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Bioactive properties of whey proteins / Lífvirkir eiginleikar mysupróteina

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Titill / Title	**Lífvirkir eiginleikar mysupróteina /** Bioactive properties of whey proteins		
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Skýrsla / Report no.	06-09	*Útgáfudagur / Date:*	Febrúar
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Ágrip á íslensku:	Rannsóknir þær sem lýst er í þessari skýrslu eru þáttur í verkefninu Nýting ostamysu í heilsutengd matvæli. Verkefnið fjallar um að bæta nýtingu og auka verðmæti mysu sem fellur til við ostaframleiðslu hjá Mjólkursamlagi KS á Sauðárkróki með því að nýta bæði prótein og mjólkursykur til framleiðslu á heilsudrykkjum og fæðubótarefnum. Með bættri nýtingu mjólkur t.d. með notkun próteina úr mysu má komast hjá óþarfa losun lífefna út í umhverfið. Ostamysa frá Mjólkursamlagi KS var aðskilin í fjóra hluta með himnusíubúnaði (Membrane Pilot Plant Type MEM11) í vinnslusal Líftækniseturs Matís á Sauðárkróki af starfsmönnum Iceprotein, annars vegar í gegnum 10 kDa himnu og hinsvegar 200 Da himnu. Efnasamsetning (raki, prótein, salt, steinefni) og lífvirkni (ACE-hamlandi virkni og andoxunareiginleikar) voru greind á rannsóknarstofu Matís í þessum fjórum sýnum auk þess sem mysan sjálf óbreytt var mæld. Niðurstöðurnar lofa góðu og sýna vel að lífvirkni er til staðar í mysunni, sem nýst getur í markfæði.		
Lykilorð á íslensku:	*Ostamysa, prótein, himnusíun, lífvirkni*		
Summary in English:	The experiment described in this report is part of the project *Utilization of Cheese whey in health based food products* which aims are to improve utilization and increase value of whey that is discarded during the cheese production at KS Sauðárkrókur, by using proteins and lactose to produce health drink and nutritional supplements. With better utilization, unnecessary disposal of bioactive components can be avoided. Cheese-Whey samples from KS were fractionated with membrane filtration equipment (Membrane Pilot Plant Type MEM11) at Matís Biotechnology centre in Sauðárkrókur with molecular weight cut-offs 10 kDa and 200 Da. Chemical composition and bioactivity properties were analyzed at Matís Laboratory. Results show that whey contains promising bioactive compounds that could be used as functional food.		
English keywords:	*Cheese-Whey, protein, membrane filtration, bioactivity*		

INTRODUCTION

Whey, a liquid by-product, is widely accepted to contain many valuable constituents. These include especially proteins that posses important nutritional and biological properties particularly with regard to promotion of health, as well as prevention of diseases and health conditions (Madureira et. al., 2007)

Milk contains two primary sources of protein, the caseins and whey. After processing occurs, the caseins are the proteins responsible for making curds, while whey remains in an aqueous environment. The components of whey include beta-lactoglobulin, alpha-lactalbumin, bovine serum albumin, lactoferrin, immunoglobulins, lactoperoxidase enzymes, glycomacropeptides, lactose, and minerals.

Many treatments have been investigated for improving whey protein functionality, including enzymatic hydrolysis (Gauthier and Pouliot, 2003; Hamada, 1994), fractionation (Morr and Ha,1993), dynamic high-pressure (Bouaouina, Desrumaux, Loisel, and Legrand, 2006; Gracia-Julia et al., 2008; Ibanoglu and Karatas¸ , 2001) and heat treatments (Bernal and Jenel, 1985; Croguennec, Renault, Beaufils, Dubois, and Pezennec, 2007; Davis and Foegeding, 2004; De Wit, 1990). Mainly functional properties are characterized by a lower molecular weight, exposure of hydrophobic groups, and by an increased number of ionic groups (Panyam and Kilara, 1996).

The objective of this work was to search for bioactive components related to blood pressure lowering effect and antioxidant effects of different whey protein fractions from by-products of the cheese industry (Mjólkursamlag KS, MKS) in Saudárkrókur, Iceland.

MATERIALS & METHODS

1.1. Fractionations of the samples

The crude whey samples were provided by MKS and ultra-filtrated using 10000Da molecular weight cut-off membranes. It was also nano-filtrated using 200 Da molecular weight cut-off membranes in Membrane Pilot Plant Type MEM 11 (GEA Wiegand GmbH, Ettlingen Germany), before laboratory analysis. All fractionation was performed by Iceprotein ehf. in Matís Biotechnology Centre, Process facility, of Matís ohf. Sauðárkókur.

1.2. Chemicals

Furanacryloyl-glycyl-glycine (FA-PGG), angiotensin converting enzyme from rabbit lungs (ACE), fluorescein sodium, 6-hydroxy-2,5,7,8-tetra-methylchroman-2-carboxylic acid (Trolox), 2,2’-azobis(2methylpropionamidine)dihydrochloride (AAPH) were obtained from Sigma-Aldrich.

1.3. Chemical composition

The protein amount, moisture content, ash, salt, carbohydrates and fat were determined following the standard methods.
All the whey fractions were set at 0.15% protein content before analysis.

1.4. Bioactive properties

1.4.1. Antihypertensive activity

The ACE inhibitory activity was performed essentially according to the method described by Shalaby *et al.* (2006) with slight modification. FA-PGG was used as substrate at 0.5 mM of solution in Tris-HCl /NaCl buffer pH 7.5. Angiotensin converting enzyme from rabbit lungs was freshly prepared before measurements, 0.2 U/ml of the enzyme solution was prepared and kept on ice. Microplate reader set (Polarstar Optima, BMG labtech) at 37°C was used. The absorbance at 340 nm was recorded each 30 seconds for 25 minutes, and the slope average over a linear interval between 10 and 25 min was taken as a measure of ACE activity. The ACE activity was expressed as the slope of the decrease in absorbance at 340 nm (Δ_{sample}), and the ACE inhibition (%) was calculated according to the following formula.

$$\text{ACE inhibition (\%)} = (1-(\Delta_{sample} / \Delta_{control}) \times 100)$$

Where Δ_{sample} is the slope in the presence of inhibitor, and $\Delta_{control}$ is the slope in the absence of the inhibitor.
The concentration of ACE inhibitory peptide that reduces ACE activity by 50% was defined as IC_{50} value.

1.4.2. Antioxidant activity

The oxygen radical absorbance capacity (ORAC) assay was performed according to Ganske F. and Dell E.J. (2006) with slight modifications. Polastar Optima (BMG labtech., Offenburg, Germany) microplate reader was used. Different dilutions of Trolox (50µM-3,125µM) and samples were prepared in phosphate buffer (10 mM, pH 7.4). In every working well of a black opaque micro-plate (200 µL, 96 wells, MJ Research, USA) the following was pipette in triplicate: 1) 75 µL of 10 nM Fluorescein solution; 2) 15 µL of Trolox dilutions for standard; 3) 15 µL of sample solution; 4) 15 µL of phosphate buffer for blank.
The micro-plate was incubated for 20 min at 37°C without shaking in the Polarstar Optima. After incubation 60 µL of 240 mM AAPH solution was quickly added manually using a multi-channel pipette. The fluorescence was recorded every 0.33 min for the first 15 cycles and every min for the last 60 cycles. The filters used for excitation was 485 nm and 520 nm for emission. The total time for the measurement was 80 min.
The antioxidant curves (fluorescence versus time) were normalized. The data from the curves were multiplied by the factor:

$$\frac{fluorescence_{blank,t=0}}{fluorescence_{sample,t=0}}$$

The area under the fluorescence decay curve (AUC) was calculated by the normalized curves with the following equation:

AUC=(0.5+f1/f0+f2/f0+ …+f20/f0)×0.1+(f21/f0+f22/f0+ …+f74/f0)×1+ 0.5×(f75/f0)

where f0 was the fluorescence reading at the initiation of the reaction and f75 was the last measurement.

The net AUC was obtained by subtracting the AUC of the blank from that of a sample or standard. The ORAC value was calculated and expressed as micromoles of Trolox equivalents per gram of protein (µmol of TE/g protein) using the calibration curve of Trolox.

RESULTS & DISCUSSIONS

2.1 Chemical composition

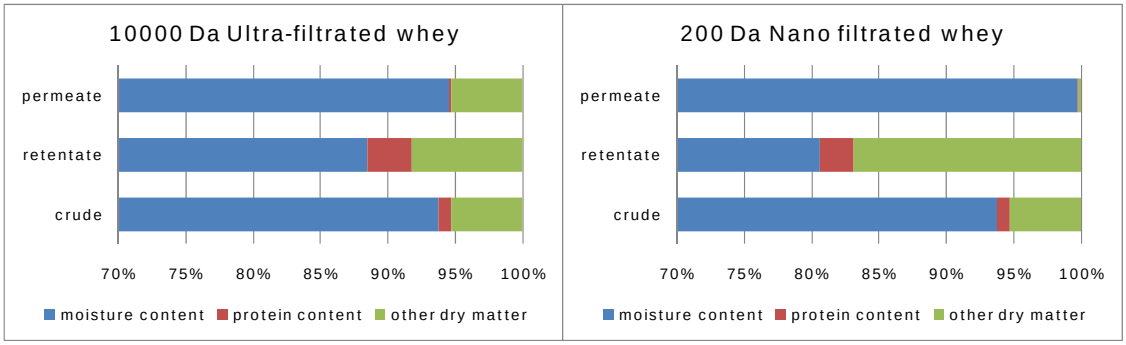

Fig. 1 and **Fig. 2** The chemical composition of ultra-filtrated whey and nano-filtrated whey using 10000 Da and 200 Da molecular weight cut-off membranes (respectively) other dry matter: carbohydrate, fat, salt and ash amounts are included.

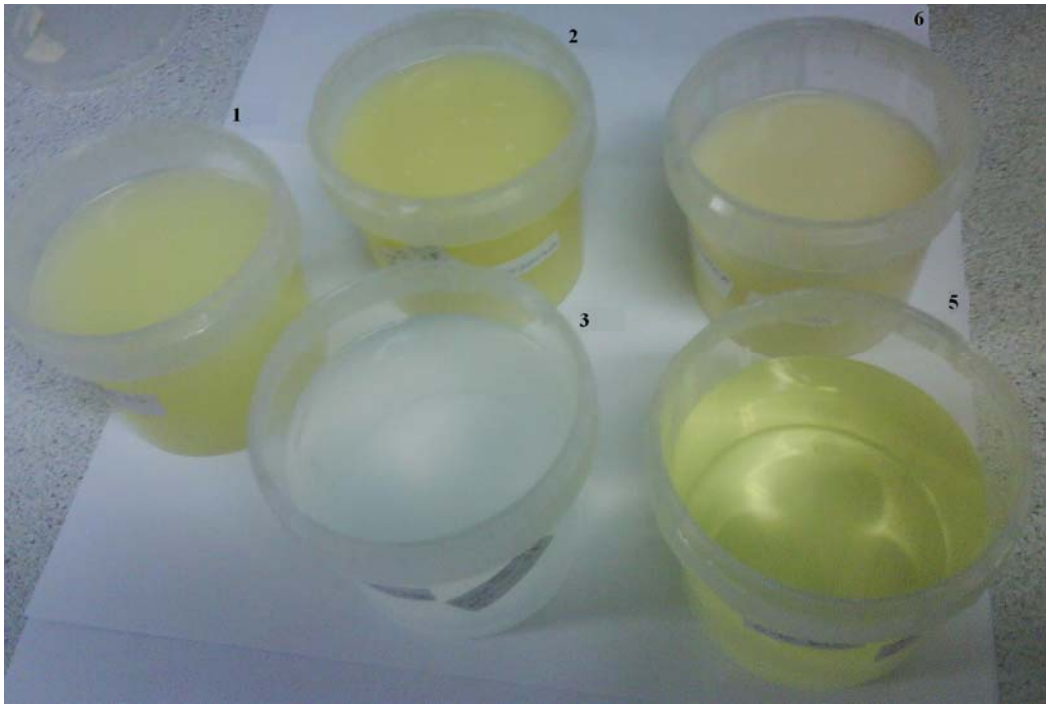

Fig. 3 Crude whey and filtrated whey. Crude 1, nano-filtrated concentrate 2, nano-filtrated permeate 3, ultra-filtrated concentrate 4, ultra-filtrated permeate 5.

The crude whey has 93.8% of moisture content, 0.9% of protein content and 5.3% of other dry matter. Using the ultra/nano filtration membranes the crude whey protein amount could be concentrated from 0.9% to 2.5 (> 200 Da) and 3.3% (> 10000 Da).
The changing in the chemical composition of ultra/nano-filtrated whey suggests that it is possible to concentrate the whey proteins for further applications or treatment.

2.2 Antihypertensive activity

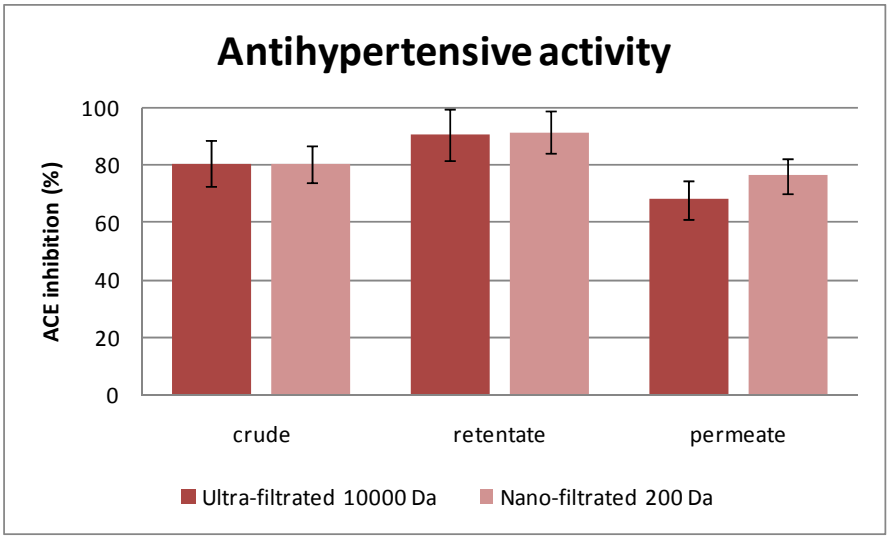

Fig. 4 Antihypertensive activity of different whey fractions.

Angiotensin I-converting enzyme has been classically associated with the renin-angiotensin system, which regulates peripheral blood pressure. ACE raises blood pressure by converting angiotensin I released from angitonsinogen by renin into the potent vasoconstrictor angiotensin II. ACE also degrades vasodilative bradykinin and stimulates the release of aldosterone in the adrenal cortex. Consequently, ACE-inhibitors may exert an inhibitory effect.

In general, all different whey fractions have very good blood pressure lowering effects which was higher than 68%. It is well known that whey peptides have good antihypertensive activity.

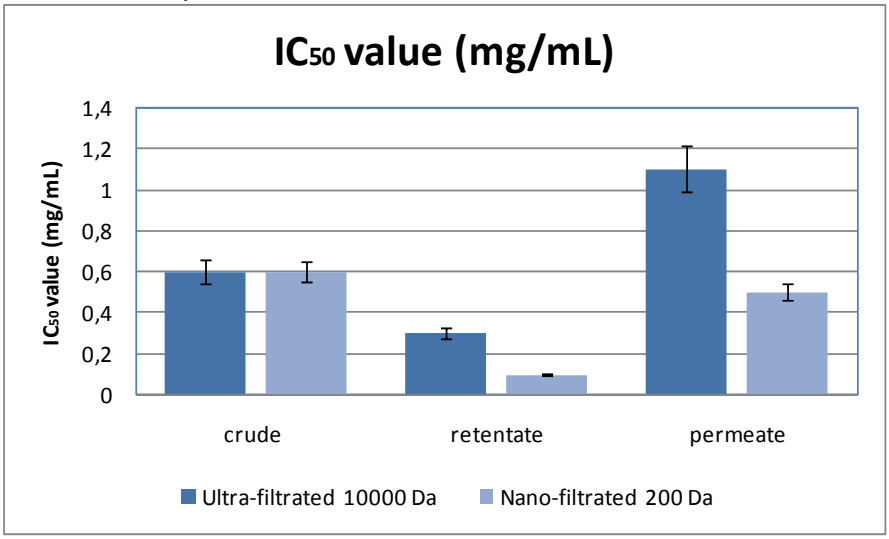

Fig. 5 The concentration of ACE inhibitory whey peptides that reduces ACE activity by 50%.

Ultrafiltration membranes have been successfully used to enrich specific peptide fractions. An ultrafiltration membrane reactor has been applied for the continuous extraction of permeates enriched with bioactive fragments, in order to produce bioactive peptide. Some scientists reported that the <1 kDa fractions from *α*-la hydrolyzed with pepsin and *β*-lg hydrolyzed either with pepsin and trypsin or with pepsin, trypsin and chymotrypsin have no opioid properties, even though they contain whey protein-derived opioid peptides (*α*- and *β*-lactorphins). In a previous study it was found that the ACE-inhibitory activity in the <1 kDa fraction was, in many cases, higher than in the other fractions tested. These results, indicate that it may be possible to exploit ultrafiltration in order to enrich ACE-inhibitory peptides derived from whey protein. Furthermore, few researchers showed that *α*-lactorphin was generated with continuous hydrolysis of goat whey in an ultrafiltration reactor. Membranes containing negatively charged materials have been used to desalt whey hydrolysates as well as to enrich cationic peptides with antibacterial properties from cheese whey. Accordingly, they assumed that this technique provides new possibilities for enriching peptides with a low molecular mass and that it is easily up-scaled to gram or even kilogram quantities.

2.3 Antioxidant activity

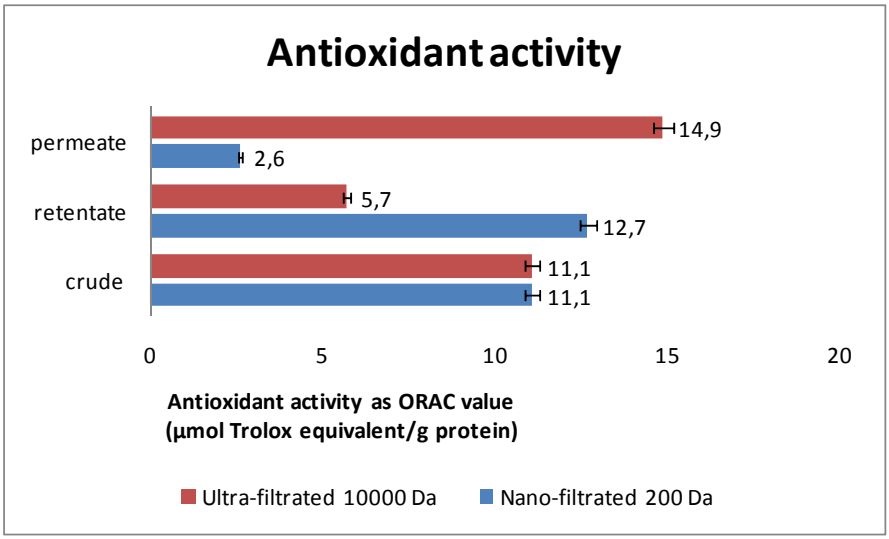

Fig. 6 Antioxidant activity of different whey fractions.

The antioxidant activity demonstrated by whey protein fractions suggest that, besides modifying physiological properties of food products, these protein have potential to enhance product stability by preventing oxidative deterioration.
The results can be used to identify possibilities for utilization of whey proteins and can be used as a base for development of hydrolysate for food ingredients.

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