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ORIGINAL PAPER

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Processing of whey from dairy industry waste

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Abstract An investigation carried out in 11 dairies in Serbia has shows that 78.75 % of whey, a by-product of cheese industry, is emitted into river systems, thus contributing to the organic pollution of the environment. This pollution can be avoided by processing of whey into food and pharmaceuticals. It is shown that low-temperature regime of whey concentration and fractionation, based on vacuum concentration and diafiltration, preserves whey proteins undenaturated, as proved by differential scanning calorimetry method. Functional native whey proteins based food products, with potentially high immunomodulatory activity, are obtained.

Keywords Whey · Pollution · Undenaturated proteins · Differential scanning calorimetry

Introduction

Whey, obtained as a by-product in cheese and casein industry, is poorly processed in Serbian dairies. Biological oxygen demand for 5 days (BOD_5) of the effluents from a big dairy, producing 50,000 l of whey/day, is 12,000 mg O_2/l, which makes 300,000 g O_2/5days. According to European standards, this is an extremely high pollution, equal to a town with 50,000 inhabitants. The most profitable solution of the ecological problem is to process waste whey into food and pharmaceutical products. After sterilization, pasteurization, ultrafiltration or spray-drying of milk and whey, proteins and vitamins are denaturated partially. Whey concentrates and isolates (lactose, proteins, minerals and vitamins) could be obtained with low-temperature procedures of whey fractioning with satisfactory degree of microbiological purity and maximal preservation of whey protein physico-chemical and bioregulatory properties. Lactose of pharmaceutical quality is used in the preparation of media for bacterial growth and in instant infant milk, and that of nutritional quality in instant soups, baked goods, confections and meat products. Whey proteins, especially undenaturated, improve emulsification, gelating and water-binding properties of food. Their isolates or hydrolysates are used in infant food (Damodaran and Paraf 1997).

The derivates of whey and casein are also used in technical and pharmaceutical products as pigments, in glues and cosmetics production. The medicinal effects of undenaturated whey proteins, obtained with low-temperature regimes, are known to stimulate antioxidant and immunoregulatory activities in cancer treatment (Kennedy et al. 1995) and beneficial effects in cancer prevention (Hakkak et al. 2001). Whey protein digests have hormonal effect in osteoporosis (Takada et al. 1996) and hypertension (Pihlanto-Lepala 2000). Besides that, whey has beneficial Ca: P and Na: K ratios, also important for the hypertension regulation, high content of B vitamins (Renner 1983), high content of cysteine and essential amino acids (Damodaran and Paraf 1997). Cysteine is known as an amino acid that regulates the *in vivo* concentration of tripeptide glutathione (γ-glutamyl-cysteinil glycine) that assumes pivotal role in the protection of cells from toxic oxygen species. The undenaturated cysteine from whey proteins is supossed to be responsible for adjuvant effect of whey in cancer treatment (Kennedy et al. 1995).

Prolonged treatment of whey at relatively low temperatures, of 55–63°C (Kennedy et al. 1995), which could reach even 70°C in certain parts of industrial vacuum evaporators, can cause whey protein denaturation, as proven by differential scanning calorimetry method (DSC) (data not shown). We have applied the low-temperature methodology of vacuum concentration of whey at 20°C and ultrafiltration at 50°C, which preserve the native structure of whey proteins in order to obtain better bioregulatory and technological effects of these products.

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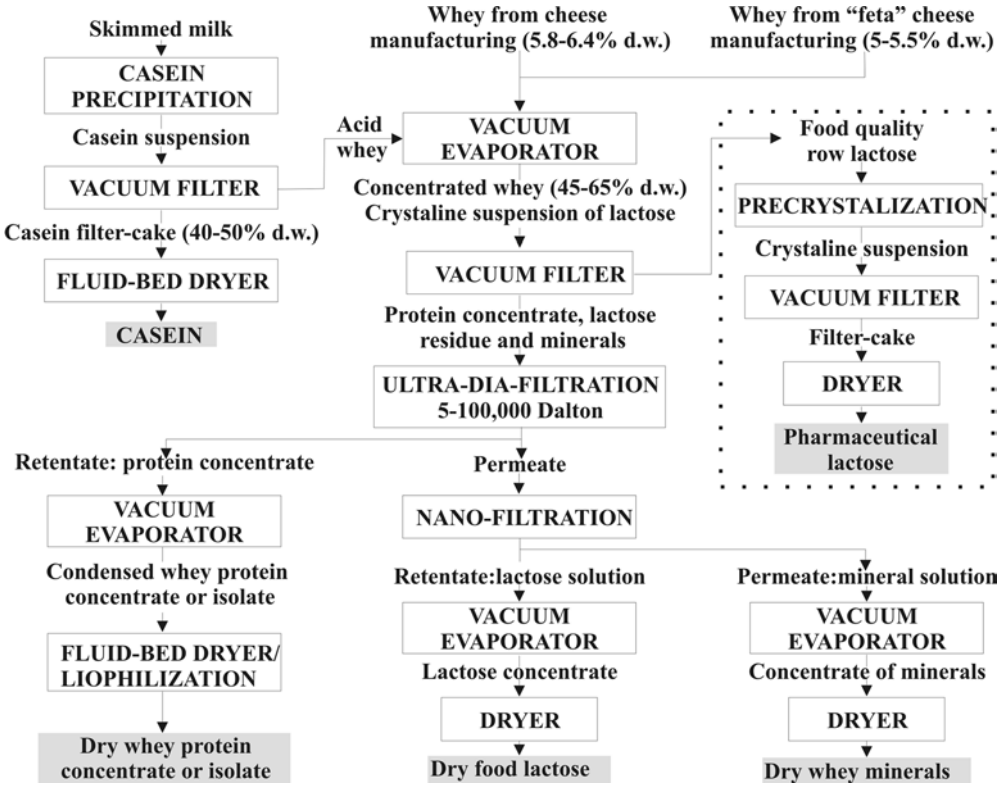

Fig. 1 Technology of skimmed milk and whey processing into food and pharmaceutical products

The optimization of fat removal, microfiltration and lactose crystallization could give products with high protein concentration, without disrupting protein-salt equilibria of whey and preserved vitamin content, which seems to be also important for the immunomodulatory effects of whey (Kennedy et al. 1995).

Experimental

Technology of whey processing

Sweet cheese whey, milk or skimmed milk (pH 5.8–6.4) were obtained from Dairy Industry "Imlek"(Padinska Skela, SCG) and pasteurised at 63°C. Sweet whey was obtained by adding rennet, and acid whey by adding 0.5 N HCl upto pH 4.6. The milk or whey was cooled at 5°C, fat was removed by centrifugation at 4000×*g* for 30 min, and concentrated on circulatory vacuum evaporator at 20°C (Fig. 1). The whey concentrate was then used as the base for various foodstuff, or processed further. Raw lactose of nutritional quality was obtained on filter-centrifuge or vacuum filter, from whey concentrate (45–65% dry weight, d.w.), after storage at 4°C for 24 h, and processed by precristalization and purified into lactose of pharmaceutical quality. Lactose, remaining in the filtrate (diluted to 20% d.w.) was separated from whey proteins by ultra filtration (U.F.) and diafiltration (D.F.), using various membrane cut-offs. Here, we applied polysulfonic 5000 D membrane "Hemomed F-6" (Hemofarm, Vrsac, SCG). The processing solution was heated to 50°C. The U.F. and D.F. processing conditions were: an average transmembrane pressure of 1 bar and permeate flow rate of 10 ml/min with distilled water as diafiltration medium. The permeate of U.F. or D.F. could be further purified from monovalent salts by nano filtration on polysulfonic 200 D membrane (DowChemicals, USA), to obtain a complex of minerals and vitamins. The obtained products were concentrated with vacuum concentration and dried on a fluid-bed dryer or lyophilized.

Investigation methods

Mineral content was determined by ion chromatography (Dionex, Sumyvale, USA) while lactose content was determined by polarimetry. Lipid content was determined by Rose-Gottlieb method. Whey protein content was determined by dodecyl Kjeldahl and Lowry method, and their molecular weight by sodium dodecil sulphate poliacrylamide gel electrophoresis (SDS-PAGE) under reducing conditions, as described by Laemmly (1970) on 12.5% running gel. All the products were tested for pathogenic microorganisms in the Institute for health protection of Serbia "Dr. Milan Jovanovic-Batut".

We followed thermal stability (enthalpy of protein unfolding, ΔH^{cal}, and temperature of denaturation, Tm) in whey or whey protein concentrates, using the differential scanning calorimetry (DSC) method, (Privalov 1980). All DSC scans were carried out on MicroCal MC-2 Scanning Calorimeter, MicroCal Inc. Northampton, MA, USA. All protein solutions (2 mg protein/ml) were degassed

Table 1 Mineral content of vacuum-concentrated sweet whey and whey fractions. The concentration of total mineral content (ash) and main cations (Na^+, K^+, Ca^{2+} and Mg^{2+}) is expressed as % of dry weight (d.w.)

Sample	d.w. (%)	Protein/d.w (%)	Ash/d.w. (%)	Na^+/d.w. (%)	K^+/d.w. (%)	Ca^{2+}/d.w. (%)	Mg^{2+}/d.w. (%)
Whey (conc. 10×)	61.68	11.60	8.24	0.611	2.688	0.399	0.097
Raw lactose	76.00	1.76	4.42	0.191	0.717	0.346	0.034
Whey filtrate after row lactose separation (whey filtrate)	29.00	16.88	19.94	1.462	5.093	0.197	0.259
Retentate of diafiltration of whey filtrate	10.40	51.90	7.31	0.231	0.356	0.067	0.856
Permeate after ultrafiltration of whey filtrate	7.70	0.55	17.04	1.182	4.234	0.195	0.156

~1 min, with gentle stirring under vacuum before loaded into the calorimeter cell. "Origin" software was used for DSC data analysis (non two-state curve fitting model, for estimating thermodynamical parameters of protein unfolding: Tm, ΔHcal). All scans were performed in the temperature range from 20°C to 100°C, with scan rate 90°C/min. After the first scan, all samples were rescanned, to obtain second scan ('reversibility'), to check whether the proteins were denaturated during the concentration steps.

Results and discussion

Processing of whey in Serbian dairies

A questionnaire on whey usage was sent to 23 dairies in Serbia, but only 11 of them responded. According to the data obtained, 11 big and medium dairies produce 43,800 ton/year of whey or milk ultrafiltration permeates, of which 3,212 ton/year (7.3%) is used for "vurda", "feta" or "fondu" cheese production, 6,096 ton/year (13.9%) as animal food and 34,493 ton/year (78.75%) is let out into rivers. In dairies producing cheese "vurda" from whey concentrate or "feta" cheese from ultrafiltrated milk, permeate, containing 70% of whey dry weight (d.w.), composed mainly of lactose, is let out as waste or used as animal food. Thus, production of these kinds of cheese is not the solution of the ecological problem, because the complete lactose content remains in the permeate.

Low-temperature regime of whey processing

Under the pilot scale conditions we have obtained several whey products from waste whey: concentrated whey (30–60 % d.w.), whey paste (over 60% of d.w., 8–10% protein in d.w.), filtrate of concentrated whey after lactose separation (28–40% d.w., 17–40% protein in d.w.), whey protein concentrates purified by ultrafiltration and diafiltration, (35–85% of protein in d.w.), lactose of nutritional quality (84% lactose in the first crystallization, 73–78% d.w., 1.5–3.3% protein in d.w.), and a complex of minerals and vitamins. The second filtrate, after washing or precristalization of raw lactose, was vacuum concentrated to obtain the protein-enriched whey concentrate (30–35% protein in d.w).

Sweet whey, (pH 6–7, 5.8–6.4% d.w.), our starting material, was free from pathogenic microorganisms, except from one sample. All samples of concentrated whey were not contaminated, which could be the effect of enhanced osmolarity and low-temperature pasteurisation regime applied. Concentrated whey (62% d.w.) was microbiologically stable at 4°C for 7 months, which indicates that it is a half-product suitable for transport or storage.

Electrophoresis of vacuum-concentrated sweet whey fractions and subsequent diafiltration fractions, (Fig. 2a) shows the same molecular weight profiles of main whey proteins as unconcentrated whey, meaning that changes in temperature, concentration and ionic strength, do not influence protein distribution after concentration and fractionation of whey. Calorimetry analysis of sweet whey and whey concentrates is represented on: (Fig. 2b) for unconcentrated whey; (Fig. 2c) for whey filtrate after row lactose separation and (Fig. 2d) for retentate of whey diafiltration after row lactose separation. From calorimetry data obtained it can be assumed that thermal denaturation process of whey proteins during the concentration steps do not differ significantly regarding Tm and ΔH^{cal}, meaning that proteins do not undergo denaturation, which was also proved by the second scans (calorimetry scans of denaturated whey proteins). The calorimetry data of retentate of whey, obtained by diafiltration only, and fluid-bed dried retentate of whey ultrafiltration after row lactose separation also showed that proteins do not undergo denaturation during the processes of concentration and drying (calorimetry data not shown). Ion-chromatography data of total mineral content and main cations in vacuum-concentrated sweet whey (conc. 10X), correspond to the literature data (Damodaran and Paraf 1997). Concentrated whey fractions and subsequent diafiltration fractions show the expected increase in mineral concentration in diafiltration permeate, (Table 1). In none of the samples toxic metals (Pb^{2+}, Cd^{2+}, As^{3+}, Hg^{2+}) were detected.

Whey concentrates or whey protein concentrates are further used as half products for both sweet and salt foodstuff: emulsions (in combination with 30–50% vegetable oil), extractction of vegetables and fruits, instant flours and additives for soups (obtained by mixing with various grain flours and fluid-bed drying). Lactose of

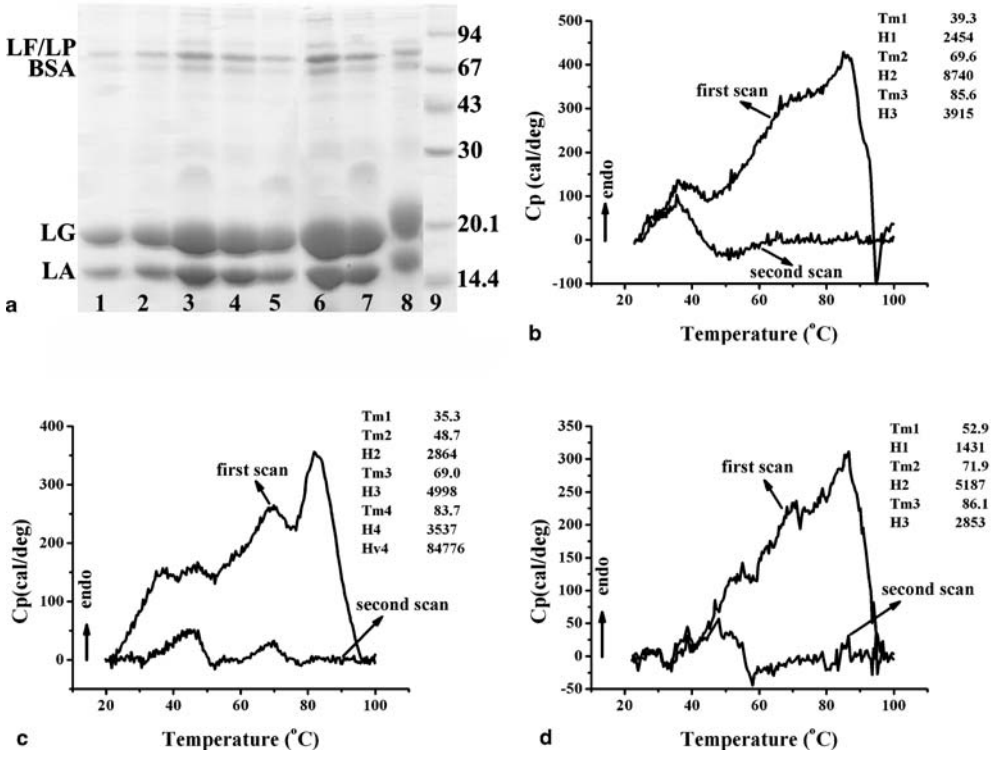

Fig. 2 Sodium dodecyl polyacrylamide gel electrophoresis (SDS-PAGE) and differential scanning calorimetry (DSC) curves (first and second scan) of whey fractions and concentrates: (**a**) Coomassie blue-stained reduced SDS-PAGE profiles of: (1) unconcentrated whey, 50 μg pr./w.; (2) raw lactose, 50 μg pr./w.; (3) whey filtrate after raw lactose separation, 250 μg pr./w.; (4) retentate of whey diafiltration after raw lactose separation (R 1) 50 μg pr./w.; (5) retentate of whey diafiltration (R2) 50 μg pr./w.; (6) R1, 650 μg pr./w.; (7) R2, 650 μg pr./w.; (8) fluid-bed dried R1, 100 μg pr./w; (9) molecular mass markers (in kD); (**b**) DSC curves of unconcentrated whey (6% d.w., 9% protein in d.w.), corresponding to electroph. line 1; (**c**) DSC curves of whey filtrate after raw lactose separation, (29% d.w., 16.88% protein in d.w.), corresponding to electroph. line 3; D) DSC curves of R1 (15.5% d.w., 80 % protein in d.w.) corresponding to electroph. line 4. *Abreviations*:d.w., dry weight; pr./w., protein per well; LF, lactoferrin; LP, lactoperoxidase; BSA, bovine serum albumin; LG, β- lactoglobulin; LA, α-lactalbumin, Cp, heat capacity; Tm, temperature maximum of thermal transition; H calorimetric enthalpy (H^{cal})

nutritional quality and a complex of minerals and vitamins (from whey filtrate ultrafiltration and nanofiltration) could be introduced in dietetic foodstuff for diabetics and hypertensic patients. Similar foodstuffs, enriched in whey proteins, have, so far, been prepared in anticipation of clinical trials (McIntosh et al. 1999).

Conclusion

We have developed "green-chemistry" products, based on whey protein concentrates, lactose of nutritional quality and a complex of minerals and vitamins in order to avoid massive discharging of waste whey from dairies in Serbia. Low-temperature regime of whey concentration and fractionation applied preserves whey proteins undenaturated and potentially bioactive, as proved by differential scanning calorimetry method. The obtained products satisfy consumer demands for food with health promoting effects, so-called functional food.

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