8 showed significant differences ($P<0.05$) in the methyl mercury concentrations among all types of fish. Shabbout, Silver carp, Bizz and Grass carp recorded lower methyl mercury concentrations, which were 0.083, 0.112, 0.158and 0.228 ppm respectively, while Common carp was contained 0.407ppm which is rather high. All results have not reached the USEPA maximal permissive level of mercury concentration (1.0ppm) but the Common carp has exceeded the maximal level of Japan standards for mercury (0.3ppm) on the basis of wet weight (FAO, 2011; Dickman and leung, 1998; Wagemann *et al*., 1998). Similar results were reported by Ahmed and Hassein (2004) who showed methylmercury concentrations 0.32, 0.17 ppm in the aquatic *M.cephalus* and *T.nilotica* respectively on the basis of wet weight. Also Little john (1984) suggested that the mercury concentrations in muscle tissue of Common carp ranged from 0.05 to 0.78 ppm based on wet weight, which mimic the current results. The variations in methylmercury are attributed to factors such as species, geographical, location, size, sex and period of catch (WHO, 1990).

Mercury is accumulated in fish as methylmercury (Al- Safy and Hamouda, 2009), Studies have shown that omnivorous fish like Common and Silver carp accumulate Hg from surrounding water. McKinney and Rogers (1992) mentioned that mercury gets into the body through the respiratory and gastrointestinal tract and is accumulated as alkyl-Hg into the liver, kidney, nervous tissues and even in central nervous system (CNS) tissues. So, theoretically it is increases within age and in the predatory fish, which may accumulate high concentration of methyl mercury resulted from preying on other fish. As WHO reported in 1990, in most species of fish, the mercury is less than 0.5 ppm but the higher levels of mercury are found in predator fish at the top of the food chain, such as sword fish, tuna and shark, that generally show

mercury concentrations between 0.2 and 1.5 ppm, which can also reach up to 5 ppm. Because of mature Bizz, which reach their sexual maturity within eight years (Ahmed, 1987), has not been sold at the period of sample collection, the Bizz samples were immature as their age were evaluated to be between 1-2 years. Thus, in Bizz the methylmercury value here may not reflect the true concentration if we also know that Bizz is a carnivore's fish. Work so should therefore be done on mature Bizz.

According to the U.S food and drug administration (FDA), heavy metals, such as mercury, occur naturally and can be added to the environment by industrial pollution (Khalifa *et al.*, 2010). It is confirmed that the major health impacts caused by mercury level effect people who are not working directly in mercury related industry, but who have a regular fish diet that has mercury above the permissible limit (Quiroga *et al.*, 2000). Generally, the differences in contaminant burdens between the different species were related to the physiological differences between different species (Goldstein *et al*., 1996) or the quantity and type of food taken by different populations of the same species (Kim,1995).

Methylmercury is toxic to the human glial cells, causing Schwann cell production of myelin, neuropsychotic, severe developmental CNS abnormalities in fetus and prolonged action potentials, as fish intake is the major source of exposure of human to mercury, mainly in the form methyl mercury, which it accumulates from the surrounding waters, this is a cause of cancer (Khalifa *et al.*, 2010).

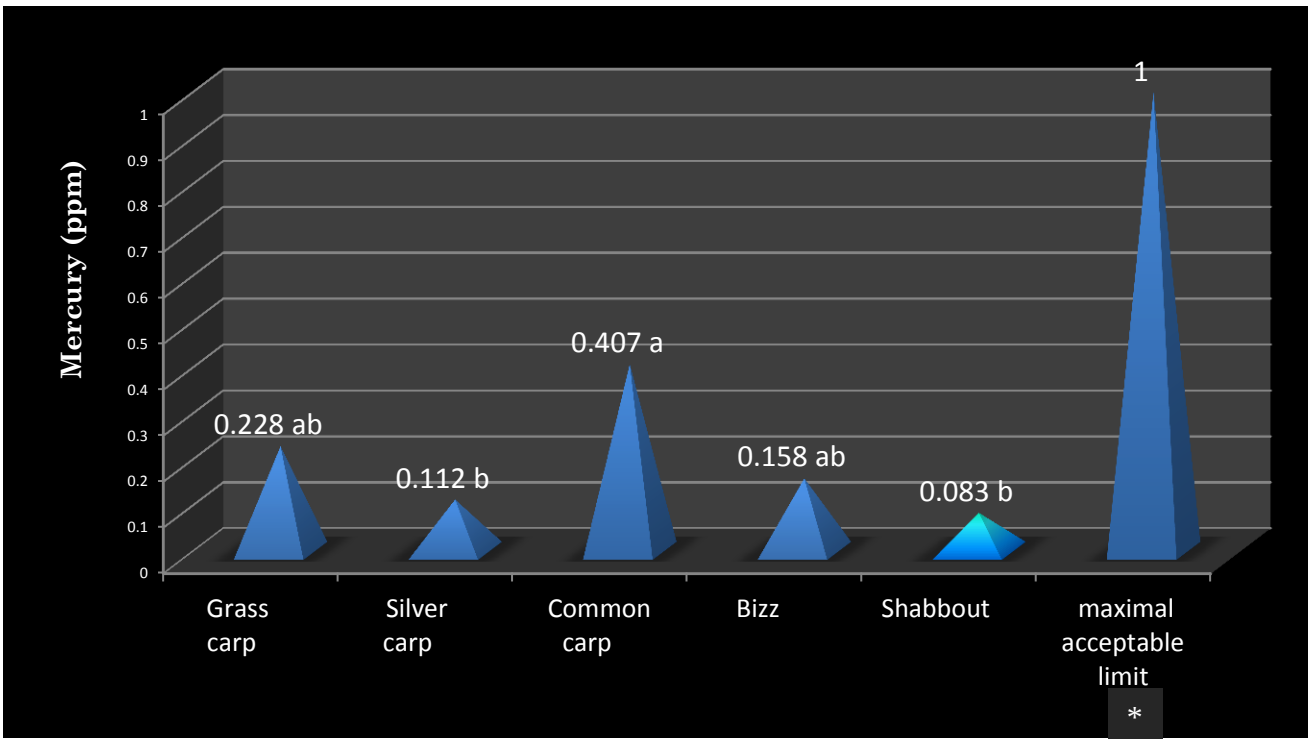


Figure 3.8: levels of Mercury for five types of local fresh fish in ppm.

Mean having different letters are significantly different.
The differences at P<0.05.
The means average of 12 replicates.
*FAO, 2011

### 3.8.2 Cadmium (Cd)

The cadmium level in all five types ranged 0.451 -0.475 ppm (figure 3.9). This range is within the legal limited of Cd of sea fish; 2.0, 0.86 and 0.77 ppm for sardines and macherel fish (WHO, 1990).

The resulted values were less than the Cd concentration of Common carp monitored by Tariq *et al*.(1994) who found the Cd concentration were 0.51ppm, but were higher than the Cd level in Common carp in Turkey, which was 0.17 ppm (Öztürk *et al*., 2009 ). However, Cd concentration Common carp ranged between 1.110 and 1.653 ppm in flesh body (Vinodhini and Narayanan, 2008). The fresh Suboor, Jaffout, Biyah and Thelah in Iraq were shown to contain Cd in concentrations of 76.30, 54.92, 54.24 and 42.11 µg/100gm respectively (Al-Shatty, 2006) while the current obtained results were less than the Cd level in the aquatic species *M.cephalus* and *T.nilotica* which were 0.60 and 0.77 ppm respectively (Ahmed and Hussein, 2004). Cadmium is of even

greater concern because of its harmful effects on plants, animal and humans. Cadmium causes itai-itai disease; this disease is known to damage the joints, cause bones to soften and the body to shrink while the affected person dies a painful death (Ademoroti, 1996).

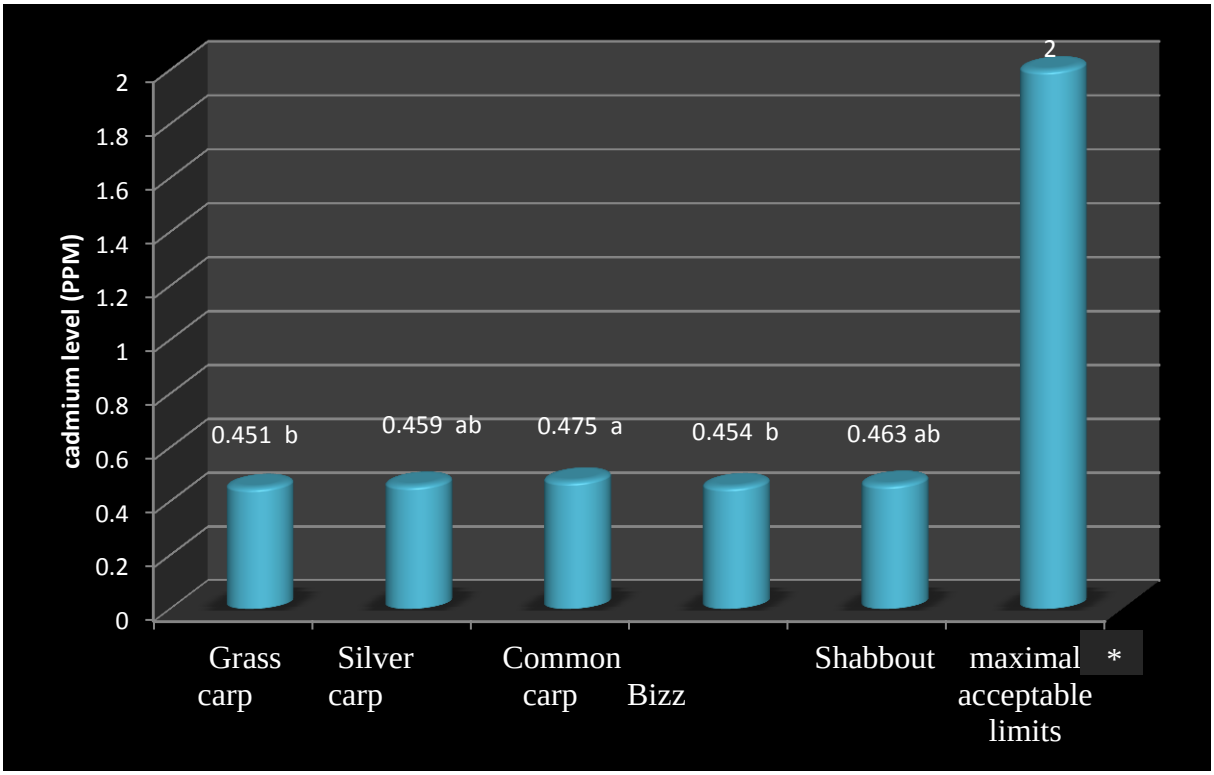


Figure 3.9: levels of cadmium for five types of local fresh fish in ppm.

Mean having different letters are significantly different.
The differences at P<0.05).
The means average of 12 replicates.
*WHO, 1990

### 3.8.3 Lead (Pb)

Lead (Pb) concentrations for the five types of fish are shown in figure (3.10). They ranged from 0.306 to 0.364 ppm, which is less than the maximal limit according to the British Pb permissible limit (2.0 ppm) for sea food (fish) (WHO, 1990). MAFF (1993) indicated that the standard concentration of lead should not exceed 2 ppm (wet weight). Also the Pb levels of this study are less than that reported by Vinodhini and Narayanan (2008) and Öztürk *et al.* (2009), who showed that lead concentrations in fish flesh in Common carp in Turkey,

were 2.39 and 2.14 ppm respectively. Similar findings were observed by Little John (1984) who recorded that lead level in Common carp ranged from 0.09 to 2.4 ppm. the source of lead was due to emission from vehicle also from batteries (Al-Shatty, 2006).

Lead is known to cause a disease called plumbism; it accumulates in aquatic biomass; and is concentrated and passed up the food chain to consumers. Lead is also known to damage the brain through the central nervous system, kidneys and liver (Ademoroti, 1996).

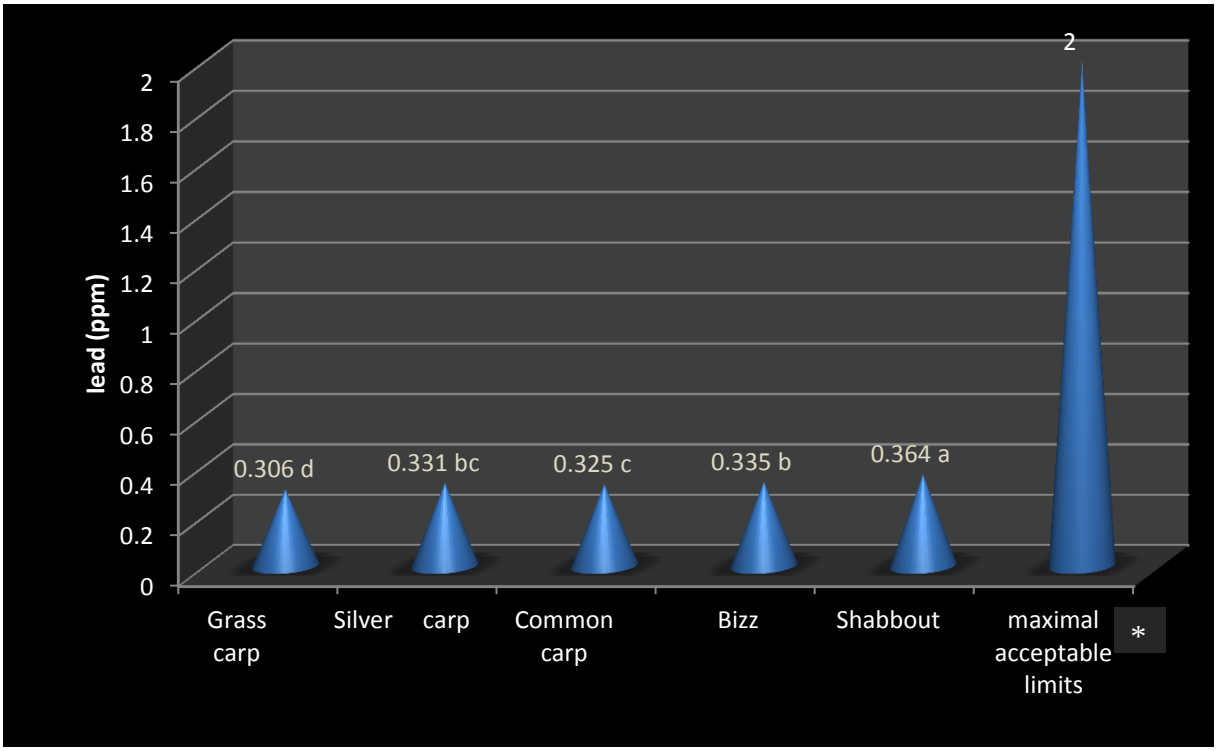


Figure 3.10: levels of lead for five types of local fresh fish in ppm.

Mean having different letters are significantly different.
The differences P<0.05.
The means average of 12 replicates.
*WHO, 1990

### 3.8.4 Heavy metals residue for farmed and wild sources

The heavy metals residue of the three farmable types (Grass, Silver and Common carps) for farmed and their wild counterpart are shown in table 3.21. There were insignificant differences in concentrations of heavy metals residue in farmed and wild Grass carp meat which valued 0.399, 0.462, and 0.310 ppm for

mercury, cadmium, and lead respectively in wild species, while farmed type recorded 0.056, 0.440, and 0.303 ppm respectively.

Silver carp meat showed a significant difference ($P<0.05$) in concentration of mercury between wild and farmed sources which were 0.143 ppm for wild and 0.081ppm for farmed. There were insignificant differences recorded between farmed and wild Silver carp meat with respect to cadmium and lead concentrations which were 0.451 and 0.329 ppm in farmed respectively and 0.467 and 0.329 ppm respectively in wild. There were significant differences in concentrations of mercury and cadmium between farmed and wild Common carp meat; mercury and cadmium concentrations were 0.099 and 0.492 ppm in farmed respectively and were 0.715 and 0.479 ppm in wild respectively. There were insignificant difference in lead concentration between farmed and wild Common carps; being 0.323 ppm in farmed and 0.327 ppm in wild.

Both farmed and wild Grass carp had similar heavy metal residues which may be related for Grass carp is being herbivores and so is not suitable to use in monitoring heavy metals concentration whereas the omnivores Common and Silver carps are more adequate for assessing heavy metals in fresh water. Furthermore, Carnivorous fish was suggested to be a good indicator for monitoring of mercury pollution while other species with other feeding habits should also be monitored for human health aspects (Vigh *et al*., 1996). These differences in mercury and cadmium between farmed and wild omnivore tested in this study (high in wild and low in farmed) indicate, without any doubt, that the natural water sources at which the tested fish were hunted are polluted.

The significant differences in concentrations of heavy metals residue between wild and farmed sources is related to industrial pollution which are discarded waste product to river runways and affected to increase concentration in wild inhabitant fish (Khalifa *et al.*, 2010). With respect to wild habitat, it was reported that variations in concentration within species may come about through the migration of fish species from unpolluted areas to relatively more polluted areas (AL-Majid and Preston, 2000).

Table 3.21: Heavy metal residue for wild and farmed sources of three types of local fresh fish.

| Type of fish | Breed | Type of test (ppm) | | |
|---|---|---|---|---|
| | | mercury | cadmium | Lead |
| Grass carp | farmed | 0.056 a | 0.440 a | 0.303 a |
| | wild | 0.399 a | 0.462 a | 0.310 a |
| Silver carp | farmed | 0.081 b | 0.451 a | 0.329 a |
| | wild | 0.143 a | 0.467 a | 0.329 a |
| Common carp | farmed | 0.099 b | 0.492 b | 0.323 a |
| | wild | 0.715 a | 0.479 a | 0.327 a |

Means having different letters in the same column are significantly different within same types
The differences at $P<0.05$.
The means average of 6 replicates.

# Chapter4. Conclusions and recommendations

## Conclusions

1. The findings related to organoleptic, chemical, physical and microbiological test as well as heavy metals residues were similar for all five types of fishes, hence, species do not constitute an important factor in all parameter which were obtained.
2. Silver carp only showed thiobarbituric acid (TBA) above the standard limits.
3. The farmable fish types showed that wild fish caught characterized with freshness criteria higher than their farmed counterparts.
4. All five types from licensed markets demonstrated to have freshness exceeded that fish sold from unlicensed markets.
5. The best fresh fish type among the five types tested was Bizz, followed by Shabbout, Grass carp, Common carp while the lowest one was Silver carp tables 4.1 and 4.2.

Table 4.1: Conclusion of all results

| Fish type | standard | Grass carp | Silver carp | Common carp | Bizz | Shabbout |
|---|---|---|---|---|---|---|
| QIM score | 1-10 | (7.00) ↑ | (10.667) ↓ | (9.167 ) ↑ | (6.667 )↑ | (8.167)↑ |
| Extract release volume ( ml) | | (13.208) ↑ | (16.271) ↓ | (15.492) ↕ | (11.150) ↑ | (14.050) ↑ |
| Water holding capacity % | | (22.833)↑ | (15.917) ↓ | (18.250)↑ | (29.083) ↑ | (21.083)↑ |
| Cooking drip % | | (31.658) ↑ | (42.994)↓ | (40.346)↓ | (28.830) ↑ | (37.732) ↕ |
| Density (floating) | | (1.008) ↕ | (1.006) ↓ | (1.017)↑ | (1.019) ↑ | (1.011)↑ |
| pH | | 6.761 | 6.605 | 6.612 | 6.978 | 6.699 |
| Total volatile nitrogen mg N /100 g | 20 mg N /100 g | (12.894)↑ | (16.414)↑ | (15.307)↑ | (12.228)↑ | (13.605)↑ |
| Trimethylamine mg N / 100 g | 10-15mg N /100 g | (3.429)↑ | (5.576)↑ | (4.52)↑ | (2.324)↑ | (3.293)↑ |
| Dimethylamine mg N / 100 g | 0.4 mg N /100 g | (0.1423)↑ | (0.3658)↑ | (0.3052)↑ | (0.1442)↑ | (0.1612)↑ |
| Ammonia mg N / 100 g | 10 mg N /100 g | (0)↑ | (0.1359)↑ | (0.0002)↑ | (0)↑ | (0)↑ |
| Free fatty acids ( %) | 1.50% | (1.23)↑ | (5.2) ↓ | (3.48) ↓ | (0.87)↑ | (2.11) ↓ |
| Peroxide value (meq oxygen/kg lipid) | 10 meq oxygen/kg lipid | (4.47)↑ | (8.86)↑ | (7.25)↑ | (3.4)↑ | (5.29)↑ |
| Thiobarbituric acid (mg malonldehyde /kg lipid) | 5 mg malonldehyde /kg lipid | (0.84)↑ | (1.36)↑ | (1.24)↑ | (0.77)↑ | (0.93)↑ |
| Biogenic amines Index | 1-10 | (1.673)↑ | (8.657) ↕ | (4.612) ↑ | (1.175)↑ | (2.177) ↑ |
| H x | | (0.0) ↑ | (0.003) ↑ | (0.002) ↑ | (0.0) ↑ | (0.0) ↑ |
| Total plate count $\times 10^5$ | $10^5$-$10^7$ | (15.75) ↑ | (25.80) ↑ | (21.71) ↑ | (8.37) ↑ | (15.49)↑ |
| Psychrophilic count $\times 10^5$ | $10^6$ | (12.73)↕ | (63.91) ↕ | (15.57) ↕ | (6.83) ↑ | (14.2)↕ |
| Psychrotrophic count $\times 10^5$ | $10^9$ | (9.1) ↑ | (20.70) ↑ | (15.05) ↑ | (7.7) ↑ | (10.18) ↑ |
| pseudomonas count ×10 | | (49) ↑ | (59.16) ↑ | (55.16) ↑ | (55.33) ↑ | (45.75) ↑ |
| overall acceptable | | (3.58) ↑ | (2.75) ↕ | (3.00)↑ | (4.00) ↑ | (3.25) ↑ |
| mercury | 0.5 ppm | (0.228) ↑ | (0.112) ↑ | (0.407) ↑ | (0.158) ↑ | (0.083) ↑ |
| cadmium | 2 ppm | (0.451) ↑ | (0.459) ↑ | (0.475) ↑ | (0.454) ↑ | (0.463) ↑ |
| lead | 2 ppm | (0.306) ↑ | (0.331) ↑ | (0.325) ↑ | (0.335) ↑ | (0.364) ↑ |

↑: Acceptable result.
↓: UN acceptable result.
↕: moderate result.

Table 4.2: Scoring of fishes

| Fish type | standard | Grass carp | Silver carp | Common carp | Bizz | Shabbout |
|---|---|---|---|---|---|---|
| QIM score | 1-10 | 4 | 1 | 2 | 5 | 3 |
| Extract release volume ( ml) | | 4 | 1 | 2 | 5 | 3 |
| Water holding capacity % | | 4 | 1 | 2 | 5 | 3 |
| Cooking drip % | | 4 | 1 | 2 | 5 | 3 |
| Density (floating) | | 2 | 1 | 4 | 5 | 3 |
| pH | | 4 | 1 | 2 | 5 | 3 |
| Total volatile nitrogen mg N /100 g | 20 mg N /100 g | 4 | 1 | 2 | 5 | 3 |
| Trimethylamine mg N / 100 g | 10-15mg N /100 g | 4 | 1 | 2 | 5 | 3 |
| Dimethylamine mg N / 100 g | 0.4 mg N /100 g | 4 | 1 | 2 | 5 | 3 |
| Ammonia mg N / 100 g | 10 mg N /100 g | 4 | 2 | 3 | 5 | 4 |
| Free fatty acids ( %) | 1.50% | 4 | 1 | 2 | 5 | 3 |
| Peroxide value (meq oxygen/kg lipid) | 10 meq oxygen/kg lipid | 4 | 1 | 2 | 5 | 3 |
| Thiobarbituric acid (mg malonldehyde /kg) | 5 mg malonldehyde /kg | 4 | 1 | 2 | 5 | 3 |
| Biogenic amines Index | 1-10 | 4 | 1 | 2 | 5 | 3 |
| H x | | 5 | 3 | 4 | 5 | 5 |
| Total plate count $\times 10^5$ | $10^5$-$10^7$ | 3 | 1 | 2 | 5 | 4 |
| Psychrophilic count $\times 10^5$ | $10^6$ | 4 | 1 | 2 | 5 | 3 |
| Psychrotrophic count $\times 10^5$ | $10^9$ | 4 | 1 | 2 | 5 | 3 |
| pseudomonas count ×10 | | 4 | 1 | 3 | 2 | 5 |
| overall acceptable | | 4 | 1 | 2 | 5 | 3 |
| mercury | 0.5 ppm | 4 | 4 | 5 | 3 | 5 |
| cadmium | 2 ppm | 5 | 3 | 1 | 4 | 2 |
| lead | 2 ppm | 5 | 3 | 4 | 2 | 1 |
| total | | 52 | 33 | 56 | 106 | 74 |
| Score | | 4 | 5 | 3 | 1 | 2 |

# Recommendations

1- For the purposes of maintaining food safety and assurance, Iraqi food quality control regulations should contained local standard limits for all tests of the parameters included in this study. This should also be applied in Kurdistan Region.

2- To fit the quality assurance, fresh fish should only be sold in licensed markets.

3- There is an urgent need to investigate the sources of heavy metals in the waters of local rivers and lakes.

4- Although caught wild fish have better quality than farmed ones, this does not mean we should the farms which are needed to ensure food security.

5- Food quality control regulation should be applied to the imported fish of different origin.

6- Improvement of fish handling and marketing.

7- Further quality assessments and keeping quality on the fishes.

8- Decide to use the rapid methods for analyzing fish for their freshness.

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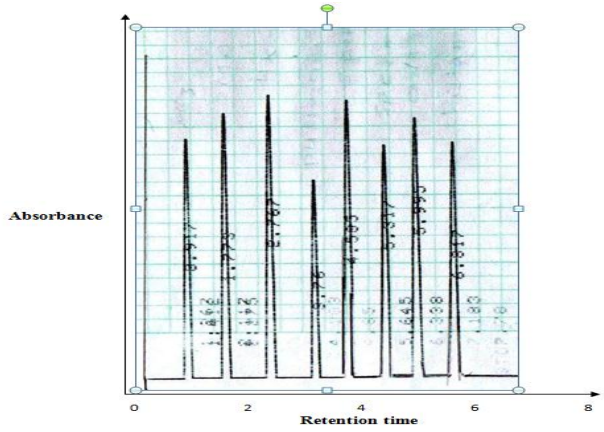
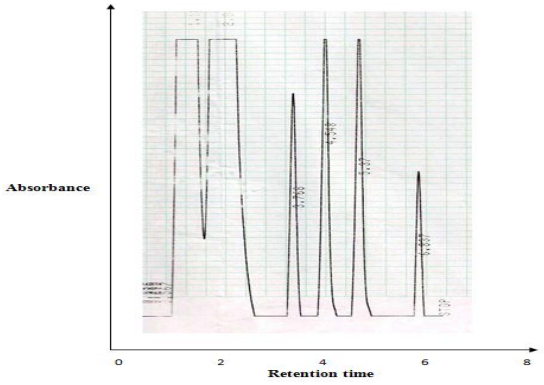
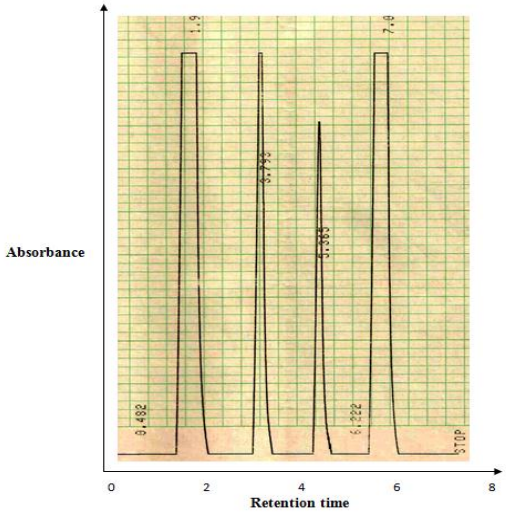
Appendix 1: Chromatogram of standard and five types of fish.



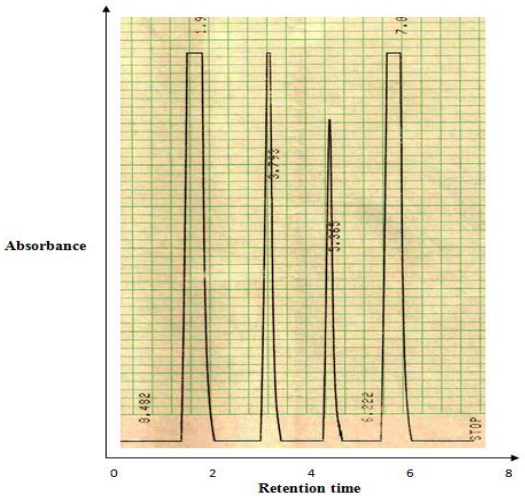

A



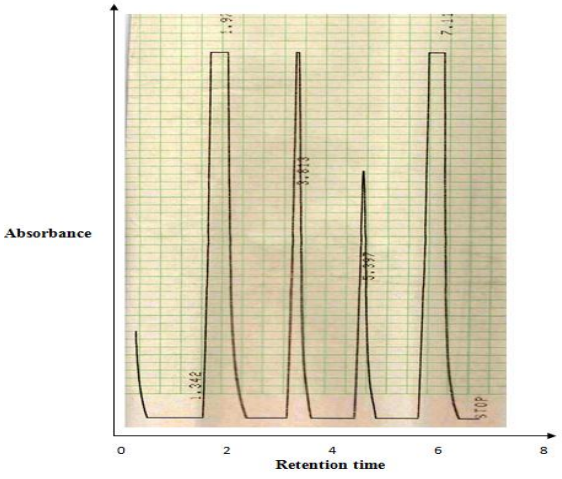

B



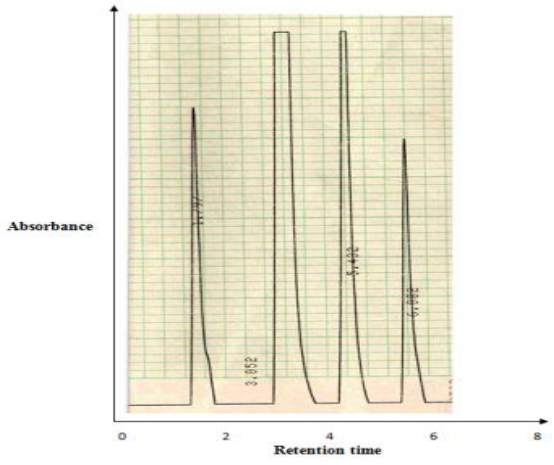

C




D




F

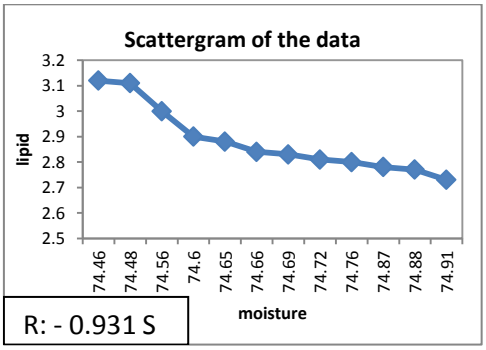



E

| Test | Retention time /min (standard) | Retention time/min (sample) | Concentration of standard µg/ml |
|---|---|---|---|
| **Ammonia** | **0.917** | **0.91** | **5** |
| **dimethylamine** | **1.77** | **1.7** | **5** |
| **Hypoxanthine** | **2.76** | **2.7** | **5** |
| **putrescine** | **3.76** | **3.76** | **5** |
| **Spermidine** | **4.5** | **4.54** | **5** |
| **spermine** | **5.31** | **5.37** | **5** |
| **Cadaverine** | **5.99** | **5.90** | **5** |
| **histamine** | **6.81** | **6.83** | **5** |

A: standard, B: Grass carp, C: Silver carp, D: Common carp, E: Bizz, F: Shabbout.

Appendix 2 : The relationship between moisture and lipids in five types of fish.

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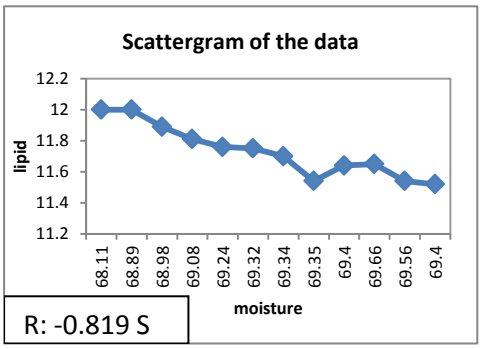


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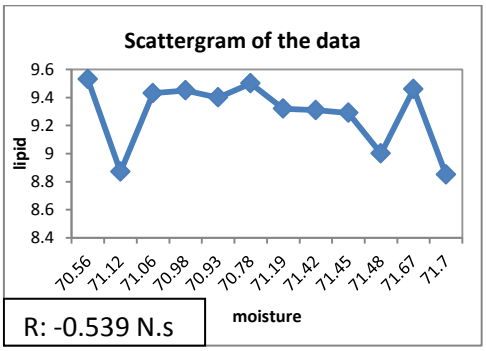


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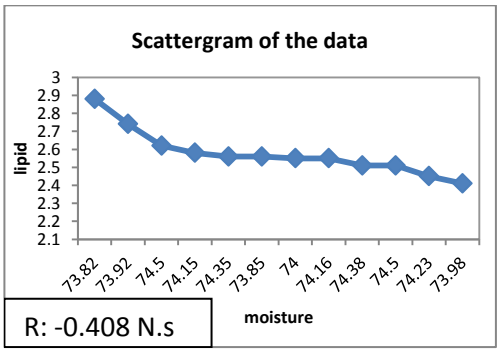
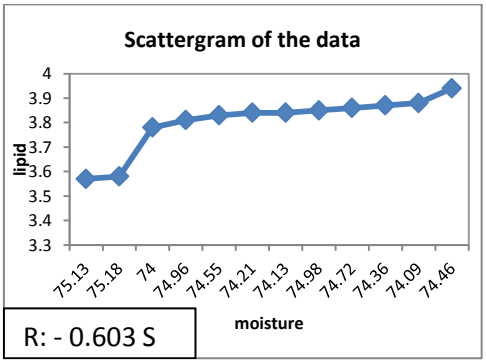



D

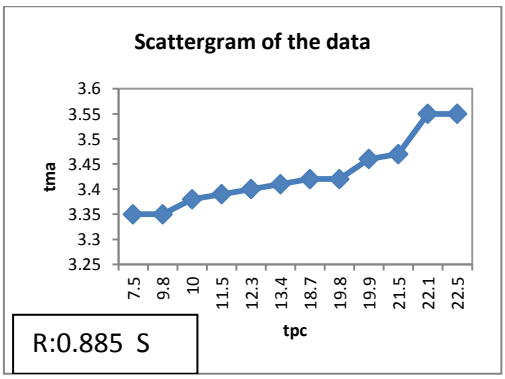



E

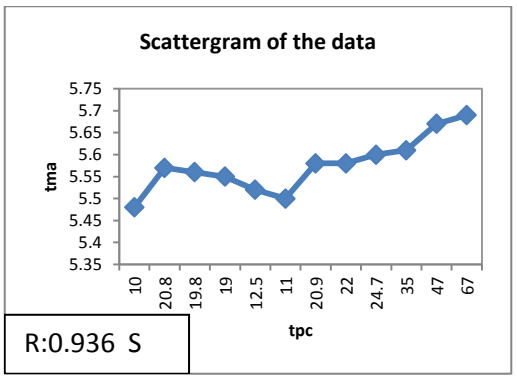
A: grass carp, B: silver carp, C: common carp, D: Bizz, E: Shabbout.

S: significant, N.s: non-significant.

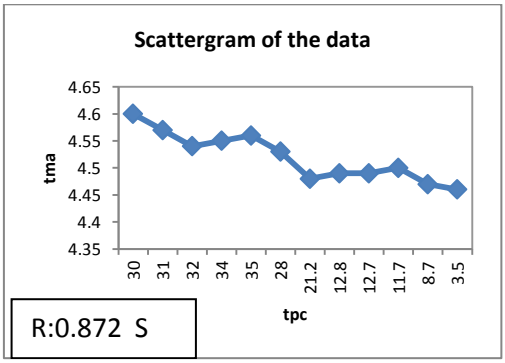
Appendix 3: The relationship between Total bacterial plate count (TPC) and trimethylamine (TMA) in five types of fish.




A




B

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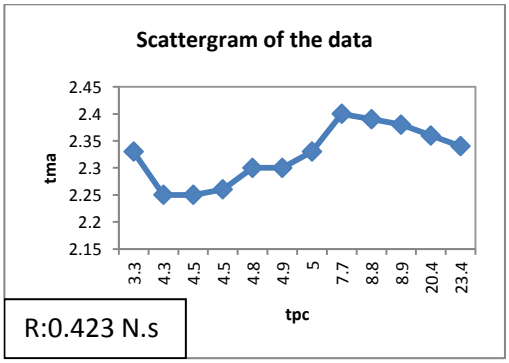
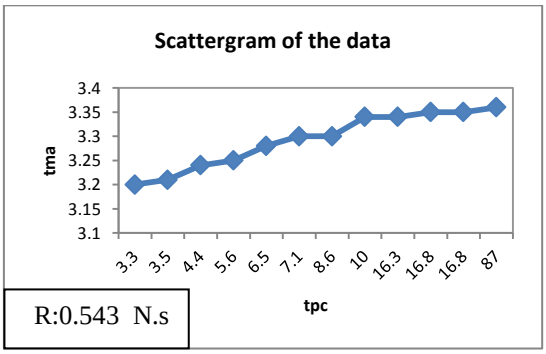



D

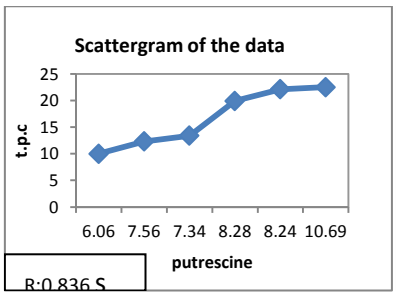


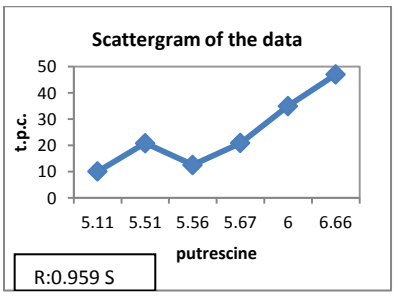

E

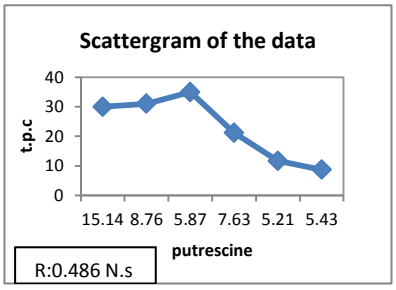
A: Grass carp, B: Silver carp, C: Common carp, D:Bizz, E:Shabbout.

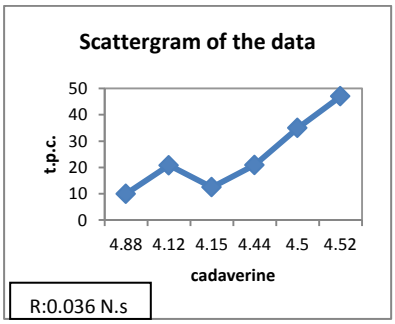
S: significant, N.s: non-significant

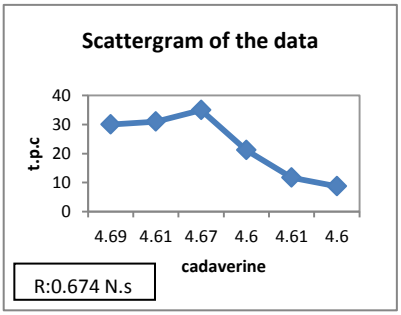
Appendix 4 : The relationship between biogenic amines and total bacterial count.

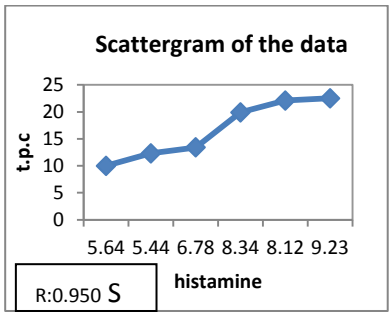


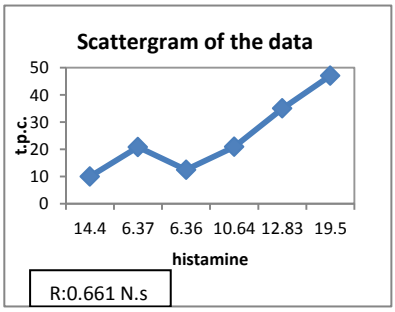



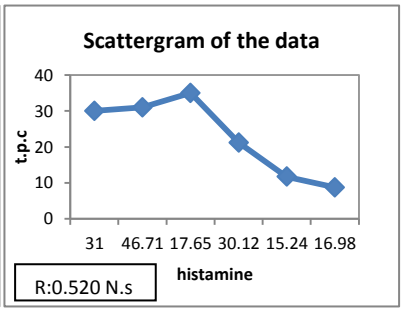



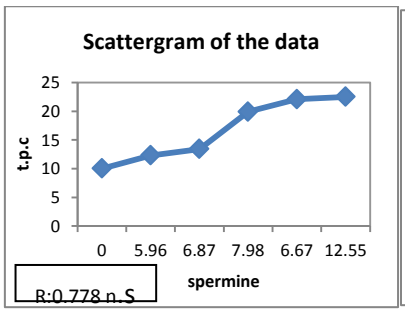



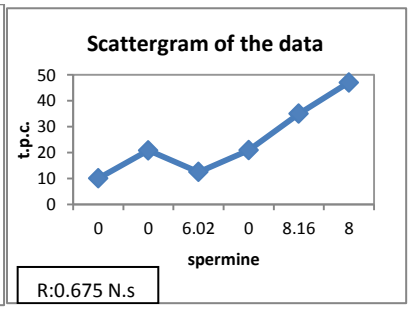



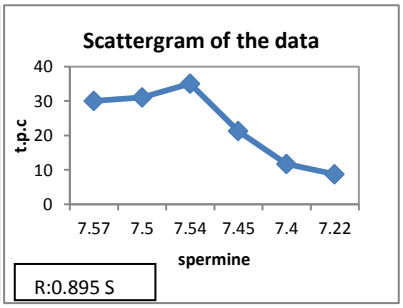



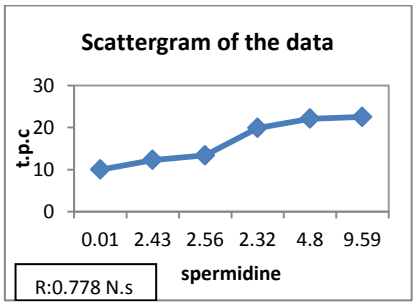



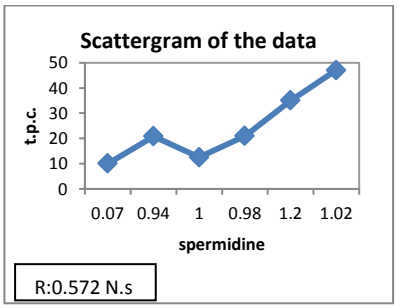



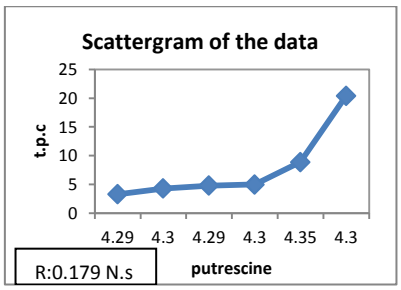



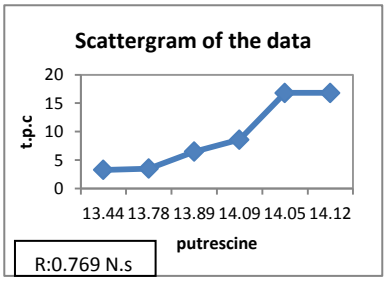



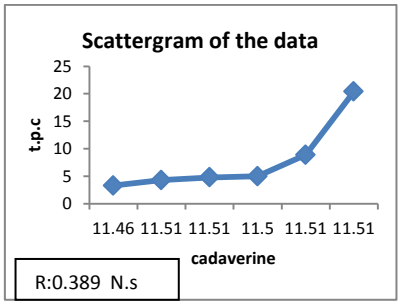



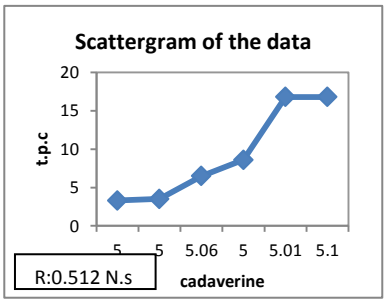



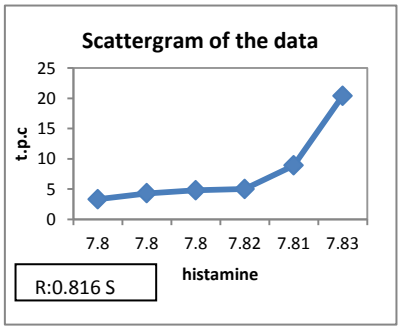

Grass carp Silver carp Common carp

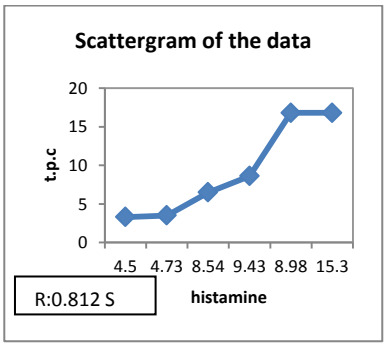


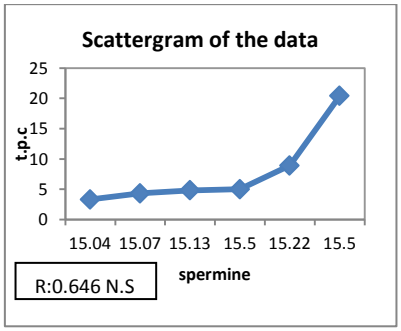



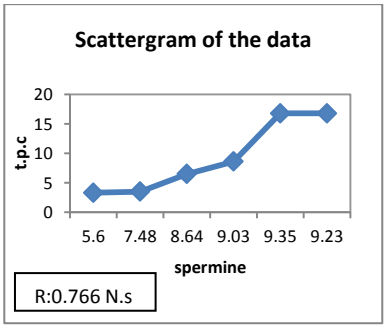



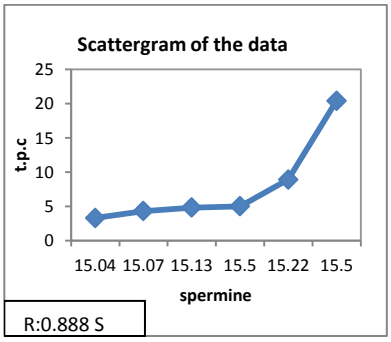



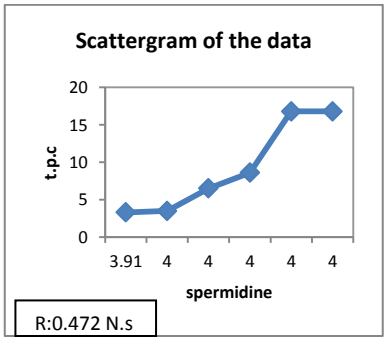




Bizz

Shabbout

**الخلاصة**

الهدف من هذه الدراسة هو تقييم نوعية خمسة انواع من الاسماك المحلية الاكثر مبيعا وانتشارا في مدينة السليمانية والتي تضمنت كارب الاعشاب (*Ctenopharyngodon idella*) و الكارب الفضي (*Hypophthalmichthys molitrix*) والكارب الشائع (*Cyprinus carpio*) والبز (*Barbus esocinus*) والشبوط (*Barbas grypus*)، جرى استخدام عدد من اختبارات التفتيش المعيارية.

سجلت جميع الانواع مؤشرا للنوعية quality index ضمن الحدود المقبولة بمدى من 6,67 للبز الى 10,67 للكارب الفضي مع وجود فروق معنوية بينها($P<0.05$).

اظهر التركيب التقريبي Proximate composition الذي تضمن محتويات الرطوبة و البروتين و الدهون والرماد نسبا مئوية مختلفة في جميع الانواع، فقد كان مدى الرطوبة من 69,19% في الكارب الفضي الى 74,69% في كارب الاعشاب، ومدى البروتين من 16,82% في الكارب الفضي الى 21,69% في البز، ومدى الدهون من 2,58% في البز الى 11,73 في الكارب الفضي، اما الرماد فقد كان بمدى من 0,99% في كارب الاعشاب الى 1,95% في الكارب الفضي مع وجود فروق معنوية بين جميع الانواع. كان المحتوى الدهني في الانواع المدجنة وهي الكارب بانواعه الثلاثة، الاعشاب و الفضي والشائع، واطئا في تلك النامية في البيئة البرية اذ كانت مختلفة معنويا عن نظيراتها المنماة في الحقل فيما كان محتوى الرطوبة على العكس من ذلك.لقد اختلفت محتويات الاحماض الامينية الاساسية معنويا في جميع الانواع.

التقييم الفيزياوي: كان مدى حجم ازالة المستخلص من 11,15 مل للبز الى 16.27 مل للكارب الفضي، ومدى سعة حمل الماء من 15,92% في الكارب الفضي الى 29,08% في البز, وكان مدى الفقدان اثناء الطبخ من 28,38% في البز الى 42,99% في الكارب الفضي، ومدى الكثافة بين 1,006 للكارب الفضي و 1.019 للبز، اما الاس الهيدروجيني فكان بمدى تراوح بين 6,605 في الكارب الفضي و 6,70 في الشبوط.اظهرت انواع الاسماك المدجنة المصطادة من البيئة البرية افضلية نوعية، فيما يتعلق بالخواص الفيزياوية، عن نظيراتها المنماة في الحقل.

اعطى التقييم الكيمياوي نتائج مقبولة ولكن باختلافات معنوية بين جميع الانواع، وكان مدى النتروجين الاساس العطري الكلي من 12,23 في البز الى 16,41 ملغم نتروجين/ 100غم في الكارب الفضي، ومدى الترايمثيل امين بين 2,35 في البز و 5,58 ملغم نتروجين/ 100 غم في الكارب الفضي، ومدى الدايمثيل امين بين 0,142 في البز و 0,365 ملغم نتروجين/ 100 غم في الكارب الفضي. اظهرت انواع الاسماك المدجنة المصطادة من البيئة البرية افضلية معنوية فيما يتعلق بالخواص الكيمياوية من نظيراتها المنماة في الحقل. واظهرت الانواع الخمسة المشتراة من الاسواق المجازة افضلية معنوية فيما يتعلق بالخواص الكيمياوية عن نظيراتها المشتراة من الاسواق غير المجازة. باستثناء الكارب الفضي الذي احتوى تركيزا واطئا جدا من الامونيا ( بمعدل 0,135 ملغم نتروجين/ 100 غم) كانت الانواع الاربعة الاخرى خالية من الامونيا.

تقييم اكسدة الدهون: باستثناء الكارب الفضي الذي اظهر حدودا غير مقبولة من حامض الثايوباربتيوريك (بمعدل 5,2 ملغم مالون الديهايد/ كيلوغرام)، فان الانواع الاربعة الاخرى اظهرت قيما ضمن الحدود المقبولة بمدى من 0,87 في البز الى 3,48 ملغم مالون الديهايد/ كيلوغرام في الكارب الشائع مع وجود فروق معنوية بينها. البيروكسيد كان بحدود مقبولة في جميع الانواع، اذ كان بمدى من 3,40 في البز و 8,86 ملي مكأفئ اوكسجين/ كيلوغرام في الكارب الفضي مع وجود فروق معنوية بينها. وكانت الاحماض الدهنية الحرة ضمن الحدود المقبولة في جميع الانواع بمدى من 0,77 في

البز و 1,36% في الكارب الفضي مع وجود فروق معنوية بين جميع الانواع، درجة النوعية من حيث نتائج اكسدة الدهون كانت بافضلية معنوية في الاسماك المصطادة في البيئة البرية عما هو عليه في نظيراتها المنماة في الحقول بالنسبة للانواع المدجنة. وفيما يتعلق باكسدة الدهون ايضا، فقد اظهرت الانواع الخمسة المشتراة من الاسواق المجازة افضلية معنوية عن نظيراتها المشتراة من الاسواق غير المجازة.

الامينات البايوجينية: لقد كان الهستامين، وهو المعول عليه في تقييم النوعية، ضمن الحدود المقبولة في الانواع الخمسة، اذ كان بمدى من 7,26 في كارب الاعشاب الى 26,28 جزء لكل مليون في الكارب الشائع وحيث كان الكارب الشائع مختلفا معنويا عن الانواع الاربعة الاخرى، اما البوتريسين و الكادافارين و السبيرمين والسبيرميدين فقد كانت ضمن مديات النوعية الجيدة للاسماك الوارد ذكرها في الادبيات. وكان مؤشر الامينات البايوجينية (BAI) ضمن الحدود المقبولة في جميع الانواع بمدى بين 1,175 في البز و 8,657 في الكارب الفضي الذي اظهر فرقا معنويا مع الانواع الاربعة الاخرى وكان قريبا من الحد الاعلى غير المرغوب. لقد جرى كشف الهايبوزانثين (Hx) في الكارب الفضي والشائع وبحدود مقبولة (0,0003 في الكارب الفضي و 0,002 جزء لكل مليون في الكارب الشائع).

التقييم الميكروبايولوجى: لقد كان العد الكلي للاطباق (TPC) وعدد البكتريا الآلفة للبرودة و عدد البكتريا المتحملة للبرودة ضمن الحدود المقبولة لجميع الانواع (مدى العدد الكلي للاطباق بين 8,37 في البز و 25,80 x 10 $^5$ وحدة تكوين مستعمرة/ غرام في الكارب الفضي، ومدى آلفات البرودة كان بين 6,83 في البز و 63,91 x 10 $^5$ وحدة تكوين مستعمرة/ غرام في الكارب الفضي، ومدى متحملات البرودة بين 7,7 في البز و 20,70 x 10 $^5$ في الكارب الفضي) ولكنها تباينت في العدد. من الناحية الميكروبايولوجية، اظهرت النتائج افضلية معنوية للانواع المدجنة المصطادة في البيئة البرية عما هو علية في نظيراتها المنماة في الحقل. وأظهرت اسماك البز و الشبوط المشتراة من الاسواق المجازة افضلية معنوية من الناحية المايكروبايولوجية بالمقارنة مع نظيراتها المشتراة من الاسواق غير المجازة. جرى الكشف عن انواع بكتريا *Pseudomonas* في جميع الانواع بمدى من 45,75 في الشبوط الى 59,16 x 10 $^5$ وحدة تكوين مستعمرة/ غرام في الكارب الفضي مع عدم وجود فروق معنوية بين جميع الانواع. لقد جرى تشخيص نوعين بكتيريين حين جرى اختيار عدد من مستعمرات بكتريا *Pseudomonas* عشوائيا وهما *P.aeruginosa* و *P.putida*. وجرى الكشف عن انواع بكتريا *Vibrio* في ثلاثة انواع، اذ كان عددها في الكارب الفضي 0,658 x 10 وحدة تكوين مستعمرة/ غرام، وفي الكارب الشائع 0,908 x 10 وحدة تكوين مستعمرة/ غرام وفي البز 1,942 x 10 وحدة تكوين مستعمرة/ غرام، وقد جرى تشخيص نوعين بكتيريين حين جرى اختيار عدد من مستعمرات بكتريا *Vibrio* عشوائيا وهما *V.metschnikovii* و *V. alginolyticus*. جرى الكشف عن انواع بكتريا *Aeromonas* في ثلاثة انواع، اذ كان عددها في الكارب الفضي 3,325 x 10 وحدة تكوين مستعمرة/ غرام، وفي الكارب الشائع 2,842 x 10 وحدة تكوين مستعمرة/ غرام وفي الشبوط 0,450 x 10 وحدة تكوين مستعمرة/ غرام، وقد جرى تشخيص نوعين بكتيريين حين جرى اختيار عدد من مستعمرات بكتريا *Aeromonas* عشوائيا وهما *A. hydrophila* و *A. caviae*.

التقييم الحسي: لقد سجلت جميع الانواع حدودا مقبولة للخواص الحسية، اذ كانت بمدى من 4,00 للبز الى 2,75 للكارب الفضي. ومن بين الانواع المدجنة، اظهرت النامية في البرية منها خواصا حسية بافضلية معنوية عن نظيراتها المنماة في الحقول. وكذلك اظهرت الخواص الحسية في جميع الانواع أفضلية معنوية للاسماك المباعة في الاسواق المجازة عما هو عليه في الاسواق غير المجازة.

متبقيات المعادن الثقيلة: سجلت الانواع الخمسة حدودا مقبولة للزئبق (بشكل مثيل زئبق)، اذ كان بمدى من 0,083 في الشبوط الى 0,407 جزء لكل مليون في الكارب الشائع،والكادميوم بمدى من 0,451 في كارب الاعشاب الى 0,475 جزء لكل مليون في كارب الشائع اما الرصاص فقد تراوح مداه بين 0,306 في كارب الاعشاب و 0,364 جزء لكل مليون في الشبوط. لقد احتوى الكارب الفضي والشائع المنميان في الحقل تراكيزا اوطأ للزئبق و الكاديميوم بالمقارنة مع نظيراتها البرية بينما لم يبدي كارب الاعشاب فرقا معنويا لكلا المعدنين في البرية والحقل.

لقد كان هنالك ارتباطا ايجابيا بين مؤشر الامينات البايوجينية والعد الكلي للاطباق وكذلك بين العد الكلي للاطباق و الترايمثيل امين فيما شوهد ارتباط سلبي بين الرطوبة والدهون.

# مقارنة تقييم نوعية خمسة انواع من الاسماك المحلية الطرية في اسواق محافظة السليمانية

رسالة مقــدمة الى

مجلس كلية الطب البيطري - جامعة السليمانية

كجزء من متطلبات نيــل

**درجة دكتوراه فلسفه**

**في السيطرة النوعية وصحة الغذاء**

**مــن قبـل**

**زيد خلف خضر**

**ماجستير صحة عامة بيطرية 2003**

**بأشراف**

**الاستاذالمساعد الدكتــور بهروز محمود امين جاف**

**الاستاذالمساعد الدكتــور حاتم حسون صالح**

**2711 ك** **2011 م**

**کورتە**

بەمەبەستی هەڵسەنگاندنی جۆرێتی پێنج جۆری ماسی خۆماڵی، کە زۆرتر باون و لة شاری سلێمانیدا دەفرۆشرێن، کە بریتیشبوون لە کاربی گیا (*Ctenopharyngodon idella*) و کاربی زیوی (*Hypophthalmichthys molitrix*) و کاربی باو (*Cyprinus carpio*) و بزۆك (*Barbus esocinus*) و سوورەماسی (*Barbas grypus*)، ژمارەیەك تاقیکردنەوەی پشکنینی پێوانەیی بەکارهێنران.

هەموو جۆرەکان پێوەری جۆرێتییان quality index لە سنووری پەسەندکراودا تۆمارکرد بە مەودای 67،6 بۆ بزۆك تا 67،10 بۆ کاربی زیوی لەگەڵ هەبوونی جیاوازی متمانەدار لەنێوانیاندا (P<0.05).

پێکهاتەی نزیك Proximate composition ،کە بڕی شێ و پرۆتین و چەوری و خۆڵەمێشی لەخۆگرتبوو، رێژەی سەدی پەسەندکراوی لە هەموو جۆرەکاندا نیشاندا، مەودای شێ لە 69،19% لە کاربی زیوی تا 74،69% لە کاربی گیا بوو، مەودای پرۆتین 16،82% لە کاربی زیوی تا 21،69% لە بزۆك بوو، مەودای چەوری لە 2،58% لە بزۆك بوو تا 11،73% لە کاربی زیوی بوو، مەودای خۆڵەمێشیش لە 0،99% لە کاربی گیا تا 1،95% لە کاربی زیوی بوو لەگەڵ هەبوونی جیاوازی متمانەدار لەنێوان هەموو جۆرەکاندا. چەوری لە جۆرە ماڵیبووەکاندا، کە ماسی کاربە بەهەر سێ جۆرەکانییەوە، گیاو زیوی وباو، رێژەی نزمی لەو ماسییانەی کە لە ژینگەی کێویدا ژیابوون نیشاندا، کە بەشێوەیەکی متمانەدار لە هاوتا کێڵگە بەخێوکراوەکانیاندا جیاوازبوو، لەکاتێکدا شێ بەڕێچەوانەی ئەوە بوو. ترشە ئەمینییە بنچینەییەکان لەنێوان هەموو جۆرەکاندا جیاوازبوون.

هەڵسانگەندنی فیزیاوی: مەودای قەبارەی گیراوەی دەرهێنراو لە 11،15 مل لە بزۆك تا 16،27 مل لە کاربی زیویدا بوو، مەودای توانای هەڵگرتنی ئاو لە 15،92% لە کاربی زیوی تا 29،08 لە بزۆکدا بوو، مەودای دڵۆپاندنی لێنان لە 28،38% لە بزۆکدا تا 42،99% لە کاربی زیوی بوو، مەودای چڕی لە نێوان 1،006 لە کاربی زیوی تا 1،019 لە بزۆکدا بوو، توانی هیدرۆجینیش بەمەودای نێوان 6،605 لە کاربی زیوی و 6،70 لە سوورەماسیدا بوو. جۆرە بەماڵیبووەکان، کە لە ژینگەی کێویدا ڕاوکرابوون باشترێکی متمانەداریان نیشاندا لەڕووی جۆرێتی فیزیاوییەوە بەبەراورد لەگەڵ هاوتا لەکێڵگە بەخێوکراوەکانیاندا. هەڵسەنگاندنی کیمیاوی ئەنجامی پەسەندکراوی نیشاندا بەڵام بە جیاوازی متمانەدار لەنێوان هەموو جۆرەکاندا. مەودای نترۆجینی بنەڕەتی فڕیوی گشتی لە نێوان 12،23 لە بزۆك تا 41،16 میللیگرام نترۆجین/ 100 گرام لە کاربی زیزیدا بوو، مەودای ترامەسیلئەمین لە نێوان 2،35 لە بزۆك تا 5،58 میللیگرام نترۆجین/ 100 گرام لە کاربی زیویدا بوو، مەودای دایمەسیلئەمین لەنێوان 0،142 لە بزۆك تا 0،365 میللیگرام نیرۆجین/ 100 گرام لە کاربی زیویدا بوو.

لەڕووی جۆرێتی کیمیاوییەوە، ئەو ماسییە بەماڵیبووەکان کە لە ژینگەی کێویدا ڕاوکرابوون باشترێکی متمانەداریان نیشاندا بە بەراورد لەگەڵ هاوتا لەکێڵگە بەخێوکراوەکانیاندا. ئەو ماسییانەی هەر پێنج جۆرەکە، کە لە بازارە مۆڵەتپێدراوەکانەوە کڕابوون، باشترێکی متمانەداریان نیشاندا لەڕووی جۆرێتی کیمیاوییەوە بە بەراورد لەگەڵ هاوتا لە بازارە بێ مۆڵەتەکانیانەوە کڕابوون. جگە لە کاربی زیوی، کە پەیتیەکی نزمی ئەمۆنیای تێدابوو (بە تێکڕای 0،135 میللیگرام نترۆجین/ 100 گرام)، هەر چوار جۆرەکەی دیکە ئەمۆنیایان تیدا نەبوو. هەڵسەگاندنی ئۆکسانی چەوری: جگە لە کاربی زیوی، کە سنووری ناپەسەندی لە ترشی سایۆباربیتیوریکی نیشاندا (بە تێکڕای 5،2 میللیگرام مالۆن ئەڵدیهاید/ کیلۆگرام). هەر چوار جۆرەکەی دیکە باییی لە سنووره پەسەندکراوەکاندا نیشاندا بەمەودای لە 0،87 لە بزۆك تا 3،48 میللیگرام مالۆن ئەڵدیهاید/ کیلۆگرام لە کاربی باودا لەگەڵ هەبوونی جیاوازی متمانەدار لەنێوانیاندا. بیرۆکسید لەهەموو جۆرەکاندا لە سنووره پەسەندکراوەکاندا بوو، کە بە مەودای لە 40،3 لە بزۆك تا 8،86 ئۆکسیجین/ کیلۆگرام لە کاربی زیویدا لەگەڵ هەبوونی جیاوازی متمانەدار لەنێوانیاندا. ترشە چەورییە ئازادەکان لە هەموو جۆرەکاندا لە سنووره پەسەندکراوەکاندا بوون بەمەودای لە 0،77 لە بزۆك تا 1،36% لە کاربی زیویدا بەهەبوونی جیاوازی متمانەدار لەنێوان هەموو جۆرەکاندا. پلەی جۆرێتی، لەڕووی ئەنجامەکانی ئۆکسیدکرنی چەورییەوە، باشترێکی متمانەداری هەبوو لەو ماسییانەی کە لە ژینگەی کێویدا ڕاوکرابوون بەبەراورد لەگەڵ هاوتا لە کێڵگە بەخێوکراوەکانیاندا لە جۆرە بەماڵیبووەکاندا. لەڕووی بەئۆکسیدکردنی چەوریش، هەر پێنج جۆرەکە کە لە

بازارە مۆڵەتپێدراوەکانەوە کڕابوون باشترێکی متمانەداریان هەبوو بەبەراورد لەگەڵ هاوتا لەبازارە بێ مۆڵەتەکانەوە کڕابوون.

ئەمینە بایۆجینییەکان: هستامین، کە پشتی پێ دەبەسترێت لە هەڵسەنگاندنی جۆرێتیدا، لە سنووورە پەسەندکراوەکاندا بوو لەهەر پێنج جۆرەکەدا بەمەودای 7،26 لە کاربی گیا تا 26،28 بەش لە ملیۆنێك لە کاربی باودا، کە کاربی باویش لەگەڵ هەر چوار جۆرەکەی تردا متمانە جیاوازی هەبوو، بەڵام پووتریسین و سپێرمین و سپێرمیدین لەسنووی جۆرێتی ماسیی باشدا بوون کە لە ئەدەبییاتدا باسیان لێوە کراوە.

پێوەری ئەمینە بایۆجینییەکان (BAI) لە سنووورە پەسەندکراوەکاندا بوو لە هەموو جۆرەکاندا بەمەودای 1،175 لە بزۆك تا 8،675 لە کاربی زیویدا کە جیاوازییەکی متمانەداری نیشاندا لەگەڵ چوار جۆرەکەی دیکەداو کە لە بەرزترین ئاستی پەسەندکراوەوە، کە حەزی لێ ناکرێ، نزیك بوو. هایۆزانسین (Hx) لە کاربی زیویدا بە سنووورێکی پەسەندکراوەوە دەستنیشانکرا (0،0003 لە کاربی زیویداو 0،002 بەش لە ملیۆنێکدا لە کاربی باودا).

هەڵسەنگاندنی مایکرۆبایۆلۆجی: ژمارەی گشتی قاپەکان (TPC) و ژمارەی بکتیریای هۆگری ساردی و ژمارەی بەکتیریای بەرگەگری ساردی لە سنووورە پەسەندکراوەکاندا بوون لەهەموو جۆرەکاندا (مەودای ژمارەی گشتی قاپەکان لەنێوان 8،37 لە بزۆك تا$25,80 \times 10^{5}$ یەکەی پێکهێنانی ئاوەدانکراوە/ گرام لە کاربی زیویدا بوو، مەودای ساردی هۆگرەکان لەنێوان 6،83 لە بزۆك تا $36،91 \times 10^{5}$ یەکەی پێکهێنانی ئاوەدانکراوە/ گرام لە کاربی زیویدا بوو، مەودای بەرگەگری ساردیەکانیش لە نێوان 7,7 لە بزۆك تا $20,70 \times 10^{5}$ لە کاربی زیویدا بوو) بەڵام لەڕووی ژمارەوە جیاواز بوون. لەڕووی مایکرۆبایۆلۆجییەوە، ئەنجامەکان دەریانخست کە باشترێکی متمانەدار بۆ جۆرە بەمالّیبووەکان کە لەژینگەی کێویدا ڕاوکرابوون هەیە بە بەراورد لەگەڵ هاوتا لەکێڵگە بەخێوکراوەکانیاندا. ماسییەکانی بزۆك و سوورەماسی کە لە بازارە مۆڵەتپێدراوەکانەوە کڕابوون باشترێکی متمانەداریان هەبووە، لەڕووی مایکرۆبایۆلۆجییەوە، بە بەراورد لەگەڵ هاوتا لە بازارە بێ مۆڵەتەکانیانەوە کڕابوون. جۆرەکانی بەکتیریای *Pseudomonas* لە هەموو جۆرە ماسییە پشکێنراوەکاندا دەستنیشانکرا، بە مەودای 45،75 لە سوورەماسی تا$59,16 \times 10^{5}$ یەکەی پێکهێنانی ئاوەدانکراوە/ گرام لە کاربی زیویدا لەگەڵ هەبوونی جیاوازی متمانەدار لە نێوان هەموو جۆرەکاندا. لەکاتی بژاردنی ژمارەیەکی ئاوەدانکراوەی بەکتیریای *Pseudomonas* دا، دوو جۆر دەستنیشانکران کە بریتیبوون لە *P.aeruginosa* و *P.putida*. جۆرەکانی بەکتیریای *Vibrio* لە سێ جۆری ماسیدا دەستنیشان کران، ژمارەکەشی لە کاربی زیویدا $0,658 \times 10$ یەکەی پێکهێنانی ئاوەدانکراوە/ گرام بوو. لە کاربی باویشدا $0,908 \times 10$ یەکەی پێکهێنانی ئاوەدانکراوە/ گرام بوو، لە بزۆکیش $1,942 \times 10$ یەکەی پێکهێنانی ئاوەدانکراوە/ گرام بوو. دوو جۆری بەکتیریاش دەستنیشان کران لەکاتی بژاردنی ژمارەیەکی ئاوەدانکراوەی بەکتیریای *Vibrio* کە بریتیبوون لە *V.metschnikovii* و*V. alginolyticus*. جۆرەکانی بەکتیریای *Aeromonas* لە سێ جۆری ماسیدا دەستنیشانکران، ژمارەکەشی لە کاربی زیویدا $3,325 \times 10$ یەکەی پێکهێنانی ئاوەدانکراوە/ گرام بوو، لە کاربی باویش $2,842 \times 10$ یەکەی پێکهێنانی ئاوەدانکراوە/ گرام بووو لە سوورەماسیش $0,450 \times 10$ یەکەی پێکهێنانی ئاوەدانکراوە/ گرام. لەکاتی بژارکردنی ژمارەیەکی ئاوەدانکراوەی بەکتیریای *Vibrio* شدا، دوو جۆر دەستنیشانکران کە بریتیبوون لە *A. hydrophila* و *A. caviae* .

هەڵسەنگاندنی هەستی: هەموو جۆرەکان سنووری پەسەندکراویان لە جۆرێتی هەستی تۆمارکرد، کە بە مەودای 4،00 بۆ بزۆك تا 2،75 بۆ کاربی زیوی. لەنێوان جۆرە بەمالّیبووەکانیشدا، ئەو ماسییانەی کە لە ژینگەی کێویدا گەشەیانکردبوو جۆرێتی هەستییان بە باشترێکی متمانەکراوەوە بوون بە بەراورد لەگەڵ هاوتا لە کێڵگەکانیاندا گەشەیانکردوە. جۆرێتی هەستیش لە هەموو جۆرەکاندا باشترێکی متمانەکراویان نیشاندا لەو ماسییانەی کە لە بازارە مۆڵەتدارەکانەوە کڕابوون بە بەراورد لەگەڵ هاوتا لە بازارە بێ مۆڵەتەکانیانەوە کڕابوون.

پاشماوەی کانزا قورسەکان: هەر پێنج جۆرەکە سنووری پەسەندکراویان تۆمارکرد بۆ جیوە (بەشێوەی مەسیل جیوە)، کە بە مەودای 0،083 لە سووورە ماسی بۆ 0،407 بەش لە ملیۆنێك لە کاربی باودا. قورقوشمیش مەودایەکی نیشاندا لە نێوان 0،306 لە کاربی گیا تا 0.364 بەش لە ملیۆنێك لە سووورە ماسیدا. کاربی زیوی و باو، کە لە کێڵگەدا کەشەیانکردووە، پەیتی کەمتریان لە جیوەو کادیمیۆمیان هەبوو بە بەراورد لەگەڵ هاوتا کێوییەکانیاندا لەکاتێکدا کاربی گیا هیچ جیاوازییەکی نەنواند بۆ هەردوو کانزاکە لەنێوان ماسییەکانی کێوو کێڵگەدا. پەیوەندییەکی

پۆزەتیڤانە لە نێوان پێوەری ئەمینە بایۆجینییەکان و هەر یەکە لە ژماردنی گشتی قاپەکان ، هەر وەهاش لەنێوان ژماردنی گشتی قاپەکان و ترایمەسیلئەمین لەکاتێکدا پەیوەندییەکی نیگاتیڤانە لەنێوان شێ و چەوریدا هەبوو.

# بەراوردكردنی هەڵسەنگاندنی جۆرێتی پێنج جۆری ماسی خۆمالّی كالّ لە بازارەكانی پارێزگای سلێمانیدا


دكتۆرانامەیەك پێشكە شە بە ئە نجومە نی

كۆلێجی پزیشكی ڤێتێرنەری- زانكۆی سلێمانی

وە ك بەشێك لە پێویستییەكانی بەبەدەستهێنانی

**پلەی دكتۆرا فیلۆسۆفی**

**لە كۆنترۆلی جۆری و دروستی خۆراك**

**لەلایەن**

**زەید خەلّەف خدر**

ما ستە ر لە تەندروستی گشتی ڤێتێرنە ری 2003

**بەسەرپەرشتی**

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