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A Modification Method for the Determination of Vegetable Oils in White Bulgarian's Cheese

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Abstract The article describes a modification of the methodology for qualitative detection of vegetable oils in traditional white Bulgarian cheese using Butyrorefractometer readings according AOAC requirements [16], of fatty acids previously extracted by Gerber-Butyrometer method [19]. Cheese-like products demonstrated higher refraction numbers (above 44.5) in comparison with white Bulgarian cheese (up to 41). The research related to the development of the method and its algorithm are presented. The modified method was simpler and less costly than other most widely used techniques.

Keywords Refraction, Detection, Palm oil, White Bulgarian's cheese

1. Introduction

White Bulgarian cheese is one of the most important dairy products marketed in Bulgaria. Cheese-like products are usually defined as products made by blending individual constituents, including non-dairy fats or proteins, to produce cheese analogues (imitations). In recent years, some white Bulgarian cheese-like products have become widespread on Bulgarian markets [1].

It is currently acknowledged that replacement of dairy fat with fats of plant origin, may confer health benefits [2-Yu and Hu, 2018). Palmitic acid is the most abundant saturated fatty acid in palm oil products used for production of cheese-like products in Bulgaria [1]. Palmitic acid is the predominant fatty acid in the human body as well [3].

Various instrumental methods have been proposed to establish the authenticity of cheese and to detect the level of its adulterations with vegetable oils. Among the methods are the PCR-based techniques [4], capillary and gel electrophoresis [5-7], immunochemical methods [8-10], HPLC [6], GC [11], fluorescence spectroscopy [12], front-face fluorescence spectroscopy [13, 14] and synchronous fluorescence spectroscopy [15].

Butyro-Refractometer (B.R.) reading, equivalent for international Butyrorefractometer readings and indices of refraction [16], is one of the quality parameters covered by legal standards for ghee in India [17]. The first report on a modification of the methodfor detection of adulterations in white Bulgarian cheese with non-milk fats appeared in 2017 [18]. The modification of the technique applied in India [17] consists in analysis of refraction of fats extracted from white Bulgarian brine cheese by the standard Gerber Butyrometer method [19], followed by determination of fat Butyrorefractometer readings [18].

In this study, additional investigations on the modification of method for detection of adulterations with vegetable oils in natural white Bulgarian cheese are presented. In addition, results were authenticated by establishing the chemical composition of fats of white Bulgarian cheese and its imitations, marketed in the town of Sliven (Bulgaria).

2. Materials and Methods

A total of 14 samples (7 ripened and 7 non-ripened) white Bulgarian cow cheese and 9 samples cheese-like products from different manufacturers near town of Sliven (Bulgaria), were studied in three laboratories.

2.1. Modified Methodology

Fats from cheese and cheese-like products were extracted in the Food Safety Laboratory at "Biopharm engineering" AD Sliven, Bulgaria, using the standard Gerber Butyrometer method [19]. Refractometric readings of extracted fats were done with Abbe-refractometer AR4 with electrical LED-lighting (Krüss Optronic, Germany; www.kruess.com), at 40°C. Refractometric results were presented as Butyrorefractometer readings [16], (Table 1).

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Table 1. Butyrorefractometer readings and indexes of refraction (AOAC INTERNATIONAL, 2005)

Reading	Index of refraction	Reading	Index of refraction
40.0	1 4524	60.0	1.4659
40.5	1.4527	60.5	1.4662
41.0	1.4531	61.0	1.4665
41.5	1.4534	61.5	1.4668
42.0	1.4538	62.0	1.4672
42.5	1.4541	62.5	1.4675
43.0	1.4545	63.0	1.4678
43.5	1.4548	63.5	1.4681
44.0	1.4552	64.0	1.4685
44.5	1.4555	64.5	1.4688
45.0	1.4558	65.0	1.4691
45.5	1.4562	65.5	1.4694
46.0	1.4565	66.0	1.4697
46.5	1.4569	66.5	1.4700
47.0	1.4572	67.0	1.4704
47.5	1.4576	67.5	1.4707
48.0	1.4579	68.0	1.4710
48.5	1.4583	68.5	1.4713
49.0	1.4586	69.0	1.4717
49.5	1.4590	69.5	1.4720
50.0	1.4593	70.0	1.4723
50.5	1.4596	70.5	1.4726
51.0	1.4600	71.0	1.4729
51.5	1.4603	71.5	1.4732
52.0	1.4607	72.0	1.4735
52.5	1.4610	72.5	1.4738
53.0	1.4613	73.0	1.4741
53.5	1.4616	73.5	1.4744
54.0	1.4619	74.0	1.4747
54.5	1.4623	74.5	1.4750
55.0	1.4626	75.0	1.4753
55.5	1.4629	75.5	1.4756
56.0	1.4633	76.0	1.4759
56.5	1.4636	76.5	1.4762
57.0	1.4639	77.0	1.4765
57.5	1.4642	77.5	1.4768
58.0	1.4646	78.0	1.4771
58.5	1.4649	78.5	1.4774
59.0	1.4652	79.0	1.4777
59.5	1.4656	79 5	1.4780

To determine the accuracy of the modified method, extracted fats were parallelly analyzed in a interlaboratory test with the same equipment (Abbe-refractometer AR4 with electrical LED-lighting (Krüss Optronic, Germany; www.kruess.com), in the research laboratory of the Department of Hygiene, Technology and Control of Foodstuffs, Veterinary Legislation and Management, Faculty of veterinary medicine, Trakia University, Stara Zagora, Bulgaria.

Statistical analyses of data were performed by the t-test (Statmost^{TM} for Windows). Butyrorefractometer readings were presented as means (X), standards deviations (S.D.), minimum (Min) and maximum (Max) values (table 2).

To verify the results from the refractometric method with the gas-chromatographic method, extracted fats were analysed in the Research laboratory of the Faculty ofAgriculture, Trakia University, Stara Zagora, Bulgaria (table 3). The fatty acid composition was determined on a gas chromatography Pay-Unicam 304 as described by Naydenova al. [20].

3. Results and Discussion

3.1. Refractometric Results

Table 1 presents comparisons between Butyrorefractometer readings from cow ripened and non-ripened white Bulgarian cheese and cheese- like products. Statistically significant differences between values of data were found out ($p<0.001$). In almost all cases, extracted fats from cheese-like products had Butyrorefractometer readings above 44.5 (Table 2).

The parallel results by refractometric method in second laboratory met the range 90% ÷ 110% and there was no need for recovery.

Table 2. Comparisons between refractive numbers from cow ripened and non-ripened white Bulgarian's cheese and cheese like products

Stat. parameters	Ripened white Bulgarian's cheese (n = 7)	Non-ripened white Bulgarian's cheese (n = 7)	Cheese-like products (n = 9)
X	40.42	39.91	47.521
S.D.	0.5427	0.031	3.215
Min.	39.97	39.87	44.5
Max.	41	39.95	51
p	0.0288		
	<0.001		

3.2. Chromatographic Results

Our study established large differences between profiles of the natural and cheese-like products. The observed fatty acid profile characterised the cheese like-products as partially replaced with non-milk fat (Table 3).

The main advantage of short-chain milk fatty acids is their easy digestibility so in this respect, they are promising for human nutrition [21]. Dairy factory imitation cheese-like products demonstrated significantly lower short-chain fatty acid (C4:0÷C10:0) levels. The very low values for this group fatty acids demonstrated great extent of substitution of the milk fat and large amount of palmitic acid (C 16:0) - 47.07% and oleic acid (C 18:1) - 30.747% from total fatty acids (Table 2).

Changes in palmitic acid intake from the diet do not influence the amount of palmitic acid present in the body. For this reason, health issues arise from parallel excessive

intake of energy and sugars and sedentary lifestyle. For some authors the imbalance among different fatty acids rather than the excessive intake of saturated fatty acids including palmitic acid, might play a role in generating a cellular environment that favors the detrimental health effects [3].

Mean fatty acid composition (% of total fatty acids) of cheese-like product samples and natural white Bulgarian cheeses, demonstrated high quantity of palmitic (47.07) and oleic (30.747) acids (Table 3).

Our findings for fatty acids composition in white Bulgarian cheese and cheese-like products are comparable with other studies. Naydenova et al. [1], studied fatty acid composition of 39 marketed white Bulgarian cheeses and imitation products from March 2009 to November 2011 and found large differences between fatty acid content of 13 imitation products and samples white Bulgarian's cheese. The concentrations of short chain fatty acids (C4:0–C10:0) of cheese analogues were <0.5% of total fatty acids, while palmitic acid proportion – 44.2%.

It could be pointed out that cheese-like products in Bulgaria were produced from 50% whole milk and 50 % palm oils. Thus, the high percentage of C4:0÷C10:0 fatty acids in cheese like products comes from milk fats in factories with specific technology (Table 3).

Table 3. Mean fatty acid composition (% of total fatty acids) of cheese-like product samples and natural White Bulgarian's Cheeses, marketed in the town of Sliven (Bulgaria)

Indices	Cheese-like products	White Bulgarian's Cheeses
C 4:0 C 6:0 C 8:0 C 10:0	7.6	14.49
C 12:0	0.715	3.7
C 14:0	2.068	9.33
C 14:1	-	0.39
C 14:2	-	0.19
C 15 iso	-	0.41
C 15:0	0.63	0.96
C 15:1	-	0.21
C 16:0	47.07	29.06
C 16:1	0.17	0.78
C 16:2	0.22	0.38
C 17 iso	-	0.53
C 17:0	1.67	2.89
C 18:0	3.25	8.96
C 18:1	30.747	23.94
C 18:2	5.85	2.23
C 18:3	-	0.24
C 20:1	-	1.195

Another study also supports our results with additional scientific information for fatty acid composition of white Bulgarian's cheese and cheese-like products, produced from all regions of Bulgaria across seasons for the period 2012–2016 [22]. A total of 670 samples produced from different manufacturers and collected from supermarkets by Official control of Bulgarian Food Safety Agency (BFSA) were examined. The palmitic and oleic acids predominated in the fatty acid composition of cheese-like products in almost equal percentages (34–36%), [22].

4. Conclusions

The study presented a modified technique using Butyrorefractometer readings according AOAC requirements [16], of fatty acids previously extracted by Gerber-Butyrometer method [19], as an alternative technique for detection of vegetable oils in traditional white Bulgarian cheese. Cheese-like products demonstrated higher refraction numbers (above 44.5) in comparison with white Bulgarian cheese (up to 41).

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