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ORIGINAL PAPER

The lactose from Ricotta cheese whey: the effect of pH and concentration on size and morphology of lactose crystals

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Abstract *Scotta* is a by-product from the Ricotta cheese production, which has been studied insufficiently, and that may be the reason of *scotta* not being widely used for the industrial purpose. The aim of the following research was to study *scotta* as a raw material for the lactose production and the process of crystallization at different pH levels and concentration factors. In the case, crystallization was carried out at six different pH levels and five concentration factors. The optimum acidity for lactose crystallization was determined close to be at pH 4; at higher or lower pH levels the growth of crystals was inhibited. The relationship between the dimensions of crystals and the concentration factor corresponded to data provided in the literature for the conventional cheese whey. The qualitative properties of the crystals obtained under the examined and recommended conditions were similar to the qualitative properties of crystals reported in the related literature about the cheese whey. It was demonstrated that *scotta* has a great potential for lactose production, and our results can be used for optimizing the industrial process.

Keywords Lactose · Crystallization · *Scotta* · Microstructure

1 Introduction

The Ricotta cheese whey or *scotta* might become a valuable source of the lactose. *Scotta* is a by-product of the Ricotta cheese production. The Ricotta processing technology consists of cheese whey heating, whey protein coagulation and its separation. Within the production process, the protein-depleted whey is separated (Jelen 2003).

Most of *scotta* is used as supplement feed for livestock. Low content of proteins (Sansonetti et al. 2009) makes *scotta* unattractive for further processing; therefore the

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liquid itself has been poorly studied so far. However, *scotta* contains lactose, which makes it possible for use in the manufacture of lactose.

The growing popularity of the Ricotta cheese in Europe, and as a consequence, the increasing amounts of *scotta*, open up a possibility for lactose manufacturers to think seriously about processing of *scotta*. In Italy alone, the *scotta* production is about 10^9 kg/year (Sansonetti et al. 2009).

Crystallization is one of the most important operations in the process of the lactose production. This process, and the factors influencing it, has been well studied for the cheese whey but not for *scotta*. Concentration and pH have the greatest impact on the morphology and the dimensions of crystals (Herrington 1934; Nickerson and Moore 1974). The form of crystals can vary from the tubular to tomahawk shaped ones, depending on the supersaturation concentration which they grow at (Hartel 2001). Also, some acids may accelerate or slow down the growth of crystals, which affect their dimensions (Jelen and Coulter 1973). The purpose of this study was to investigate *scotta* as a raw material for further processing and the lactose crystallization process that takes place at different levels of pH and the concentration factors of the samples. The yield of lactose and losses during the process were not investigated in this work.

2 Materials and methods

Scotta for this study was provided by a local cheese factory, Põltsamaa Juustutööstus Ltd., Estonia. The lactose, fat and protein content were measured at the Milk Analysis Laboratory of the Estonian Animal Recording Centre using an automated infrared milk analyzer (CombiFoss FT+, Foss Electric, Hillerød, Denmark). The ash content was estimated using the International IDF Standard 42B:1990; pH of *scotta* was measured by the Mettler-Toledo pH-meter (Mettler-Toledo International Inc., Greifensee, Switzerland). Dry matter content was measured using the Estonian Standard EVS 641:1994. Viscometer Brookfield RVDV-III U EZ (Brookfield Engineering Labs. Inc, Middleboro, USA) with the spindel YULA-15 EK (min 0.01 RPM (round per minute); max 250 RPM) was used for viscosity determination of samples.

For investigation of pH influence, crystallization was carried out at six different pH values: 10, 8, 6, 4, 3 and 2. pH of the provided *scotta* was approximately 4. For the other values, lactic acid (Sigma-Aldrich Chemie GmbH, Steinheim, Germany) (for pH 3 and 2) and sodium hydroxide (Sigma-Aldrich Chemie GmbH, Steinheim, Germany) (for pH 10, 8 and 6) were added. Five different concentration factors (5, 6, 8, 10, and 12) at pH 4 were analyzed to investigate influence of concentration factor. The above-mentioned concentration factors of *scotta* were received via water evaporation at low temperature (60 °C). Samples of 500 ml were evaporated until the desired concentration was reached (time for the evaporation of each sample see in Table 1). Viscosity of samples before evaporation was 10.8 mPa•s (at torque 42.21%). Concentrated *scotta* was poured into test tubes, 10 ml in each tube. Crystallization was carried out at temperature of 23 °C (at the indoor temperature) during 24 h without agitation. Some additional information of crystallization parameters at different

Table 1 Time of evaporation, total solids content after evaporation, viscosity with torque and pH for samples at different concentration factors. Viscosity and pH analysis was carried out at the temperature of 23 °C

Concentration factor	5	6	8	10	12
Time of evaporation, min	90	120	160	200	240
Total solids after evaporation, %*w*/*w*	28.20	31.32	43.05	49.58	69.04
Viscosity at 23 °C, mPa•s	11.57 (at torque 45.17%)	13.62 (at torque 53.24%)	549.12 (at torque 85.76%)	405.12 (at torque 63.32%)	12,672 (at torque 99.01%)
pH after evaporation	4.20	4.34	4.30	4.39	4.35

Torque is the measured ability of a rotation element, as of gear or shaft, to overcome turning resistance

concentration factors is given in Table 1. In the tests, only unsalted *scotta* was used.

The shape and the size of crystals were examined with a microscope "Nikon SMZ 1000" (Nikon Corporation, Tokyo, Japan), equipped with the digital camera "Nikon DS-U2/L2 USB" (Nikon Corporation, Tokyo, Japan). The dimensions of crystals, such as the height and the projection surface area were estimated using the software "NIS-Elements D3.1" (Nikon Corporation, Tokyo, Japan). Projection surface area was defined as the area of the crystal's projection on the focal plane of the microscope. Height of crystals was defined by measuring the perpendicular from the top to the opposite base of crystal. (See Fig. 1)

A scanning electron microscope "Leo 1430VP" (LEO Electron Microscopy Ltd, Cambridge, England) was used for studying the surface of crystals.

The statistical significance of *scotta* pH level effect on height and projection surface of crystals was tested using analysis of variance and the pair wise comparison of different *scotta* pH levels was performed using Tukey test. The linear and

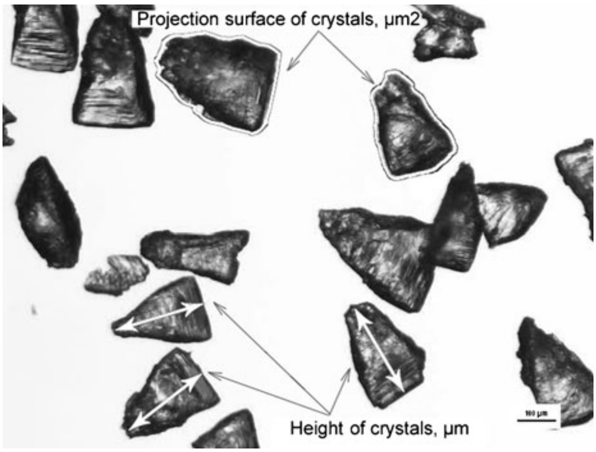

Fig. 1 Exemplification of lactose dimensions investigation using a microscope "Nikon SMZ 1000" with digital camera "Nikon DS-U2/L2 USB" and "NIS-Elements D3.1" software

nonlinear regression analysis was used to study the relationship between *scotta* concentration factor and the average height and the projection surface area of crystals.

3 Results

3.1 *Scotta* composition

The content of dry matter, fat, protein, and lactose in *scotta* is given in Table 2.

The dry matter, lactose, and protein content obtained in this study (Table 2) were consistent during the experiments and were lower than those of the normal cheese whey (Jelen 2003).

3.2 Influence of *scotta* pH

The crystal's dimensions varied depending on the pH level of *scotta*. Influence of *scotta* pH on the crystals dimensions is shown in Table 3.

At pH 4, the average dimensions of crystals were the largest (average height was 274 μm, projection surface area was 45,789 μm^2), with clear pyramidal shapes. The smallest difference between variations of height and projection surface area was observed at pH levels 2 and 3 (Table 3). The crystals received from *scotta*, pH of which was 2 and 3, were significantly smaller than the crystals obtained in the other trials. Most of the crystals were of the elongated and trapezoidal shape. The average height values correspondingly equalled to 82 and 65 μm and the projection surfaces to 6,605 and 2,981 μm^2. Larger variances in data of projection surface area were traced at *scotta* pH 6 and 8. In the solution with pH 6, the crystals were short, though with a larger projection surface. At pH 8, crystals were elongated (214 μm; 29,992 μm^2). At pH 10, the average heights and the projection surface area decreased to 172 μm and 17,812 μm^2. Figure 2 summarizes the afore-said changes in the crystals' dimensions depending on pH level of crystallization process.

The experiments showed that the pH level affects not only the size of crystals, but also the morphology of crystals. On the decrease of pH level (within the range from 4 to 2), crystals lost their regular shape and clearly distinguished faces. The surface became rough, as if it was covered with scales, and upon the surface a lot of small crystallized formations were found (Fig. 3a).

Table 2 Content of dry matter, fat, protein and lactose in native *scotta* (%w/w), ash content in salted and unsalted *scotta* (%w/w), pH of native *scotta.* 15 measurements were considered for each data set

	Dry matter	Fat	Protein	Lactose	pH	Ash content	
						Unsalted *scotta*	Salted *scotta*
Average	5.43	0.26	0.50	4.14	5.51	0.44	0.62
Standard deviation	0.24	0.10	0.02	0.11	0.12	0.03	0.02
Max	5.82	0.36	0.54	4.31	5.69	0.51	0.64
Min	5.19	0.11	0.48	4.00	5.36	0.42	0.61

Table 3 Average ± standard deviation (minimum—maximum) height and projection surface area of crystals at different *scotta* pH levels

Scotta pH level	Height of crystals, μm	Projectional surface area of crystals, μm^2
pH 2	81.8±33.5^{ab}	6,605±1,732^{a}
	(40—120)	(4,095—9,353)
pH 3	65.4±14.9^{a}	2,981±993^{a}
	(49—98)	(1,661—4,664)
pH 4	274.0±74.1^{d}	45,789±26,053^{b}
	(129—379)	(8,483—94,220)
pH 6	129.5±44.1^{abc}	22,961±41,848^{ab}
	(77—266)	(5,778—161,700)
pH 8	214.1±125.3^{cd}	29,993±31,417^{ab}
	(102—557)	(6,533—114,440)
pH 10	171.6±70.3^{bc}	17,812±12,623^{ab}
	(86—336)	(4,352—46,356)
p value	<0.001	0.002

p value shows the statistical significance of *scotta* pH level (ANOVA), means with different superscript letters are statistically significantly different ($p<0.05$, Tukey test)

On the increase of *scotta* pH value (in range from 4 to 10), more common pyramid-shaped crystals with the sharp edges and smoother surface appeared (Fig. 3).

3.3 Influence of the *scotta* concentration factor on the crystals' dimensions

There was a strong linear correlation between the *scotta* concentration factor and dimension of crystals (See Fig. 4).

Low *scotta* concentration led to emergence of the large pyramidal-shaped crystals with clear faces and smooth surface. At the same time, at concentration factor 12, the

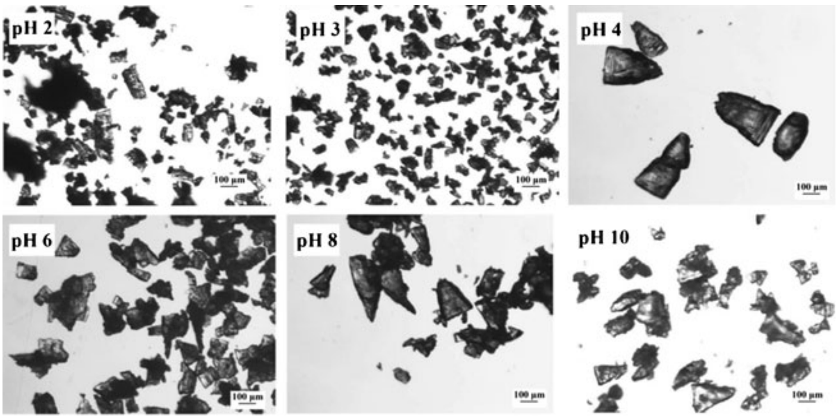

Fig. 2 Lactose crystals dimension's depending *scotta* pH level at the temperature of 23 °C. Taken with stereomicroscopy

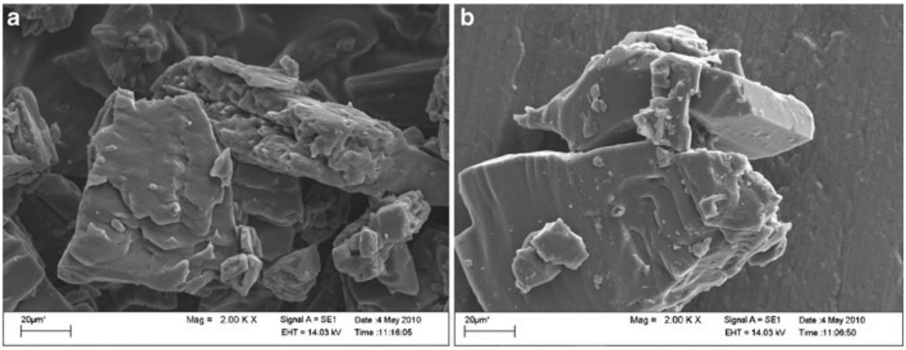

Fig. 3 The SE micrographs of lactose microstructure depending on *scotta* pH level: **a** crystals obtained from *scotta*, which pH was 2; **b** crystals obtained from *scotta*, which pH was 10

irregular crystals appeared. At *scotta* concentration factor 5, the average length of crystals equalled to 650 μm, by increasing a concentration factor up to 12, the average length of crystals decreased to 107 μm. The average projection surface also decreased from 224,087 to 7,719 μm^2. There was a low content of pyramidal-shaped crystals at concentration factor 12. Their faces were poorly distinguished and a lot of smaller, poorly developed crystals were found too. The changes as described above are illustrated in Fig. 5.

In addition, during the detailed study of the crystal surface it was detected that the surface of crystals received from the low concentration *scotta* (concentration factor 5) appeared to be partially covered with holes (Fig. 6a). At the same time, it proved that the crystals received from the high concentration *scotta* just consisted of scales (Fig. 6d).

4 Discussion

Lower content of dry matter and protein is caused by characteristics of Ricotta manufacture, in the process during which the significant part of whey proteins is removed from whey. It is worth noting that the protein content obtained in this study was higher than in *scotta* used by Sansonetti et al. (2009). It can depend on technique of protein precipitation. At the same time, the fat content appeared to be higher than in cheese whey as described in the literature (Jelen 2003). This can be caused by milk added to whey during Ricotta production (Kosikowski 1982; Modler 1988). The *scotta* pH was lower than that of whey (Jelen 2003) because for Ricotta production a certain amount of acid is used (an aqueous solution of citric acid at ca 2.5%) (Modler 1988). Adding of salt during Ricotta manufacture increases the salt content of the *scotta*. Therefore *scotta's* ash content, received during manufacture of the salted Ricotta, was higher than ash content of *scotta*, received during manufacture of Ricotta without adding salt. *Scotta* initial viscosity was higher than reported by Morison and Mackay (2001) for the fresh retentate and permeate of whey as the concentration of lactose in *scotta* was higher than in comparable samples.

The current study confirms the theory of Nickerson and Moore (1974) that pH 4 is close to optimum for obtaining the high quality crystals (Table 3). The form of crystals at

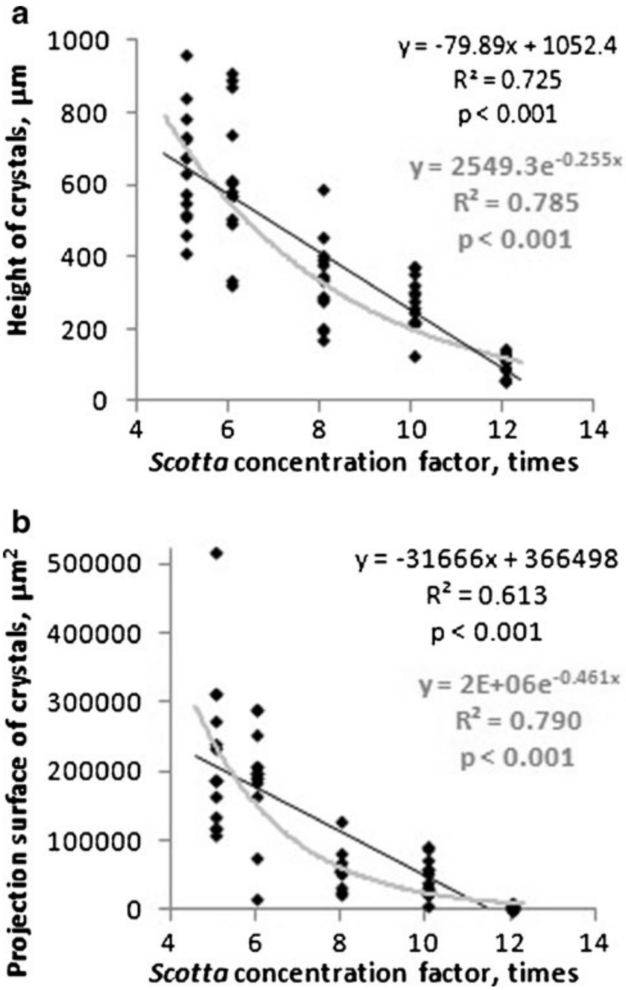

Fig. 4 Relationship between *scotta* concentration factor and **a** height of crystals and **b** projection surface area of crystals. The results showed strong and statistically significant ($p<0.001$) linear relationship between *scotta* concentration factor and crystals dimensions

pH 4 compared with tall pyramid with bevel faces at the base by Herrington (1934). When using pH 6 or 8, the variation of the crystals' dimensions is too wide. This will result in obtaining of the heterogeneous mass with very large and very small crystals. When decreasing the pH level lower than 4, the difference between the crystals' dimensions is minimal, but the crystals themselves are small. Studies by Jelen and Coulter (1973) show that lactic acid slows down the crystallization velocity, therefore, the crystals obtained at pH 2 and 3 were significantly smaller than the crystals obtained at other pH levels. Also, the crystals size at low pH levels can be affected by the presence of denatured whey proteins in the mother liquor. pH 2 and 3 are lower than isoelectric point (pH 4.75 to 4.80) of whey proteins and their additional precipitation increases viscosity, which, in turn, retarded growth of lactose crystals (Modler 1986). Reducing of crystals' size at pH levels higher than 4 can be explained by the fact, that alkali is destructive for carbohydrates and some of degradation products will inhibit

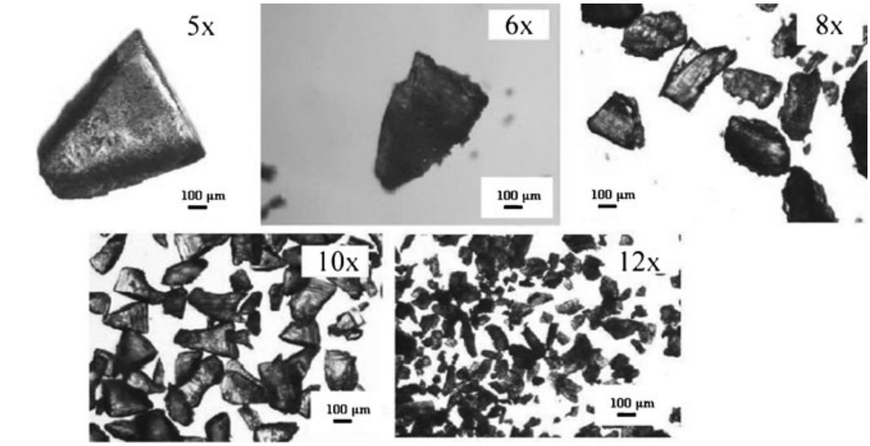

Fig. 5 Lactose microstructure depending on *scotta* concentration factors (5; 6; 8; 10 and 12 times). Taken with stereomicroscopy

crystallization (Nickerson and Moore 1974). Surfaces covered with flakes can be explained by the presence of certain impurities (Hartel 2001). It may also mean that the crystals have not been fully developed, because lactic acid inhibits the growth of crystals, but due to supersaturation the formation of the new crystals remains high (Jelen and Coulter 1973).

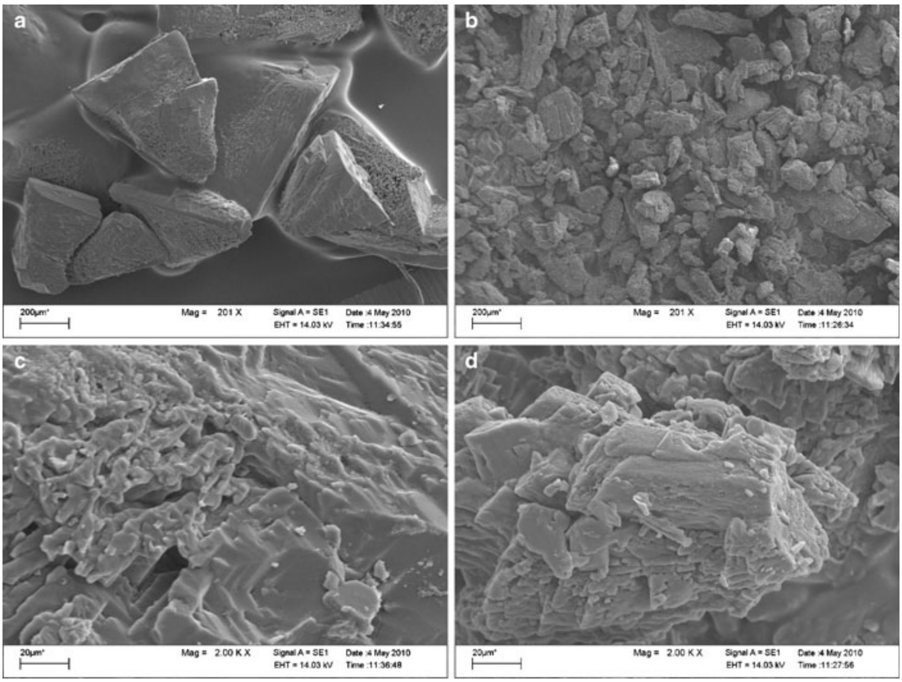

Fig. 6 The SE micrographs of lactose microstructure depending on *scotta* concentration factors: **a** concentration factor 5, magnification ×200; **b** concentration factor 12, magnification ×200; **c** concentration factor 5, magnification ×2,000; **d** concentration factor 12, magnification ×2,000

Crystallization of lactose at different concentrations of whey was studied in details by many other authors (Herrington 1934; Hartel 2001). Nearly the same behavior with the same variances was observed during our experiment with *scotta* (Fig. 5). During the elaborate research with a use of SEM (at magnification ×2,000) the holes appear to be small folds (Fig. 6c). The presence of folds on the crystals surfaces can be caused by presence of some organic compounds in *scotta*. It can be riboflavin (Lifran et al. 2007), contained in whey, and, accordingly, it might be found in *scotta*, too. Riboflavin may adsorb on growing lactose crystals and it will cause modification of their shapes. The most effective adsorption of riboflavin on the crystals surface occurs at low supersaturation of samples (Holsinger 1988). This fact may explain the presence of folds exactly on the crystals obtained at concentration factor 5. Presence of protein gave a sharp increase of sample viscosity at concentration factors 8, 10, and 12. According to Alizadehfard and Wiley (1995), this is a consequence of hydrodynamic and molecular interactions between protein molecules in solution which reaches a maximum at concentration of total solid content more than 35%*w*/*w*. Inasmuch as in this experiment total solid content was higher than 35%*w*/*w* starting at concentration factor 8 (Table 1), a sharp increase of the solution viscosity occurs, accompanied by reduce in crystals' dimensions (Fig. 5).

Sufficiently big crystals can be explained by the fact that crystals in deproteinated whey grow similarly well as in a pure solution of lactose (Jelen and Coulter 1973). However, according to Miumoni et al. (2005), the presence of whey proteins at 5 g× 100 g^{-1} water reduces the final size of the crystals almost three times, which contradicts the results obtained during this experiment (with the same protein content ~30 μm against ~300 μm at the same concentration factor). Such difference may be due to the fact that in Miumoni experiment stirring was applied during the whole period of crystallization, which led to an increase in a number of crystals with different dimensions. In this experiment the crystals grown without agitation and results agree with data, obtained by Zeng et al. (2000). So, Zeng reports that crystals prepared from Carbopol gel (growth without stirring) had the uniform elongated tomahawk shapes, in contrast to crystallization under constant stirring. Also, the size of crystals from a control group was smaller than the size of the crystals prepared from gel (Zeng et al. 2000). $CaCl_2$ also had a positive impact on growth of crystals (Jelen and Coulter 1973). The appearance of big crystals is consistent with the theory of additive's actions at low super saturations in general (Jelen and Coulter 1973; Holsinger 1988).

5 Conclusion

As the topic of the lactose crystallization from *scotta* has been examined scantily, the aim of the study was to study the influence of the concentration factor and the pH level on the crystals dimensions. The optimum acidity for the lactose crystallization was determined to be close to pH 4; while at higher or lower pH levels, the growth of crystals was inhibited. The relationship between the crystals' dimensions and the concentration factor corresponds to data provided in literature for conventional cheese whey. The qualitative properties of the crystals obtained in this study do not differ much from the qualitative properties of the crystals reported in the related literature in regarding to cheese whey. Since *scotta* can be used as the additional source of the lactose production, our results can be used to implement the crystallization process for manufacture of lactose from *scotta*.

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