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KINETICS OF CHANGES IN THE GRANULOMETRIC COMPOSITION OF THERMODENATURED WHEY PROTEINS

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ABSTRACT

The study objective was to determine the optimal modes of temperature-induced denaturation of whey proteins under conditions of complete refolding to enhance the efficiency of subsequent enzymatic release of biologically active peptides. Whey samples obtained after acid, acid and rennet, and rennet coagulation were identified on the basis of physico-chemical parameters and thermal stability using multidirectional methods. The kinetics of denaturation and aggregation of particles, changes in their mean diameter depending on the physico-chemical whey composition, as well as heat treatment modes were studied. The temperature 95 °C with the exposure time of 120 minutes should be considered as the most optimal mode in terms of maximum protein denaturation and minimum mean particle diameter. At the same time, the rate of protein denaturation and the size of aggregated particles varied depending on the deviation of pH away from pI. For sweet whey, a slightly different mechanism of temperature-induced aggregation of whey protein was noted, characterized by the predominance of hydrophobic interactions.

1. Introduction

Whey is the main secondary raw material resource of the dairy industry resulting from the use of various casein deposition technologies (Bardone *et al.*, 2018). It is internationally accepted to classify whey into two types: sweet whey (pH>5.6) obtained as a result of chymosin/pepsin-induced precipitation of casein in hard cheeses production, and acid whey (pH<5.2) formed as a result of casein isoelectric coagulation in the production of curd, fresh cheese or technical casein. The physico-chemical composition of acid whey differs from the composition of sweet one in a lower content of protein and lactose, as well as a higher level of calcium, phosphorus and lactic acid (Papademas *et al.*, 2019; Zandona *et al.*, 2021).

Today, the volume of the global whey market is about 180-190 million tons showing a constant upward trend. On average, no more than half of the produced whey is subject to industrial processing, of which 50% is processed in liquid form, 30% is dried, and the remaining share is used for protein fractionation, production of lactose and its derivatives (Paladii *et al.*, 2021; Sáenz-Hidalgo *et al.*, 2021). Unprocessed whey is often disposed with industrial wastewater at municipal wastewater treatment plants or in aeration fields. The environmental standards for the management of food waste and by-products consider whey to be harmful to the environment due to high biological oxygen demand (BOD 35-60 g/l) and chemical oxygen demand (COD 60-80 g/l), all of this combined with low pH (Das *et al.*, 2016; Bosco *et al.*, 2018; Mehri *et al.*, 2021). Therefore, tightening of environmental standards and assignment of producer personal responsibility for by-products disposal should contribute to the transition to a closed production cycle in the long run (Fermoso *et al.*, 2018). This could also be facilitated by

accessibility and intensification of technologies for deep whey processing, including those that were introduced with the advent of a new generation of dairy products with added value.

Recently, biologically active peptides obtained from secondary dairy raw materials are of considerable interest. This interest is justified by the high potential of whey proteins biological activity, especially in the manifestation of ACE-, DPP4- and renin-inhibitory effects. In the profile of potential biological activity, such functions as inhibition of alpha-glucosidase in all whey proteins and opioid-agonistic in β–lactoglobulin are also noted (Minkiewicz *et al.*, 2019; Sultan *et al.*, 2017). A wide range of positive potential effects of bioactive whey peptides encouraged the international scientific community to pay close attention to this topic (Patil *et al.*, 2022).

Bioactive peptides initiate various biological reactions in the human body along the receptor pathway and have an effect comparable to that of medicinal or hormonal drugs (Sultan *et al.*, 2017). At the same time, bioactive peptides derived from natural raw materials are known to have a number of advantages over synthetic agents in terms of therapeutic action (Kaur *et al.*, 2020). In most cases, they do not show toxic effects and do not cause other adverse effects (Zambrowicz *et al.*, 2012). Bioinformatic tools existing at the stage of planning the release of bioactive peptides allow assess the possible adverse effects, including their toxicity, and finished bioactive hydrolysis products, which, if necessary, allows changing the cleavage conditions (Kruchinin & Bolshakova, 2022). It is worth noting that biologically active peptides isolated from secondary dairy raw materials are capable of absorption with minimal degradation and entering directly into the bloodstream. This is also one of the advantages in their prospective use (Sultan *et al.*, 2017).

Commercialization of the bioactive peptides production process and the prevalence of their use in various industries, in particular in pharmaceutical and food industry, is impeded by number of reasons: insufficient clinical studies, lack of evidence base of bioactivity of isolated peptides, lack of proper systematization of their mechanisms of action and optimal methods of scaling their production (Chakrabarti *et al.*, 2018).

One of the problems when it comes to bioactive peptides extraction from secondary dairy raw materials may be the complex conformational structure of whey proteins, which, being in a globular form, are not able to undergo complete hydrolysis under the action of enzymes (Abadía-García *et al.*, 2021). It is possible to increase the availability of protein to hydrolytic enzymatic cleavage through temperature-induced protein refolding. It is noted that the complete refolding of β–lactoglobulin is achieved by heat treatment of dairy raw materials at a temperature of 95-97 °C with an exposure of more than one hour without observation of subsequent protein renaturation (Gunkova P.I. *et al.*, 2015). In studies (Halder *et al.*, 2012; Vetri & Militello, 2005) the temperature optimum of such a targeted effect is provided, which is at the level of 80 °C with an exposure time of up to 120 minutes. α-lactalbumin, being a metalloprotein, has a higher thermal stability compared to β–lactoglobulin and the ability to renature. At the same time, the irreversibility of the denaturation of α-lactoalbumin during thermal denaturation is possible under the condition of protein decalcification and destruction of all disulfide bonds (Bernal & Jelen, 1984; Salamanca & Chang, 2005).

This study objective was to determine the optimal thermal denaturation modes under conditions of complete refolding of the main protein fractions of whey, in which the protein yield from secondary dairy raw materials reaches the maximum value with the minimum possible protein aggregation, which in turn can reduce the effectiveness of controlled hydrolysis by proteolytic enzymes and hinder the release of biologically active peptides.

2. Materials and methods

2.1 Materials and Preparation of Whey

Samples of sweet whey were obtained in the industrial conditions of the company "Italian

Traditions" (Russia) from the black-and-white cow milk produced at the "Lenin State Farm" (Russia). The whey was collected at the end of the technological process of Montasio (rennet coagulation) and Mozzarella cheese production (acid-rennet coagulation).

Samples of acid whey were obtained in the production and experimental workshop of the "All-Russian Dairy Research Institute" ("VNIMI", Russia) from the black-and-white cow milk produced at the "Lenin State Farm" (Russia). The whey was collected as a result of curd production by acid coagulation of milk to a pH of 4.5-4.6 A starter culture with pure cultures of *Lactococcus lactis* strains 79 $_{5}$, 79 $_{10}$, 79 $_{13}$ (VNIMI, Russia) was used for this purpose. The acid whey was also obtained as a result of curd production by acid-rennet coagulation of milk to the final pH level of 5.0-5.2. The product was fermented using *Lactococcus lactis* strains 79 $_{5}$, 79 $_{10}$, 79 $_{13}$ (VNIMI, Russia) and the enzyme preparation Clerici 96/04 (Caglificio Clerici, Italy).

The samples obtained were delivered to the laboratory within 10 minutes, immediately heated in an incubator to a temperature of 40 ± 5 °C and separated from milk fat and casein dust on a MilkyDay FJ 90 PP separator (Austria). Purified whey samples were cooled to a temperature of 4±2°C and stored.

2.2. Experimental Design

The experiment was carried out according to the plan presented in Figure 1.

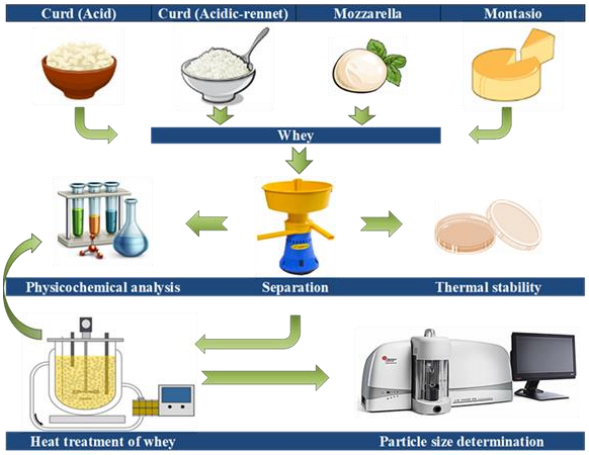

Figure 1. The experimental design

2.3. Physicochemical Analysis

The evaluation of the physicochemical parameters of sweet and acid whey types was carried out by well-known methods: humidity was determined by the thermogravimetric method according to GOST ISO 6731/IDF 21-2012; the mass fraction of fat was determined by the Gerber method GOST R ISO 2446-2011; the mass fraction of protein was determined by the total nitrogen by the Kjeldahl method in accordance with ISO 1871:2009; the mass fraction of casein and whey fractions proteins were determined according to ISO 17997-1:2004; the mass fraction of lactose was determined in accordance with ISO 26462:2010; calcium content was determined by titrimetric method according to ISO 12081:2010; active acidity (pH) was measured by the potentiometric method using an InoLab pH Level 1 high-precision pH meter equipped with a Sen Tix 61 pH. The fractional composition of proteins was determined by electrophoresis in polyacrylamide gel with sodium dodecyl sulfate (SDS-PAGE), followed by densitometry of the intensity of staining of the tracks in accordance with the technique (Bavaro *et al.*, 2019).

2.4. Thermal Stability

The protein system resistance to denaturation during heat treatment was determined using multidirectional methods: an alcoholic sample, a chlorocalcium sample, a thermoacid sample and a thermal sample described earlier in the work (Vafin *et al.*, 2021).

2.5. Whey Heat Treatment

Whey samples were subjected to thermal denaturation in the temperature range of 75 to 135 ^{o}C with interval of 10 ^{o}C in jacket bioreactors (Alkhitekh, Russia) connected in series with a circulating glycerin bath (Alkhitekh, Russia). Heating and aging were carried out without stirring, in order to neutralize of the flocculation effect to the denaturation and aggregation of whey proteins. Protein denaturation was evaluated over a span of 120 minutes with 20 minutes interval. At the end of protein thermodenaturation the whey was

cooled to 25 °C by feeding cold water into the jacket (Figure 2).

Figure 2. Bioreactor for thermally induced denaturation of whey protein

2.6. Determination of whey proteins denaturation

The mass fraction of denatured whey proteins was determined by the method described in (Pan *et al.*, 2022). The mass fraction of the protein not denatured was determined by the Kjeldahl method in the filtrate after whey sample centrifugation at 3500×g for 10 min. The mass fraction of denatured proteins was determined as the difference between the mass fraction of protein in the initial whey and the mass fraction of protein in the fugate after centrifugation.

2.7. Particle Size Determination

The size distribution of aggregated particles was analyzed using an LS 13 320 XR laser diffraction analyzer (Beckman Coulter, USA) equipped with a universal liquid module. Distilled degassed water at room temperature was used as a dispersion medium in the module chamber. The selected averaged whey sample was introduced into the module chamber with an automatic single-channel pipette (5-10 ml) with an increased diameter of the tip inlet (5 mm). The sample was introduced until the reading signal levels of 50% were reached on the PIDS photodetectors (differential intensity of polarized light scattering). At the end of the analysis, the area of the obtained graph in the range from 0.01 to 3000 microns was evaluated. The results were calculated with refractive indices of 1.33 for water and 1.54 for the samples of different types of treated whey studied.

2.8. Statistical Analysis

Statistical data analysis was performed using the Statistica 2010 software package. All measurements were carried out in 3 independent repetitions, the results are presented as the mean (±) standard deviation (SD). Statistical analysis was performed using single-factor analysis of variance (ANOVA) at a significance value of $P < 0.05$.

3. Results and Discussions

3.1. Physicochemical characteristics of Milk Whey

The average physicochemical composition of milk whey samples as a result of curd and cheese production is presented in Table 1. The whey samples studied were characterized by significant differences in level of active acidity (pH) and content of dry substances, including total protein, lactose, minerals. This was directly related to the mechanism of coagulation of milk proteins and the technology of processing curd. Data provided in Table 1 demonstrate that the acidity of whey AW(A) (pH 4.50) obtained during acid coagulation of milk is below the isoelectric point of casein. 0.55% of proteins consisting of 0.47% whey proteins and 0.08% casein fractions are transferred to AW(A) whey. This particular content of protein in whey is associated on the one hand with the inclusion of some denatured whey proteins in the curd matrix due to complexation with k-casein, and on the other hand, the formation of casein dust due to the processing of a curd with a low density and small size of aggregated particles formed under the action of lactic acid (Dalgleish & Corredig, 2012). As a result of acid coagulation, calcium phosphate and structure-forming calcium also cleave from the casein micelle, accompanied by its transition to a soluble state, followed by partial migration to whey (93.40 mg/100 g).

The physicochemical composition of AW(A/R) whey obtained as a result of mixed milk coagulation differs from AW(A) by higher values of active acidity (pH 5.09) and lactose (3.94%). This is associated with isoelectric coagulation of milk at pH 5.20 as a result of the hydrolytic effect of pepsin on k-casein, and,

accordingly, with a lower intensity of lactose fermentation by lactic acid microorganisms. Acid-rennet isoelectric coagulation is accompanied by the transition to a soluble form of only part of the calcium-containing salts migrating into the whey (76.80 mg/100 g). The whey produced by acid-rennet isoelectric coagulation is characterized by a low content of total protein (0.39%) and, in particular, k-casein (0.02%).

Table 1. Physicochemical analysis of milk whey characteristics

Name of parameter	Curd whey		Cheese whey	
	Acid	Acidic-rennet	Acidic-rennet	Rennet
	AW(A)	AW(A/R)	SW(A/R)	SW(R)
Total solids, %	5.89±0.15	6.07±0.19	6.22±0.15	6.54±0.12
Fat, %	0.05±0.01	0.05±0.01	0.05±0.01	0.05±0.01
Protein, %	0.55±0.11	0.39±0.07	0.72±0.04	0.89±0.10
Whey protein, %	0.47±0.04	0.37±0.07	0.69±0.04	0.82±0.08
β-lactoglobulin	0.28±0.03	0.22±0.04	0.47±0.03	0.57±0.05
α-lactoalbumin	0.14±0.01	0.12±0.02	0.17±0.01	0.19±0.03
BSA	0.046±0.003	0.047±0.003	0.043±0.002	0.05±0.004
Lactoferrin	0.005±0.001	0.006±0.001	0.005±0.001	0.008±0.001
Casein, %	0.08±0.02	0.02±0.01	0.03±0.01	0.07±0.02
αs_1-casein	0.03±0.01	0	0	0
αs_2-casein	0	0	0	0
β-casein	0.03±0.01	0	0	0
κ-casein	0.02±0.01	0.02±0.01	0.03±0.01	0.07±0.02
Lactose, %	3.55±0.14	3.94±0.11	4.46±0.09	4.62±0.13
pH	4.50±0.10	5.09±0.08	5.84±0.05	6.43±0.09
Ash,%	0.72±0.08	0.59±0.06	0.54±0.05	0.46±0.03
Calcium, mg/100 g	93.40±3.10	76.80±2.70	68.89±1.50	61.30±2.20

SW(A/R) whey with a pH of 5.84 obtained during acidification of the mixture with lactic acid formed as a result of lactic acid fermentation (residual lactose level of 4.46%) followed by rennet coagulation, is characterized by a high protein content (0.72%, including 0.03% k-casein) and low calcium content (68.89 mg/100 g).

SW(R) whey obtained during rennet coagulation of milk has a slightly acidic pH of 6.43, a high content of lactose (4.62%) and total protein (0.89%) with a minimum calcium content (61.30 mg/100 g), which is fully consistent with the theory of rennet protein coagulation (Lucey, 2017). With this mechanism of coagulation, whey proteins and lactose almost completely transfer into the whey together with an insignificant amount of the fraction of k-casein and soluble calcium.

3.2. Milk Whey thermal stability

Table 2 demonstrates assessing results of the thermal stability of whey types obtained during acidic, acidic-rennet and rennet coagulation of milk using multidirectional methods.

Table 2. Milk whey thermal stability

Name of test	AW(A)	AW(A/R)	SW(A/R)	SW(R)
Alcohol test	< 68%	< 68%	68%	85%
Calcium chloride test	±	–	–	±
Phosphate test	–	±	±	+
Acid-boiling test	1.2 mL*	0.8 mL*	0.8 mL*	0.5 mL*

– negative result; ± conditionally positive result; + positive result

*amount of 0.1N HCl withstood by whey, mL

Analysis of data obtained as a result of the study of thermal stability by alcohol test method showed high resistance to denaturation under the action of an 85% solution of SW(R) whey alcohol, while the SW(A/R) sample withstood the test with only 68% alcohol concentration. The samples of AW(A) and AW(A/R) whey types failed the alcohol test.

Thus, the concentration of the alcohol solution causing the denaturation of whey proteins correlates with a decrease in pH in the samples of milk whey types. The strong denaturing effect of alcohol solutions at low pH values is accounted for by electrostatic and hydrophobic interactions due to decrease in the negative charge of the protein (as a result of decrease in the pH of the medium) and increase in the hydrophobicity of its surface due to the contact of the solvent with nonpolar amino acid residues (Nikolaidis & Moschakis, 2018; Wagner *et al.*, 2021). The data obtained fully correlated with the results of the thermal stability of whey types determined by the phosphate test method.

The assessment of thermal stability by calcium chloride test method showed that protein precipitate in the samples of AW(A) and SW(R) whey types was poorly visualized, while in the samples of AW(A/R) and SW(A/R) there was clearly pronounced denaturation and aggregation of protein, accompanied by the separation of transparent whey. This effect is assumed to be associated with a change in the solubility of proteins as a result of increase in the concentration of calcium ions at pH 5.0 and is generally consistent with the results of studies (Dissanayake et al., 2013). The results of the acid-boiling test showed that the protein denaturation in the SW(R) whey required minimum amount of 0.1N HCl (0.5 mL), while for the AW(A) whey - maximum amount (1.2 mL). Whey samples AW(A) and AW(A/R) were denatured with the addition of 0.8 mL of 0.1N HCl. Inversely proportional dependence of the amount of 0.1N HCl required for protein denaturation on the initial acidity of the whey and the proximity of pH to pI of the main whey proteins was noted.

3.3. Thermodenaturation of Milk Whey

Differences in the physicochemical composition (Table 1) and thermal stability of milk whey samples suggest different intensity and degree of protein thermodenaturation. Figure 3 shows the kinetics of changes in the degree of denaturation of whey proteins under different heat treatment modes.

In the process of heat treatment of milk whey types with different physicochemical composition at temperature of 75 ^{o}C low percentage of protein denaturation was observed. After 120 minutes of incubation, only 15.7% of the protein from their initial whey content was denatured in the SW(R) sweet whey sample. With decrease in the pH of the studied samples, the percentage of denatured protein increased and reached 22.2% and 26.4% for SW(A/R) and AW(A/R) whey samples, respectively. The maximum percentage of denatured protein of 36.5% was observed in the AW(A) sample. Thus, the denaturation of proteins under this regime is local in nature, mainly dependent on the level of acidity of the sample.

Data analysis in the temperature range of 85-135 ^{o}C with an exposure of 120 minutes showed

a slightly different dependence of the degree of protein denaturation on pH and temperature. The sensitivity of proteins to thermal denaturation, regardless of the coefficient of thermal exposure, decreased in the following sequence AW(A)>SW(R)>AW(A/R)>SW(A/R). The maximum rate of thermally induced denaturation/aggregation of whey proteins was observed at pH equal to or close to pI (AW(A)) due to electrostatic and covalent interactions. An increase in pH (5.09 and 5.84) and its distance from the pI of the main whey proteins led to a significant slowdown in this process. However, at pH 6.43, the rate of denaturation /aggregation of sweet whey proteins, which occurs mainly due to the formation of disulfide bonds with more reactive sites (Gulzar et al., 2011), was insignificantly lower than in samples with a pH close to pI. Similar results on model whey protein systems were also obtained in the works (Nicolai et al., 2011; Nishanthi et al., 2017).

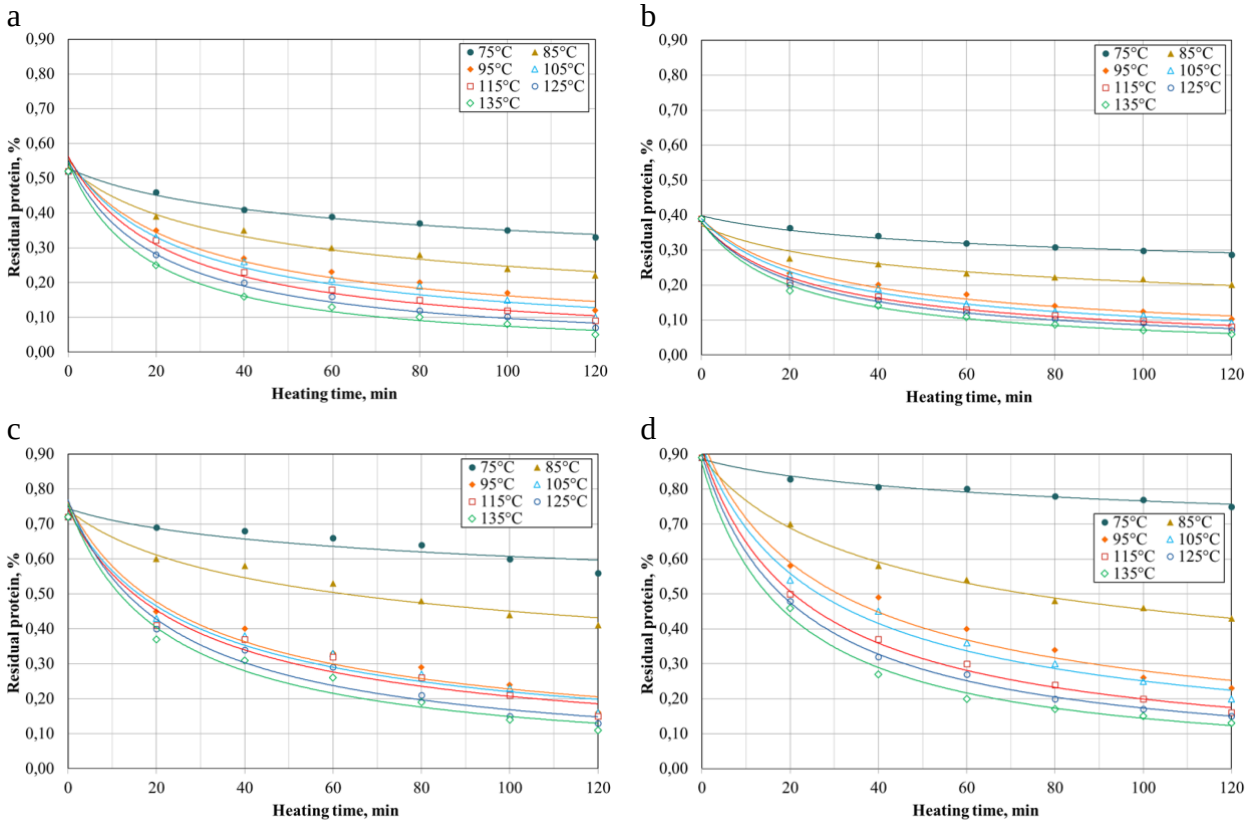

Figure 3. Changes in whey protein content at different modes of thermodenaturation process (a - AW(A); b - AW(A/R); c - SW(A/R); d - SW(R))

The degree of denaturation of whey proteins also directly depends on the temperature and duration of heat treatment. So, at a temperature of 85 ºC and after 120 minutes of denaturation, 43-58% of the protein from the initial content in the whey was subjected to denaturation. Temperature rise to 95 ºC and 105 ºC led to an increase in the degree of denaturation to 73.6-75.0% and 76.4-80.0%, respectively. While the transition to temperatures critical for dairy raw materials led to a maximum degree of denaturation of 115 °C (79.2-82.7%), 125 °C (81.9-86.5%) and 135 °C (84.7-90.4%) with a maximum average speed of 0.072 g/min (AW(A)), 0.070 g/min (SW(R)) and 0.068 g/min (AW(A/R) and SW(A/R)). At the same time, due to exposure to high temperatures, the Maillard reaction mechanism was activated and accelerated. Aggregated proteins were characterized by a coarser structure due to the

probable formation of intermolecular isopeptide and lysinoalanine cross-links at high temperatures, which according to (Zhang *et al.*, 2021) it may limit the availability of enzymes to cleavage sites both during digestion and during directed hydrolysis. Therefore, these temperature regimes were not considered in further studies.

3.4. Particle size determination of thermodenatured whey protein

The physicochemical composition, temperature, and duration of thermally induced protein denaturation can have a direct effect on the size and structure of the resulting aggregates (Nicolai *et al.*, 2011). Therefore, the next researches were aimed at establishing the cumulative effect of these factors on the size of the protein aggregates formed under different temperature and time regimes of whey processing (Tables 3-4).

Data analysis (Tables 3 and 4) showed that at temperature of 75 °C the volume-weighted average diameter (D[4;3]) of denatured proteins and their median values (D 50) did not change significantly within120 minutes. This indicates the absence of conditions for mass aggregation of protein and fully correlates with the data presented in Figure 3. The average size of whey protein aggregates and their distribution enhanced significantly with increase in temperature and duration of thermal exposure, which is explained by a decrease in electrostatic interactions due to the predominance of hydrophobic interactions (Nicolai *et al.*, 2011). The influence of the acidity factor on the size of the denatured protein aggregates formed was observed. Whey with a pH of 4.50 (AW(A)) was characterized by the highest values of D[4;3] and D 50 aggregated proteins for all temperature-time treatment modes, as well as a high aggregation rate.

Table 3. Size distribution of aggregated protein particles (microns) in the process thermally induced denaturation of acid whey types

Parameter of heat treatment		AW(A)		AW(A/R)	
Temperature, °C	Heating time, min	$D_{[4;3]}$	D_{50}	$D_{[4;3]}$	D_{50}
0	0	1.07±0.02	0.70±0.01	0.98±0.03	0.61±0.01
75	20	1.89±0.03	1.43±0.02	1.36±0.01	0.76±0.03
75	40	3.91±0.04	2.83±0.03	1.73±0.04	0.91±0.02
75	60	4.26±0.01	3.23±0.01	1.98±0.02	1.29±0.04
75	80	5.43±0.03	4.08±0.02	3.14±0.05	2.70±0.06
75	100	6.62±0.02	4.85±0.05	4.21±0.03	3.37±0.05
75	120	7.38±0.09	5.62±0.08	5.10±0.05	4.11±0.03
85	20	3.79±0.05	2.16±0.08	2.65±0.02	2.23±0.04
85	40	4.44±0.03	3.15±0.06	3.87±0.07	2.26±0.03
85	60	14.97±0.11	9.75±0.09	9.63±0.10	7.02±0.05
85	80	23.12±0.19	11.75±0.08	16.12±0.12	9.64±0.08
85	100	29.19±0.22	15.94±0.15	23.58±0.18	11.87±0.09
85	120	42.42±0.31	26.83±0.20	34.93±0.10	21.63±0.19
95	20	6.67±0.04	4.86±0.08	4.48±0.06	3.59±0.03
95	40	10.56±0.12	7.74±0.09	7.04±0.06	5.11±0.07
95	60	30.09±0.21	23.11±0.15	23.67±0.11	11.95±0.10
95	80	53.65±0.27	27.09±0.15	39.95±0.19	24.36±0.13
95	100	87.97±0.43	32.92±0.21	57.24±0.25	26.91±0.16
95	120	94.32±0.51	40.03±0.28	66.83±0.28	32.06±0.17
105	20	9.60±0.05	7.99±0.08	8.11±0.04	7.03±0.03

105	40	19.34±0.15	13.45±0.12	15.23±0.09	11.42±0.05
105	60	35.92±0.19	20.28±0.10	29.66±0.13	18.46±0.16
105	80	60.57±0.23	33.17±0.18	48.87±0.32	32.64±0.21
105	100	91.23±0.47	45.93±0.25	63.03±0.27	37.19±0.30
105	120	112.87±0.62	65.01±0.27	74.21±0.31	42.74±0.24

The average diameter of aggregated protein particles and their median values in sera AW(A/R) and SW(A/R) decreased significantly with pH (5.09 and 5.84) moving away from pI protein. This dependence is generally consistent with the results (Buggy et al., 2018). It is worth noting that in the whey SW(R) (pH 6.43) at processing temperature of 75-95 ^{o}C values of D[4;3] and D50 were slightly higher than in the SW(A/R) sample. As far as the processing temperature increased up to 105 ^{o}C the growth of aggregates in the SW(R) whey, unlike in other types of whey, has noticeably accelerated. This is most likely related to more intensive formation of disulfide bonds alongside with the resulting flotation effect due to intense vaporization in the whey.

Table 4. Size distribution of aggregated protein particles (microns) in the process thermally induced denaturation of sweet whey types

Parameter of heat treatment		SW(A/R)		SW(R)	
Temperature, ^{o}C	Heating time, min	$D_{[4;3]}$	D_{50}	$D_{[4;3]}$	D_{50}
0	0	0.76±0.03	0.18±0.01	0.88±0.02	0.50±0.02
75	20	1.00±0.05	0.48±0.02	1.25±0.03	0.73±0.01
75	40	1.35±0.04	0.78±0.03	3.62±0.02	3.05±0.02
75	60	1.62±0.03	1.19±0.02	4.01±0.04	3.48±0.03
75	80	2.07±0.03	1.08±0.02	4.36±0.05	3.49±0.04
75	100	3.25±0.02	2.75±0.01	5.74±0.03	4.88±0.03
75	120	4.23±0.05	3.82±0.03	6.72±0.07	5.74±0.02
85	20	2.19±0.01	1.91±0.01	3.43±0.04	2.94±0.02
85	40	3.68±0.03	3.12±0.02	4.10±0.04	3.01±0.07
85	60	7.27±0.06	5.51±0.05	10.86±0.08	8.13±0.04
85	80	12.82±0.12	9.07±0.08	14.91±0.14	9.90±0.12
85	100	17.39±0.15	9.91±0.06	19.03±0.17	11.18±0.05
85	120	21.05±0.12	10.68±0.7	25.06±0.14	12.92±0.09
95	20	3.56±0.04	2.88±0.02	5.64±0.04	3.58±0.05
95	40	6.67±0.02	4.35±0.03	7.91±0.06	4.99±0.02
95	60	12.48±0.10	8.93±0.07	17.23±0.12	9.85±0.06
95	80	19.74±0.16	11.66±0.11	24.96±0.18	12.32±0.10
95	100	25.65±0.19	13.57±0.12	31.51±0.19	18.46±0.16
95	120	32.78±0.23	19.80±0.15	38.94±0.26	24.22±0.13
105	20	7.35±0.06	6.17±0.04	8.67±0.04	7.62±0.02
105	40	10.61±0.09	8.48±0.05	13.86±0.12	9.94±0.07
105	60	16.75±0.17	11.34±0.09	25.36±0.19	17.51±0.14
105	80	23.04±0.22	15.42±0.14	42.15±0.25	29.67±0.16
105	100	31.10±0.25	19.19±0.11	54.81±0.23	34.13±0.19
105	120	40.86±0.26	28.54±0.17	68.97±0.31	39.00±0.21

4. Conclusions

The high capacity of whey as a natural raw material for the production of biologically active peptides draws attention to the concern of increasing the efficiency of whey proteins hydrolysis. Deployment of globular structures along with reduced degree of protein aggregation will increase the accessibility of sites to enzymatic hydrolysis. This research shows that different combinations of temperature-time modes of heat treatment of whey types with different physicochemical composition formed protein structures with different average diameter and particle distribution, as well as the degree of denaturation. According to the results of the research, temperature of 95 ^{o}C and exposure time of 120 minutes should be considered as the optimal mode in terms of maximum protein denaturation and minimum average particle diameter. It was found that the rate of protein denaturation and the size of aggregated particles varied depending on the removal of pH from pI, in all samples except SW(R), where the mechanism of hydrophobic interactions prevailed. However, further studies in this domain are required taking into account the factors of lactose content and salt composition, as well as additional methods of whey processing: neutralization and acidification.

5. References

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