

XXIII. ULUSAL BİYOKİMYA KONGRESİ

Biyomoleküllerin Yaşamdaki Önemi ve Klinik Kararda Laboratuvar



29 Kasım - 2 Aralık
Hilton Hotel - Adana



SÜTTEKİ ŞEYTAN: HASTALIKTA VE SAĞLIKTA A1 VE A2 SÜT

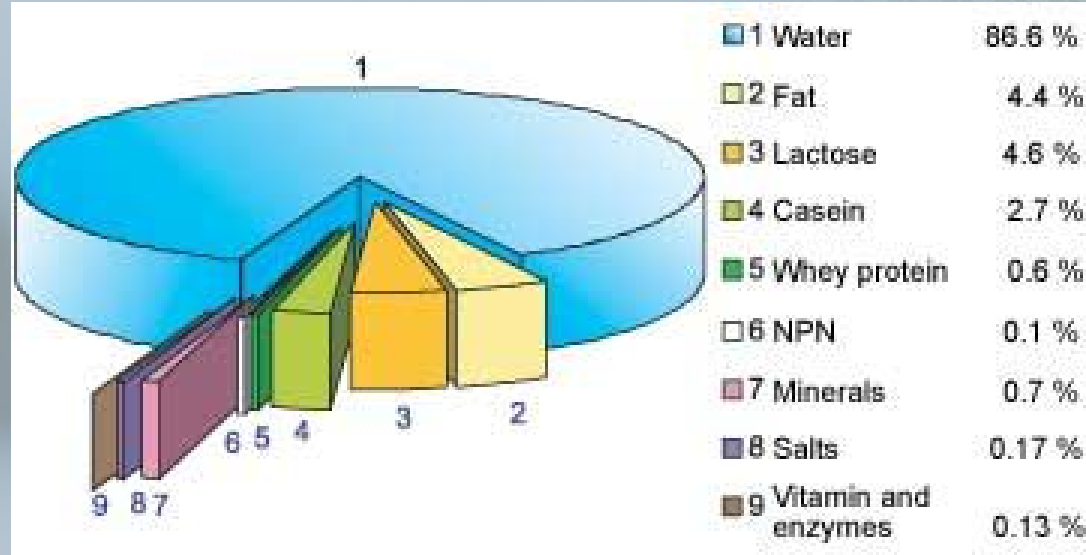
Ebru SAATÇİ*, Aysel ERASLAN, Havva DİNÇ
Mesude İŞCAN, İnci TOGAN

*Erciyes Üniversitesi, Fen Fakültesi, Biyoloji Bölümü, 38030, Kayseri

İnek Sütü'nün Özellikleri

- Süt teknolojisi = inek sütü (kazeinli süt)
- Besleyici temel gıda maddesi
- Koruyucu gıda (antikorlar, mineraller, vitaminler)
- Kullanım alanları:
 - Yeni doğan yavru beslenmesinde
 - Temel gıda maddesi
 - Kazein ve laktoz gibi maddelerin üretiminde ham madde
 - Bir çok sütlü yan gıda maddesinin üretiminde

İnek Sütü'nün İçeriği



- İnek sütü: su (% 86.6), proteinler (% 3.4), yağ (% 4.4), laktoz (% 4.7), vitamin, tuzlar, mineraller ve süt enzimleri
- Proteinler: Kazeinler (% 80) ve whey proteinleri (% 20)
- Kazeinler: α s1-kazein, α s2-kazein, β -kazein ve κ -kazein
- Whey proteinleri: β -laktoglobulin ve α -laktalbumin

*http://en.delaval.cn/Dairy_Knowledge/EfficientCooling/Milk.htm

Protein İeriđi

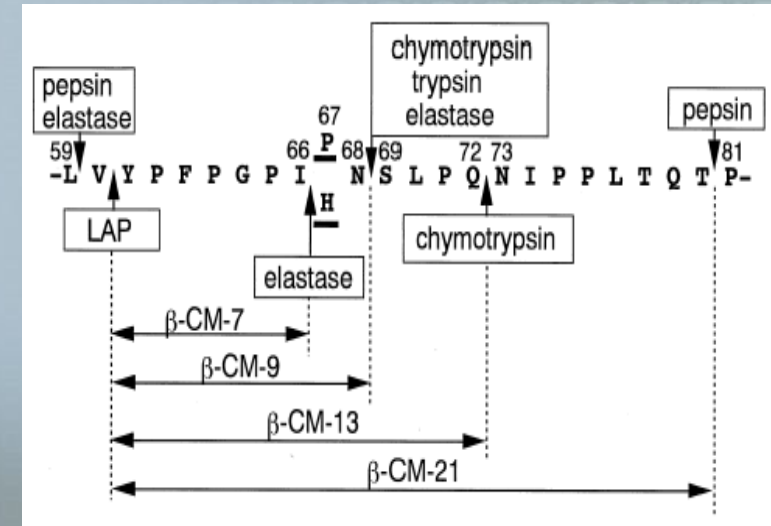
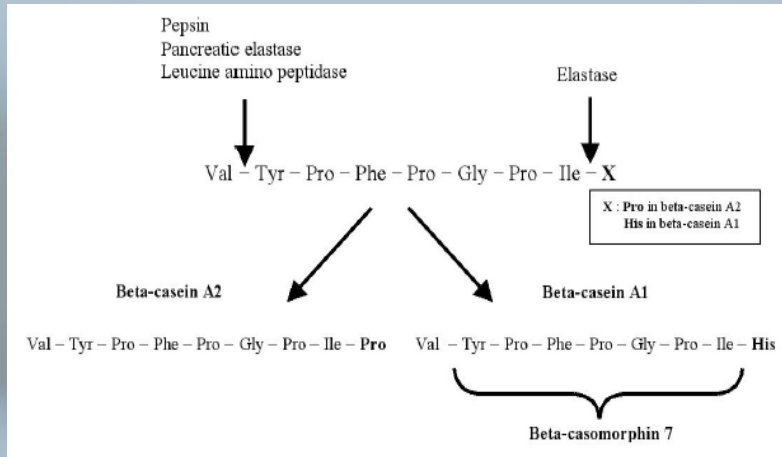
Protein Fraksiyonları		%
Kazein (% 2.70)	Beta-kazein	1.10
	Alfa s1- kazein	1.00
	Alfa s2- kazein	0.30
	Kappa-kazein	0.30
Whey Proteinleri (% 0.70)	Beta-laktoglobulin	0.40
	Alfa-laktalbumin	0.15
	İmmünglobulinler	0.10
	BSA	0.04
	Glikomakropeptitler	0.01
Minor Proteinler	Laktoferrin	0.01
Enzimler	Laktoperoksidaz	0.01

İnek Sütündeki Kazein Fraksiyonları

Kazein Fraksiyonları	Konsantrasyon Aralığı (mg/ml)
Kappa-kazein	0.038-0.377 mg/ml
Beta-kazein	0.151-1.506 mg/ml
Alfa-kazeinler (Alfa s1- ve Alfa s2- kazein)	0.188-1.883 mg/ml

*Veloso et al., (2002). Journal of Chromatography A

A1 ve A2 Tip Süt Arasındaki Fark Nedir?

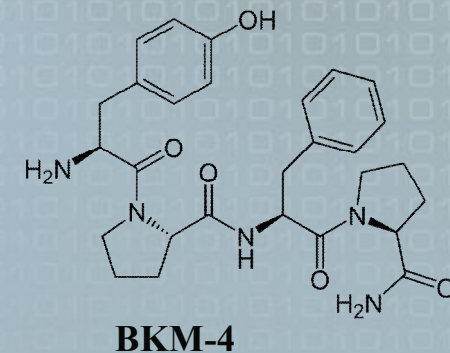
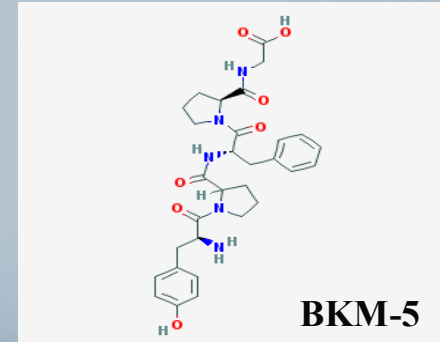
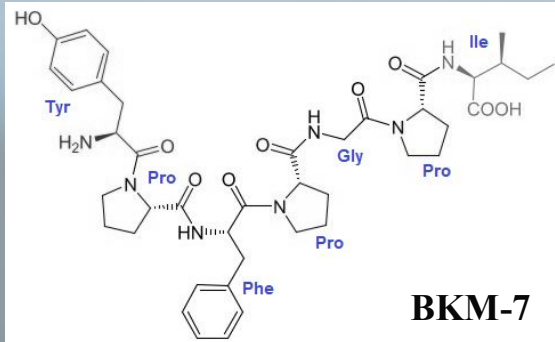


- A1 tip (Pro-His mutasyon) süt β -kazeinin gastrointestinal sindirimi sonucunda BKM-7 oluşmaktadır.
- A2 β -kazeinin parçalanması sonucunda BKM-7 açığa çıkmamaktadır.

*Jinsmaa *et al.*, Peptides, 1999.



Beta-Kazomorfinler

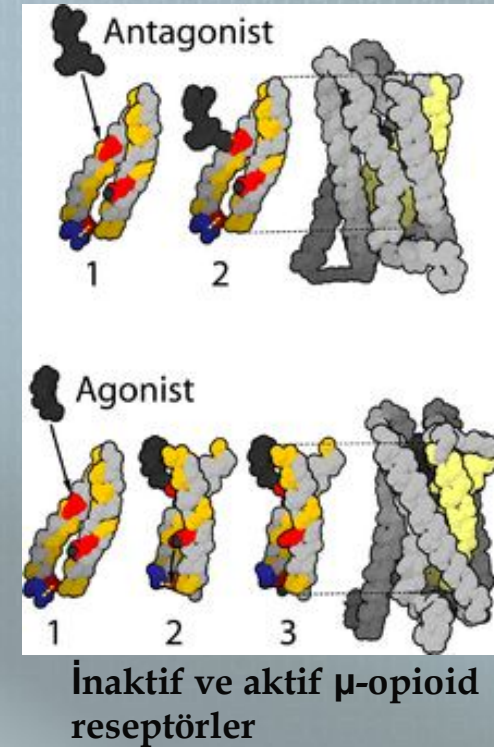


- **İnek β-kazomorfin 1-4**
H-Tyr-Pro-Phe-Pro-OH (MA = 522.61 g/mol)
- **İnek β-kazomorfin 1-4, amid ([morfiseptin](#))**
H-Tyr-Pro-Phe-Pro-NH₂ (MA = 557.64 g/mol)
- **İnek β-kazomorfin 5**
H-Tyr-Pro-Phe-Pro-Gly-OH (MA = 594.66 g/mol)
- **İnek β-kazomorfin 7 (BKM-7)**
H-Tyr-Pro-Phe-Pro-Gly-Pro-Ile-OH (MA = 789.9g/mol)
- **İnek β-kazomorfin 8**
H-Tyr-Pro-Phe-Pro-Gly-Pro-Ile-Pro-OH (MA = 887.00 g/mol)

İnsan BKM-7: Tyr-Pro-Phe-Val-Glu-Pro-Ile

Beta-Kazomorfinler

- β -kazomorfinler morfine benzer opioid agonist etkilerini vücuttaki opioid reseptörlere bağlanarak göstermektedirler.
- A1 tip inek sütü tüketimi =
 - Nörolojik bozukluklar
 - Alzheimer, Parkinson
 - Otizm, şizofreni,
 - Damar sertliği gelişimi,
 - İskemik kalp hastalığı,
 - Yeni doğan bebeklerde ani bebek ölümü sendromu (SIDS) etkeni
 - Çocuklarda Tip I diyabet,
 - Öğrenme ve hafıza bozukluğu



Tanıda Beta-Kazomorfinler

- Otizm tanısında, çocuklarda idrarda **Gluten/Kazein** peptitlerinin (kazomorfin ve gliadorfin) tayini yapılmaktadır.
- **Gluten/Kazein** testi aynı zamanda
 - AD(H)D, MS,
 - Alzheimer
 - Şizofreni,
 - Down sendromu,
 - Beslenme ve sindirim bozuklukları gözlenen çocuklarda da başvurulan bir yöntem haline gelmiştir.

AD(H)D: Attention Deficit Hyperactivity Disorder



The Great Plains Laboratory, Inc.
11813 West 77th Street, Lenexa, KS 66214
Phone: (913) 341-8949 Fax: (913) 341-6207
E-mail: customerservice@GPL4U.com

What Are Peptides?

Specimen Requirements: 10 mL of first morning urine (most concentrated) – Follow instructions provided in the test kit.

Innovations in Testing

With the recent attention on the gluten-free, casein-free diet and its positive affects for those with gastrointestinal, neurological, and movement disorders, The Great Plains Laboratory, Inc. offers a test which can help to determine whether the diet should be part of an overall integrated treatment plan.

This test measures levels of two opiate peptides derived from dairy and gluten products. Peptides are small fragments of proteins; many peptides engage in cell-to-cell signaling and have neurological effects. Casomorphin is produced by digestion of casein, the major protein in all mammalian milk, including human milk. The opiate effect of milk may help soothe and quiet the nursing infant, whether a puppy or a child. Gluten in wheat produces another peptide of the same size called gliadorphin. Grains like rye and barley contain similar prolamine proteins like gluten, and are assumed to yield bioactive peptides.

Peptide problems are more severe among individuals who have difficulty breaking down opiate peptides, which requires an enzyme nicknamed DPPIV. This enzyme can be inhibited by both yeast byproducts and mercury exposure. These incompletely digested peptides are then absorbed into the body and bind to opiate receptors – the physiological effect can alter behavior and contribute to lack of focus and attention, sleepiness, or even aggression and self-abuse can result.

Dr. Kalle Reichelt in Norway and Dr. William Cade at the University of Florida found high levels of the casomorphin peptide in urine samples from people with gastrointestinal, neurological, and movement disorders. Many

parents have stated that before taking their child off of gluten and casein the child acted as if he/she were on hallucinogenic drugs. Children and adults with gastrointestinal, neurological, and movement disorders may incompletely digest wheat and dairy products. Because these peptides are so structurally similar, they are presumed to affect the brain similarly.

Both casomorphin and gliadorphin are composed of seven amino acids, which are abbreviated below. Both casomorphin and gliadorphin are 7-amino acid peptides which have a beginning N-terminal sequence tyr-pro (for tyrosine and proline) with additional pro in positions 4 and 6 of both peptides as indicated below. Similarities are indicated by bold print.

	1	2	3	4	5	6	7
Casomorphin:	tyr	pro	phe	pro	gly	pro	ile
Gliadorphin:	tyr	pro	gln	pro	gln	pro	phe

Because these peptides are so structurally similar, they are presumed to affect the brain similarly.

The problem arises if gluten and casein foods are consumed exclusively, which many children, particularly those on the autistic spectrum, would prefer to do. Individuals with gastrointestinal, neurological, and movement disorders frequently seem addicted to wheat and dairy products. They crave the foods that cause their problems. Dr. William Shaw remembers a mother complaining that her child would only drink milk.

Recommended for the following disorders:

- AD(H)D
- AIDS
- Alzheimer's Disease
- Arthritis
- Autism Spectrum Disorders
- Chronic Fatigue Syndrome
- Colitis & Crohn's Disease
- Depression
- Diarrhea/Constipation
- Down Syndrome
- Fibromyalgia
- Movement Disorders
- Multiple Sclerosis
- Obsessive-Compulsive Disorder
- Psychoses
- Schizophrenia
- Tic Disorder
- Tourette Syndrome



"Our services prove that we care about our client's continuous well-being and progress after doing our tests"

Gluten / Casein Peptides Test

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BKM-7: Tyr-Pro-Phe-Pro-Gly-Pro-Ile
Gliadorfin: Tyr-Pro-Gln-Pro-Gln-Pro-Phe

Aralık 2011

Beta-Kazomorfinlerin Genetik Varyansı

Milk Protein Genes	Alleles	Exons	Nucleotide Substitutions	Amino Acid Position	Amino Acid Substitutions	References
Beta-casein	A2→A1	VII	CCT→CAT	67	Pro→His	Lien <i>et al.</i> , 1992
	A2→A3	VII	CAC→CAA	106	His→Gln	
	A2→B	VII	CCT→CAT AGC→AGG	67 122	Pro→His Ser→Arg	
Kappa-casein	A→B	IV	ACC→ATC GAT→GCT	136 148	Thr→Ile Asp→Ala	Malik <i>et al.</i> , 2000
	A→E	IV	AGC→GGC	155	Ser→Gly	
BLG	A→B	IV	GAT→GGT GTC→GCC	64 118	Asp→Gly Val→Ala	Medrano and Aguilar-Cordova, 1990

Süt proteinleri genetik varyansları

Populations	Beta-casein Genotype and Allele Frequencies								
	A2A2	A2A3 or A3A3	A2A1	A2B	A1A1	A1B or BB	A2	A1	B
Turkish Grey (34)	0.324	0	0.382	0.059	0.235	0	0.544	0.426	0.029
Eastern Anatolian Red (25)	0.760	0	0.160	0.080	0	0.074†	0.880	0.080	0.040
Anatolian Black (16)	0.625	0	0.125	0.188	0.062	0	0.781	0.125	0.094
Southern Anatolian Red (30)	0.534	0	0.233	0.233	0	0	0.766	0.117	0.117
Turkish Holstein (22)	0.091	0.043†	0.682	0.045	0.182	0	0.454	0.523	0.023
Holstein Candidate Bulls (18)	0.444	0	0.556	0	0	0	0.722	0.278	0

California, 1991 and Finland, 2001



*Windsor Dairy, LLC

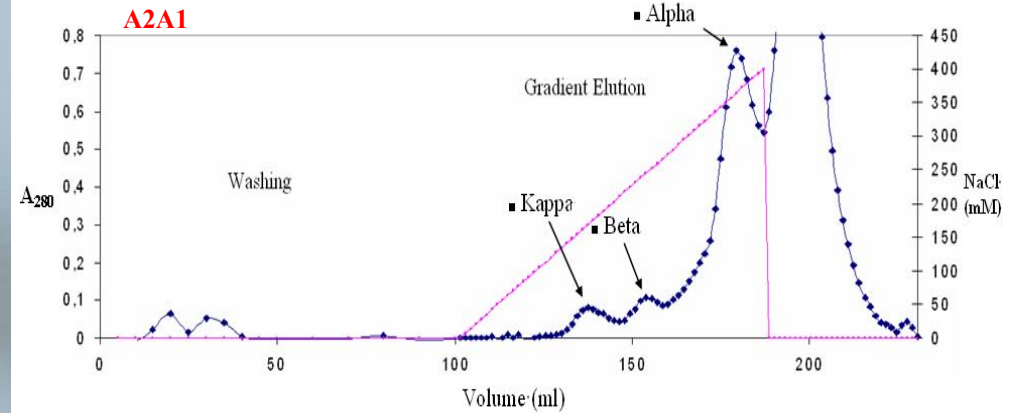
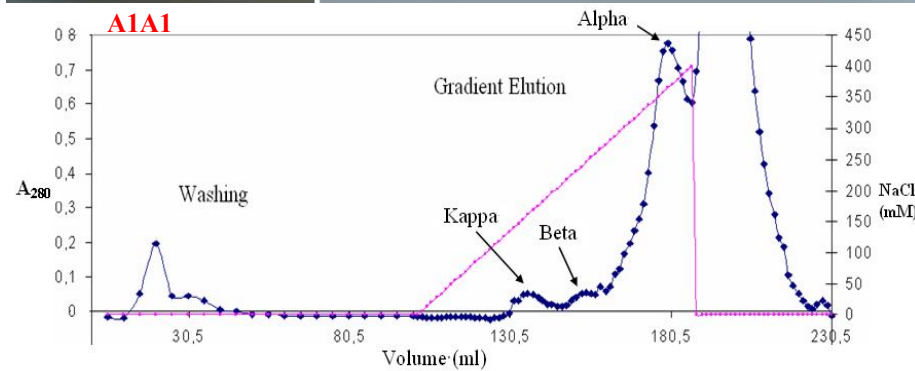
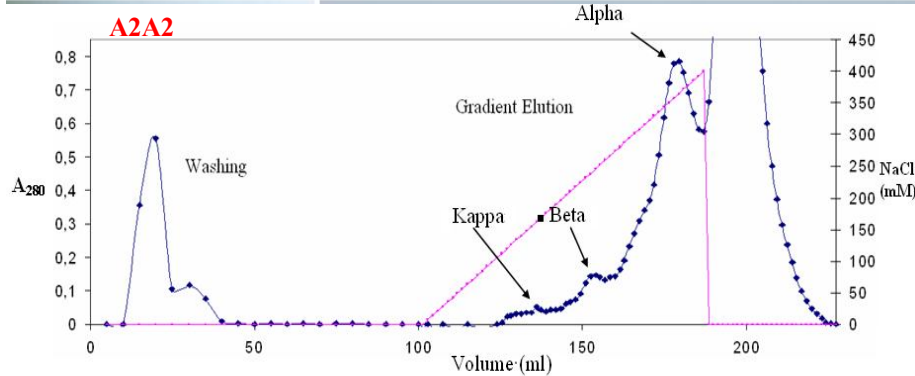
Türkiye'deki Beta-kazein genotipleri ve 6 sığır popülasyonundaki allel frekansları

*Havva Dinç, METU, PhD Thesis, 2009.

Sütten Total Kazein Eldesi

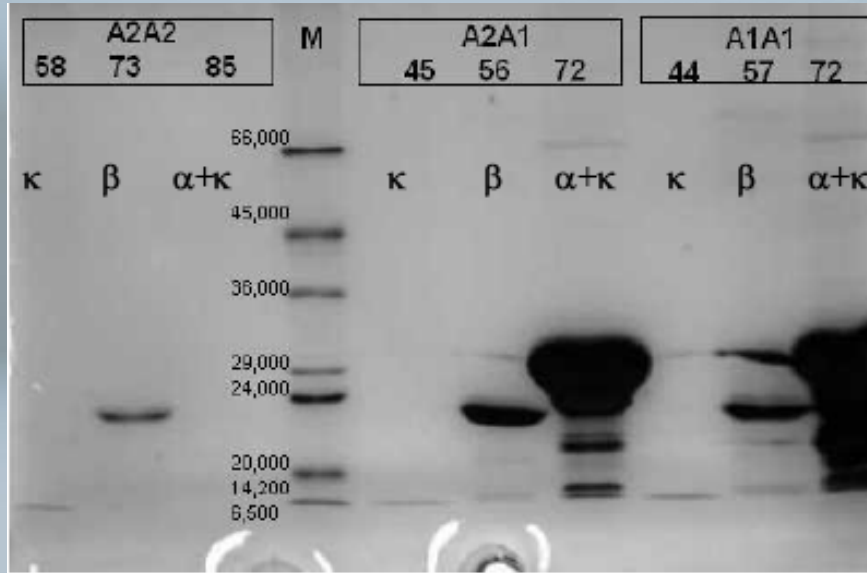
1. Sütler, Edirne ve Kırklareli'den, genotipleme yapılmış 8 Holstein inekten toplandı.
2. Sütlerden öncelikle asetik asit, eter-alkol ve üre presipitasyonları ile total kazein elde edildi.
3. Presipitatlardan, FPLC, HiPrep 16/10 Q XL anyon değişim kolonu ile kazein fragmentasyonu (NaCl gradienti ile) gerçekleştirildi.
4. Toplanan beta-kazein fragmentları enzimatik kesilime (pepsin, porsin pankreatik elastaz ve lösin aminopeptidaz) tabii tutularak kazomorfin peptidleri elde edildi.
5. Enzimatik kesim sonrası peptit fragmentları, HPLC'de Cosmosil 5C18-AR kolonunda asetonnitrile gradienti uygulanarak saflaştırıldı.

FPLC ile Ayırıştırma- A2A2, A2A1, A1A1 Tip Sütler



- A2A2, A2A1 ve A1A1 genotiplerine sahip sütlerin Kazein (kappa, beta ve alfa) fraksiyonlarının, 0-400mM NaCl gradienti FPLC kromatogramı.

FPLC Fraksiyonları: SDS-PAGE



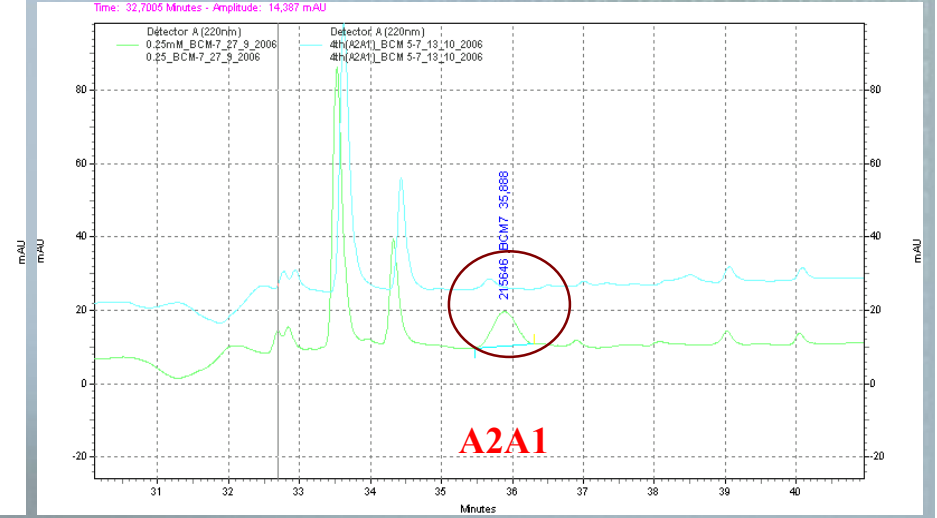
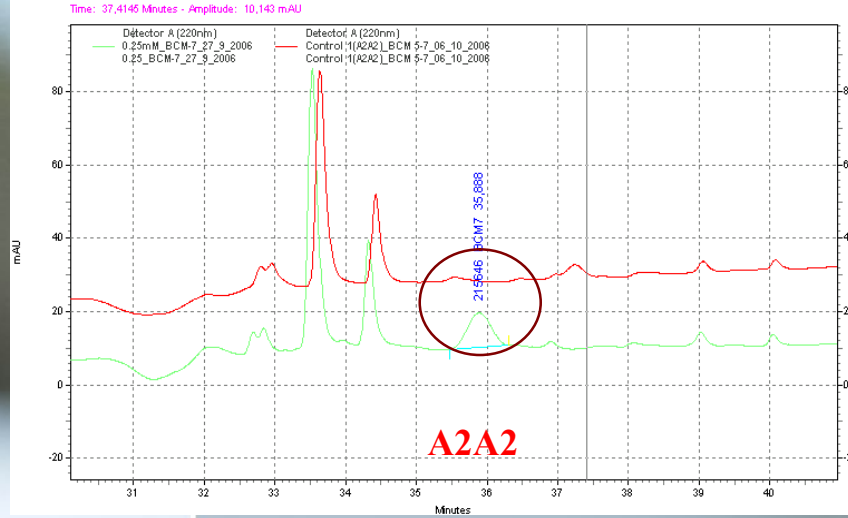
Kazeinler	Molekül Ağırlığı
Kappa-kazein	19,000 Da
Beta-kazein	24,000 Da
Alfa-kazein	s1: 23,000 Da s2: 25,000 Da

- FPLC elüsyonundan elde edilen kazein fraksiyonlarına ait SDS-PAGE jeli.
Protein Std MA: 6,500 - 66,000 Da
κ: kappa-kazein, β: beta-kazein ve α+κ: alfa + kappa kazeinler

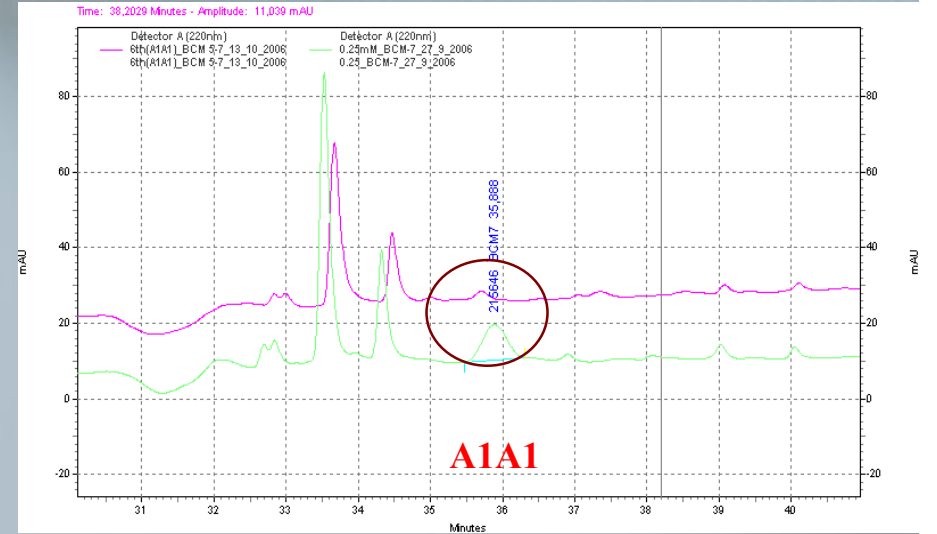
*Havva DINC, Evren KOBAN, Ebru SAATCI, Emel OZKAN, Mesude ISCAN and Inci TOGAN.
Proceedings of International Conference on Environment: Survival and Sustainability, 2: 345-351. 2009.

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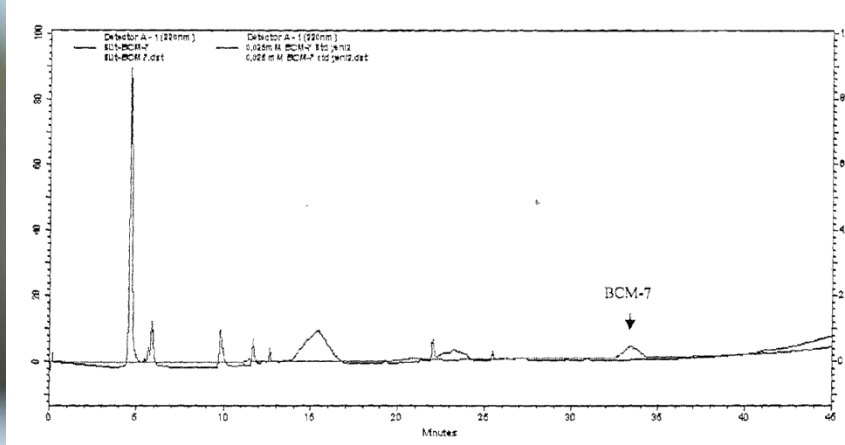
A2A2, A2A1, A1A1 Tip Süt HPLC Kromatogramları



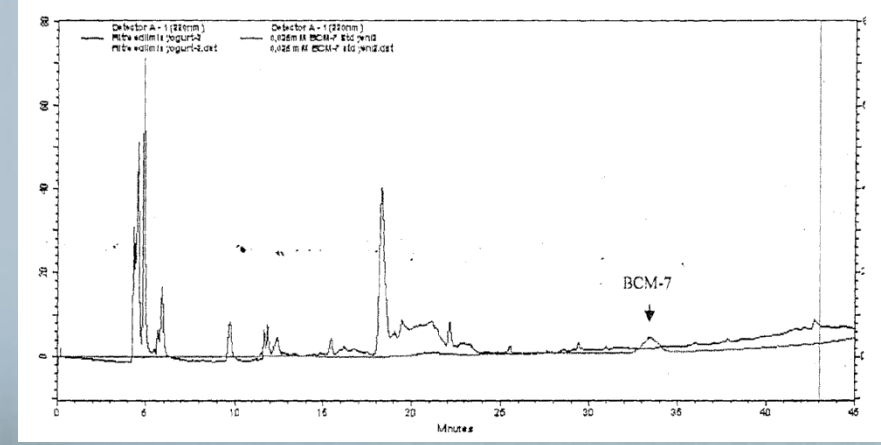
•A2A2, A2A1 ve A1A1
HPLC Kromatogramları
(40 dk içinde % 0-40
asetonitril gradienti ile)



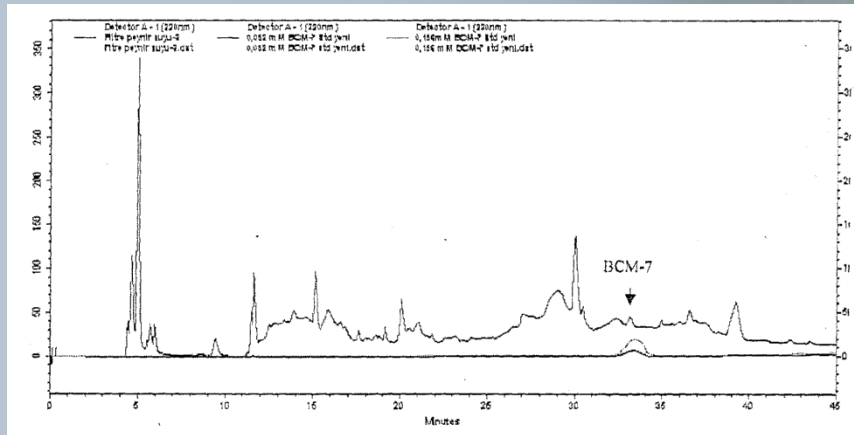
Şarküteri Ürünleri-HPLC



Şarküteri Süt HPLC Kromatogramı



Şarküteri Yoğurt suyu HPLC Kromatogramı



Şarküteri Peynir suyu HPLC Kromatogramı

- Amicon (MWCO 30000) Reservoir/base ultra santrifüj tüpleri ile konsantrasyon.

İleri Çalışmalar

- Sütte ve idrarda ELİSA kazomorfin testi
- Beta-kazomorfin-7 İmmunoamperometrik Biyosensör Dizaynı

Destekler:

- A1Tip İnek Sütü ve Süt Ürünlerinde Beta-kazamorfin Peptidleri için Moleküller Arası İlişkiye Dayalı Tayin Yöntemi Geliştirilmesi . ODTÜ, BAP-2006-07-02-03. 2006-2008.
- Beta-Kazomorfin-7 Amperometrik İmmünosensörü Yapımı. KOSGEB, Ar-GE ve İnovasyon Projesi,. Erciyes Üniversitesi, Teknopark, MOBETU şirketi ortak proje. 2010-2011.
- A1 Tip İnek Sütü ve Süt Ürünlerinde Beta-Kazomorfin Peptitleri için HPLC'ye Dayalı Tayin Yöntemi Geliştirilmesi. Yüksek Lisans Tezi, Aysel ERASLAN, FBA-11-3614, 2011-2012.

A2 Süt Logosu ve Kitaplar



A2 Logo



Amerika



Yeni Zellanda



Avusturalya

