Beacon Analytical Systems Inc.

www.beaconkits.com

Determination of progesterone in dairy products by immunoassay

Byungchul Kim, Jingping Xie and Titan Fan

Beacon Analytical Systems, Inc., Saco, ME, USA

Abstract

A competitive enzyme-linked immunosorbent assay (ELISA) for the detection of progesterone was developed. The sensitivity of the assay was 0.1 ppb and the assay range is from 0.1 to 10 ppb in buffer solution. This kit uses rabbit polyclonal antibodies against progesterone and progesterone-HRP (horseradish peroxidase) conjugate. Progesterone in dairy products were simply extracted by methanol and diluted with PBS buffer before running the assay. Progesterone levels in dairy products are correlated with the fat contents. In this study, we found high progesterone levels (73.5 – 127.1 ppb) from high fat content dairy products such as butter, sour cream, heavy cream and ice cream. On the contrary, very low levels of progesterone (≤1 ppb) were found from skim milk. Progesterone were spiked in the dairy products and determined with good recoveries. The results indicate that the immunoassay kit developed in this study can detect progesterone in various dairy products with simple extraction methods. The assay is sensitive enough to detect low progesterone levels in low fat content product as well.

Introduction

Progesterone is a naturally occurring hormone and involved in pregnancy and embryogenesis. In veterinary medicine, progesterone is used for therapeutic purposes such as disorders of the reproductive systems. Analytical methods to determine progesterone such as gas chromatography (GC) and liquid chromatography (LC) coupled with mass spectrometry (MS) or tandem mass spectrometry (MS/MS) are most popular. However, enzyme-linked immunosorbent assay (ELISA) is a promising alternative due to its simplicity and accuracy. Most of the commercial progesterone ELISA assays are used for biological samples such as milk, urine and serum. Indeed, many of them are used for detection of pregnancy in cows. Progesterone and other hormones in milk and milk products have been concerned if they affect public health. Adebamowo et al. found that teenage acne may be associated with intake of milk due to hormones. Ganmaa et al. concluded that increased consumption of milk and dairy products may have adverse effects on the development of hormone-dependent cancers. Goodson found that salivary progesterone increased 22 to 116% after 24 hours of a high-fat dairy product diet, and he concerned that consumption of milk products containing high progesterone may affect the health of young persons. Jouan et al. reviewed the levels of hormones from milk and milk products, and they were suspicious whether those hormones can bring health benefits to humans or not. Even though there is no regulation on progesterone level in dairy products, high progesterone levels in foods may attract people's attention due to its possible relationship to health problems. In this study, we developed a competitive ELISA assay and successfully determined progesterone levels in dairy products such as milk, cheese, yogurt, whipped cream, sour cream, ice cream, heavy cream and butter.

Methodology

Polyclonal antibody production:

In the development of these assays, progesterone was coupled to a protein carrier, which served as an immunogen, and horse radish peroxidase (HRP) which served as the enzyme conjugate. The antibody was produced through the immunization of New Zealand white rabbits. The serum was collected monthly.

Methodology

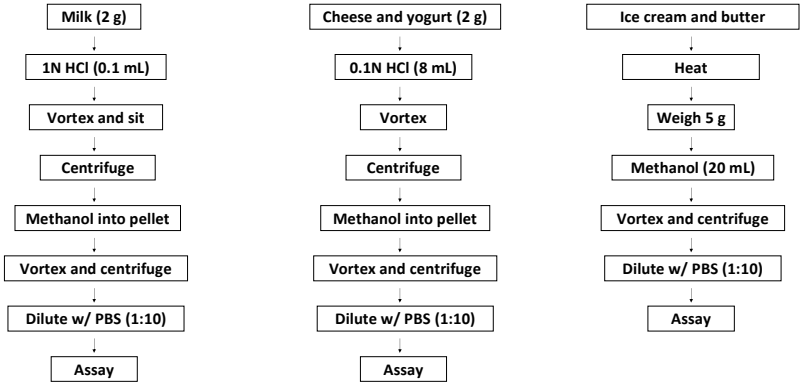


Figure 1. Flow charts for sample extraction procedure for dairy products.

Assay protocol:

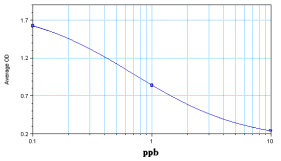
50 µl of HRP conjugate is added to each well followed by 50 µl of calibrator or sample extract. Lastly 50 µl of antibody solution is added to the wells and mixed gently for 30 seconds and incubated for 30 minutes. The well contents are then decanted and washed 4 times with PBST wash solution. Substrate (100 µl) is added to the wells and incubated for 30 minutes. The reaction is stopped with 100 µl of stop solution and the contents of the wells are read at 450nm-650nm. The concentration is calculated from a calibration curve applying dilution factors.

Results

Table Progesterone contents in dairy products and recoveries with different spike levels.

Sample	Spike level (ppb)	Progesterone (ppb) before spike	Progesterone (ppb) after spike	Recovery (%)
Whipped cream	10	1.4	12.2	108
Skim milk	10	2.3	14.9	126
Yogurt	10	8.3	21.9	119
Cottage cheese	10	10.0	25.9	132
Whole milk	10	12.7	17.0	86
Ice cream (1)	20	73.5	91.0	88
Ice cream (2)	20	80.4	101.4	105
Sour cream	20	80.5	101.9	107
Heavy cream	50	98.3	145.3	94
Butter	50	127.1	171.1	88

Results



4-P Fit; y = (A – D)/(1 + (x/C)^B) + D

A	B	C	D	R^2
1.87	0.931	0.703	0.0992	1

IC 50: 0.8 ppb, IC 85: 0.12 ppb

Figure. 2. Progesterone calibration curves.

Discussion

A competitive immunoassay was developed to detect progesterone in dairy products. It was found that removal of aqueous solution followed by dissolving in organic solvent (methanol) is a necessary procedure for high-protein foods. Acid (HCl) was used for this purpose. After treated with HCl, sample was centrifuged to remove aqueous portion (supernatant), and the precipitate was dissolved in methanol to extract progesterone. Solid sample such as cheese must be homogenized by breaking solid matter. Butter and creams must be melted by microwave heating. Methanol extraction should be done as early as possible before the samples re-solidify.

It has been known that progesterone levels in milk products are correlated with fat content. Low-fat dairy foods such as whipped cream and skim milk had the lowest progesterone levels of 1.4 and 2.3 ppb, respectively. High-fat dairy foods such as sour cream, heavy cream, ice cream and butter contain high progesterone ranging from 73.5 to 127.1 ppb. Known concentrations of progesterone were spiked into the dairy products to investigate recovery of progesterone. Different levels of progesterone were spiked depending on the existing progesterone levels in the dairy products (10, 20 and 50 ppb). Recoveries from all the tested dairy products ranged from 86 to 132%.

A competitive ELISA assay for determination of progesterone from dairy products was successfully developed and evaluated. This immunoassay can provide fast and reliable results for progesterone determination in dairy products.

References

Adebamowo, C.A.; Spiegelman, D.; Danby, F.W.; Frazier, A.L; Willett, W.C; Holmes, D. High school dietary dairy intake and teenage acne. J. Am. Acad. Dermatol. 2005, 52, 207-214.

Ganmaa, D.; Sato, A. The possible role of female sex hormones in milk from pregnant cows in the development of breast, ovarian and corpus uteri cancers. Medical Hypotheses, 2005, 65, 1028-1037.

Goodson, W.H. Milk products are a source of dietary progesterone. San Antonio Breast Cancer Symposium (SABCS), 2007, Abstract #2028.

Jouan, P.N.; Pouliot, Y.; Gauthier, S. F.; Laforest, J.P. Hormones in bovine milk and milk products: A survey. International Dairy Journal, 2006, 16, 1408-1414.