THE BOUILLON TABLES FROM TURKEY: DETERMINATON OF CIS-*TRANS* FATTY ACID PROFILES BY CAPILLARY GAS CHROMATOGRAPHY

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Abstract. Nowadays, commmercial bouillon tablets are mostly used as a flavor enhancer substant or an instant product in Turkish cuisine. As chemical structure, *Trans* FAs are unsaturated fatty acids having at least one double bond in its *trans* geometric configuration. *Trans* FA are occured by the partial hydrogenation of vegetable oils in the manufacturing of margarine and vegetable shortening. In this study, industrially produced commmercial bouillon tablet (n=14) samples from Turkey were analyzed by capillary (DB 23 column) gas chromatography method and an under controlled temperature oven programm with particular emphasis on cis–*trans* fatty acid profiles. The bouillon samples were collected as two goups (first group containing animal additives [AAB n=12] and second group including vegatable additives [VAB n=2]). There is no detailed information on fatty acid (FA) compounds, including *trans* fatty acids (TFAs), of consumed several commercial boullions in Turkey. There is no "*trans* fatty acid-free" declaration on various bouillon labels in Turkey. Large variations were observed among the cis–*trans* FA profiles of the boullion samples from Turkey,despite the fact that the samples are produced in the same production conditions.The palmitic (PAM) levels (predominant cis saturated FAs for all samples) of AAB samples (32.63–44.44%) were more high rather than the VAB samples (47.62–50.64%). Other major saturated FAs for AAB and VAB samples were determined stearic ((5.28–9.90% AAB and 6.33–6.73% VAB), lauric (0.19– 5.84% AAB and 1.13–1.30% VAB) and myristic acids (0.86–2.61% AAB and 1.39–1.45% VAB).The changes of oleic (OLA) acid (from other predominant cis monounsaturated FA) of VAB samples (30.75–33.45%) were high than those of AABs (12.64–29.35%). The ranges of LO, an essential /nutritional fatty acid and the predominant FA of PUFAs, and PUFAs of AAB – except two samples– commercial bouillon samples (0.45–9.82% and 0.45–9.97%) were low rather than VAA samples (7.97–8.14% and 8.14–8.35 %). Conjugated Linoleic (CLA) FAs, having nutritional FAs having anti-carcinogenic, antioxidative and anti-atherosclerotic effects, were determined in small amounts for only six samples (0.03% to 0.06%). Elaidic acid (C18:1 *trans* acid) content, the mainly *trans* FA in all boullion, was within the range of 20.00–27.32% in the AAB samples, and it was significantly higher than the range in VAB samples (0.08–0.14%). Total *trans* fatty acids (TFAs) were another important major (second) FAs for all bouillon samples. The changes of *Trans*/Cis ratio ranged between 0.26 and 0.50 for AAB samples but VB samples has a little values from 0.002 to 0.003. Turkish vegetable boullion. samples have low total *trans* FA contents (0.19–0.26%) than the animal boullion samples (20.19–28.78%).There are a large variation and significant ($P<0.05$) differences were statistically determined among major cis FAs (PAM, SA, OLA, LO and LN) and their involved parameters (SFAs, MUFAs and PUFAs). The consumed several commercial boullions in Turkey were classified and characterized chemometric method (Principal Component Analysis, PCA) based on some fatty acid profiles and their parameters. Applying PCA to the all bouillon samples data determined the percentage of total variance explained by the first two PCs were 49.3% and 21.8% (totally 71.1%), respectively.

Keywords: bouillon tablets, cis- *trans* fatty acids, GC-capillary column, Turkey, chemometry.

Introduction. Formulation of the problem

A bouillon cube or tablet, a dehydrated flavor product from dehydrated vegetables, includes meat/chicken stock, a small portion of hydrogenated fats (partially hydrogenated vegetable oils, PHVO) and salt – sometime MSG [1-5] and they are mostly used as a flavor enhancer substant or an instant product in Turkish cuisine [6]. The bouillon cube or " known as a trade name Maggi cube" was firstly introduced to cusines in 1908 by Swedish Julius Maggi. This commercial product included the meat substitute and these products were developed to use of the local cuisines in many different countries, for example as undername Maggi herb in German and Dutch [3,5].

Analysis of recent research and publications

The production procedure of bouillon cubes includes blending with fats hardened by hydrogenation and these fats have higher fraction of saturated rather than unsaturated fatty acids. Hydrogenation process provides to reduce their level of unsaturation and enhance their resistance to oxidation. Coconut, palm and palm-nut oils having a higher fraction of saturated have been using for hydrogenation process in lipid technology. Also, addition of these fats make the bouillon solid or semi-solid at room temperature due to the high of their melting point [1,2,3,5,7,8].

As chemical structure, *Trans* FAs are unsaturated fatty acids having at least one double bond in its *trans* geometric configuration. *Trans* FA are occured by the partial hydrogenation of vegetable oils in the manufacturing of margarine and vegetable shortening [9,10]. As a remarkable dietary source in the daily nutrition is important industrial hydrogenated vegetable oils having *trans fatty acids* (TFA) in different levels and it can reach up to 60% in the fat. These FAs are mainly formed by industrial hydrogenation of economically important vegetable oils such as sun flower, soybean, coconut, palm and palm-nut oils and TFA content of industrially hydrogenated fats is dependent on diverse parameters of the technological process. PHVO containing industrial TFA (ITFA) are semi-solid, have a higher oxidative stability and a longer shelf life. TFAs have some risks in the view of human health because of increase serum levels of LDL cholesterol and decrease those of HDL cholesterol, especially the risk of coronary heart disease. Globally, PHVO are commonly used in processed food products (margarines, deep-fried foods, bakery and instant/confectionery products [especially bouillon cubes]). The bouillon cubes which known to contain PHVO, are great of as a TFAs source in the daily diet [1,2,7,8,9].

The chemical/main nutritional composition of bouillon cubes were reported by Akpanyung [3] and Al-Subhi [5]. Akpanyung [3] the main food components (moisture, protein, oil, ash) and minerals (sodium, iron and zinc) of commercial bouillon cubes produced in Nigeria were analyzed whereas Al-Subhi [5] performing the study of evaluation (crude protein,fat, ash, total fiber ans sensorial propreties) of mushroom broth cube and comparison with Maggi Broth Cube Products in Saudi Arabia. There have been some limited studies on the cis-*trans* fatty acid (FA) profiles of bouillon cubes from different countries and Turkey [1,2,6,7,8]. There is no detailed information on fatty acid (FA) compounds, including *trans* fatty acids (TFAs), of consumed several commercial boullions in Turkey. There is no "*trans* fatty acid-free" declaration on various bouillon labels in Turkey.

The purpose of this study is to determine the cis – *trans* fatty acid profile of commercial samples taken from various firms in Turkey based on nutritional quality of the lipid fraction of bouillon tablets. There are limited studies on the cis– *trans* fatty acid profile of commercial bouillon samples, a remarkable popular and commonly food additives in the diet of Turkish people is known.

For this purpose, it is necessary to achieve the following **objectives**:

1. To detect the nutritional fatty acids (especially n3 and n6) of bouillons;
2. To determine the level of trans fatty acids in bouillons,which are important for nutritional physiology;
3. To characterize the bouillon samples based on the fatty acid profile by multiple data analysis.

Research materials and methods

Materials and Industrial production of bouillon tables. In the present work, totally 14 industrially produced in Turkey are collected from various markets. The commmercial bouillon tablet samples are two goups first group (with beef extract n= 6 [BB], with lamb extract n=1 [LB] with chicken extract n=5 [CB] and with butter extract [BT] n= 1) containing animal additives (AAB) and second group (with vegatable extract n=2) including vegatable additives (VAB). The AAB samples (n=12) were coded according to their firms Maggi Beef Boullion (MG BB), Knorr Beef Boullion (KNR BB), Halk Beef Boullion (HLK BB), Ülker i Beef Boullion (ULK BB), Hürrem Beef Boullion (HR BB), Ülker Lamb Extract Boullion (ULK LB), Ülker Butter Extract Boullion (ULK BTB), Maggi Chicken Extract Boullion (MG CB), Knorr Chicken Extract Boullion (KNR CB), Ülker Chicken Extract Boullion (ULK CB), Halk Chicken Extract Boullion (HLK CB) and Hürrem Chicken Extract Boullion (HRM CB). The VAB (n=2) samples to the firms were coded Knorr Virgin Olive Oil Boullion (KNR VBO) and Knorr Vegetable Extract Boullion (KNR VB). All these samples (AAB and VAB, n=14) were analyzed in cis – *trans* fatty acid profiles, in particular essential fatty acid (omega 3 and 6 or n3 or n6 PUFA's) levels and other nutritional parameters. Industrial scale bouillon cube manufacturing process is shown in Figure 1.

All the dry ingredients are mixed together (followed by mixing/granulation with molten fat followed by addition of colour and flavours (step 1). The cooling (mostly referred as maturation becauese of solidifying of the molten fat) (step 2) and then shaped (step 4), wrapped and packed. These processes include the following temperatures respectively, step 1 about 45 °C and 55 °C, step 2 cooling at 30 °C and it can take up to 20–30 h in 50 lt – 200 lt bins (even longer during summer time) [4].

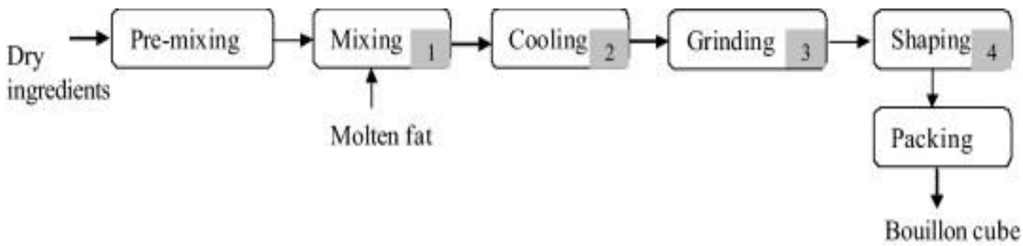

Figure 1. The industrial production of bouillon cubes [4]

Oil Extraction from Bouillon Cube Samples. The fats of bouillon cube samples were obtained by cold extraction (4-5 cube bouillon dissolved in 150 ml heptane at room temperature for 12 h) method using hexan. The hexane was removed with a rotary evaporator (40 ºC) and then the oil residue was stored at – 10 ºC in dark until cis – *trans* fatty acid composition could be determined [11**]**.

Cis – *Trans* Fatty Acid Profile Determination in Bouillon Samples. The *cis–trans* fatty acid contents of bouillion cube samples were determined using a standard capillary gas chromatographic method described in the IUPAC [12]. Fatty acid methyl esters (FAMEs) were prepared by a cold methylation technique (using hexan solvent and 2 M methanolic potassium hydroxide) (IUPAC,1987). Fatty acid analyses were carried out by gas chromatography (HP 6890) using a DB-23 capillary column (30 m x 0.25 mm ID and 0.25 µm film thickness J & W Scientific, Folsom, CA, USA). The conrolled oven temperature ranged from 170 °C to 210 °C with an increase of 2 °C/min and then was held at 210 °C for 10 min. Fatty acid profile data were calculated by the HP 3365 Chemistation program and recorded as a percent of the total peak area [13].

FA standards had linear calibration curves through the origin (R2 = 0.99). The GC method used were validated for cis – *trans* FA determination of bouillion cube samples within the 95% confidence limits. Mean analytical recoveries determined from individual FA in bouillion samples changed from 99.7% to 100%. The results were calculated as percentage peak area. The identification of FAMEs of samples was performed using a standard FAMEs mixture (Sigma-Aldrich Chemicals 189-19).

Statistical and Chemometric Analysis. All data from bouillion samples were presented as mean ± SD (n = 4) and were subjected to ANOVA. Among groups means were compared by Duncan multiple range tests at $\alpha = 0.05$ level (n – 1 = 14). The statistical analysis was performed using the SPSS 10 statistics software [14]. Characterization and classification of oils from all bouillion samples were carried out using chemometric methods, PCA (Ward Method) using Student's test in JMP11 (2014) program (SAS Institute, ISBN:978-1-62959-560-3) at a significance level of $p < 0.05$. Pearson's correlation coefficients (r) were determined. PCA and biplot analysis was performed to identy and calssify the relationships existing between all all bouillion samples based on their cis- *trans* fatty acid profiles.

Results of the research and their discussion

Cis – *trans* fatty acid (FA) profile is a critical and remarkable parameter used to characterize oil from boullion samples in terms of nutritional physiology. The DB-23 column, having high polarity, exhibited clear and excellent separation in all of short and long chain cis – *trans* FAs on a boullion sample (Fig. 2, HLK CB sample).

A total of 22 major – minor FAs were determined by capillary GC in various commercial boullion samples from Turkey (Table 1). The major and minor cis – *trans* fatty acid profiles of the 14 commercial boullion samples (containing animal additives [AAB] and including vegatable additives [VAB]) produced in Turkey is shown as percentages of total lipids in Table 1

Major – minor cis fatty acid profiles in commercial bouillon samples. As shown in Table 1, palmitic (PAM,C16:0), oleic (OLA,C18:1 n-9 and C18:1n-7), stearic (SA,C18:0), lauric (LA, C12:0), myristic (MA, C14:0) and linoleic (LO, 18:2 n-2) were measured as major cis fatty acids for all boullion samples. Other cis FAs, from long chain cis FAs, palmitoleic (POA,C16:1 n-7), linolenic (LNO, C18:2 n-2), arachidic (AA, C20:0), behenic (BA, C22:0) and lignoceric (LG, C 24:0) acids were detected in small amounts.

All Turkish boullion samples have a remarkable high (42.80 – 60.54 %) levels of saturated FAs and the level of palmitic acid (16:0), the major fraction of SFA in all samples, ranged from 32.63 to 50.64 % (Table 3). The palmitic levels of AAB samples (32.63 – 44.44%) were more high rather than the VAB samples (47.62 – 50.64 %) (Table 1). As shown in Table 1, the contents of other major SFAs for all bouillion samples were stearic ([C18:0] (5.28–9.90 %), lauric (LAU [C12:0] (0.19– 5.84%) and myristic acids (MA [C14:0] (0.84– 2.61%) (Table 1). Butyric acid (BA [C4:0]) was determined only one sample (BTB from AAB) as 0.14 %. In addition, there were in a little amounts of capric (CA) and caprylic (CPR) FAs for all samples. Among firms for levels of SFAs, considerable differences were observed according to the types of bouillion samples. The presence of a considerable amount of LAU (1.1 – 5.3 %) and MA (1.7 – 2.6 %) in some bouillon samples (HLK BB, HR BB, KNR CB, ULK CB, HLK CB, HR CB, KNR VB and KNR VBO) is an indication that coconut (coco nut) and palm kernel oil are used in production [8]. These findings on major cis SFAs (PAM, SA and others) and their involved parameters (total SFAs levels) were generally found similar to similar to those reported by some studies [1,2,6,7,8] for bouillions and also Kuhnt *et al*., [8], Arıcı *et al*., [9] and Demir and Taşan [10] data for margarine and Kuhnt *et al*., [8] results for butter.

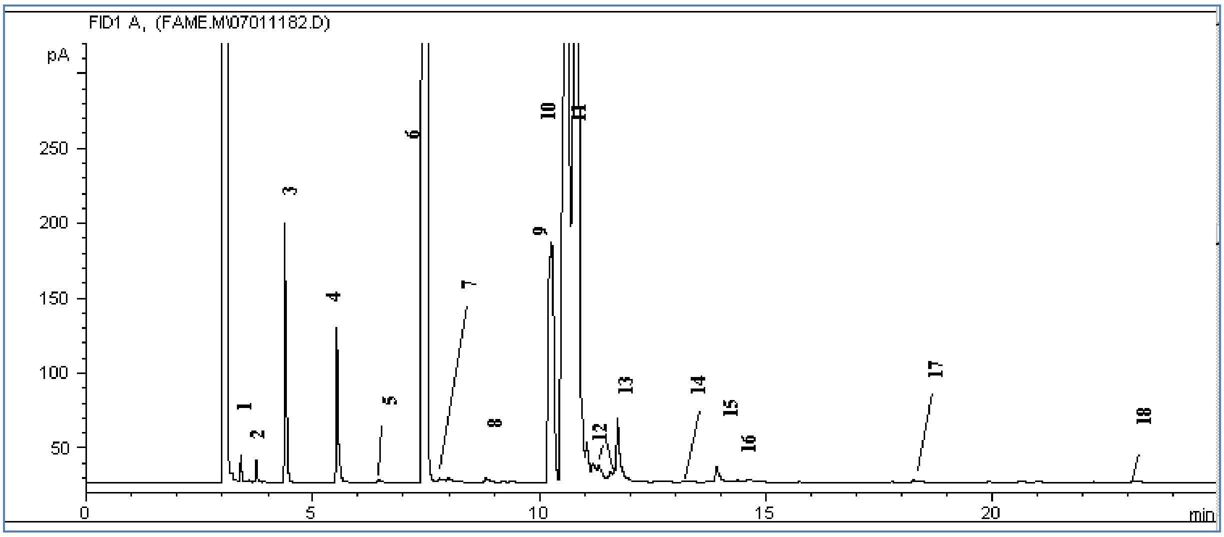

Figure 2. A typical chromatogram of commercia bouillion sample (HLK CB), cis–*trans* fatty acids
(1.Caproic [CP], 2.Caprylic [CPR], 3.Lauric [LAU], 4.Myristic [MA],5.Pentadecanoic, 6.Palmitic [PAM],7.Palmitoleic [POLA], 8.Margaric, 9.Stearic [SA], 10.Elaidic [EA], 11.Oleic [OLA], 12.*trans*-linoleic [tLO], 13.Linoleic [LO], 19.*trans* Linolenic [tLN],14.Linolenic [LN], 15.Aracidic [AA], 16. Gadoleic [GA],17.Behenic [BA],18.Lignoceric [LG])

Table 1 – The major and minor cis – *trans* fatty acid profiles commercial boullion samples produced in Turkey

Fatty acid	Samples													
	MG BB	KNR BB	HLK BB	ULK BB	HRM BB	ULK LB	ULK BTB	MG CB	KNR CB	ULK CB	HLK CB	HRM CB	KNR VBO	KNR VB
CP	0.02	0.09	0.53	0.36	0.38	0.39	0.14	0.02	0.2	0.19	0.44	0.42	ND	ND
CPR	0.04	0.08	0.43	0.21	0.4	0.28	0.24	0.02	0.17	ND	0.47	0.4	0.14	0.07
LA	0.78 ±0.08 f	1.21 ±0.20 e	5.11 ±0.80 b	0.87 ±0.08 f	5.01 ±0.50 b	0.57 ±0.07 gh	0.69 ±0.09 fg	0.19 ±0.02 ı	2.39 ±0.55 c	1.67 ±0.30 d	5.84 ±0.60 a	4.80 ±0.45 b	1.30 ±0.35 e	1.13 ±0.25 e
MA	1.26 ±0.08 g	1.17 ±0.05 h	2.43 ±0.08 b	1.31 ±0.08 g	2.51 ±0.10 a	1.21 ±0.04 h	1.58 ±0.10 e	0.86 ±0.08 ı	1.76 ±0.08 d	1.47 ±0.09 f	2.61 ±0.08 a	2.31 ±0.03 c	1.45 ±0.08 f	1.39 ±0.09 f
PAM	44.44 ±0.45 c	38.41 ±0.50 f	38.98 ±0.75 e	37.16 ±0.45 g	40.35 ±0.55 e	32.63 ±0.15 ı	37.88 ±0.25 g	36.52 ±0.20 h	42.70 ±0.55 d	38.52 ±0.35 fg	38.20±0 .55 fg	39.05 ±0.65 ef	47.62 ±0.35 b	50.64 ±0.55 a
POLA	0.04	0.05	0.05	0.07	0.03	0.1	0.13	0.36	0.03	0.05	0.03	0.03	0.16	0.10
MG	0.1	0.1	0.08	ND	0.07	0.2	0.12	0.09	0.1	0.06	0.09	0.08	0.11	0.10
MGO	ND	ND	ND	ND	ND	0.11	ND	0.01	ND	ND	ND	ND	0.03	ND
SA	8.10 ±0.15 c	7.85 ±0.55 cd	8.55 ±0.65 bc	5.28 ±0.45 g	8.83 ±0.25 b	6.80 ±0.5 e	7.59 ±0.53 d	5.95 ±0.45 f	6.97 ±0.44 e	5.82 ±0.25 f	9.90 ±0.75 a	9.16 ±0.45 ab	6.33 ±0.15 f	6.73 ±0.35 e
OLA	15.18 ±0.65 fg	22.06 ±0.78 d	15.97 ±0.85 f	28.00 ±0.85 c	14.63 ±0.15 g	29.35 ±0.35 c	22.50 ±0.65 d	20.03 ±0.35 e	21.21 ±0.75 de	28.27 ±0.65 c	12.64 ±0.45 h	14.78 ±0.45 g	33.45 ±0.43 a	30.75 ±0.75 b
LO	0.50 ±0.02 g	1.13 ±0.05 ef	1.05 ±0.09 ef	1.18 ±0.08 ef	0.53 ±0.02 g	1.43 ±0.11 d	6.72 ±0.95 c	9.82 ±0.95 a	1.24 ±0.15 e	1.70 ±0.15 d	0.45 ±0.02 g	0.61 ±0.03 g	8.14 ±0.85 b	7.97 ±0.90 b
LN	ND	0.03	0.04	ND	0.36	0.05	0.03	0.12	0.02	ND	ND	0.36	0.18	0.17
18:2 CLA	ND	0.04	0.03	ND	ND	0.06	0.03	0.03	ND	ND	ND	ND	0.03	ND
GA	0.17	0.23	0.33	0.13	0.05	0.19	0.13	0.23	0.15	0.07	0.27	0.04	0.12	0.07
AA	0.44	0.52	0.46	0.29	0.05	0.46	0.35	0.40	0.36	0.27	0.50	0.05	0.37	0.33
BA	0.07	0.08	0.06	0.09	0.08	0.1	0.13	0.10	0.05	0.07	0.11	0.04	0.09	0.06
LG	0.07	0.08	0.06	0.07	0.06	0.06	0.08	0.07	0.04	0.05	0.10	0.05	0.06	0.05

ND: Not Detected

The monounsaturated fatty acids (MUFAs, especially oleic acid [OLA] for bouillon oils) are of great importance because of their high nutritional value and contribution to the oxidative stability of oils. Other predominant cis fatty acids were oleic acid [OLA, C18:1n9] (12.64 – 29.35 % for AAB samples and 30.75 – 33.45 % for VAB samples, respectively) (Table 1) and MUFAs (12.94 – 29.75 % for AAB samples and 30.72 – 33.76 % for VAB samples, respectively) **(**Table 3). The changes of OLA and MUFAs of VAB samples were high than those of AABs. These findings on major cis MUFAs (OLA and others) and its involved parameter (total MUFAs levels) were generally found to be similar and compatible with the findings of bouillon [6,7,8] with soft type margarine [9] Our OA and MUFAs amounts were lower than those of reported by Caponio *et al.*, [1,2] for bouillons and Arici *et al*.,[9] for hard type margarines.

As shown in Table 1, linoleic acid [LO, C18:2 n-6], which is an essential /nutritional fatty acid and the predominant FA of PUFAs, was also present in all boiullion oils at lowest levels (0.45 – 9.82 %). The changes of PUFAs based on samples types (AAB and VAB) were between 0.45 – 9.97 % and 8.14 – 8.35 %, respectively (Table 3). The ranges of LO and PUFAs of AAB – except ULK BTB and MGG CB – commercial bouillon samples were low rather than VAA samples. Linolenic acid (LN, C18:3 n3) levels for samples were a little amount (0.03–0.36%) and also, there was no LN in four samples (MG BB, ULK BB, ULK CB, HLK CB). These results on major cis PUFAs (LO and LN) and its involved parameter (total PUFAs levels) were generally agree with the findings of bouillons [6,7] but our data on PUFAs were lower than those of reported for bouillons [1,2,8] and for margarines [9,10].

In addition, Conjugated Linoleic (CLA) FAs, as known nutritional FAs having anti-carcinogenic, antioxidative and anti-atherosclerotic effects, were determined in small amounts for only six samples (mostly AAB [BB, LB, BTB and CB also, VAB] types) and CLA values were ranged from 0.03 % to 0.06 % (Table 1). Milk, dairy products and meat of ruminant animals is a remarkable and primer source for CLA. Thermal treatments during refination or hydrogenation of oils/fats used in bouillon formulations, might elevate the content of CLA and more isomers might also occur [6]. Our CLA contents were lower than those of Kuhnt *et. al.*, [8] butter samples.

The range of total unsaturated fatty acids (UFAs [MUFAs + PUFAs]) for all commercial bouillon samples were between 13.39 % and 42.11 %. The range of UFAs of VAB commercial bouillon samples were low than AAB samples.

The variation and significant ($P < 0.05$) differences were determined among major cis FAs (PA, SA, OA, LO and LN) and their involved parameters (SFAs, MUFAs and PUFAs), according to the results of the Duncan Multiple Range Test (Table 1). The variations between the cis SFA and UFA (MUFA + PUFA) profiles and their distribution in bouillon samples (AAB and VAB) may possibly be due to the raw material properties (vegetable/animal origin) of the oils / fats used for bouillon production [1,2,6,7].

Trans **fatty acid (TFA) profiles in commercial bouillon samples**

Total *trans* fatty acids (TFAs) were another important major (second) FAs for all bouillon samples. The analysis of individual *trans* C18:1 isomers is important for determining the origin of TFA in foods. These FAs content, made up mainly elaidic acid ([EA] *trans*-9-octadecenoic acid, *t* 9-C18:1), ranged as elaidic from (20.00 – 27.32 % for AAB samples and 0.08 – 0.14% for VAB samples) when the changes of TFAs amounts for AAB and VAB samples were 20.19 – 28.78% and 0.19 – 0.26 %, respectively (Table 2). The EA (C18:1*trans* acid) and TFAs content in the AAB samples was significantly higher than those of VAB samples (Table 2). As shown in Table 2, particularly high levels of trans isomers (especially of trans oleic acid or EA) were determined in the fat fraction of the commercial bouillon (AAB and VAB) samples analysed. Distinctly lower contents of trans oleic Acid (EA) were found in VB samples (KNR VBO and KNR VAB) contained only non-hydrogenated vegetable fat.

The levels of *trans* linoleic (TLO) acid (linoeladic or C:18:2n6 *t*) values, from other *trans* fatty acids, were ranging from 0.16% to 2.55% for AAB whereas these values for VAB were between 0.08 and 0.14%. Also, only five samples contained *trans* linolenic (C:18:3 n3 *t*) acid and they were 0.01–0.04% (Table 2).As another trans fatty acid, *trans* palmitoleic (C16: 1 *t*) contents for all (AAB and VAB) were determined and their ranges were 0.03–0.12% and 0.01–0.02, respectively (Table 2).

The variations of elaidic acid (EA), linoelaidic [*trans* linoleic] (TLO) and trans linolenic (TLNO) and total Trans Fatty Acid (TFA) in bouillon samples were compared with the literature findings. There is no information on *trans* palmitoleic (C16: 1*t*) findings of bouillon samples in the literature. All *trans* fatty acid results (EA, TLO, TLNO and TFA) in bouillon samples were generally found similar to some studies [1,2,8]. The data of Karšulínová *et al*., [7] were more lower than those of our results. There was no EA reported by Karabulut [6] in meat bouillion samples but his TLO and TLNO findings were agree with our data. In addition, hard-type margarine data given by Arici *et al.* [9] were agree with our results on EA and TFA but the data reported by Kuhnt *et al*. [8] and Demir Taşan [10] were more lower than those of our findings. Determination of the *trans* isomers of UFAs in all commercial bouillon samples (AAB and VAB) differentiated the tables on the basis of the type of fat (especially hydrogenated vegetable fats) added.

Table 2. The major and minor profiles commercial boullion samples produced in Turkey

***Trans* fatty acid**	**Samples**													
	MG BB	KNR BB	HLK BB	ULK BB	HRM BB	ULK LB	ULK BTB	MG CB	KNR CB	ULK CB	HLK CB	HRM CB	KNR VBO	KNR VB
t Palmitoleic (C 16:1 t)	0.12	0.09	0.11	0.06	0.12	0.08	0.03	0.15	0.07	0.06	0.11	0.11	0.02	0.01
Elaidic (C 18:1 t)	27.32 ±0.67 a	25.07 ±0.80 ab	24.62 ±0.55 b	22.81 ±0.75 c	24.88 ±0.65 ab	23.00 ±0.55 c	20.00 ±0.09 e	23.29 ±0.45 c	21.18 ±0.55 d	20.22 ±0.11 e	25.71 ±0.75 a	26.00 ±0.98 a	0.08 ±0.01 f	0.14 ±0.03 g
t Linoleic (C 18:2 *t*)	1.32 ±0.16 d	1.61 ±0.20 c	1.05 ±0.11 e	2.16 ±0.09 b	1.61 ±0.13 c	2.55 ±0.25 a	0.16 ±0.05 f	1.64 ±0.20 c	1.16 ±0.08 e	1.64 ±0.18 c	2.46 ±0.33 a	1.66 ±0.15 c	0.08 ±0.05 g	0.14 ±0.05 f
t Linolenic (C 18:3 *t*)	ND	ND	0.01	ND	ND	ND	ND	0.04	ND	ND	ND	0.03	0.06	0.08

ND: Not Detected

The parameters calculated based on fatty acid profiles in commercial bouillon samples. The nutritional (LO /LN or n6/n3) fatty acid ratios of commercial bouillon samples were changed from 1.47 (HRM BB) to 224.0 (ULK BTB) and this ratio was not calculated in four examples (MG BB, ULK BB, ULK CB and HLK CB) (Table 3).The ranges of LO/LN of AAB – except ULK BTB, MGG CB and KNR CB – commercial bouillon samples were generally low rather than VAB samples. The limiting the intake of LO in order to decrease the ratio of LO (n6) to LN (n3) PUFAs has been stated by some medical researchers to reduce inflammation and for prevention of obesity and obesity-related chronic diseases [15].

Total SFA / Total Unsaturated FA (SFA/UFA) ratios were recorded between 1.35 and 4.35 for AAB samples whereas the changes of this ratio ranged from 1.55 to 6.91 for VB samples (Table 3). The all data of Arici *et al*. [9] on soft and hard types margarines were more lower those of our results.

A PUFA/SFA ratio of 1 is recommended in current dietary guidelines. This ratio for all fat of bouillion samples was ranged from 0.008 to 0.145 and the PUFA/SFA ratio of analyzed bouillion samples was lower than recommended values (Table3). Our findings were agree with Karabulut [6] data.

The changes of *Trans*/Cis ratio, calculated parameters based on fatty acid profile for all bouillion samples, has a large of variation from 0.002 to 0.5 (Table 3). This ratio ranged between 0.26 and 0.50 for AAB samples but VB samples has a little values from 0.002 to 0.003. There is no information in the literature on *Trans*/Cis ratio of commercial bouillions.

Chemometric Analysis based on fatty acid profiles in commercial bouillon samples. In this study, the commercial bouillon samples were classified and characterized chemometric method (Principal Component Analysis, PCA). Some fatty acid compounds (Figure 3) played a role in the characterization of Turkish commercial bouillon samples. The data matrix of variables analysed (commercial bouillon samples) was subjected to PCA.

Table 3 – The parameters calculated based on fatty acid profiles of commercial bouillon samples produced in Turkey

UFA	**Samples**													
	MG BB	KNR BB	HLK BB	ULK BB	HRM BB	ULK LB	ULK BTB	MG CB	KNR CB	ULK CB	HLK CB	HRM CB	KNR VBO	KNR VB
SFA	55.36 ±0.47 d	49.63 ±0.73 e	56.73 ±0.28 c	45.64 ±0.55 g	57.77 ±0.65 d	42.80 ±0.68 h	48.93 ±0.85 e	44.26 ±0.65 g	54.79 ±0.55 d	47.80 ±0.48 ef	58.30 ±0.55 b	56.37 ±0.38 c	57.51 ±0.69 b	60.54 ±0.58 a
MUFA	15.18 ±0.85 f	22.06 ±0.75 d	15.97 ±0.88 f	28.00 ±0.55 bc	14.63 ±0.68 f	29.35 ±0.75 b	22.50 ±0.64 d	20.03 ±0.85 e	21.21 ±0.55 e	28.27 ±0.85 b	12.64 ±0.59 g	14.78 ±0.35 f	33.76 ±0.95 a	30.92 ±0.68 b
PUFA	0.50 ±0.09 h	1.20 ±0.65 ef	1.12 ±0.80 ef	1.18 ±0.08 ef	0.85 ±0.03 g	1.49 ±0.09 d	6.78 ±0.95 c	9.97 ±0.92 a	1.26 ±0.12 e	1.70 ±0.15 d	0.45 ±0.02 h	0.97 ±0.15 g	8.35 ±0.45 b	8.14 ±0.33 b
UFA	15.89 ±0.55 g	23.54 ±0.15 e	17.60 ±0.75 f	29.38 ±0.66 cd	15.60 ±0.75 g	31.24 ±0.89 c	29.60 ±0.45 cd	30.80 ±0.77 c	23.65 ±0.15 e	30.09 ±0.65 c	13.39 ±0.15 h	15.82 ±0.35 g	42.11 ±0.90 a	39.06 ±0.53 b
TFA	28.72 ±0.65 a	26.77 ±0.60 ab	28.78 ±0.57 a	25.03 ±0.55 bc	26.61 ±0.55 ab	25.63 ±0.65 b	20.19 ±0.30 e	25.22 ±0.40 b	22.41 ±0.50 d	21.92 ±0.60 d	28.28 ±0.68 a	27.69 ±0.80 a	0.19 ±0.07 f	0.26 ±0.05 g
LO/LN	NC	37.70±0 .90 f	26.25 ±0.80 gh	NC	1.47 ±0.50 ı	28.60 ±0.75 g	224.0 ±0.90 a	81.83 ±0.90 b	62.00 ±0.70 c	NC	NC	1.69 ±0.70 h	45.22 ±0.80 de	46.88 ±0.70 d
SFA/UFA	3.50 ±0.30 b	2.11 ±0.50 c	3.22 ±0.55 b	1.55 ±0.55 d	3.70 ±0.55 ab	1.37 ±0.35 d	1.65 ±0.40 d	1.44 ±0.30 de	2.42 ±0.70 c	1.55 ±0.50 d	4.35 ±0.70 a	3.56 ±0.20 ab	1.36 ±0.09 e	1.55 ±0.30 d
PUFA/SFA	0.01	0.03	0.02	0.03	0.02	0.04	0.14	0.23	0.02	0.04	0.01	0.28	0.15	0.13
Trans/Cis	0.40 ±0.03 b	0.37 ±0.04 b	0.50 ±0.03 a	0.34 ±0.03 b	0.36 ±0.02 b	0.35 ±0.03 b	0.26 ±0.04 c	0.34 ±0.03 b	0.28 ±0.03 c	0.28 ±0.03 c	0.39 ±0.04 b	0.36 ±0.03 b	0.002 ±0.00 d	0.003 ±0.00 d

NC: Not calculated

Applying PCA to the all bouillon samples (n= 14) data determined the percentage of total variance explained by the first two PCs were 49.3 % and 21.8 % (totally 71.1%), respectively (Figure 3), The results were graphically represented by PCA score and loading plots (Figure 3). Visualization of the discrimination among all bouillon samples on the plane of the first two PCs led to a fairly good separation (Figure 2). According to PCA biplot analysis, MUFA, OLA, UFA and LO/ LN parameters were effective on the characterisation of BTB (no:7) sample whereas the BB2 (no:2) sample was classified with the GA. The CB5 (no:12) sample was characterized with the MA parameter while the SA and LAU parameters were discriminative in classification of BB 5 (no:5) sample. PUFA and LO with PUFA/ SFA parameters were played a role in discrimination of VBO (no:13) and VB (no:14) samples while the SFA / UFA parameter was for characterizing CB4 (no:11) sample (Figure 3). In this study, significant correlations ($p<0.05$) were determined between some fatty acid variables and their some parameters. These significance correlations are MA–LAU= 0.971, SFA–PAM = 0.737, *t*LO –PAM = -0,729, EA–UFA=-0.814, trans/cis- OLA = - 0.733, *trans*/cis – TFA = 0.975. Some parameters based on fatty acid profile (especially nutritional omega-3 and omega-6 fatty acids, TFA) could be used to identify commercial bouillon samples for nutritional studies.

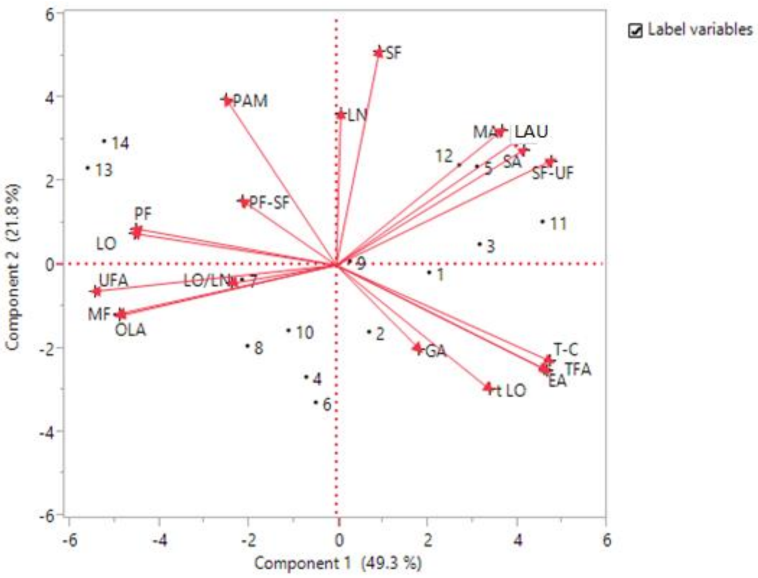

Figure 3.Biplot of scores and loadings for the first two PC (PC1 and PC2) for the Turkish commercial bouillon samples based on cis-trans fatty acid profiles

(the first two PCs which accounts for 71.1 % of the variability in the data) Sample no: 1 (MG BB); 2. (KNR BB); 3. (HLK BB); 4 (ULK BB); 5.(HRM BB); 6 (ULK LB); 7 (ULK BTB); 8 (MG CB) ; 9 (KNR CB); 10 (ULK CB); 11 (HLK CB); 12 (HRM CB); 13 (KNR VBO); 14 (KNR VB).

Abr: SF (SFA); MF (MUFA); PF (PUFA); SF-UF (SFA/PUFA); PF-SF (PUFA/SFA); T-C (*trans*/cis)

Conclusion

It was determined that the commercial bouillion samples, commonly consumed as a flavor enhancer substant or an instant product, in Turkish cusine, have rich in mainly SFAs (PAM,SA) and MUFA's (OLA). Also, other major FA profile of bouillon samples (AAB and VAB) was *trans* fatty acids (TFA) and its predominant level contained from maximum mainly elaidic acid or *trans* octadecenoic [oleic] acid (C18: 1 *t*) to minimum linoelaidic (C18: 2 *t*) and *trans* linolenic (C18: 3 *t*). Essential fatty acids (linoleic [omega-6] and linolenic acids [omega–3], PUFAs), as an important source in terms of human nutrition, were a little contents for all bouillon samples. Considering the negative effect having *trans* isomers of unsaturated fatty acids on consumer health, non-hydrogenated vegetable fats should be used in the preparation of bouillon cubes.

Newly, we may see an increase in demand for Turkish boullions with greatly reduced *trans* isomer content because of questions occured about the biological utilization and undesirably effects of *trans* FAs. The hardened margarines required for this type of boullions could be made by interesterification. In general, because of the declining tendency of the TFA content in foods and by a concurrent reduction of fat intake on the basis of daily diet, TFA depletion could decrease**.**

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Compliance with ethical standards. The author declare that they have no conflict of interest

References:

1. Caponio F, Gomes T, Delcuratolo. 2002. Qualitative and quantitative characterisation of lipi fraction of bouillon cubes. *Eur J Lipid Sci Technol*.215 (3):202- 203. https://doi.org/10.1007/s00217-002-0542-x
2. Caponio F, Gomes T, Bilancia M T. 2003. Bouillon cubes: Assessment of the state of degradation of the lipid fraction. *J Sci Food and Agric* 83 (13): 1331 – 1336 https://doi.org/10.1002/jsfa.1544
3. Akpanyung, E.O. 2005. Proximate and mineral element composition of bouillon cubes produced in Nigeria. *Pak. J. Nutr*. 45(5): 327-329. https://doi.org/10.3923/pjn.2005.327.329
4. Gupta S,Bongers P.2011.Bouillon cube process design by applying product driven process synthesis. *Chemical Engineering and Processing* 50 : 9–15. https://doi.org/10.1016/j.cep.2010.10.008
5. Al-Subhi F MM.2013. Evaluation of mushrooms broth cube and its compared with maggi broth cube products in Saudi Arabia . *Journal of American Science*, 9(5):250-255
6. Karabulut I. 2007. Fatty acid composition of frequently consumed foods in Turkey with special emphasis on trans fatty acids . *Int J Food Sci Nutr*. 58(8):619-28.
7. Karšulínová L, Folprechtová B, Doležal M, Dostálová J, Velíšek J.2007. Analysis of the lipid fractions of coffee creamers, cream aerosols, and bouillon cubes for their health risk associated constituents. *Czech J Food Sci*., Vol. 25, No. 5: 257–264. https://doi.org/10.17221/679-CJFS
8. Kuhnt K, Baehr M, Rohrer C, Jahreis G. 2011.Trans fatty acid isomers and the trans-9/trans-11 index in fat containing foods. *Eur J Lipid Sci Technol*.113(10): 1281-1292. https://doi.org/10.1002/ejlt.201100037
9. Arıcı M, Taşan M, Geçgel Ü, Özsoy S (2002). Determination of fatty acid composition and total *trans* fatty acids of Turkish margarines by Capillary Gas-Liquid Chromatography. *J. Am. Oil Chem Soc.*, 79: 439-441. https://doi.org/10.1007/s11746-002-0502-x
10. Demir, B.A., Taşan, M.2019. Trans yağ asidi içermez beyanı bulunan bazı endüstriyel gıdaların yağ asidi profilleri. *Tekirdağ Ziraat Fakültesi Dergisi,* 16(1), 23-33. https://doi.org/10.33462/jotaf.288733
11. Özgul-Yücel S., 2005. Determination of conjugated linolenic acid content of selected oil seeds grown in Turkey. *J.Am. Oil Chem Soc*., 82:893-897 https://doi.org/10.1007/s11746-005-1161-7
12. IUPAC, 1987. *Standard Methods for Analysis of Oils, Fats and Derivates*, 7th ed. IUPAC Method 2.301, IUPAC Method 2.3012.303. Blackwell Sci. Publ., Oxford,UK.
13. Dıraman, H. 2010.Characterization of important domestic and foreign olive cultivars from the national olive collection orchard of Turkey by chemometry. *Grasas Aceites*. 61 (4): 341- 351 https://doi.org/10.3989/gya.111609
14. SPSS. (2001) Base 12.0 applications guide. Chigago, IL: Author.
15. Simopoulos AP. 2016. An increase in the omega-6/omega-3 fatty acid ratio increases the risk for obesity. Nutrients; 8 (3):128 -145 https://doi.org/10.3390/nu8030128