Current knowledge of the adulteration of milk and milk products – proof method

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Abstract

The aim of the work was to overview main issues and aspects of milk adulteration. Milk is certainly belonging to the staple type of food that is broadly worldwide consumed due to its nutritional properties. These nutritional properties are favorable for consumers when they consume milk as drink product or when milk is included to other food products adding additional value to these products. Nutritional properties of milk are expressed as protein content, fat and vitamins contents. Behind milk production and market is high profit that leads to certain adulteration. The importance of finding new methods for milk adulteration can be overviewed by the fact that milk adulteration is not only cheating of consumers, but also milk adulteration very often represents health risk for consumers due to the use of hazardous compounds such as nitrogen rich melamine. At the present time can be stated that there are good enough methods for the detection of milk adulteration, though less time consuming, operating and less expensive methods should be invented in the future.

Artificial milk, inexpensive, melamine, nitrogen rich compounds

Introduction

Milk is a highly nutritious food due to its valuable composition and the fact that it is easy digestible and absorbed by the human organism (El-Loly et al. 2013). Average milk is composed of 84.7% water and 12.6% milk solids. Milk solids consist of 3.7% fat and 8.9% milk solids – non-fat (protein 3.4%, lactose 4.8% and minerals 0.7%). Milk proteins consist of casein (80%), lacto albumin (18%) and lacto globulin (0.05% – 0.07%) (Ayub et al. 2007).

Milk producers try to increase their profits by adding various substances to milk, such as urea, starch, carbonate, flour, skimmed milk powder, gelatin, cane sugar, vegetable oils, detergents, hydrogen peroxide, etc. The nutritional value of milk is reduced by the addition of these adulterants, and its nutritional value is also decreased by the removal of valuable substances. Producers try to convince consumers that their product is more valuable and of better quality. At the same time, adulterated milk represents a risk to consumer health. Milk which has been adulterated with the addition of urea is very dangerous for young girls because urea accelerates the process of puberty. Added starch can cause diarrhea and can be very hazardous for diabetic people. Hydrogen peroxide is added to milk to prolong its freshness, but hydrogen peroxide is harmful to the gastrointestinal cells and its consumption can lead to gastritis and inflammation of the intestine. This is why it is extremely important that consumers should be protected from adulterated milk by adequate analysis being conducted with the usage of reliable methods (El-Loly et al. 2013; Kandpal et al. 2012; Ayub et al. 2007; Singulari and Sukumaran 2014).

Adulteration caused by mixing different types of milk

Ewe and goat milk are very often mixed with cheaper bovine milk. This mixing is also considered milk adulteration and producers make a bigger profit at the expense of customers in this way. Bovine, ewe and goat milk can be differentiated according to their chemical

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composition. A higher content of fat, crude protein, lactose, ash and dry matter is found in ewe milk than in goat milk, with the content being lowest in bovine milk. Goat milk has the highest amount of non-protein nitrogen, while bovine milk has the lowest. It is possible to detect mixing by protein analyses, though the protein content can vary considerably even for a single type of milk in relation to breed and lactation level. The casein content can also be one of the markers for this type of adulteration, though there are only small differences in casein content between buffalo, bovine, ewe and goat milk. The proteins in ewe, buffalo, bovine and goat milk consist of casein up to 82, 87, 80 and 77%, respectively (Borkova and Snaselova 2005).

The adulteration method consisting of one type of milk being mixed with another can be detected by electrophoresis, isoelectric focusing (IEF), capillary electrophoresis (CE), reverse-phase high-performance liquid chromatography (RP HPLC) and ion-exchange high-performance liquid chromatography (IE HPLC), hydrophobic interactive chromatography (HIC), immunochemical methods (ELISA), PCR techniques, and mass spectrometry (Borkova and Snaselova 2005). Immunochemical methods and methods based on DNA analysis are considered the best choice for the detection of this type of adulteration because these analyses make it possible to detect 0.5 and 0.1% adulterants (Borkova and Snaselova 2005).

All of these abovementioned analyses have disadvantages in that they are too slow to perform and are laborious, meaning they are not suitable for routine industrial screening. In contrast, Fourier-transform infrared (FT-IR) spectroscopy is an extremely fast and not time-consuming biochemical fingerprinting technique. FT-IR allows the analysis of a sample within one minute and requires minimal sample preparation. This method is perhaps the best solution for the quick and reliable detection and quantification of adulterants in milk when it is combined with partial least squares (PLS) regression (Nicolaou et al. 2010).

This type of adulteration is also present in the production of various kinds of cheese. Cow milk is added very often to water buffalo Mozzarella cheese. Cow milk is cheaper than buffalo milk, which is why producers try to increase their profit by means of this fraud. Methods based on the isoelectrofocusing of g-caseins after plasminolysis and on HPLC can detect the presence of as little as 1% bovine milk. The PCR method can also be used for the determination of bovine milk present in water buffalo Mozzarella cheese. The method is fast and the detection limit is 0.5% (Feligini et al. 2005).

Detection of plant proteins in milk

Plant proteins are widely used for milk adulteration, with the plant proteins used most often being soy, pea and soluble wheat proteins. Soy proteins are the main plant proteins used for milk and milk product adulteration due to inexpensive soy products such as flours, textured flours, protein concentrates, isolates and hydrolysates (Haasnoot and Du Pre 2007).

European Union reports in 2002 and 2007 also indicated that plant proteins are very often used to adulterate skimmed milk powder. The problem is even more serious in view of fact that adulterants such as soy and almond proteins are potential allergens (Scholl et al. 2014).

Recently, chromatography, the enzyme-linked immunosorbent assay (ELISA), fast protein liquid chromatography (LC), near-infrared (NIR) spectroscopy and mass spectrometry (MS) have been used for the detection of soy proteins in milk. These methods are good for the detection of specific foreign proteins, but are not adequate for quick and low-cost evaluation of economically motivated adulteration with numerous cheap plant proteins (Scholl et al. 2014).

Electrophoretic methods and immunochemical methods for plant protein detection in milk products have the disadvantage that they are highly specific and sensitive and require many samples. Plant proteins cannot be detected by capillary zone electrophoresis (CZE).

In contrast, comparative liquid chromatography – mass spectrometry (LC-MS) methods have great potential as they are detailed and precise for the detection of proteins originating from soy and pea. LC-MS methods in combination with a borate enrichment step can detect many proteins originating from soy or pea due to the fact that proteins from these plants are not soluble in borate (Cordewener et al. 2009 and Luykx et al. 2007).

The sodium dodecyl sulfate capillary gel electrophoresis (SDS-CGE) method is more accurate than the immunochemical method enzyme-linked immunosorbent assays (ELISA), though ELISA is more suitable for the determination of soluble wheat proteins that cannot be detected by SDS-CGE. Plant protein adulterants in milk treated with an ultrahigh temperature (UHT) can also be detected with the ELISA method, though the concentrations found are lower than they are in reality due to partial heat denaturation of the antigens. Pasteurization does not affect the plant protein content in milk as much as UHT. It is hard to detect plant proteins in milk when it is not known how the sample was treated before analysis, i.e. by pasteurization or UHT (Haasnoot and Du Pre 2007).

Lipid adulteration in milk

Adulteration of butter has a long history, as is shown by the fact that as early as in 1877 the Bureau of the Leipzig Pharmaceutical Union offered a prize of 800 marks for the discovery of practical methods that would make it easy to detect the adulteration of butter with other lipids (Nollet and Fidel 2010).

The fatty acids profile is used for the identification of possible adulteration with other lipids, though the fact that the fatty acids profile can also be influenced by breed, season, climate, geographical origin and technological processes has to be taken into consideration. Butyric acid is the most characteristic fatty acid in milk and its content ranges from 2.4% to 4.22%. Relatively simple analysis can be used for the evaluation of the fatty acids profile, such as the iodine value (total unsaturation of a fat) and the Polenske value (determination of volatile fatty acids extracted through saponification), though these methods can only detect massive adulteration of milk fat. Capillary gas chromatography is used for the determination of the fatty acids content in milk. The combination of four fatty acids ratios can also be used: C18:0/C8:0 < 7.63; C14:0/C18:0 > 1.02; (C6:0+C8:0+C10:0+C12:0)/ C18:0 > 0.95; C18:1/C18:0 < 2.34; these ratios are for genuine bovine fat milk. In some cases, the authenticity of milk can also be determined because the ratio C14:1/C15:0 is 1.00 for cow milk and 0.20 for sheep milk (Nollet and Fidel 2010).

The infrared spectroscopic technique and proton-transfer reaction mass spectrometry are also used for the detection of lipid adulteration in milk, though they are still not widely used. These methods do not require long preparation of samples and are not destructive for samples (Nollet and Fidel 2010).

Milk adulteration with water and whey

The addition of water and whey to milk is called “economic adulteration”. This kind of adulteration is practiced very often. Water and whey adulteration of milk are not considered hazardous for consumers, though the addition of whey has been shown to increase the absorption of trace minerals (Oancea 2009).

Whey is very often used as an adulterant due to its price. In some cases it is very hard to detect whey in milk because HPLC cannot detect whey in adulterated milk if it is produced by direct acidification of milk (Kartheek et al. 2011).

Direct and indirect methods are used for the detection of the presence of whey in milk. Indirect methods are based on the evaluation of the casein/whey protein ratio and direct methods involve separating protein mixtures. Direct detection methods include electrophoretic, chromatographic and immunoturbidimetric methods. These are effective, though extremely laborious and time-consuming. Whey can also be detected by the

analysis of glycomacropeptide (GMP). GMP is composed of 64 amino-acid residues which are released enzymatically in whey by reaction with chymosin during cheese processing. Quantitative determination of GMP can be performed by chromatographic, electrophoretic and capillary electrophoretic techniques. An immunochromatographic method can also be used for the detection of GMP, and a study has shown that 70% of analyzed samples had been adulterated with whey (Oancea 2009).

Some producers try to increase the volume of milk and enhance their profit by adding water to milk, though the nutritive value of the milk is lowered by water addition and producers try to maintain natural milk composition with substances such as starch, flour, urea, cane sugar, vegetable oil, etc. Milk adulteration with water occurs so often that some studies have shown that more than 90% of investigated milk samples had been adulterated by the addition of water (Singulari and Sukumaran 2014 and Lateef et al. 2013).

The EU directive for fresh and unadulterated goat milk states that the freezing point should be -0.52 ºC. The freezing point can be used for the detection of added water to milk, though the problem is that up to 7% water can be added without changing the freezing point (Csanadi et al. 2013).

Synthetic milk

Unscrupulous producers have gone so far in their pursuit of bigger profit that they have invented the production of totally artificial milk. Synthetic or artificial milk is prepared by mixing urea, caustic soda, refined oil (inexpensive cooking oil) and common detergents. The frothy solution and white color of synthetic milk are achieved by the addition of detergents. Refined oil replaces the fats in milk. Caustic soda is added to neutralize acidity and to prevent the possible creation of a sour taste during transportation. Urea/sugar is used to achieve the natural taste of milk and its white color. Synthetic milk could be very dangerous for pregnant women, fetuses and people with cardiovascular and kidney problems (Kandpal et al. 2012).

Simple methods can be used for the detection of synthetic milk. If a drop of pure milk is put on a smooth vertical surface it should stop or flow slowly leaving a white trace, but milk adulterated with water will not leave a white mark. The presence of urea in milk is estimated by mixing bromocresol blue solution with milk – the test is positive if a blue color appears after 10 minutes (Kandpal et al. 2012).

The adulteration of milk with nitrogen-rich compounds

Adulteration is also a big issue in relation to human health, and is not merely a case of producers committing fraud to make more profit. Milk adulteration can even cause the death of consumers, as is proved by incidents in 2004 and 2008 when melamine was found to be widely used by milk producers in China. Six and twelve children, respectively, died during these incidents and numbers in excess of thousands were probably harmed. Melamine causes kidney failure, particularly in infants. Melamine is added to milk because it enhances the nitrogen content in food. Melamine is mainly used for the production of synthetic resins (Lakashmi et al. 2012; Zhang et al. 2009 and Yang et al. 2009).

In addition to melamine, substances such as ammonium sulfate and urea are also used as nitrogen-rich compounds and, like melamine, they increase the nitrogen level in food when the Kjeldahl method is practiced. The Kjeldahl method is not suitable for the adulteration test since it detects these substances as proteins due to their analytical characteristics. These incidents involving the use of melamine as an adulterant in milk have led to increased interest in the detection of this substance. Gas chromatography, liquid chromatography and Raman spectrometry are used for the detection of melamine in milk, and capillary zone electrophoresis is used as a supplement to chromatographic techniques. Mass spectrometry techniques are not widely used because they demand costly instrumentation, skillful staff

and the necessary infrastructure for their implementation. An attempt has been made to detect the presence of melamine, ammonium sulfate and urea using the four common spectrophotometric methods (Biurete, Lowry, Bradford and Markwell), but this attempt was not successful. A positive finding was that it is possible to measure these nitrogen-rich substances with a combination of Bradford and Markwell spectrophotometric methods (the most laborious and time-consuming) and with the Kjeldahl method (Klampfl et al. 2009; Finete et al. 2013).

The World Health Organization (WHO) has set a limit for the daily dose of melamine for humans that should not exceed 0.2 mg per kilogram of body weight. The Food and Drug Organization has set limits for melamine in food that must be lower than 2.5 parts per million and lower than 1 part per million in infant formula (Zhang et al. 2009).

Melamine in food can be detected by mass spectrometry or an enzyme-linked immunosorbent assay (ELISA). Methods based on mass spectrometry are suitable for melamine detection, though they are very time-consuming. As an example, the U.S. Food and Drug Administration has published a method for the detection of melamine in pet food which requires 3 hours for one sample. These methods are not suitable for occasional screening within industrial processes. Surface desorption atmospheric pressure chemical mass spectrometry (DAPCI-MS) is a sensitive method and suitable for various milk products, both for powdered milk and liquid milk. The DAPCI-MS method does not require any sample preparation (Yang et al. 2009).

Conclusions

Milk is one of the most consumed foods in the world due to its nutritive value, and is necessary for the healthy development of the human body. At the same time, milk is one of the most frequently adulterated foods in the world. Milk producers have been trying constantly to increase their profit by adding adulterants to milk and by the introduction of new methods for milk adulteration. Unscrupulous producers are even producing totally synthetic or artificial milk with a combination of substances that can be harmful for consumers. Some incidents in which milk has been adulterated by nitrogen-rich substances such as melamine have even had fatal consequences for consumers.

It can be concluded that there are reliable and effective methods for the detection of milk adulteration, though the problem with some of them is that they require expensive equipment and well-educated staff, and some of them are also time-consuming. To protect consumers, milk screening should be performed more often within industrial processing and within market chains. This means that more facilities should be equipped with the necessary instrumentation and more members of staff should be educated to work with this equipment.

There is also constant demand for more reliable, faster and inexpensive methods for the detection of milk adulteration and some of these demands will certainly be adequately met with the invention of new methods.

More information about milk originating from different animals and also milk originating from the same animal but different breeds would also be very helpful, because it would reveal possible detection markers.

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