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Detection of Raw Milk Adulterated with Cheese Whey by Ultrasound Method

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Abstract

The potentiality of the ultrasound method to identify adulteration of pasteurized milk with cheese whey was evaluated. Milk samples were mixed with different concentrations of whey cheese (0, 0.5, 1, 2.5, 5, 10, 15 and 20% v/v), resulting in eight levels of adulteration (500 mL for each one). This procedure was repeated six times totaling 48 samples. Cheese whey was obtained from the manufacturing of fresh cheese under laboratory conditions. Samples were examined for conventional method and ultrasound method by lipids, cryoscopy index, density and non-fat solids. The results of fat were higher from ultrasound than conventional method. However, a significant difference between control and adulterer samples was observed by conventional method while ultrasound showed differences in samples adulterer with 5% of whey cheese onwards. For non-fat solid, only the ultrasound method showed differences in samples adulterer with 2.5% onwards. While no differences in density and cryoscopy index were shown in both methods for any level of adulteration. Although none of the methods shown to be better for the determination of adulterated milk with whey cheese, it is suggested that others physicochemical parameters will evaluate by both methods in order to find parameters indicative of adulteration in pasteurized milk adulterer with whey cheese.

Keywords: Adulteration; Fraud; Milk; Official analysis; Ultrasound

Introduction

Food adulteration is a point of great concern around the world. Adulteration in milk with other dairy or non-dairy ingredients has become very common [1,2]. In fact, milk is one of the seven top foods that is adulterated at all levels of the production process, and this fact has been widely recorded [3]. Recently, in Brazil was reported that commercial ultra-high temperature milks available in the Brazilian market presented at least one adulterant, such as starch, chlorine, formaldehyde, hydrogen peroxide and urine [4]. Moreover, the addition of cheese whey in fluid milk has already been reported [5]. This adulteration could be identify by individual analytical procedures such as phosphor partition [6], western blot immunoassay [7], liquid chromatography–electrospray–tandem mass spectrometry analysis [8], but these procedures are not accuracy, take a long time and are expensive [9].

In this context, the use of ultrasonic sensors is a potential alternative method to provide multiple parameters results in a single evaluation, requires a small amount of samples, minimize the use of chemical reagents, perform the analysis without destroying the sample and is not expensive because not require a specific procedure to prepare the samples [10]. Previous studies have used different dairy matrices, such as conventional and organic milk, and fermented milks to study the addition of adulterants and results have shown a good correlation between the ultrasound and conventional methods [11].

Based on these considerations, the present study aimed to evaluate the sensitivity of conventional methods and the ultrasound method to detect adulteration of raw milk intentionally added with cheese whey at different concentrations.

Materials and Methods

Experimental design and sample preparation

Fifty litters of pasteurized milk, purchased in markets of Rio de Janeiro (Brazil), were used for the experiment. In order to adulterate the milk, samples were mixed with different concentrations of whey (0, 0.5, 1, 2.5, 5, 10, 15 and 20% v/v), resulting in eight levels of adulteration (500 mL for each one). This procedure was repeated six times totaling 48 samples. Cheese whey was obtained from the manufacturing of fresh cheese under laboratory conditions as a follow: Twenty-five litters of milk was submitted to enzymatic clotting, the formed gel was cut and the whey was separated by filtration (Approximately 20 L). Whey cheese was stored at 4°C until used [12].

Physical and chemical analyses

The adulterated milk samples were analyzed for cryoscopy index, density, non-fat solids, and lipids by conventional methods [9]. Simultaneously, same samples were analyzed by the ultrasound method (BOECOLAC 50, Boeco, Hamburg, Germany). The ultrasonic device has a cannula that aspirates approximately 20 mL of sample. Prior to analysis, the device was calibrated using cow's milk. The milk samples were continuously homogenized during the analysis. For each sample, aliquots of about 80 ml were used. After a period of 60-90 seconds per sample, the display indicated the values of the physicochemical parameters evaluated. All analyses were performed in triplicate.

Statistical analysis

The data of conventional and ultrasound methods was subjected to one-way analysis of variance (ANOVA) to check for differences among treatments and methods. Data were analyzed using GraphPad Prism® (version 5.00 for Windows, GraphPad Software, San Diego, California, USA).

Results and Discussion

Table 1 demonstrates the mean results for physicochemical analysis obtained by the two tested methods. No significant differences ($p>0.05$) were obtained in density and cryoscopy between ultrasound and conventional methods in different percentages of cheese whey addition. Fat content evaluated with conventional method showed a significant difference ($p<0.05$) in non-adulterer samples (3.02 ± 0.09) compared with the samples adulterated with cheese whey regardless of the percentage. On the other hand, the ultrasound method showed a significant difference ($p<0.05$) in fat content in samples adulterer with 5% onwards compared with no adulterer samples.

Addition of cheese whey (%) in milk	Density		Cryoscopy index (°H)		Fat (%)		Non-fat solids (%)	
	UM	CM	UM	CM	UM	CM	UM	CM
0	1.030 ± 0.0008^{Aa}	1.030 ± 0.0006^{Aa}	0.571 ± 0.0156^{Aa}	0.538 ± 0.0041^{Aa}	3.25 ± 0.2570^{Aa}	3.02 ± 0.0983^{Ab}	8.74 ± 0.1407^{Aa}	8.55 ± 0.1712^{Aa}
0.5	1.034 ± 0.0005^{Aa}	1.031 ± 0.0008^{Aa}	0.569 ± 0.0155^{Aa}	0.536 ± 0.0045^{Aa}	3.26 ± 0.2902^{Aa}	2.85 ± 0.1761^{Bb}	8.71 ± 0.1235^{Aa}	8.54 ± 0.1060^{Aa}
1	1.034 ± 0.0008^{Aa}	1.031 ± 0.0005^{Aa}	0.566 ± 0.0147^{Aa}	0.536 ± 0.0039^{Aa}	3.27 ± 0.3147^{Aa}	2.89 ± 0.1306^{Bb}	8.68 ± 0.1269^{Aa}	8.47 ± 0.1794^{Aa}
2.5	1.034 ± 0.0005^{Aa}	1.031 ± 0.0005^{Aa}	0.563 ± 0.0164^{Aa}	0.538 ± 0.0047^{Aa}	3.19 ± 0.2626^{Aa}	2.87 ± 0.1506^{Bb}	8.63 ± 0.1412^{Ba}	8.48 ± 0.2076^{Aa}
5	1.034 ± 0.0005^{Aa}	1.031 ± 0.0010^{Aa}	0.563 ± 0.0163^{Aa}	0.541 ± 0.0036^{Aa}	3.12 ± 0.2544^{Ba}	2.80 ± 0.0000^{Bb}	8.65 ± 0.1375^{Ba}	8.52 ± 0.1408^{Aa}
10	1.034 ± 0.0008^{Aa}	1.031 ± 0.0009^{Aa}	0.558 ± 0.0176^{Aa}	0.549 ± 0.0110^{Aa}	2.99 ± 0.3137^{Ba}	2.63 ± 0.0516^{Bb}	8.60 ± 0.1852^{Ba}	8.48 ± 0.1023^{Aa}
15	1.034 ± 0.0008^{Aa}	1.031 ± 0.0008^{Aa}	0.550 ± 0.0166^{Aa}	0.555 ± 0.0135^{Aa}	2.90 ± 0.3560^{Ba}	2.53 ± 0.0816^{Bb}	8.49 ± 0.1779^{Ba}	8.39 ± 0.1084^{Ba}
20	1.033 ± 0.0014^{Aa}	1.030 ± 0.0008^{Aa}	0.548 ± 0.0149^{Aa}	0.551 ± 0.0135^{Aa}	2.81 ± 0.3653^{Ba}	2.53 ± 0.0816^{Bb}	8.42 ± 0.1157^{Ba}	8.46 ± 0.0492^{Aa}

Table 1: Results of physicochemical analysis (Density, cryoscopy index, fat, and non-fat solids) from ultrasound method (UM) and conventional method (CM) in milk samples adulterated with different percentages of the cheese whey. Results express in mean ± standar deviation. For each physicochemical analysis: Different small letters in a line indicate significant difference between methods. Different capital letters in a column mean significant difference among different percentages.

Results express in mean ± standard deviation. For each physicochemical analysis: Different small letters in a line indicate significant difference at the 5% level between conventional method (CM) and ultrasound method (UM). Different capital letters in a column mean significant difference at the 5% among different percentages of the cheese whey.

Among the parameters analyzed in this study, the fat content was proved to be more influenced by the adulteration. Although both conventional and ultrasound methods can employed to detect adulteration in milk, the conventional method was more efficient, presented a lower detection limit as compared the ultrasound method ($p<0.05$) especially between control and adulterer samples. This result can be explained because the conventional method (Gerber method) is based on the selective attack of organic matter by sulphuric acid followed by fat separation by centrifugation [9]. Although this method is simple, it is time consuming, requires sample destruction and temperature control of the sample and reagents is critical [13,14]. Although the conventional method was more efficient in detect milk adulterer by whey cheese, the analysis of milk fat by ultrasound method, directly based in the dispersion of sound waves in non-homogeneous samples such as emulsions and suspensions, is considered a non-invasive and on-line measuring systems [15-17]. However, this method is subject to interfering variables (temperature, fat content, defatting process etc.) that can mask detection of an adulterant [18].

Results of non-fat milk solids by the conventional method remained stables when different concentrations of cheese whey were added. However, a significant difference was observed only in the addition of 15% cheese whey. It suggests a limitation of conventional method (Ackermann disk) to detect adulteration in pasteurized milk. This method is indirect and requires the results of total lipids and density, these facts increasing the uncertainty and the imprecision of the results [10]. On the other hand, the ultrasound method detects differences in non-fat milk when cheese whey was added above 2.5%. A possible explanation of this is the presence of few solid consisted by lactose, whey protein, ash, lactic acid and fat, present in whey cheese [18]. These solids could change the way of wave sound through [19].

From the cryoscopy index values, it is possible to assume that the adulterated samples presented mean values close to that of raw cheese whey, suggesting the identification of an increased amount of soluble matter rather than the presence of water. It is noteworthy that despite there was not a significant difference by means of this methodology after adding a concentration equal or higher than 15%, the milk would have been rejected for presenting a cryoscopy index lower than the standard [20].

The electronic cryoscopy, in turn, is an Official Analysis influenced by the number of molecules and/or dissolved ions in the aqueous phase, particularly lactose and mineral salts [20]. The addition of aqueous substances dilutes such components, making the freezing point reach 0°C [21]. The cheese whey, despite having a high content of water, presents a significant amount of soluble substances, showing

thus a low cryoscopy index from -0.555 to -0.565°H [22]. The ultrasound method is based on the assessment on how certain chemical constituents behave in relation to a high frequency of sound waves propagated through a liquid sample [15].

The solid dissolved in water produces an increase in the speed of sound. Moreover, the milk fat produces the opposite effect [11]. For this reason, in samples with higher percentage of cheese whey, the sound waves are more easily propagated, detecting the increase in milk components. Therefore, the way of evaluating the cryoscopy index results for each method should be differentiated in order to study the presence of adulterants (cheese whey) in milk. Although no statistically significant difference between the methods was obtained, the results of the cryoscopy index obtained by the ultrasound method were lower than the value allowed by an International Legislation [9] for the control sample.

Conclusion

Our findings suggest that conventional and ultrasound methods have limitations in detecting adulterations in milk added with cheese whey. However results of fat and non-fat solids by ultrasound method could be used such as parameters of adulteration in milk whit more that 2.5% of whey cheese. It is suggested that others physicochemical parameters will evaluate by both methods in order to find another parameters indicative of adulteration in pasteurized milk adulterer with whey cheese.

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