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ORIGINAL ARTICLES

Vitamins Contents of some Commercially Important Fish Species from South Caspian Sea

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ABSTRACT:

The fat and water soluble vitamin contents (A, D, E, K, B_1, B_2, B_6, B_{12}, niacin, biotin, ascorbic, folic and pantothenic acids; in mg/kgfw) were analyzed in important fish (South Caspian Sea mullet, carp, kilka, kutum, perch). The results showed significant difference in 5 species ($p \leq 0.01$), and the maximum content was found to be: A in kutum (5); E, K, D, B_{12} in kilka (81, 0.46, 6.5, 0.72); C, pantothenic and folic acids in perch (24.23, 23.3, 0.28); B_2, B_1, B_6, biotin in mullet (7.95, 7.76, 12.6, 0.15); niacin in carp (104). The cluster results showed that with respect to the fat soluble vitamins, carp and perch, followed by the mullet, and in the other group kutum and kilka have a good similarity. Concerning the water soluble vitamins, kutum and perch have a good similarity followed by the mullet and kilka, and carp has no similarity with the others.

Key words: Fish. Fat soluble vitamins. Water soluble vitamins. High-performance liquid chromatography. Caspian Sea

Introduction

Fish and other marine species give rise to products of great economic importance in many countries. Aubourg S.P., 1999. and have become increasingly pursued due to the increasing population (Benjakul *et al.*, 2003). Fish is considered as a valuable source of proteins with high biological value in the human diet rich in certain minerals and vitamins and low in saturated fatty acids and cholesterol. The significance of long chain poly unsaturated fatty acids has gained attention because of their prevention of human cardiovascular diseases. Fishes are the main contributors of n-3 PUFA for human diet according to the American Heart Association. Fish consumption frequency should be at least twice a week to result in cardioprotective effects. Therefore, consumption of both freshwater and seawater fish is recommended (Aro *et al.*, 2005; Ozogul *et al.*, 2006; Sioen *et al.*, 2007; Nielsen *et al.*, 2007; Domingo, 2007).

As vitamins are organic compounds that are indispensable in very small amounts in the diet, and food is the main source of vitamins for human and animals, we need to determine the vitamin content of foods. Vitamins form a heterogeneous group of substances and are vital nutrients, and the absence of vitamins causes serious physiological problems. They have specific and individual functions to promote growth or reproduction or maintain health and life. Vitamins regulate metabolic processes, control cellular functions and prevent diseases such as scurvy and rickets. Conventional nutritional science now recognizes 13 vitamins divided into two categories: four fat-soluble vitamins, and nine water-soluble vitamins (Leskova *et al.*, 2006. Lebiedzi *et al.*, 2007).

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Fish is the most important dietary source of vitamin D and the vitamin content is dependent on the species (Benjakul *et al.*, 2003) Martin *et al*., 1990; Mattila *et al.*, 1999; Aubourg, 1999).

Fish muscle is a good source of niacin, pyridoxine, vitamin B_{12} (especially fatty fish and shellfish), whereas the concentration of riboflavin is quite variable, with the dark meat containing more riboflavin than the white meat. Concentration of riboflavin in fish is comparable to that found in terrestrial animals, but the vitamin K content of fish is relatively low (Martin *et al*., 1990. Ostermeyer U. and Schmidt T., 2001).

The highest concentration of pantothenic acid in fish appears to be found in the gonads, whereas vitamin A in fish is found concentrated in the viscera, especially the liver (Martin *et al*., 1990).

The aim of this study, undertaken at Tarbiat Modares University, was to evaluate the vitamin content of the most important fish species caught in South Caspian Sea (Anonymous. 2004).

Materials and methods

Fish species

The fish species used for this study were golden grey mullet (*Liza Aurata*), common carp (*Caprinus Carpio*), common kilka (*Clupeonella Cultriventrif Caspea*), Caspian kutum (*Rutilus Srisii Kutum*) and pike perch (*Sander Lucioperea*). These species (in the same genus, weight and size; November 2006; 25-30 specimens; middle size value) were purchased from three different harbors (Anzalii, Babolsar and Turcoman harbors located in north of Iran, represented by South West, Middle South and South East of Southern Caspian Sea, respectively). On board the fish specimens were beheaded, gutted and edible sections were selected. Then, the edible sections of each species from all harbors were mixed (for representation by Southern Caspian Sea), and transferred to the laboratory 5 hours post-capture on arrival in ice and were used for the analysis.

Materials

α-tocopherol was purchased from Fluka (Buchs, Germany); B_{12} (cyanocobalamin), B_1, B_2, B_6, biotin, niacin, pantothenic acid, folic acid, ascorbic acid from Sigma Chemical Co. (MO, USA); and vitamins D_2, D_3, K_1, all trans retinol and other chemical reagents having analytical grade with highest purity from Merck (Darmstadt, Germany).

Preparation of samples

The sample preparation and analysis of vitamins A, E and D was performed according to the modified method of Salo-Väänänen *et al.*, 2000. as for the analysis of vitamin K, the modified method of Ostermeyer, and Schmidt (Ostermeyer. and Schmidt, 2001). was used; the analysis of biotin was conducted according to the modified method of Lahely *et al.,* 1999. the analysis of B_{12} was done in line with the modified method of Heudi *et al.,* 2005. and finally the analysis of other water soluble vitamins was carried out according to the modified method of Ekinci, 2005.

HPLC analysis

HPLC is the preferred technique for vitamin separation because of its high selectivity (Ekinci, 2005). Therefore, we used HPLC system for the vitamin analysis of fishes. All HPLC analyses were performed at 25 °C on Waters 600E high performance liquid chromatograph (Waters, Millford, USA) equipped with the Eurospher 100-5 C_{18} (250×4.6mm i.d; 5 µm particle size) column for A, E, D and water soluble vitamins, and also equipped with Perfectsil (250×4.6mm i.d; 5 µm particle size) column with corresponding guards for vitamin K and clean-up of vitamin D.

The stock solutions of vitamins D_2, D_3, all trans retinol and α-tocopherol were prepared by dissolving appropriate amounts of each standard in ethanol; those of K_1 in hexane, and the stock solution of water soluble vitamins was prepared in an aquatic solution.

For determining vitamin A and E values, the mobile phase was methanol, and its flow rate was 1ml/min. Detection was accomplished with a UV (at λ_{max}=325nm) and fluorescence (at λ_{ex}=292 nm and λ_{em}=325 nm) detector for vitamins A and E, respectively. The injection volume was 50 µl.

As for determining vitamin K, D and clean-up of vitamin D, the mobile phase was 2% (v/v) tert-butyl methyl ether in hexane, 93% methanol and 7% water; and n-hexane, tetrahydroforan, and 2-propanol (98:1:1), respectively. Detection was accomplished with a UV detector and chromatogram was recorded at 248, 265 and 265nm for vitamins K, D and clean-up of D, respectively. The injection volume was 50 µl.

Finally, for determining biotin, B_{12} and other water soluble vitamins, the mobile phase was aqueous 0.050% tri fluoroacetic acid and 100% acetonitrile, 0.025% tri fluoroacetic acid in water; and 100% acetonitrile, K_2HPO_4 (0.01M and pH 7) and methanol, respectively. Detection was accomplished with a UV detector and chromatogram was recorded at 220, 361 and 220nm for biotin, B_{12} and other water soluble vitamins, respectively. The injection volume was 50 µl. Chromatogram samples of all vitamins are shown in Fig 4.

Recovery of vitamins

Recovery tests were carried out by spiking the samples with standard solutions of vitamins before extractions to determine the recovery of the procedures. The average percentage of (n=2) recoveries of vitamin A (0.75 and 1.25 mg were spiked), E (0.750 and 0.875 mg were spiked), D (0.500 and 0.750 mg were spiked), K (0.0030 and 0.0075 mg were spiked), C (25.0 and 37.5 µg were spiked), B_1 (2.5 and 7.5 µg were spiked), B_2 (2.5 and 7.5 µg were spiked), B_6 (2.5 and 7.5 µg were spiked), B_{12} (2.5 and 7.5 µg were spiked), niacin (62.5 and 87.5 µg were spiked), pantothenic acid (12.5 and 25.0 µg were spiked), folic acid (2.5 and 7.5 µg were spiked) and biotin (2.5 and 7.5 µg were spiked) were 92, 95, 101, 95%, 95, 92, 92, 95%, 98, 100, 95, 92 and 85%, respectively.

Statistical analysis

One-way ANOVA and t-test were used; via SAS software (SAS Institute, 1990). to assess the statistically significant differences resulting from vitamin content in fish species. The results were presented as mean values from triplicates and the significant variations were considered at $p<0.01$. Cluster analysis was displayed by Ward's method. The linkage in a dendrogram shows the order of dissimilarity designated as a distance index (Krzanowski., 1995).

Results and discussion

The lipid content of golden grey mullet, common carp, common kilka, Caspian kutum and pike perch were determined by Bligh and Dyer method (Bligh and Dyer., 1959). and their values were 4.93±0.03, 3.61±0.03, 10.23±0.09, 6.72±0.01 and 1.97±0.01 % wet weight, respectively.

Statistical results showed significant differences in all 5 species for both fat and water soluble vitamins presented in Table 1 and 2, respectively.

Table 1: Fat soluble vitamins content (mg/kfw) of Caspian Sea fish species.

Vitamin	Golden grey mullet	Common carp	Common kilka	Caspian kutum	Pike perch
Vitamin A	1.4±0.4 ^{c}	1.2±0.2^{c}	4.3±0.3^{b}	5±0.8^{a}	0.69±0.08^{d}
Vitamin E	12.5±2.5^{c}	1.9±0.24^{d}	81±3.5^{a}	61±3^{b}	1.3±0.15^{d}
Vitamin D	1.47±0.22^{c}	1.4±0.3^{c}	6.5±0.7^{a}	5.2±0.45^{b}	0.83±0.13^{c}
Vitamin K	0.39±0.022^{a}	0.23±0.04^{c}	0.46±.02^{a}	0.3±0.035^{b}	0.1±0.01^{d}

Values with different letter in the same column are significantly different ($p \leq 0.01$)

Table 2: Water soluble vitamins (mg/kg fw) of Caspian Sea fish species.

Vitamin	Golden grey mullet	Common carp	Common kilka	Caspian kutum	Pike perch
Vitamin C	2.52±0.37^{c}	3.15±0.35^{c}	9.2±1.3^{b}	21.73±2.52^{a}	24.23±1.75^{a}
Vitamin B_1	7.76±0.25^{a}	2.35±0.35^{b}	ND	2.25±0.15^{b}	ND
Vitamin B_2	7.95±0.15^{a}	1.6±0.3^{c}	2.45±0.45^{b}	0.52±0.12^{d}	1.95±0.15^{b}
Vitamin B_6	12.6±1.6^{a}	1.8±0.3^{c}	2.53±0.55^{c}	2.35±0.55^{c}	9±0.3^{b}
Vitamin B_{12}	0.13±0.017^{c}	0.23±0.025^{c}	0.72±.075^{a}	0.54±0.087^{b}	0.18±0.01^{c}
Niacin	45.7±2.75^{bc}	104±5^{a}	6.65±0.45^{d}	51.5±2^{b}	42.3±3.25^{c}
Pantothenic acid	20.2±1.25^{a}	21.6±1.85^{a}	9.55±0.55^{b}	7.18±0.6^{b}	23.3±1.65^{a}
Folic acid	0.13±0.015^{b}	0.16±0.017^{b}	ND	0.15±0.04^{b}	0.28±0.03^{a}
Biotin	0.15±0.027^{a}	0.13±0.03^{a}	0.047±0.005^{b}	0.025±0.006^{b}	0.015±0.0046^{b}

Values with different letter in the same column are significantly different ($p \leq 0.01$)
ND = not detected.

The wide diversity in the determined vitamin A content of all the fish species ranged from 0.69 to 5.00 mg/kgfw, and the highest level was measured in Caspian kutum. According to this item, there is statistical difference between these species, except for common carp and golden grey mullet. The previously reported vitamin A content ranges from 500 to 1500 RAE/100g raw Common Cambodia fish species (RAE =retinol activity equivalents). Those differences may be attributed to the variability of the studied species (Roos *et al*., 2007).

α-tocopherol as vitamin E is above the range of values from 1.3 to 81 mg/kgfw. The best source of vitamin E among the samples is common kilka (81mg/kgfw) with Caspian kutum (61mg/kgfw) as the other good source of this vitamin.

Cholecalciferol as vitamin D in these fishes varied from 0.83 to 6.5 mg/kgfw. The results showed the statistical differences of vitamin D content, except between common carp, golden grey mullet and pike perch. Common kilka has the highest content of vitamin D (6.5mg/kgfw). Our data do not corroborate previous studies which showed no correlation between the fat and cholecalciferol content of fish (Mattila *et al*., 1999). However, our results are in accord with those studies enunciating that oily fish, such as a mackerel and herring, contain a higher level of vitamin D than leaner fish like flounder and sea trout (Martin and Flick, 1990).

In all the studied fish species, vitamin K_1 was considered as vitamin K and varied within the range 0.1 to 0.46mg/kgfw. As a result, there was significant statistical difference among the fish species, except for common kilka and golden grey mullet. The maximum content of vitamin K was found in common kilka (0.46 mg/kgfw) which confirms the results that there is a relationship between fat and vitamin K content in fish samples. Fish with high content of fat had a high content of vitamin K as well (Ostermeyer and Schmidt, 2001).

As expected, the highest content of fat soluble vitamins is in fatty fishes. This finding further corroborates those previously reported by USDA (Anonymous, 2007).

Ascorbic acid content of 5 fish species was determined. Vitamin C was over the range 2.52 to 24.23 mg/kgfw, and the highest content was in pike perch (24.23 mg/kgfw). There are significant statistical difference among vitamin C contents of the studied samples except between Caspian kutum and pike perch, and between common carp and golden grey mullet.

The vitamin B_1 contents of fish species are in the range of 2.25 to 7.76 mg/kgfw. The highest amount of vitamin B_1 was in golden grey mullet (7.76 mg/kgfw). With our analytical procedure, no vitamin content was detected in common kilka and pike perch. Statistical analysis of data showed that there is no difference between Caspian kutum and common carp but golden grey mullet showed difference from the others. The vitamin B_1 contents of fish species (cod, saithe and salmon) are in the range of present study (Liaset. and Espe, 2008).

The vitamin B_2 content ranged from 0.52 to 7.95mg/kgfw. Amongst the analyzed samples, golden grey mullet was the best source of vitamin B_2 (7.95 mg/kgfw). The Statistical analysis of the given data showed that pike perch and common kilka have no difference with each other, but in other cases there were differences between the fish species.

The vitamin B_6 contents showed a range of 1.8 to12.6 mg/kgfw vitamin content with Golden grey mullet having the highest vitamin content. According to the analyses, there was no difference among golden grey mullet, common carp and Caspian kutum. In addition, there was statistical difference between golden grey mullet and pike perch.

Concentration of vitamin B_{12} in the selected fish species showed that of all species, common kilka was characterized by the highest vitamin B_{12} content (0.72 mg/kgfw). The range of vitamin content was between 0.13 to 0.72 mg/kgfw. Pike perch, common carp and golden grey mullet showed a very close range of vitamin B_{12} concentration, and there was no significant statistical difference among them. However, common kilka and Caspian kutum had a statistically significant difference. The vitamin B_{12} contents of the fish species (cod, saithe and salmon) were within the range of the present study (Liaset. and Espe, 2008).

It was showed that the values of niacin concentration in the studied fish species were above the range of 6.65 to 104mg/kgfw. Among the fish species analyzed, Common carp was the richest in niacin (104mg/kgfw) compared to the others. The data analysis showed that there were no statistical difference between Caspian kutum and golden grey mullet; and between golden grey mullet and pike perch. Nevertheless, \in other instances the fish species showed significant difference.

Pantothenic acid concentration of fish species ranged from7.18 to 23.3mg/kgfw. Among the studied species, the highest pantothenic acid content belonged to pike perch. The Statistical analysis of pantothenic content showed that there were no difference among pike perch, common carp and golden grey mullet. In addition, Caspian kutum and common kilka showed no significant difference.

In all samples, except for common kilka, folic acid was detected with values ranging from 0.13 to 0.28 mg/kgfw. Among the studied species, pike perch was the most important fish with respect to the folic acid content. The Statistical analysis of the obtained data showed that Caspian kutum, common carp and golden grey mullet had no difference, while pike perch was showed difference from the others.

Levels of biotin analyzed for the studied fish species had a range of 0.15 to 0.47mg/kgfw. According to the studied samples, common kilka was the best source of biotin (0.47mg/kgfw). The statistical analyses showed that common carp and golden grey mullet had no significant difference. In addition, common kilka, pike perch and Caspian kutum were similar, yet in other situations significant difference was observed.

Our data are similar or lower for the vitamins B_2, B_6 and Pantothenic acid contents; lower in the niacin and folic acid contents and higher in the niacin contents of fish species (cod, saithe and salmon). This might be due to the species used in the different experiments (Liaset. and Espe, 2008).

Our data fall well within the range published by USDA for the water soluble vitamin contents of some fishes (Anonymous, 2007).

Based on our literature studies, there were no information on the amount of other vitamins in fish and, therefore, we could not compare our results with those of other studies.

The results of cluster analysis are shown in Figure 1 for all vitamins, Figure 2 for fat soluble and Figure 3 for water soluble vitamins. The dendrogram was classified into groups by randomly applying a rescaled distance cluster Ward's method. The cluster analysis results showed that with respect to all vitamins, common carp and pike perch had a good similarity followed by golden grey mullet. In addition, Caspian kutum and common kilka showed a good similarity with each other, while having no similarity with the other species. As for the fat soluble vitamins, common carp and pike perch have noticeable similarity, followed by the golden grey mullet; and in the other group, Caspian kutum and common kilka showed noticeable similarity. Finally, with respect to the water soluble vitamins, Caspian kutum and pike perch manifest a good similarity followed by the golden grey mullet and common kilka, respectively; and common carp has no similarity with the others.

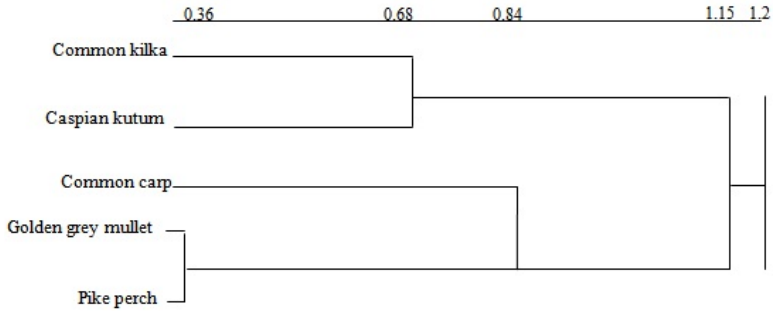


Fig. 1: Cluster analysis of fish species using Ward's method based on total vitamins.

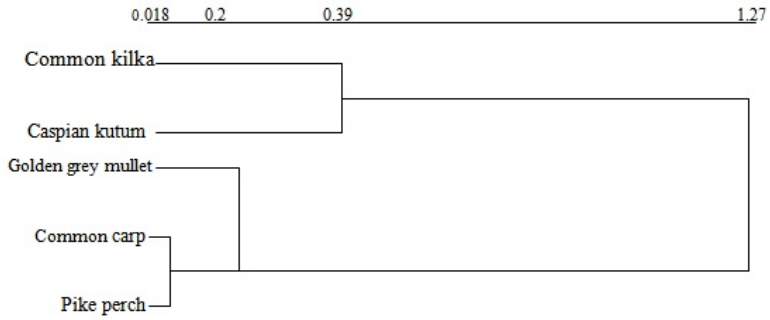


Fig. 2: Cluster analysis of fish species using Ward's method based on fat soluble vitamins.

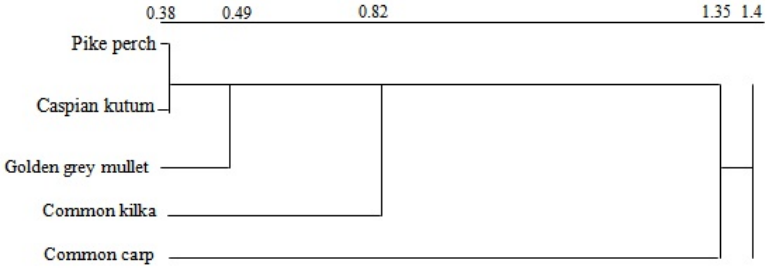
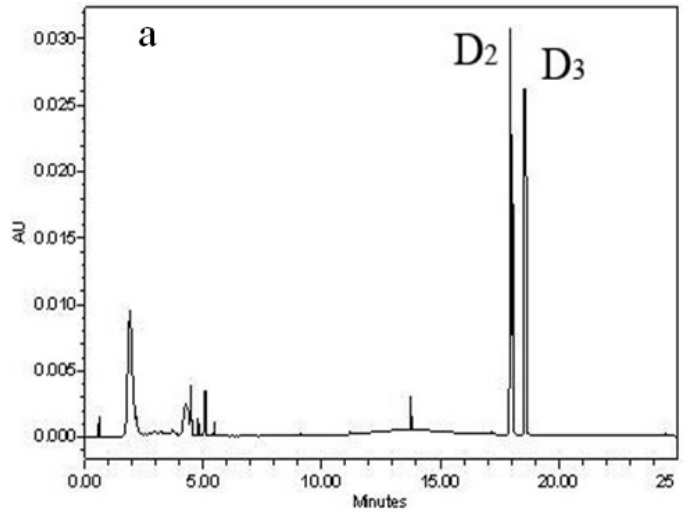


Fig. 3: Cluster analysis of fish species using Ward's method based on water soluble vitamins.

Conclusion

Among the most important fish species of South Caspian Sea, the richest source of vitamin content was as follows: Caspian kutum has the maximum vitamin A content; common kilka had the highest content of E, K, D and B_{12} vitamins; pike perch has the highest content of vitamin C, pantothenic acid and folic acid; golden grey mullet has the maximum B_2, B_1, B_6 and biotin vitamins; and common carp has the highest content of niacin. Furthermore, the clustering analysis showed the highest similarity between golden grey mullet and pike perch, common carp and pike perch, Caspian kutum and pike perch for all the fat soluble and water soluble vitamins, respectively.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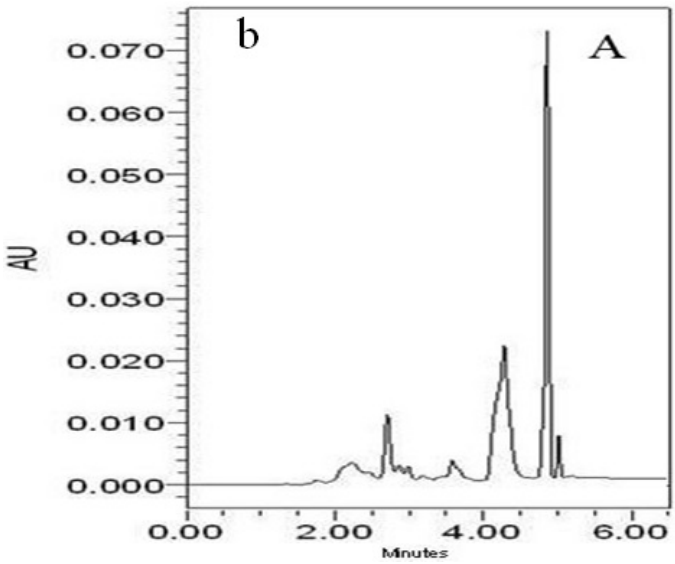
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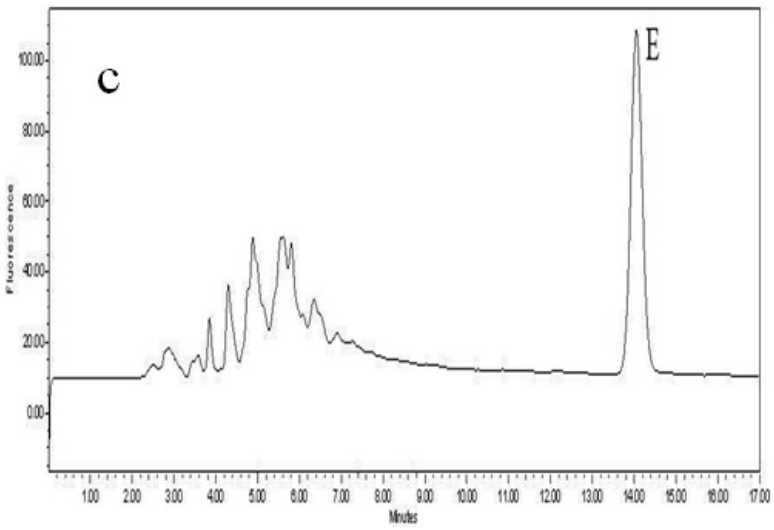
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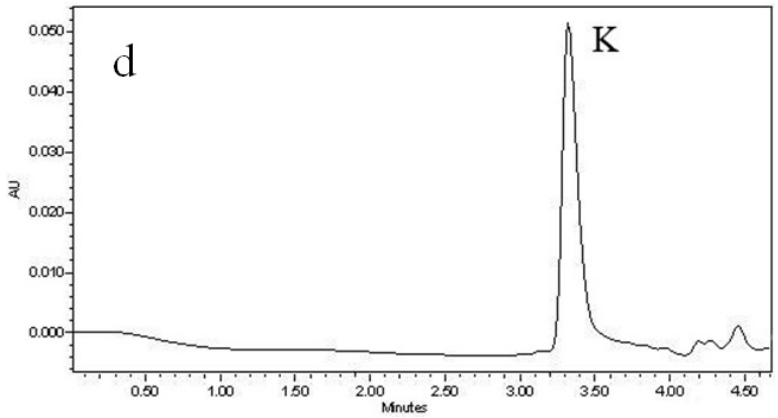
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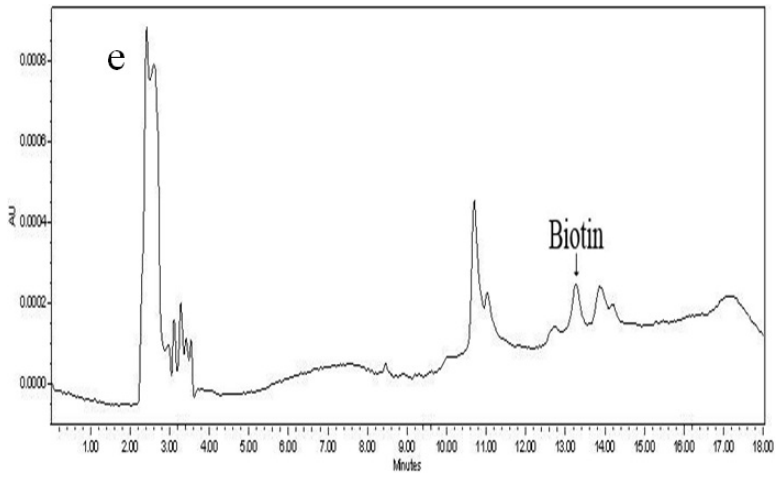
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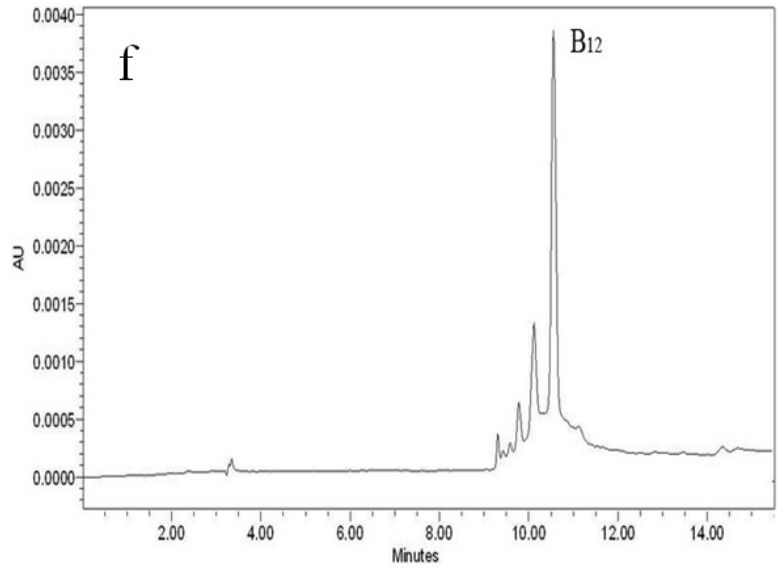
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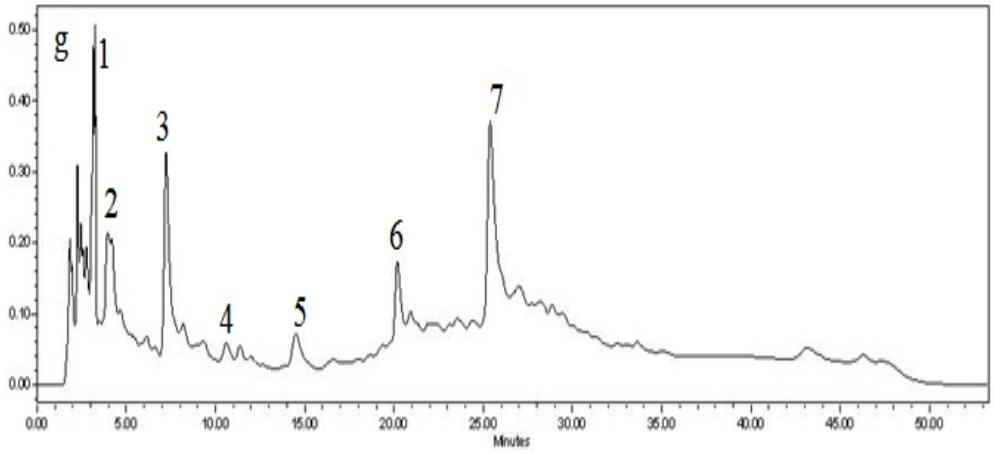

Fig. 4: hromatograms of some vitamins.

a: vitamin D in Caspian kutum; b: vitamin A in common kilka; c: vitamin E in Caspian kutum; d: vitamin K in golden grey mullet; e: biotin in common carp; f: vitamin B_{12} in common kilka; g: some water soluble vitamins in golden grey mullet (1: ascorbic acid; 2: pantothenic acid; 3: niacin; 4: folic acid; 5: B_1; 6: B_2; 7: B_6).

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References

Aubourg, S.P., 1999. Lipid damage detection during frozen storage of an underutilized fish species. J. Food Res. Inter., 32: 497-502.

Benjakul, S., W. Visessanguam, C. Thomgkeaw, and M. Tanaka, 2003. Comparative study on physicochemical changes of muscle proteins from some tropical fish during frozen storage. J. Food Res. Inter., 36: 787-795.

Aro, L.T., S.P. Larmo, H.C. Bäckman, P.K. Heikki and R.L. Tahvonen, 2005. Fatty acid and fat-soluble vitamins in salted Herring (*Clupea harengus*) products. J. Agric. Food Chem., 53: 1482-1488.

Özogul, Y., F. Özogul, A. Esmeray Kuley, S. Özkutuk, C. GÖkbulut, and S. KÖse, 2006. Biochemical, sensory and microbiological attributes of wild turbot (*Scophthalmus maximus*) from the Black sea during child storage. Food Chem., 99: 752-758.

Sioen, I., S. De Henauw, F. Verdonck, N.V. Thuyne and J.V.. Camp, 2007. Development of a nutrient database and distributions for use in a probabilistic risk–benefit analysis of human seafood consumption. J. Food Comp. Anal., 20: 662-670.

Nielsen, N.S., D. Debnath and C. Jacobsen, 2007. Oxidative stability of fish oil enriched drinking yoghurt. Inter. Dairy J., 17: 1478-1485.

Domingo, J.L., 2007. Omega-3 fatty acids and the benefits of fish consumption: Is all that glitters gold? Envir. Inter., 33: 993-998.

Leskova, E., J. Kubikova, E. Kovacikova, M. Kosicka, J. Porubska and K. Holcikova, 2006. Vitamin losses: Retention during heat treatment and continual changes expressed by mathematical models. J. Food Comp. Anal., 19: 252-276.

Lebiedzi, A., M.L. Marszall, J. Kuta and P. Szefer, 2007. Reversed-phase high-performance liquid chromatography method with coulometric electrochemical and ultraviolet detection for the quantification of vitamins B1 (thiamine), B6 pyridoxamine, pyridoxal, pyridoxine) and B12 in animal and plant foods. J. Chrom. A. (in press).

Martin, R.E. and G.J. Flick, 1990. The Sea Food Industry. Van Nostrand Reinhold, New York. 445.

Mattila, P., R. Ronkainen, K. Lehikoinen and V. Piironen, 1999. Effect of household cooking on the Vitamin D content in fish, eggs, and Wild Mushrooms. J. Food Comp. Anal., 12: 153-160.

Ostermeyer, U. and T. Schmidt, 2001. Determination of vitamin K in edible part of fish by high-performance liquid chromatography. J. Euro. Food Res. Tech., 212: 518-528.

Anonymous, 2004. Statistical Fishery Year Book, *Naghshe Bayan,* Tehran, 42.

Salo-Väänänen P., V. Ollilainen, P. Mattila and K. Lehikoinen, 2000. Simultaneous HPLC analysis of fat-soluble vitamins in selected animal products after small-scale extraction. Food Chem., 71: 535-543.

Lahèly, S., S. Ndaw, F. Arella. and C. Hasselmann, 1999. Determination of biotin in foods by high-performance liquid chromatography with post-column derivatization and fluorimetric detection. Food Chem., 65: 253-258.

Heudi, O., T. Kilinc, P. Fontannaz and E. Marley, 2005. Determination of vitamin B_{12} in food products and in premixes by reversed-phase high performance liquid chromatography and immunoaffinity extraction. J. Chrom. A, 1101: 63-68.

Ekinci, R., 2005. The effect of fermentation and drying on the water-soluble vitamin content of tarhana, a traditional Turkish cereal food. Food Chem., 90: 127-132.

SAS Institute, 1990. SAS/STAT^{R} user's guide, release 6.03 edition, SAS Institute INC. Cary, NC.

Krzanowski, W.J., 1995. Multivariate Analysis, Oxford: Oxford University Press US.

Bligh, E. and W. Dyer, 1959. A rapid method of total lipid extraction and purification. Canadian J. Biochem Physio., 37: 911-917.

Roos, N., C. Chamnan, D. Loeung, J. Jakobsen and S.H. Thilsted, 2007. Freshwater fish as a dietary source of vitamin A in Cambodia. Food Chem., 103: 1104-1111.

Anonymous. 2007. USDA national nutrient database for standard reference. http// www. Nalusda.gov/finc/foodcomp/.

Liaset, B. and M. Espe, 2008. Nutritional composition of soluble and insoluble fractions obtained by enzymatic hydrolysis of fish-raw materials. Process Biochem., 43: 42-48.