Determination of Manganese, Iron, Zinc, Copper and Nickel in Tilapia and *Cyprinus carpio* Fish Consumed in Syria by Flame Atomic Absorption Spectrometry

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The present work describes a microwave digestion procedure by using new convenient digestion mixture (nitric acid and hydrogen peroxide) to determine manganese, zinc, copper, iron and nickel in (Tilapia and *Cyprinus carpio*) fish samples, breeding in artificial lakes, employing flame atomic absorption spectrometry. The optimization conditions involving the factors: amount of fish, microwave power and power time were studied. The experiments of this factorial were carried out using two species of local fish which are common consumed in Syria. The proposed method was used to determine the precedent metals in muscle, skin and tail in the two species fish (Tilapia and *Cyprinus carpio*). The relative standard deviations of the method were found to be less than 5 % for the five studied elements. The concentration results for manganese, iron, zinc, copper and nickel in *Cyprinus carpio* fish species varied between 1.37-3.09, 41.61-52.77, 53.44-200.71, 2.53-5.83 and 0.90-1.11 ($\mu\text{g/g}$) in muscle, 1.63-4.03, 54.81-62.45, 99.22-177.31, 2.84-7.43 and 0.74-1.15 ($\mu\text{g/g}$) in skin and 8.65-51.69, 8.48-20.60, 282.98-309.41, 0.89-1.67 and 0.77-0.96 ($\mu\text{g/g}$) in tail respectively. The variation of precedent metals in tilapia fish species varied between 0.86-2.34, 68.48-125.74, 103.36-152.68, 3.25-5.31 and 1.08-2.51 ($\mu\text{g/g}$) in muscle, 5.20-9.90, 73.12-183.62, 200.39-241.70, 2.13-5.69 and 1.09-1.90 ($\mu\text{g/g}$) in skin and 12.75-28.77, 75.39-119.75, 215.03-289.13, 1.65-3.42 and 0.99-2.45 ($\mu\text{g/g}$) in tail, respectively.

Keywords: Microwave digestion, Tilapia, *Cyprinus carpio*, Flame atomic absorption spectrometry.

INTRODUCTION

Fish are one of the most important and the largest groups of vertebrates in the aquatic system. Trace metals can be accumulated *via* both food chain and water in fish. Fish have been considered good indicators for heavy metal contamination in aquatic systems because they occupy different trophic levels with different sizes and ages. Meanwhile, fish are widely consumed in many parts of the world by humans and polluted fish may endanger human health¹.

For years, the American Heart Association has recommended eating an average of two to three fish meals each week to help reduce cholesterol, high blood pressure and hardening of arteries. Research shows that consuming fish increases high quality protein with fewer calories and it is rich in omega-3 fatty acids. Omega-3 fatty acid helps to reduce the risk of coronary artery disease, helps in the treatment of bipolar disorder/depression and helps reduce inflammation in autoimmune diseases. Fish are also low in sodium and a good source of potassium².

Fish meat is a perfect foodstuff which is up to standard of rational nourishment. It is source of healthy and good digestible material rich in proteins, minerals and vitamins. Fish muscles especially back and lateral muscles are the most important parts of fish organism consumed for excellent chemical composition. Proteins in fish meat are rich on high amino acids content. The content of fish fat is usually low with the high proportion of unsaturated fatty acids. Also minerals and vitamins A, B and D are very important components of this foodstuff. According to rational nourishment the fish meat should be consumed minimal two times weekly³.

Several trace elements are found in fish, including aluminum, boron, cadmium, cobalt, chromium, copper, iron, manganese, nickel, lead and zinc. Several of these elements are beneficial as nutrients for humans⁴. Wide range of digestion methods for food have been published such as dry digestion, wet digestion with different mixtures of reagents or conventionally heating procedures, digestion using an electromagnetic heating column and microwave dissolution⁵. These methods generally show both good accuracy and precision. But the dry

and wet digestion procedures take long time⁶. Microwave digestion offers many advantages over conventional digestion procedures for food analysis as: short time, using acids in a closed high-pressure polytetrafluoroethylene, temperatures above the boiling point of nitric acid and almost no loss of any amount of the sample (good recovery)⁷.

A wide range of techniques have been used for determining of trace heavy metals such as inductively coupled plasma-atomic emission spectrometry (ICP-AES)⁸, inductively coupled plasma-mass spectrometry (ICP-MS)⁹, inductively coupled plasma-optical emission spectrometry (ICP-OES)¹⁰, X-ray fluorescence spectrometry, capillary electrophoresis (CE)¹¹, atomic absorption spectrometry by graphite furnace (AAS-GF)¹² and Flame atomic absorption spectrometry (FAAS)¹³. Flame atomic absorption spectrometry (FAAS) has been shown to be a promising technique for the determination of trace heavy metals in view of its low costs and easy usage¹⁴.

EXPERIMENTAL

A Phoenix 986 AAWin V2.1 atomic absorption spectrometer with self-reversal background correction mode (SR lamp-BGC mode) was used in this study. The operating parameters for working elements were set as recommended by the manufacturer. The elements were determined by using air-acetylene flame. Microwave digestions were carried out in an Ethos D (Milestone, Sorisole, Italy) with maximum pressure 1450 psi and maximum temperature 300 °C suggested heating program for the digestion procedure is given in Table-1.

Step	Time (min)	Power (W)
1	2	250
2	2	0
3	2	250
4	4	400
5	8	600
Ventilation	10	0

All used reagents in the presented work were of analytical grade. Double distilled deionized water was used for all dilutions. Analytical reagent grade HNO₃ 65 % (ww⁻¹) and H₂O₂ 30 % (ww⁻¹) (Merck, Germany) were used for sample digestion. All the plastic and glassware were cleaned by soaking in dilute HNO₃ and were rinsed with distilled water prior to use. The standard solutions, used for calibration were

produced by diluting a stock solution of 1000 mg L⁻¹ of the given element supplied by (Merck, Germany). The calibration curves for analyte metals were drawn after setting various parameters of FAAS including wavelength, slit width, lamp current at an optimum level

Fish samples were purchased from supermarkets from Aleppo-Syria during the year 2013.

Microwave digestion: After the optimization of the digestion conditions, the selected factors (the optimum conditions) were applied to an oven-dried fish samples. About 1 g of dried fish was digested with 6 mL of concentrated HNO₃ and 2 mL of concentrated H₂O₂ in microwave refill and then it closed tightly and put in microwave and digested by heating program given in Table-1. Digested sample was transferred to beaker and evaporated to 5 mL and then sample was transferred to standard flask 10 mL and completed by distilled deionized water to volume. A blank digestion was also carried out in the same way.

RESULTS AND DISCUSSION

Determination of the experimental factors for microwave digestion: The factors that concern microwave digestion were studied: amount of fish, microwave power and power time. These factors were studied on samples of muscle *Cyprinus carpio* fish, weight 320 g and 31 cm length.

Amount of fish: Digestion efficacy of amount dried muscle fish was studied by using acid mixture (HNO₃-H₂O₂) and using previous digestion program. The concentration metals stayed constant until Amount of fish 1 g. The obtained results of digestion efficacy of amount dried muscle fish are presented in Table-2 and Fig. 1. We obtained similar digestion efficacy in the case of skin and tail.

Fig. 1. Effect of amount fish (AF) on digestion efficacy in muscle *Cyprinus carpio* fish

Amount fish	Concentration elements (µg/g dry weight)									
	Ni	RSD (%)	Cu	RSD (%)	Zn	RSD (%)	Fe	RSD (%)	Mn	RSD (%)
0.1	ND	ND	4.28	9.49	26.71	3.35	41.55	4.21	4.89	10.57
0.2	ND	ND	4.33	8.03	26.00	3.07	41.26	3.70	4.93	10.10
0.3	ND	ND	4.74	7.75	26.09	3.16	41.69	2.71	4.51	9.87
0.4	ND	ND	4.62	7.46	25.41	1.99	42.12	2.88	5.02	7.45
0.5	2.00	8.45	4.53	6.02	26.32	2.05	40.15	2.68	5.16	5.24
0.6	2.15	7.36	4.31	5.43	26.04	2.11	41.44	2.54	5.27	4.25
0.7	2.24	4.64	4.58	3.90	26.17	2.34	41.15	3.96	5.12	3.91
0.8	2.11	4.04	3.99	2.67	25.94	1.74	40.24	2.37	4.48	3.12
0.9	2.13	5.69	4.02	3.99	25.43	1.91	40.66	2.13	4.39	2.23
1.0	2.17	4.93	3.85	2.96	25.76	1.27	40.62	3.04	4.56	3.03

TABLE-3
EFFECT OF MICROWAVE POWER ON DIGESTION EFFICACY IN MUSCLE *Cyprinus carpio* FISH (n = 5)

Microwave power	Concentration elements ($\mu\text{g/g}$ dry weight)									
	Ni	RSD (%)	Cu	RSD (%)	Zn	RSD (%)	Fe	RSD (%)	Mn	RSD (%)
300	1.50	3.12	3.27	4.29	17.77	1.84	30.13	1.37	3.16	2.7
350	1.61	3.44	3.40	3.56	18.80	2.10	31.44	1.64	3.48	2.14
400	1.72	3.15	3.61	4.63	20.75	1.92	33.25	1.22	3.75	1.91
450	1.85	3.46	3.75	3.61	21.99	2.43	35.30	2.33	4.02	1.22
500	2.01	3.50	3.90	3.12	23.11	2.63	37.10	1.60	4.22	1.13
550	2.10	2.17	4.14	2.90	25.01	1.44	38.98	1.40	4.53	1.46
600	2.30	3.42	4.53	2.44	26.02	2.56	41.31	2.53	5.03	1.33
650	2.36	2.71	4.58	2.87	26.10	2.77	41.79	1.46	5.10	2.10
700	2.41	3.71	4.60	3.13	26.12	1.95	41.91	2.34	5.15	1.13

TABLE-4
EFFECT OF POWER TIME ON DIGESTION EFFICACY IN MUSCLE *Cyprinus carpio* FISH (n = 5)

Power time	Concentration elements ($\mu\text{g/g}$ dry weight)									
	Ni	RSD (%)	Cu	RSD (%)	Zn	RSD (%)	Fe	RSD (%)	Mn	RSD (%)
2	1.56	4.15	2.72	4.29	23.05	3.62	38.71	2.30	3.51	1.35
4	1.80	3.22	3.74	3.56	24.01	3.95	39.77	2.01	4.07	1.64
6	2.24	3.47	4.25	4.65	26.20	3.28	41.80	1.60	5.10	2.16
8	2.20	2.92	4.28	3.67	26.15	2.13	41.75	1.53	5.13	1.18
10	2.15	2.61	4.15	3.18	26.25	2.54	41.86	1.68	5.19	1.29
12	2.23	3.19	4.25	3.20	26.30	2.33	41.90	1.48	5.15	1.39

Microwave power: Microwave power was studied by using dried muscle fish amount of 1 g and power time 8 min. Expedience microwave power for good digestion was 650 watt (Table-3, Fig. 2).

Fig. 2. Effect of microwave power (MP) on digestion efficacy in muscle *Cyprinus carpio* fish

Power time: Power time was studied by using dried muscle fish amount of 1 g and microwave power 600 watt. Expedience power time for good digestion was 8 min, as it is given in Table-4 and Fig. 3.

Fig. 3. Effect of power time (PT) on digestion efficacy in muscle *Cyprinus carpio* fish

Samples: The proposed microwave digestion procedure was applied for determining of manganese, iron, zinc, copper and nickel in muscle, skin and tail of Tilapia and *Cyprinus carpio* fish samples, breeding in artificial lakes and consumed in Syria.

***Cyprinus carpio* Fish:** Six *Cyprinus carpio* fish of different weight and length are selected as samples to determine five heavy metals *i.e.*, manganese, iron, zinc, copper and nickel, as shown in Table-5.

TABLE-5
WEIGHT AND LENGTH IN *Cyprinus carpio* FISH

Sample	Weight (g)	Length (cm)
1	302	25
2	310	25
3	926	40
4	930	42
5	3350	55
6	3412	58

Five heavy metals *i.e.*, manganese, iron, zinc, copper and nickel were determined in three organs muscles, skin and tail in the *Cyprinus carpio* fish for the six precedent samples. The results are presented in Table-6.

Tilapia fish: Six tilapia fish of different weight and length are selected as samples to determine heavy metals *i.e.*, manganese, iron, zinc, copper and nickel, as presented in Table-7.

Five heavy metals *i.e.*, manganese, iron, zinc, copper and nickel were determined in three organs muscles, skin and tail in the tilapia fish for the six precedent samples. The results are presented in Table-8.

Recovery: The recovery test was applied on muscle, skin and tail for one representative of *Cyprinus carpio* and Tilapia fish samples. The obtained results are presented in Table-9.

TABLE-6
MANGANESE, IRON, ZINC, COPPER AND NICKEL CONCENTRATIONS IN DIFFERENT PARTS OF *Cyprinus carpio* FISH

Sample	Concentration elements ($\mu\text{g/g}$ dry weight)									
	Ni	RSD (%)	Cu	RSD (%)	Zn	RSD (%)	Fe	RSD (%)	Mn	RSD (%)
Muscle										
1	0.94	3.53	2.53	3.46	161.05	2.23	41.61	2.82	2.27	2.77
2	1.11	1.01	2.76	3.86	200.71	0.86	44.21	1.20	2.31	2.39
3	0.91	6.94	5.83	2.50	67.41	1.90	50.74	1.20	2.75	0.67
4	1.11	1.07	5.00	1.51	53.44	5.45	52.77	2.96	3.09	2.51
5	0.90	0.94	2.64	3.14	78.45	2.20	43.59	1.07	1.37	5.16
6	0.90	0.96	3.13	2.70	73.79	1.73	50.42	0.70	2.69	3.06
Skin										
1	0.95	3.03	2.84	2.76	172.61	1.42	58.35	1.40	3.57	4.62
2	0.97	4.34	3.01	2.60	177.31	0.81	56.36	1.31	3.51	1.08
3	0.74	5.17	5.44	1.52	111.81	0.93	54.81	2.50	3.72	2.85
4	1.15	5.53	4.94	2.99	99.22	0.94	58.98	1.75	4.03	1.44
5	0.97	1.35	4.19	1.40	124.39	1.10	57.17	2.99	1.63	1.43
6	0.90	1.53	7.43	1.12	145.95	2.07	62.45	2.42	2.05	4.84
Tail										
1	0.91	1.86	1.67	4.68	287.91	1.14	14.31	7.10	51.69	1.94
2	0.81	2.32	1.25	2.67	308.92	0.83	20.60	2.58	46.04	0.39
3	0.78	2.95	0.97	4.47	282.98	0.94	8.48	5.29	45.47	4.17
4	0.83	13.69	1.17	7.91	297.61	0.71	16.07	3.46	42.06	3.44
5	0.77	2.01	0.89	2.49	309.41	0.27	11.86	5.77	9.94	1.21
6	0.96	1.33	1.14	2.38	294.45	1.78	15.93	5.48	8.65	1.18

TABLE-7
WEIGHT AND LENGTH TILAPIA FISH

Sample	Weight (g)	Length (cm)
1	240	23
2	245	24
3	630	35
4	640	37
5	1050	44
6	1075	46

Conclusion

The concentration of manganese, iron, zinc, copper and nickel in every six various samples of *Cyprinus carpio* fish varied between 1.37-3.09, 41.61-52.77, 53.44-200.71, 2.53-5.83 and 0.90-1.11 ($\mu\text{g/g}$) in muscle, 1.63-4.03, 54.81-62.45, 99.22-177.31, 2.84-7.43 and 0.74-1.15 ($\mu\text{g/g}$) in skin and 8.65-51.69, 8.48-20.60, 282.98-309.41, 0.89-1.67 and 0.77-0.96 ($\mu\text{g/g}$) in tail, respectively. The variation of precedent metals

TABLE-8
MANGANESE, IRON, ZINC, COPPER AND NICKEL CONCENTRATIONS IN DIFFERENT PARTS OF TILAPIA FISH

Sample	Concentration elements ($\mu\text{g/g}$ dry weight)									
	Ni	RSD (%)	Cu	RSD (%)	Zn	RSD (%)	Fe	RSD (%)	Mn	RSD (%)
Muscle										
1	1.08	5.38	3.35	1.45	103.36	2.28	68.48	1.51	0.86	4.94
2	1.43	6.45	3.25	3.59	108.19	1.52	75.13	2.04	1.06	4.32
3	2.51	5.27	4.62	3.06	123.18	1.68	94.54	3.00	1.26	3.51
4	2.34	6.20	4.78	2.59	122.28	1.73	97.87	2.20	1.35	2.78
5	1.51	5.60	5.31	1.14	148.15	1.21	120.45	1.46	2.11	3.26
6	1.23	5.98	5.23	4.38	152.68	0.99	125.74	1.48	2.34	2.27
Skin										
1	1.09	4.45	2.13	4.35	200.39	1.05	73.12	3.49	5.68	3.73
2	1.16	5.47	2.39	4.18	202.59	2.44	80.56	3.27	5.20	4.90
3	1.88	4.61	3.13	3.86	222.89	1.01	155.27	2.27	7.90	3.30
4	1.90	4.14	3.38	3.11	223.89	1.51	147.09	1.58	7.82	2.74
5	1.37	4.91	5.30	3.54	239.34	2.94	170.51	2.10	9.43	2.26
6	1.37	3.66	5.69	4.57	241.70	2.72	183.62	1.17	9.90	3.38
Tail										
1	0.99	3.18	1.71	4.24	215.03	1.76	111.67	2.17	12.75	1.40
2	1.08	4.07	1.65	3.88	222.93	2.26	119.75	3.82	13.15	4.03
3	1.68	3.79	2.17	2.64	258.30	2.44	75.39	3.36	18.16	3.92
4	1.65	4.15	2.42	3.12	267.47	0.98	75.40	2.40	20.80	4.25
5	2.09	5.18	3.17	1.31	280.40	2.10	95.85	2.61	25.33	2.15
6	2.45	4.65	3.42	1.77	289.13	2.56	101.67	2.811	28.77	4.49

TABLE-9
ADDITION-RECOVERY TEST FOR DIFFERENT PARTS OF *Cyprinus carpio* AND TILAPIA FISHES (N = 5)

Cyprinus carpio fish				Tilapia fish		
Element	Added (µg/g)	Found (µg/g)	Recovery (%)	Added (µg/g)	Found (µg/g)	Recovery (%)
Muscle				Muscle		
Zn	–	78.45 ± 1.73	–	–	148.15 ± 1.79	–
	40	106.24 ± 3.18	94.93	100	244.40 ± 5.40	96.25
	60	137.89 ± 3.32	99.07	200	344.63 ± 5.84	98.24
	80	155.48 ± 3.42	96.28	300	457.31 ± 13.00	103.05
Cu	–	2.64 ± 0.08	–	–	5.31 ± 0.06	–
	4	6.45 ± 0.29	95.25	4	9.41 ± 0.15	102.48
	6	8.71 ± 0.13	101.125	6	11.27 ± 0.19	99.34
	8	10.54 ± 0.29	98.70	8	13.06 ± 0.31	96.94
Fe	–	43.59 ± 0.46	–	–	120.45 ± 1.75	–
	40	82.30 ± 5.22	96.78	100	217.61 ± 3.52	97.16
	60	101.76 ± 1.96	96.95	200	313.95 ± 6.13	96.75
	80	124.26 ± 3.05	100.84	300	419.25 ± 6.74	99.60
Ni	–	0.90 ± 0.01	–	–	1.51 ± 0.08	–
	1	1.85 ± 0.09	94.95	1	2.45 ± 0.11	93.96
	2	2.83 ± 0.11	96.24	2	3.41 ± 0.12	95.23
	3	3.83 ± 0.11	97.74	3	4.41 ± 0.16	96.85
Mn	–	1.37 ± 0.07	–	–	2.11 ± 0.06	–
	1	2.33 ± 0.14	96.68	2	4.18 ± 0.20	103.45
	2	3.23 ± 0.17	93.24	4	5.98 ± 0.21	96.74
	3	4.23 ± 0.15	95.28	6	7.87 ± 0.27	96.14
Skin				Skin		
Zn	–	124.39 ± 1.37	–	–	239.34 ± 7.03	–
	100	225.71 ± 10.86	101.32	200	439.26 ± 6.34	99.46
	200	318.91 ± 5.73	97.26	300	532.62 ± 9.28	97.76
	300	429.31 ± 4.28	101.64	400	615.38 ± 6.04	94.01
Cu	–	4.19 ± 0.05	–	–	5.30 ± 0.18	–
	4	8.21 ± 0.20	100.56	4	9.38 ± 0.28	101.96
	6	10.35 ± 0.27	102.71	6	11.35 ± 0.35	100.9
	8	12.16 ± 0.19	99.64	8	13.23 ± 0.30	99.18
Fe	–	57.17 ± 1.71	–	–	170.51 ± 3.58	–
	40	94.84 ± 2.92	94.17	100	266.05 ± 4.74	95.54
	60	114.93 ± 3.75	96.26	200	366.39 ± 4.29	97.94
	80	134.87 ± 1.72	97.12	300	454.34 ± 5.32	94.61
Ni	–	0.91 ± 0.01	–	–	1.37 ± 0.06	–
	1	1.91 ± 0.08	99.45	1	2.42 ± 0.09	104.95
	2	2.89 ± 0.13	99.01	2	3.48 ± 0.12	105.77
	3	3.85 ± 0.17	97.94	3	4.29 ± 0.13	97.32
Mn	–	1.63 ± 0.02	–	–	9.43 ± 0.21	–
	1	2.60 ± 0.15	97.38	10	19.31 ± 0.59	98.82
	2	3.59 ± 0.11	101.87	20	30.08 ± 0.21	103.24
	3	4.56 ± 0.19	97.55	30	38.65 ± 0.80	97.41
Tail				Tail		
Zn	–	309.41 ± 0.86	–	–	280.40 ± 5.90	–
	100	411.86 ± 6.22	102.45	200	476.50 ± 8.13	98.05
	200	513.53 ± 3.60	102.06	300	569.84 ± 6.57	96.48
	300	615.17 ± 3.52	101.92	400	670.96 ± 3.54	97.64
Cu	–	0.89 ± 0.02	–	–	3.17 ± 0.04	–
	1	1.87 ± 0.03	98.44	4	7.06 ± 0.15	97.19
	2	2.87 ± 0.13	99.16	6	8.95 ± 0.25	96.43
	3	3.81 ± 0.16	97.60	8	10.99 ± 0.19	97.71
Fe	–	11.86 ± 0.68	–	–	95.85 ± 2.50	–
	40	51.55 ± 2.83	99.22	100	199.69 ± 2.48	103.84
	60	71.07 ± 1.38	98.69	200	290.41 ± 2.43	97.28
	80	92.45 ± 2.02	100.74	300	385.32 ± 6.64	96.49
Ni	–	0.77 ± 0.01	–	–	2.09 ± 0.10	–
	1	1.73 ± 0.08	96.37	4	6.02 ± 0.25	98.24
	2	2.67 ± 0.12	95.12	6	8.01 ± 0.25	98.66
	3	3.63 ± 0.15	95.38	8	9.73 ± 0.23	95.50
Mn	–	9.94 ± 0.12	–	–	25.33 ± 0.54	–
	4	13.72 ± 0.29	94.42	40	67.19 ± 2.11	104.65
	6	15.57 ± 0.19	93.87	60	85.47 ± 0.71	100.24
	8	17.69 ± 0.19	96.90	80	102.76 ± 1.85	96.79

in Tilapia fish species varied between 0.86-2.34, 68.48-125.74, 103.36-152.68, 3.25-5.31 and 1.08-2.51 ($\mu\text{g/g}$) in muscle, 5.20-9.90, 73.12-183.62, 200.39-241.70, 2.13-5.69 and 1.09-1.90 ($\mu\text{g/g}$) in skin and 12.75-28.77, 75.39-119.75, 215.03-289.13, 1.65-3.42 and 0.99-2.45 ($\mu\text{g/g}$) in tail, respectively. By comparison among the six different weights and lengths of two species (*Tilapia* and *Cyprinus carpio*) fish, we did not observed any significant variation of studied heavy metals in relation to their weights and lengths. It is due to breeding studied fish in artificial lakes, where there is no metals contamination or pollution. Raised recoveries values were reflected to good digestion efficacy by microwave technique. The relative standard deviation for digestion efficacy of the amount of dried muscle fish begins to be acceptable from 0.7 g for nickel and copper, 0.1 g for copper, zinc and iron, 0.6 g for manganese.

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