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Production of novel dairy products using actinidin and high pressure as enzyme activity regulator

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ABSTRACT

Milk clotting for the production of novel dairy products, alternative or complementary to cheese and yogurt type products can be achieved using plant sulfhydryl proteases. The objective was to apply the protease actinidin, from *Actinidia chinensis*, as the milk clotting agent, and High pressure (HP) technology to control excessive proteolysis. The effect of the dairy substrate and the process parameters on the coagulation rate and the texture and sensory properties of the end product, were studied. Selected values of design parameters were 25% total solids, 6.49 adjusted pH, 0.35 U activity of the clotting agent actinidin, 40 ºC process temperature and 2 h time. The selected pressure-temperature conditions, 600 MPa at 40 ºC, were applied to stop the potentially detrimental further proteolytic action of the enzyme. Results indicated that use of actinidin for milk clotting and HP to stop the enzyme activity in the final product, leads to a "fresh cheese" type dairy product.

Industrial relevance: Alternative clotting methods for novel dairy products, complementary to cheese and yogurt type products, are of interest to the industry. Plant proteases can be a viable approach, provided that excessive proteolysis after structure formation is regulated. High hydrostatic pressure can be used for controlling proteolytic activity in the final products without affecting their texture and sensory characteristics.

1. Introduction

Plant proteolytic enzymes have been used for various applications in the food industry. Applications include their use as meat tenderizers and plant milk clotting enzymes for dairy products (Lopes, Teixeira, Liberato, & Pais, 1998; Vieira de Sa and Barbosa, 1972; Lewis and Luh, 1988; Barbosa, Corradini, & Battistotti, 1981). Actinidin, found in kiwi fruit (*Actinidia chinensis*) (McDowall, 1970) is a plant sulfhydryl protease that could be used in similar applications (Masahiro, Toshiro, Yukio, & Tadao, 2002). Actinidin has a wide substrate specificity, hydrolyzes most strongly the amide and ester bonds at the carboxyl side of a lysine residue and it is active at pH range 4-10 (Arcus, 1959; McDowall, 1970; Hussain et al., 2003; Morimoto, Furuta, Hashimoto, & Inouye, 2006). It is composed of 220 amino acid residues with molecular mass of 23.5 kDa (Carne and Moore, 1978; Baker, 1980; Watts and Brocklehurst, 2004). Most of the applications of plant proteolytic enzymes on dairy products refer to the use of ficin from fig latex, as reported in several traditional recipes from the Mediterranean area. There is little published research on such use of these proteases. Production of immobilized ficin and its application for making of teleme cheese was reported (Fadyloglu, 2001). The application of proteolytic enzymes of fig latex was investigated for the production of Gaziantep cheese (Oner & Akar, 1993). One requirement frequently mentioned is the control of the proteolytic activity because uncontrolled hydrolysis may lead to protein fragments and peptides of undesirable functional and organoleptic properties. Heat inactivation of the enzymes after milk clotting by thermal pasteurization of the final dairy product has not been reported but would be impractical to apply, since the treatment would be time and energy consuming due to heat transfer limitations and could be detrimental to the texture and flavor of the products. High Pressure (HP) technology can potentially inactivate enzymes in the final food system at low temperatures (Cheftel & Culioli, 1997). The work reported in the literature for thermal and pressure inactivation of plant sulfhydryl proteases is limited. Katsaros, Katapodis, and Taoukis (2009b) reported that kiwi juice proteolytic activity can be controlled by HP processing at low to moderate temperatures (up to 40 °C). At conditions of 600 MPa and 40 °C, the D-value was 16.8 min. Papain and ficin inactivation was investigated and it was concluded that both enzymes showed a high thermal and pressure stability requiring intense process conditions for adequate inactivation (Katsaros, Katapodis, & Taoukis, 2009a). Oxygen concentration was an important factor, indicating that oxidation of the thiolate at the active site plays a role in the pressure inactivation (Gomes, Summer, & Ledward, 1997). Studies of milk processing using plant proteases as clotting agents, in combination with subsequent controlled inactivation with HP have not been reported.

The objective of this work was to study the development of a fresh-cheese like product using the protease actinidin, found in *Actinidia*

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Chinensis, as milk clotting agent and HP technology to regulate the enzyme activity.

2. Materials and methods

Actinidin was obtained directly from ripe fresh kiwifruits (*Actinidia chinensis*), Hayward var., acquired from a selected producer in Northern Greece. After peeling, the kiwifruits were pulped. The pulp was centrifuged at 10000 rpm for 15 min. The supernatant was filtered through muslin cloth and the clear juice, rich in actinidin, was used in milk coagulation experiments.

2.1. Partial purification of actinidin

Milk coagulation experiments were also conducted using a more purified actinidin. The purification procedure was used for the removal of flavors and compounds that might affect sensory and other characteristics of the final products. For the purification of actinidin, a modification of the method described by Brocklehurst, Baines, and Malthouse (1981) was used. After the series of treatments and centrifugations of 1 kg kiwi pulp as described, the final enzyme solution (300 ml, in 0.1 M CH_3COONa, 0.3 M NaCl and 1 mM EDTA at pH 4.4) was stirred at 4 °C for 24 h, centrifuged (11,000 rpm for 30 min at 4 °C) and the supernatant was filtered using a 0.45 μm filter. The filtrarte was ultrafiltered using a 30,000 MW membrane (PLCTK 30, Pellicon, Millipore Co., USA). The retentate was freeze dried (Alpha 1-4 LD plus, Christ GmbH, Germany) to obtain a powder rich in actinidin.

2.2. Measurement of enzyme activity

For the measurement of actinidin activity the method described by Katsaros et al. (2009b) was used. The catalytic activity of clear kiwi juice was 0.42 ± 0.02 U/ml, while for the freeze dried powder the corresponding activity was estimated as 520 ± 20 U/g.

2.2.1. Coagulated milk product manufacturing

For the coagulation of products, pasteurized, whole milk (3.5% fat, 3.2% proteins, 2.45% caseins, calcium content 120 mg/100 ml), enriched in solids by addition of full fat (26% fat) milk powder (25.5% proteins, 22.3% caseins) (Arla Foods Ingredients, Denmark) was used. Dairy products were manufactured using either homogenized or non-homogenized milk for comparison of the final products. For the adjustment of the pH value of the products citric acid (0.5 M) was added. Samples were packed in 100 ml screw cup soft and flexible polypropylene bottles within which both incubation and subsequent HP treatment took place.

2.2.2. Texture analysis measurements

Texture analysis measurements were performed using a Texture Analyser TA.XT2i (Stable Micro Systems, England) equipped with a cylinder probe (0.5 cm diameter). A two-bite cycle was employed and the stress that developed in the product samples was measured as the samples were compressed. The resistance during deformation of the products was monitored throughout this two-bite cycle. Hardness of the products was estimated as the maximum force of the stress-strain curve, monitored during the first bite (Rosenthal, 1999). All the experiments were carried out at 25 ± 0.2 ºC. In order to avoid the effect to subsequent measurements of the texture analysis procedure different samples were used for each measurement. Analytical software was used to compare the force, distance and time data of the stress-strain curve. The hardness of the products expressed in Newton (N) was measured instrumentally every 30 min over 5 hrs, during coagulation. The high sensitivity of the texture analyzer (measuring 0.05 N difference in hardness between samples) allows the use of this method as a good alternative to conventional rheology. The rate of the hardness increase (N/min) was directly correlated to the product coagulation rate. The products were considered to be coagulated when hardness value, measured instrumentally, reached 1.32 N. Above this value syneresis was observed. This texture of the products (after coagulation and before HP treatment) was similar to the texture of 'fresh cheese' (fromage frais) products.

2.2.3. Sensory analysis

Sensory analysis was used to evaluate the overall quality of the milk coagulated products. All samples were scored in a 1 to 9 hedonic scale for their texture, color, odor, flavor and the overall impression from 8 trained panelists. A mean value equal to 5 was determined as the acceptance limit of the products. The target product after HP treatment was assumed to be a white cheese with texture similar to 'soft feta cheese'. All organoleptic characteristics were scored based on this target.

2.2.4. Measurement of syneresis

The syneresis of the products was used as one of the indices of the degree of the product coagulation and it was expressed as the volume of the whey (ml) per total mass (100 g) of the milk coagulated product.

2.2.5. Measurement of pH value

The pH value of the milk coagulated products was determined with 0.005 accuracy, using an Amel 338 pH-meter (Amel Instruments, Milano, Italy).

2.2.6. High pressure experiments

High pressure treatments were conducted using a high pressure unit (Food Pressure Unit FPU 1.01, Resato International BV, Roden, Holland), comprising a pressure intensifier, a multivessel system consisting of six vessels of 45 mL capacity each, and a vessel of 1.5 lt capacity, with a maximum operating pressure and temperature of 1000 MPa and 90 °C. The 1.5 lt volume vessel was used for the processing of the fresh-cheese like products, to control further actinidin action in the products. The pressure transmitting fluid was polyglycol ISO viscosity class VG 15 (Resato International BV, Roden, Holland). Process temperature in the vessel was achieved by liquid circulation in the outer jacket controlled by a heating-cooling system. For the inactivation of actinidin within the milk products the desired value of pressure was set and after pressure build up (20 MPa/s), the pressure vessel was isolated. Pressure of the vessel was released after a preset time interval (according to the experimental design) by opening the pressure valve. The initial adiabatic temperature increase during pressure build up was taken into consideration in order to achieve the desired operating temperature during pressurization. Pressure and temperature were constantly monitored and recorded (in 1 s intervals) during the process. Processing at 600 MPa and 40 °C resulted in an increase of temperature of approximately 12 °C, but 2 min after pressure build up the temperature equilibrated with the temperature of the jacket of the vessel (40 °C). The necessary time for achieving 600 MPa ranged from 54 sec to 58 sec.

3. Results and discussion

Homogenization of milk for fresh cheese and yoghurts manufacture helps to prevent fat separation during storage, improves consistency, increases whiteness and reduces whey separation. It is considered that for these types of product, homogenized full-fat milk produces a firmer gel than those made from skim milk (Becker and Puhan, 1989). This was confirmed by enzyme induced coagulation experiments of both homogenized and raw milk, using actinidin powder and kiwi juice. Non homogenized milk tended to form a thin top layer of separated fat therefore homogenized milk was further used for the experiments.

3.1. Effect of milk solids on product coagulation

The total solids (TS) of the milk substrate varied from 13 to 40% w/w. Addition of 1 ml of the rich in actinidin juice or 0.8 mg freeze dried powder