TRANS FAT, FATTY ACIDS AND CHARACTERISTICS OF SLAUGHTERED BUFFALO WASTE FAT BY EDIBLE RENDERING

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ABSTRACT

The lipids were extracted from waste fat after edible rendering of slaughtered male buffalo (*Nili-Ravi* breed) raising in Punjab, Pakistan. The *trans* fats were identified by FTIR and Ag-TLC (2.4% ±0.17). The average fatty acids were found as; $C_{12:0}$ (1.53%), $C_{14:0}$ (28.62%), $C_{16:0}$ (2.60%), $C_{16:1}$(1.58%), $C_{18:0}$ (0.49%), $C_{18:1}$ (20.95%), $C_{18:2}$ (35.76%), $C_{18:3}$ (4.30%) and $C_{20:0}$ (4.05%). The fatty acids profile and physical values *ie.,* Iodine value (14), saponification value (203), FFA (0.23) and slip melting point (42.4ºC) indicates waste fats characteristic hardness greater than Palm kernel oil stearin and comparable hardness to Palm oil-stearin fraction at normal conditions. However, it contains higher contents of mono, poly and essential fatty acids as compared to commercial fractionated vegetable fats and hydrogenated oils. Although, these processed fats have not recommended *trans* fats but it may be processed to maintain *trans* contents and to achieve higher nutritional values of edible products, margarines and confectionery products etc.

Keywords: animal fats, vegetable fats, hydrogenated fats, confectionery fats, cosmetics, toiletries, fuel

INTRODUCTION

Buffalo is playing a leading role in the national economy of Pakistan by producing milk, meat and draught power. Due to high fat contents of buffalo milk, it is the most preferred species in Pakistan, buffalo not only fulfil the protein requirements of the human population by milk and meat, but are also have a great share in providing the traction power for various agricultural purposes. Bilal *et al*. (2016). No doubt, we have the best breeds (Nili-Ravi and Kundi) at world level due to their versatile qualities they are rightly called as Black Gold of Pakistan. Buffalo are also a source of leather and dung for manure or fuel (Bilal *et al.*, 2016). The buffalo breed is the main dairy and live stock animal in Pakistan found in the Punjab region, and in east Punjab of India as well. The home tract of the buffalo includes Lahore, Sheikhupura, Faisalabad, Okara, Sahiwal, Pakpattan and Vehari districts of Central Punjab; and Multan and parts of Bahawalpur and Bahawalnagar districts of southern Punjab. However, because of their well-recognized dairy qualities, these animals are now found all over the country.Buffalo are in great demand in several other countries as well. This domestic animal belongs to the family *Bovidae*, Tribe Bovini, and Asian buffalo group as described by Maison (1974). The Nili-ravi is a water buffalo breed. Nili and Ravi were two

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different breeds until 1950, but after this period it was difficult to distinguish between the two breeds probably due to an overlapping selection criteria of breeders. Thus, the common name Nili-Ravi became popular (Moioli and Borghese, 2005). The *Nili-Ravi* breed is well adapted to a hot and humid climate with temperatures ranging from 0°C (32°F) in the winter to 30°C (86 F) and over in the summer (Borghese, A and Mazzi, 2005). Buffalo meat is popular in a number of developing countries including Pakistan. In general the meat is richer in protein in comparison to meat of beef. Meat from much elderly animals have a poor flavor, while from young ones, it is lean, tender, less fatty, palatable and considered a delicacy. Buffalo meat contains white fat as the beta carotene (a precursor of vitamin A), which is golden yellow in color, is fully converted into vitamin A, which is colorless (Bilal *et al*., 2016).A large number of male buffalo are not only slaughtered to meet local consumption but also for export purposes as especial beef cuts. These well known animals are also sacrificed at the religious annual event of Muslims Eid ul Azha, and huge quantities of their byproducts are available. The waste fatty parts are generally processed through edible rendering to achieve pure fats. The best class as edible fat is of heart and kidney areas. However, it's a common practice that the fat of major meat cuts areas is consumed through direct edible usage, due to characteristic taste. Keeping in view of public health and awareness, the thorough parametric investigations regarding nature waste fat and fatty acids have been carried out. The *trans* fats are identified after conversion into methyl esters of fatty acids, purified by thin layer chromatography and later on separated into saturated fatty acids, *trans* fatty acids, and monoenoic fatty acids by the use of $AgNO_3$ impregnated thin layer chromatography (Ag-TLC), the *cis* and *trans* fatty acids are identified by gas chromatographic (GC) analysis (Ali *et al.*, 2007). The fat providing the *trans* fatty acids can easily be metabolized and provide energy in the major meat cuts at the cost of unsaturated fatty acids (Gurr, 1985). The negative effect of *trans* fatty acids have been reported by number of workers. The *trans* fats appear to increase the risk of CHD more than any other micronutrient (Mozaffarian *et al.*, 2005). There are various factors causing cardiovascular diseases, but one of them is related to *trans* fatty acids. The *trans* fats raise the low density lipoprotein (LDL) cholesterol and lower the good high density lipoprotein (HDL) cholesterol when consumed at a sufficient level. The lowering of HDL cholesterol makes *trans* fats worse than a typical saturated fat. One of the researchers concluded that the increase in LDL cholesterol is directly proportional to the amount of *trans* fatty acids consumed (Gatto *et al*., 2002; Judd, 2002; Katan *et al.,*1995). The research work on the effect of *trans* fatty acids on human health has been carried out since a long time. The animal derived foods like major meat cuts and dairy products also contain natural *trans* fats, a source of ruminant milk fat. Several studies have shown a connection between factors causing cardiovascular diseases at *trans* fatty acids and the presence of natural *trans* fats from animal products interacts with *trans* fats still demands further research work (Kallio *et al*., 2006).

MATERIALS AND METHODS

Materials

The samples of waste fats after fat rendering process at Lahore slaughter house of Nili-Ravi breed aged (18-21 months) were selected; these animals (10) were not specifically raised for beef

production and grown under ordinary conditions of feeding and management. Normally, grass, clover, straw make up the bulk of a buffalo diet and no bone meals, fish meals or genetically modified feeds are ever fed to our buffaloes (Bilal, 2016). The waste fatty parts were rendered separately in open pan type vessel at 50-55°C, the homogenized pure fat sample (1 L) of each animal was collected for further studies. All of the solvents/reagents used were of analytical-grade mostly purchased from Merck-Darmstadt, Germany and Riedel-de-Haën, Germany. Silica gel HF_{254}, Merck Ref. 7739 was used for *Ag-TLC*. The silver nitrate & borax of Winlab, UK were also used for the preparation of TL-chromatograms. The standards; fatty acids' methyl esters, were attained from Supelco®, USA for *TLC* & *GLC* analysis. The non-destructive locating reagent 2,7-dichlorofluorescein (Merck, Germany) used to have colored spots of lipid compounds under ultra violet light (λ 366 nm).

Extraction of lipids and purification

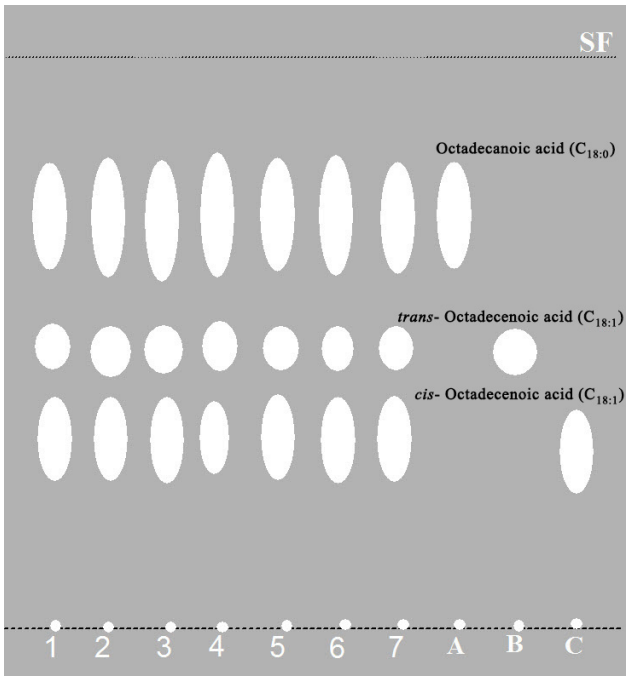
The pure rendered fat samples was homogenized after heating in oven at 55°C and 50 g of each ten animals were taken in a flask separately and the mixture of solvent 500 mL of chloroform-methanol (2:1) employed for extraction of pure lipids (Kallio *et al.,* 2006). The process was revised with the same solvent mixture (2×250 mL) for maximum extraction. The oil was dried over anhydrous sodium sulfate and distilled by rotary thin film evaporator under vacuum to get lipids prior to its treatment with a mixture of chloroform\methanol\0.9% sodium chloride solution (3:48:47 v/v) to remove impurities by a separator funnel (Kallio, 2006). The extracted lipids were physicochemicaly analyzed by standard methods (AOCS, 1997).

Methylation of fats, Purification and Fractionation

Each extracted lipid sample (500 mg) was refluxed with 40 mL mixture of methanol\benzene\ acetyl chloride (20:4:1 v/v) for 1½ hour to prepare methyl esters (Kumar and Tsunoda, 1978). The methyl esters after removal of solvent were purified by five thin layer chromatograms (20cm × 20cm) of thickness 0.5 mm by developing solvent system n-hexane-diethylether (90:10). Fractionation of 120 mg methyl esters of fat was carried out by using TLC chromatograms (0.5 mm), prepared by making slurry of silicagel (36 g), with 7.2 g of $AgNO_3$ (20 %) and 77 ml of distilled water and chromatograms were activated at 105°C for an hour. The solvent system; n-hexane\diethylether (96:5) was used for the separation of methyl esters into saturated, *trans* and monounsaturated fatty acids (Ali *et al.*, 2007). The non-destructive locating reagent 2,7-dichlorofluorescein was used to have purple yellow colored bands under ultraviolet light (λ 366 nm) in case of silica gel plates whereas pink coloration was observed by the use of silver nitrate impregnated thin layer chromatograms.

Infrared Spectroscopy and Ag-TLC

The *trans* fatty acids as methyl esters, separated by $AgNO_3$ impregnated thin layer chromatography was also confirmed by infrared spectroscope (Beckman IR model 5A), IR spectroscopy is a simplest method for identification of *trans*-fat. The absorptions were observed at 1360 cm^{-1} (CH_3 bend), 1460 cm^{-1} (CH_2 bend), 2850 cm^{-1} (CH_3 stretch), 2930 cm^{-1} (CH_2 stretch), 1740 cm^{-1} (C=O stretch) and 966 cm^{-1} (*trans* stretch). The *trans* fat contents of extracted lipids was identified at 966 cm^{-1} and quantified by Ag-TLC (Raie and Rehman, 1992).



Solvent system→ nHexane-diethylether; 95 / 5

Figure 1. Ag-TLC Fractionation of buffalo waste fats into saturated, *trans* and unsaturated fat.

Gas Chromatography

The gas chromatograph Shimadzu GC-14A and data processor C-R-4A was used for the identification of methyl esters by using polar column of dimensions (2.5 m × 3 m ID) having coating material GP-10 %-SP-2330 on supporting media 100-120 chromosorb WAW. The hydrogen flame ionization detector FID was used with the requisite temperature of detector and injector at 250ºC and 230ºC respectively. It was operated under temperature programming 180-210ºC at the rate of 4ºC/minute. The fatty acids as methyl esters were identified by the comparison of their corresponding retention times with standard methyl esters under same conditions (Ali *et al.,* 2009).

Statistical Analysis

Fatty acids' profile of each animal waste fat was conducted in triplicate by Gas Chromatograph and data are reported as mean ± Standard deviation (SD). The total saturated fat, total *trans* fat and total unsaturated fat of each animal waste fat was averaged, SDEV and RSD values calculated through MS Excel.

RESULTS AND DISCUSSION

Higher contents of free fatty acids were found out in buffalo meat fats (BMF) (Table 1) and the fat was comparable with vegetable fats. Palm oil stearine (POS) and Palm kernel oil stearine (PKOS) are well known commercial vegetable fats, these are used as it is for specific applications or blended with other oils as per requirements of specific food products (Gunstone, 2002). The peroxide values are exceeding the limits, these fats require certain processing to minimize free acids and to meet the

standard values of desi ghee (anhydrous milk fats) and banaspati ghee (hydrogenated vegetable oils). The higher contents of myristic acid (28.62 %) in waste fat as shown in Table 2, makes the waste fat hard and differentiate it with commercially well known vegetable fats having higher contents of palmitic acids in fractionated stearin of of POS and PKOS. The higher slip melting point of waste fat (42.4ºC) indicates hard waste fat characteristics like PKOS (31.3-33.1ºC) and soft fat characteristics like POS (44.5-56.2ºC) as described (Gunstone, 2002).

However, the *trans* fat/fatty acids content is a critical parameter for the assessment of such semisolid fats. The *trans* fat contents of extracted lipids was identified by FTIR (966 cm^{-1}) and quantified by Ag-TLC (Raie and Rehman, 1992). The *trans* fat contents of extracted lipids ranged as ; 2.22 - 2.81 % and mean results (2.46 %) with standard deviation value (±0.17) as given in Table 3.

The waste fats may be derivatized for eco-friendly fuel purposes through tranesterification (Ali *et al.*, 2016, Punsuvon *et al.,* 2015) but the waste fat contains higher percentage of total saturated fatty acids (34.25 %) as shown in Table 3 as compared to conventional vegetable oils *ie*., Cotton seed (28.00 %), Soybean (15.50 %), Canola (6.80 %), Sunflower (12.60 %) and Peanut (20.10 %) (Ortheoefer, 1996). Edible vegetable oils are hydrogenated to produce vegetable ghee for cooking and confectionery fats for the preparation of bakery products. The partially hydrogenated vegetable fats are extensively used for the preparation of food like doughnuts, french fries, cookies, breads and cakes. The hydrogenation process produces *trans* fats, and *trans* fats are quiet functional for preparation of foods on large scale. The resistance of fat produced under the process of hydrogenation is improved to deterioration through oxidation (Swern, 1982). Although the natural animal fats have higher contents of saturated fatty acids as found out in the current studies, they are solids at normal temperatures and needs not to be hydrogenate.

The hydrogenation process is carried out

Table 1. The physicochemical parameters of buffalo waste fat.

Sr. No.	Parameters	Results	
		Mean	±SDEV
1	Acid value	0.50	0.20
2	FFA [as oleic acid %]	0.23	0.15
3	Iodine value	14	2.0
4	Moisture-105 ± 2°C [%]	2.75	0.15
5	Peroxide value [meq/Kg]	9.26	0.45
6	Rancidity [Kries test]	< 3R	0.0
7	Refractive index [40°C]	1.4543	0.0004
8	Saponification value	203	5.0
9	Sliding point [°C]	42.4	1.50
10	Specific gravity [25°C]	0.8995	0.0005

SDEV → standard deviation value of different buffaloes (10 samples).

Table 2. Fatty acids methyl esters composition of waste fats after edible rendering by Gas Chromatography (GC).

Sr. No.	Fatty Acids	Average [%]	Sample Numbered [%]										SDEV	RSD [%]
			1	2	3	4	5	6	7	8	9	10		
1	$C_{12:0}$	1.53	1.43	1.66	1.4	0.99	1.18	1.51	1.58	1.38	2.31	1.84	0.36	23.73
2	$C_{14:0}$	28.62	30.16	25.93	27.12	28.17	29.26	29.77	31.54	29.18	27.12	28.85	1.66	5.80
3	$C_{16:0}$	2.60	2.88	2.47	2.60	2.73	2.28	2.35	2.76	2.63	2.39	2.86	0.22	8.27
4	$C_{16:1}$	1.58	1.91	1.16	1.53	1.82	1.99	1.63	1.11	1.75	1.43	1.29	0.31	19.62
5	$C_{18:0}$	0.49	0.40	0.40	0.57	0.51	0.44	0.45	0.48	0.50	0.53	0.54	0.05	10.82
6	$C_{18:1}$	20.95	19.17	21.55	20.46	22.88	23.16	18.57	19.81	20.88	21.37	21.63	1.49	7.11
7	$C_{18:2}$	35.76	35.97	38.28	37.66	33.49	33.18	37.41	34.26	35.88	36.69	34.80	1.78	4.98
8	$C_{18:3}$	4.30	4.15	4.44	4.23	4.71	4.33	4.26	4.28	4.11	4.26	4.27	0.17	3.93
9	$C_{20:0}$	4.05	3.87	4.10	4.42	4.69	4.17	4.04	4.17	3.68	3.89	3.91	0.29	7.21

SDEV →standard deviation value, **RSD** →relative standard deviation.

Table 3. Fractionation of *cis/ trans* composition of waste fats (200.0 mg) after edible rendering by *Ag-TLC*, developing solvent system; n hexane-diethyl ether (95/5).

Fat Fractions	R_f Values	Average [%]	Buffalo Sample Numbered [%]										SDEV	RSD [%]
			1	2	3	4	5	6	7	8	9	10		
ΣTSF	0.58	34.25	34.51	34.44	35.13	33.71	34.27	34.36	33.75	35.13	34.35	32.82	0.70	2.01
ΣTTF	0.48	2.46	2.40	2.51	2.22	2.41	2.36	2.45	2.31	2.45	2.81	2.67	0.17	7.00
ΣTUF	0.36	63.29	63.09	63.05	62.65	63.88	63.37	63.19	63.94	62.42	62.84	64.51	0.65	1.02

ΣTSF*→total saturated fat, **ΣTTF**→total *trans* fat, **ΣTUF** →total unsaturated fat,

SDEV →standard deviation value, **RSD** →relative standard deviation.

by passing hydrogen through the oil in the presence of nickel catalyst and specific conditions applied for and the process is known as selective hydrogenation (Raie *et al.*, 1990). So, it is advantageous to substitute vegetable fats for different applications with the major meat cuts fat of buffalo as it is also highly saturated and doesn't need processing like for vegetable oils to increase saturation and sliding points by the process of hydrogenation. The process of hydrogenation also produce *trans* acids (Katan *et al.*, 1995) which are not recommended for edible purposes (Booyens *et al.,* 1988, Willett and Ascherio, 1994). The oleic acid ($C_{18:1}$) is the major acid in vegetable ghee which is converted into $C_{18:1}$ *trans* oleic acid known as elaidic acid.

Intake of dietary *trans* fat perturbs the body's ability to metabolize essential fatty acids (EFAs including Omega 3) leading to changes in the phospholipid fatty acid composition in the aorta, the main artery of the heart, thereby increasing risk of coronary heart disease (Kummerow *et al.,* 2007). The research work on the effect of *trans* fatty acids on human health has been carried out since a long time. There is a controversy on the merits and demerits of animals and hydrogenated fats. The animal derived foods like major meat cuts and dairy products also contain natural *trans* fats, a source of ruminant milk fat.

Several studies have shown a connection between factors causing cardiovascular diseases at *trans* fatty acids. However, large variations in different edible fats standards are observed in all over the world ranged from 0.1to 1.0 % (Ali *et al.*, 2007) and European countries targeted to achieve *trans* free food products. Food and Agriculture Organization of the United Nations and World Health Organization came up with the recommendation that the contents of TFA in human dietary fats should be reduced to less than 4% (WHO, 1994). Denmark was the first country to introduce ban on the consumption of partially hydrogenated oils. It is generally believed that because of Danish Government's efforts in reducing *trans* fatty acids consumption from 6 gm to 1 gm per day over a period of 2 decades, the deaths among Danes due to CHD have dropped by 50% (Stender and Dyerberg, 2004).

So, the current study does not recommend buffalo fats for direct usage and in food products. The *trans* fats contents of buffalo waste fats may be reduced by specific processes and blending with low *trans* hydrogenated vegetable fats for their applications in confectionery, butter fats, margarines, cooking fats and number of food products. The required processing to maintain the *trans* fat contents in buffalo meat waste fats may also be avoided as they are a good source of fatty acids and have higher percentages of saturated fatty acids as compare to unsaturated fatty acids. The oils, fats and fatty acids are also a potential source for the development of number of consumer products. Keeping in view of their fatty acids profile they may be use for the developments of soaps, shaving creams and also in number of cosmetic/ personal care products inspite of ecofriendly fuel production through *trans*esterification (Ali *et al*, 2016, Punsuvon *et al.,* 2015).

CONCLUSION

It is concluded that buffalo waste fat processed through edible rendering is not recommended for direct edible usage, such fats may be used as confectionery fats after certain industrial processing, blending and after depleting *trans* fat contents, which are injurious to health. However, its fatty acids and higher contents of myristic acid

reveals its characteristic direct applications in surfactants, cosmetics and household products.

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