Adulterants

Definition

1. An adulterant is a chemical substance which should not be contained within other substances for legal or other reasons. The addition of adulterants is called adulteration.

2. Adulterants need to be relatively odorless, colorless and tasteless to avoid negative impact upon consumer acceptance of the fraudulent product.

3. If used as a protein substitute, the adulterant(s) also need to be commercially available in large quantities.

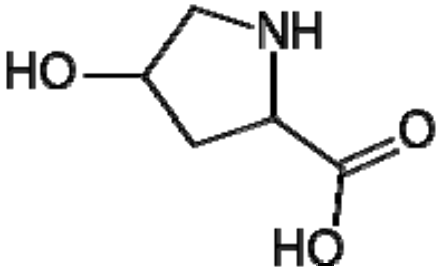
4. The ultimate goal with adulteration is economic fraud, not to injure consumers; hence food producers would be unlikely to deliberately use an acutely toxic chemical as an adulterant. However, as could be proven with melamine adulteration, even relatively non-toxic chemicals can cause unforeseen health effects.

5. Addition of adulterants must generally result in a product that costs less than the authentic food product, or otherwise there is no real economic incentive for adulteration.

6. Adulterants when used in illicit drugs are called cutting agents, while deliberate addition of toxic adulterants to food or other products for human consumption is known as poisoning.

Further reading on Food Contaminants & Adulteration:
http://www.fda.gov/food/foodsafety/foodcontaminantsadulteration

Non-native amino acids in milk
"leather milk"



L-Hydroxyproline

Incidents: China 2011

Amino acids are molecules with both amine and carboxylic acid functionalities and a varying side-chain that defines their properties into being; hydrophilic or hydrophobic, weak acid or base. Amino acids are important to life, and have many functions. Eight amino acids are classified as "essential" for human and cannot be synthesized via metabolism from other endogenous compounds, therefore they must come from our food intake..

Amino acids serve as building blocks of proteins (chains of amino acids). For this reason, amino acid composition analysis is a classical protein analysis method, used in medical and food science research. Composition analysis of proteins is complex, comprising two steps, hydrolysis of the substrate and chromatographic separation and detection. The hydrolysis is commonly conducted with very high concentration of acid.

Recently hydrolysis of protein has been utilized for tampering of milk, i.e. "leather milk".

Leather Milk

Milk is very important to mammals. Among human it is the only source of nutrition for infants, hence quality is important. Dishonest dairy producers can unfortunately increase their profit substantially by diluting milk with water as in the melamine scandal, and consumers suffer and potentially become exposed to harmful agents. With an economical motive in hand new cunning attempts are conducted.

An alternative tampering technique is to add protein hydrolysate to diluted milk. Unscrupulous Chinese dairy producers have, for years, been collecting the scraps left over from the leather softening process at local tanneries, and putting it into milk, thereby boosting the milk's protein content as measured with the standard Kjeldahl test. This toxic milk has been named "leather milk", and again the most vulnerable victims are infants.

The consumer risk factor is not acute but those who consume leather milk are at risk of developing osteoporosis and cancer after long-term exposure. In fact, as early as 2005, this was reported in Chinese media. Addition of leather hydrolyzed protein is more difficult to detect than nitrogen-rich compounds like melamine, because it is of protein origin itself. The Chinese Ministry of Agriculture has recently advised manufacturers to check milk for L-hydroxyproline (L-Hyp). This amino acid is a good marker of hydrolyzed animal collagen as it is formed from the hydrolysis of connective tissue protein (the content is about 13%) and is not present naturally in lactoprotein. Another possibility is to check for sodium and potassium dichromate; chemicals used for softening leather, which eventually end up in the milk via the collagen hydrolysis procedure, and where chromium exist in its most toxic form; hexavalent chromium ($CrVI^{+}$).

In this compilation, a new sensitive method is presented based on hydrophilic interaction liquid chromatographic separation and tandem mass spectrometric detection (HILIC-MS/MS) suitable for positive identification and quantitative analysis of L-Hydroxyproline in dairy products. A qualitative method based on HILIC and evaporative light scattering detection (ELSD) or single MS detection has also been developed. Herein, we present the new method and illustrate the effectiveness of having simultaneous qualitative and quantitative methods at hand for L-hydroxyproline to check milk quality.

Detection of L-Hydroxyproline in Milk using HILIC-MS/MS

Recommended column

SeQuant® ZIC®-HILIC (5 µm, 200Å) PEEK 150×2.1 mm	(1.50454.0001)

Alternative column

SeQuant® ZIC®-HILIC (3.5 µm, 200Å) PEEK 100×2.1 mm	(1.50447.0001)

Recommended solvents and reagents

Acetonitrile:	Hypergrade for LC-MS LiChrosolv®	(1.00029)
Water:	Water for chromatography LiChrosolv® or freshly purified water from Milli-Q® water purification system	(1.15333)
Acetic acid:	(glacial) for analysis EMSURE® ACS,ISO,Reag. Ph Eur	(1.00063)
Ammonium acetate:	for analysis EMSURE® ACS,Reag. Ph Eur	(1.01116)
Hydrochloric acid:	32% for analysis EMSURE®	(1.00319)
Sodium hydroxide pellets:	EMSURE® ACS, Reag. Ph Eur	(1.06469)
L-Hydroxyproline:	for biochemistry	(1.04506)

Recommended filtration tools

Mobile phase filtration:

PTFE coated with funnel, base, stopper clamp	(XX1004720)
Omnipore PTFE membrane filter 0.45µm	(JHWP04700)

Sample filtration:

Millex-LCR Filter, 0.45 µm, PTFE, 13 mm, non-sterile	(SLCRT13NL)
Samplicity™ starter bundle with filter 0.45µm	(SAMPLCRBL)

Detection of L-Hydroxyproline in Milk using HILIC-MS/MS

Column: SeQuant® ZIC®-HILIC (5 µm, 200Å) PEEK 150x2.1 mm (1.50454.0001)
Alt. Column: SeQuant® ZIC®-HILIC (3.5 µm, 200Å) PEEK 100x2.1 mm (1.50447.0001)

Mobile phase: A: 100 % ammonium acetate (50 mM, pH 5.6)
B: 100 % acetonitrile

Gradient profile:

Time (min)	Solution A (%)	Solution B (%)	Flow rate (mL/min)	Elution
0.0-10.0	15	85	0.800	isocratic
10.0-12.0	15→45	85→55	0.800	gradient
12.0-20.0	45	45	0.800	isocratic
20-25.0	15	85	0.800	equilibration

MS/MS parameters:

Compound	Precursor Ion (m/z)	Product Ion (m/z)	Collision Energy (eV)
L-Hydroxyproline (L-Hyp)	132.1	86.0* 68.2	22 18
L-Leucine (L-Leu)	132.1	86.10 43.2	16 35
L-Proline (L-Pro)	116.0	70.0	25
L-Histidine (L-His)	156.0	109.9 93.0	25 35
L-Arginine (L-Arg)	175.2	116.0 70.3	21 34
L-Valine (L-Val)	115.2	72.2 55.2	19 33

Sample preparation

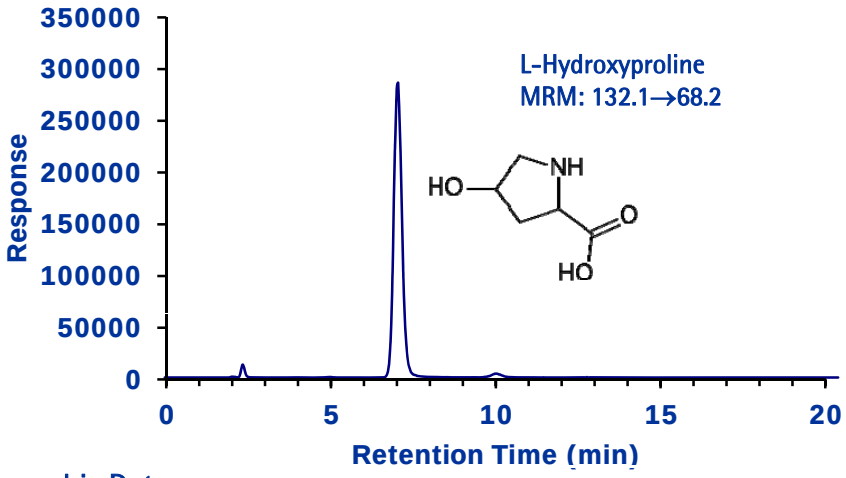
Local brand milk (2.0 g) was digested with a solution of 10 N HCl (6mL), placed under vacuum and sealed, then boiled for 12 hours. The mixture was evaporated to remove the solvent, followed by addition of 5ml of water and pH adjustment to 7 by NaOH solution (1N) before transfer to a volumetric flask and a final addition of water to bring the total volume to 25mL. The hydrolyzed mixture was centrifuged for 3min (8000 rpm) to give a clear solution. 1mL of the sample solution was transferred to a volumetric flask (10mL) and a solvent combination of ammonium acetate (50mM, pH5.6) and acetonitrile (20:80 v/v) was added to the scale. The diluted solution was filtered by 0.45 PTFE filter prior LC-MS/MS analysis

Detection of L-Hydroxyproline in Milk using HILIC-MS/MS

SeQuant® ZIC®-HILIC

Chromatographic Conditions

Column:	SeQuant® ZIC®-HILIC (5 µm, 200Å) PEEK 150x2.1 mm	1.50454.0001
Injection:	2 µL	
Detection:	Agilent 1200 LC system equipped with an Applied Biosystems API3200 MS/MS. Electrospray ionization was performed in positive ion mode, and multiple-reaction monitoring mode (MRM) for detection, see Table 2.	
Flow Rate:	0.8 mL/min.	
Mobile Phase (v/v):	A: 100% ammonium acetate (50 mM, pH 5.6) B: 100 % acetonitrile	
Gradient profile:	See gradient table on page 30.	
Temperature:	25 °C	
Diluent	Mobile phase	
Sample:	Local milk sample treated as per sample preparation method.	



Chromatographic Data

No.	Compound	Time (min)	Transition (m/z)
1	Void volume (t0)	0.6	
2	L-Leucine	3.3	132.1→86.1; 132.1→43.0
3	L-Valine	4.7	115.2→72.2; 115.2→55.2
4	L-Proline	4.8	116→70.0
5	L-Hydroxyproline	7.1	132.1→86.0; 132.1→68.2
6	L-Histidine	15.3	156.0→109.9; 156.0→93.0
7	L-Arginine	15.9	175.2→116.0; 175.2→70.3