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ZIGER WHEY CHEESE AND ITS MANUFACTURE

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Tiivistelmä – Referat – Abstract

Whey is a significant side stream in the cheese making industry, and it can be utilized in several ways especially because whey proteins are nutritionally valuable. Whey cheeses are produced all around the world and mostly in small scale and traditional ways. The production of Ziger whey cheese has a long tradition in Switzerland, and it requires fresh whey derived from rennet-induced cheese making. The whey proteins are precipitated by high manufacturing temperature, the addition of acid and NaCl. The goal of this master's thesis is to develop a Ziger whey cheese that can be applied in the Finnish dairy industry including optimization of the manufacturing process emphasizing the manufacturing temperature. The thesis also investigates and compares the effect of the temperature on the moisture, protein, and fat content and the yield of the Ziger whey cheese. Also, this thesis aims to examine the effect of the manufacturing temperature on the shelf life by monitoring microbial and physicochemical parameters of the Ziger whey cheese over 21 days of storage. The manufacturing temperatures examined in the production of Ziger were 88 °C and 93 °C, and the production was repeated three times for both temperatures which resulted in altogether six production series. The temperature had a significant effect on the yield which concluded, that the higher the temperature was during the manufacture, the higher the yield was. The microbiological quality was not affected by the manufacturing temperature. However, during the storage there was a significant correlation between the growth of lactic acid bacteria and the pH value *i.e.*, the bacterial count increased as the pH value decreased. After 21 days of storage, every Ziger whey cheese sample crossed the threshold of spoilage for the total viable count which was set at 10^7 CFU/mL. The manufacturing temperature had no impact on the protein and fat contents. Instead of the manufacturing temperature, there was a strong correlation between the moisture and the fat content. Based on the fat content, the Ziger whey cheese can be categorized as creamy and soft whey cheese. For future reference of research, the quality and shelf life of Ziger whey cheese could be improved by modified atmosphere or vacuum packaging, high packaging temperatures, and shorter drainage time. Moreover, the production could be made more efficient by a continuous process in comparison to batch production.

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Whey cheese, whey, shelf life, product development

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Tiivistelmä – Referat – Abstract

Meijeriteollisuuden yksi mittavimmista sivuvirroista on hera, jota voidaan hyödyntää monin tavoin johtuen heraproteiinien ravitsemuksellisesta arvosta. Herajuustoja tuotetaan monissa maissa ympäri maailmaa, ja tuotanto on yleensä suppeaa ja perinteistä. Sveitsissä on pitkät perinteet Ziger herajuustolla, jonka valmistaminen vaatii tuoretta heraa ja jossa heraproteiinit saostetaan korkealla lämpötilalla sekä happo- ja suolalisäyksellä. Tämän maisterintutkielman tavoite oli kehittää Ziger herajuusto, jonka tuotanto sopii suomalaiseen meijeriteollisuuteen keskittyen varsinkin heraproteiinien saostamislämpötilaan tuotantoparametrina. Tavoitteena oli myös tutkia ja vertailla saostamislämpötilan vaikutusta saantoon, kosteuspitoisuuteen sekä proteiini- ja rasvapitoisuuksiin. Lisäksi tutkittiin saostamislämpötilan vaikutuksia hyllyikään mikrobiologisten ja fysikaaliskemiallisten parametrien avulla 21 päivän säilytyksen ajan. Heraproteiinien saostamislämpötilat olivat 88 ^{o}C ja 93 ^{o}C, jotka olivat kirjallisuudesta löytyneet ääripäät valmistusprosessin saostamislämpötilalle. Molemmista lämpötiloista valmistettiin kolme erää, mikä tarkoitti yhteensä kuutta valmistuserää. Saostamislämpötilan ja saannon välillä oli merkittävä korrelaatio, mistä voitiin päätellä, että mitä korkeampi saostamislämpötila oli, sitä suurempi oli Ziger herajuuston saanto. Saostamislämpötila ei vaikuttanut mikrobiologiseen laatuun. Säilytyksen aikana maitohappobakteerien kasvu korreloi pH-arvon kanssa, kun maitohappobakteerien pesäkelukumäärän kasvaessa pH-arvo laski. 21 päivän säilytyksen jälkeen kokonaispesäkelukumäärä jokaisessa Ziger herajuustonäytteessä ylitti pilaantumisen kynnysarvon, joka oli 10^7 pmy/mL. Saostamislämpötila ei vaikuttanut rasva- ja proteiinipitoisuuksiin, mutta kosteuspitoisuudella ja rasvapitoisuudella huomattiin olevan merkittävä korrelaatio. Rasvapitoisuuden perusteella Ziger herajuusto voitiin kategorisoida kermaiseksi ja pehmeäksi herajuustoksi. Hyllyikää voisi parantaa tutkimalla esimerkiksi suojakaasu- ja vakuumipakkaamista, korkeampaa pakkaamislämpötilaa sekä lyhyempää heranpoistoaikaa. Lisäksi valmistusprosessin voisi optimoida sovittamalla se jatkuvatoimiseksi nykyisen erätuotannon sijaan.

Avainsanat – Nyckelord – Keywords
Herajuusto, hera, hyllyikä, tuotekehitys

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Preface

This Master's thesis was done for the Department of Food and Nutrition at the University of Helsinki as an independent project. For funding, this thesis received an EMFOL-stipend from the Faculty of Agriculture and Forestry. Apart from the lactose analysis, which was conducted at Metropolilab Oy, all research was conducted at the University of Helsinki premises. This thesis was supervised by Professor Tapani Alatossava.

I would like to thank Professor Tapani Alatossava for the supervision and guidance. Furthermore, I would like to thank DI Antti Alavuotunki and University Lecturer Pekka Varmanen for their guidance. I would also like to thank other University staff; Research technicians Miikka Olin and Kati Hakala. I would also like to express my gratitude to fellow-student Emma Sinkkonen for help and support throughout the thesis.

Content

1. Introduction

Whey is a significant side stream in the cheese making industry. According to the Finnish Food and Drink Industries' Federation (ETL), the amount of whey as a side stream in Finland was 884 000 tons in 2014. Whey can be utilized in several ways including animal feed and energy production (Berg, 2016). Proteins and other elements can be isolated as well and used otherwise in the food industry. In addition, whey can be used to produce whey cheeses that originate from different countries and maintain a status in the given traditional cuisine. Some whey cheeses in Europe include Italian Ricotta, Norwegian Mysost, and Swiss Ziger (Pintado, et al., 2001). The Codex Alimentarius describes whey cheeses as follows: Whey cheeses are solid, semi-solid, or soft products which are produced by the concentration and molding of whey derived products or by coagulating whey by heat with or without the addition of acid (CODEX STAN 284-1971). The role of the heating process in the production of Ziger whey cheese makes it crucial to understand the behavior of the whey proteins of which over half is β-lactoglobulin.

1.1 Whey proteins

Whey proteins are nutritionally more valuable than casein proteins (Božanić, et al., 2014). This is due to the presence of essential amino acids in them *i.e.,* lysine, cysteine, and methionine. Therefore, whey cheese can be considered a nutritionally valuable product. The two major proteins of whey are β-lactoglobulin (β-lg) and α-lactalbumin (α-la), but other proteins are present as well which include bovine serum albumins and immunoglobulins (Hettiarachchy, et al., 2012). α-lactalbumin is a small protein that includes 123 amino acids and four disulfide bridges. It has a low number of organized structures of α-helix and β-sheets which gives the molecule excellent properties of flexibility (Cayot & Lorient, 1997).

β-lactoglobulin comprises ca. half of the protein in whey. β-lactoglobulin contains 162 amino acids with two disulfide bonds and the majority of the structure resembles β-sheets *i.e.,* the structure is well organized (Cayot & Lorient, 1997). In the molecular structure, nine β-sheets are packed on top of each other, and the disulfide bond bridges hold the structure together. At the pH of milk, β-lactoglobulin exists as a dimer in which two stacked molecular cones are

present. Furthermore, the association properties of β-lg depend on the pH value. At the pH value of 5–8 β-lg exists as a dimer but at the pH value of 3–5 β-lg dimers form octamers together and at extreme conditions of the pH value, β-lg exists as a monomer.

1.2 Denaturation of whey proteins

When α-lactalbumin is heated together with β-lactoglobulin, which is the case in standard whey, α-lactalbumin is easily irreversibly denatured (Cayot & Lorient, 1997). At temperatures above 70 °C and at a neutral pH value the two proteins form complexes due to a disulfide exchange reaction between the proteins. Generally, α-lactalbumin is more severely heat denatured in comparison to β-lactoglobulin because it possesses a lower denaturation temperature. When sweet whey is heated above 70 °C, β-lactoglobulin forms dimers, and copolymers with α-lactalbumin. When whey is further heated and the temperature increased, immunoglobulins and bovine serum albumin denature and form different copolymers and complexes with other whey proteins.

The solubility of the proteins is inversely proportional to protein denaturation (Pelegrine & Gasparetto, 2005). Usually, the proteins' solubility is at its lowest at the isoelectric point where the proteins have the most interaction with each other and are more likely to aggregate and precipitate. When the pH value is shifted from the isoelectric point, the proteins then have a positive or a negative charge *i.e.,* there is more interaction with water and charge repulsion between the protein molecules. Gasparetto et al. (2005) concluded that when heating a whey protein and water mixture at the temperature range of 40–60 °C, the solubility rates of whey proteins were the lowest at the pH value of 4,5. This is not quite the isoelectric point of β-lactoglobulin, but the whey protein mixture also contains several other proteins with varying isoelectric points which could explain the phenomenon. Dissanayake et al. (2013) also noticed a substantial increase in the rate of thermal denaturation of whey proteins close to their isoelectric point which was at the pH value of 5. The size of the aggregated and heat-denatured β-lactoglobulin polymers was studied by Hoffmann et al. (1996) and it was concluded that at the pH value of 6,4 the aggregates were the largest on a pH scale of 6,2 to 8. Donato et al. (2009) discovered that stable aggregates of β-lactoglobulin can be achieved at the pH value of 5,7–5,9 while heating at 85 °C.

In the process of whey cheese making, however, the goal is to achieve insoluble aggregates that can eventually be separated from the remaining liquid whey. Parris et al. (1993) noticed that during a heating process with a temperature above 65 °C, lowering the pH value of sweet whey to below 6,6 increased specifically the number of insoluble aggregates. The same phenomenon was observed by Zúñiga et al. (2010), too. They noticed that the decrease of the pH value of a β-lactoglobulin and water mixture (5 % w/v) from 6,8 to 6 produced a higher degree of denaturation of β-lactoglobulin dispersions when heated at 80 °C. This concurs with the notion of shifting the pH value towards the isoelectric point to aggregate and precipitate whey proteins. Apart from the pH, the solubility of the whey proteins can be decreased by increasing the temperature (Pelegrine & de Moraes Santos Gomes, 2008).

The addition of sodium chloride (NaCl) to a β-lactoglobulin solution does not per se affect the β-lg conformation but it promotes heat denaturation and aggregation which emphasizes the fact that salts, in general, induce aggregation via ionic strength (Cayot & Lorient, 1997). Schmitt et al. (2007) heated whey protein concentrates with and without NaCl and concluded that the whey protein aggregates formed in the presence of NaCl were denser.

It can therefore be said that the aggregation of β-lg is very sensitive to the pH value for the pH values which are further away from the isoelectric point (for β-lg at pH 5,3) promote instability within the conformations (Verheul, et al., 1998; Hoffmann, et al., 1999). The aggregation is further enhanced by increasing the ionic strengths of whey with NaCl for high concentrations of NaCl can modify the conformation of the β-lg as well as decrease their thermal stability (Xiong, et al., 1993). However, many studies focus on pure β-lg-solution and *e.g.,* whey protein concentrate (WPC) which lack the versatile environment that whey is.

1.3 Whey cheeses

Whey cheeses are produced all around the world and mostly in small scale and traditional ways. The German whey cheese is called *Molkenkäse* or *Ziger* and the French *fromage de serum* (Kammerlehner, 2009). Traditionally, whey cheeses are made in open cheese kettles where the whey is heated and partly concentrated by evaporation. In comparison to expensive and challenging membrane- or chromatography-based protein separation, the

heat-induced processing of whey is an economical alternative for small dairy industries (Pintado, et al., 2001). Although today, the industrial methods of whey cheese production include *i.a.,* vacuum evaporation (Kammerlehner, 2009). In the production of whey cheeses, whey can be pre-concentrated before the actual whey cheese production. The addition of other milk-derived raw materials is allowed to the extent where the whey proteins still hold the majority in the finished product. Whey cheeses can be ripened or fresh. The high lactose content of whey causes the whey cheeses to have a yellowish or brown color and a sweet, cooked, or caramelized flavor (CODEX STAN 284-1971). Whey cheeses can be used in a variety of ways from appetizers to desserts. In the USA, Ricotta whey cheese is mainly used in the production of desserts and pasta-type dishes such as lasagna and manicotti (Pintado, et al., 2001). In Portugal, the Requeijão whey cheese is mostly used in desserts such as puddings and cakes, and in Greece, the Myzithra whey cheese is popular in local cheese pies.

Next, Ricotta whey cheese manufacture is scrutinized for Ricotta is perhaps the most well-known cheese in which whey is used. Its manufacturing process is similar compared to the production of Ziger whey cheese. Ricotta, however, differs in the raw materials used since Ricotta can be made out of whole milk, skim milk, sweet whey (which traditionally originates from the Mozzarella production), or a mixture of the above mentioned (Fox, 2004). Traditionally, the milk or the whey and milk mixture is heated to 80–85 ^{o}C and at this point, acid is added to the milk and whey mixture. In the manufacture of Ricotta, a slow heating process and mixing contributes to the coagulum of the proteins. At about 40–50 ^{o}C 0,1 % NaCl is added to the whey (Pintado, et al., 2001). When the denatured proteins, or in other words, the curd rises to the surface the mixing is stopped and the holding time lasts for 5 minutes until the Ricotta is taken out from the kettle with a scoop or a similar instrument. The Ricotta is allowed to rest in the cheese forms for about 4–6 hours.

The cheese yield for a typical Ricotta with a whey and milk mixture is 5–6 % (Pintado, et al., 2001). The yield of plain cheese whey is even smaller which is partly the reason why milk is added to the whey. The addition of milk improves the recovery of the proteins in the process. However, the addition of milk or cream increases the fat content of the final product which can be an unwished property. Another way to increase the yield of Ricotta is to concentrate the whey beforehand or in some cases, skim milk powder is added to the whey. It is also

researched that acetic acid in the acidification of the whey causes higher yields than citric acid or lactic acid. In industrial continuous processes, other factors that affect the yield include the pH value, quality of the whey, and pumping rates.

1.4 Ziger whey cheese

Traditional production of Ziger whey cheese has a long tradition in Switzerland and its manufacture's predominant aim was to preserve milk's nutritional factors before rennet-induced cheese production overpowered it (Hösli & Schläpfer, 2012). The traditional production of Ziger requires fresh whey derived from rennet-induced cheese making. The heating temperature of the whey can vary between 91–93 °C and 88–92 °C (Ernst & Gonzalez, 2015; Zufferey, 2012). In order for the whey proteins to precipitate, acid (which is mostly acetic acid) is used (Hösli & Schläpfer, 2012). In order to further catalyze the aggregation process, NaCl is used. Whey cheeses, in general, are fresh cheeses with a high moisture content, a pH value close to neutral, and a low salt content which result in a relatively good matrix for bacterial growth (Pintado, et al., 2001). Therefore, the evaluation of the shelf life of Ziger whey cheese is crucial.

Ziger whey cheese was not a product that could be preserved for longer periods of time, so during the 16th and 17th century rennet-induced cheese making overpowered the Ziger production more or less (Hösli & Schläpfer, 2012). Ziger could then only be produced from whey and sometimes from a mixture of whey and buttermilk. The production stayed very marginal and Ziger was produced mostly for personal use. Nowadays, the production is small-scale and traditional which makes Ziger a regional specialty. Ziger has not reached significant demands on the market since its manufacture is considered inefficient due to high energy demands. However, the problem might as well be the lack of product development, research, and marketing. In Switzerland, only the Ziger produced in the canton of Glarus, also called the Schabziger, has reached widespread popularity.

Traditional whey cheeses have a limited amount of literature although there are studies of certain whey cheeses in regard to their production and shelf life. Rarely such studies exist for

Ziger whey cheese and therefore this study mimics the studies of other whey cheeses. In this study, the Italian Ricotta whey cheese was often used as a comparison.

2. Experimental research

2.1 Aims of the study

The aim of this thesis was to produce a Ziger whey cheese that is suitable for the Finnish dairy industry. One of the most significant parameters that affects the properties of Ziger whey cheese is the temperature at the time of protein precipitation. The effect of the manufacturing temperature on physicochemical properties, chemical composition, and microbiological quality was evaluated.

The main objectives of this thesis were:

1. To develop a Ziger whey cheese that can be applied in the Finnish dairy industry including optimization of the manufacturing process emphasizing the manufacturing temperature.
2. To investigate and compare the effect of the manufacturing temperature on the yield, moisture content as well as the protein, and fat content.
3. To investigate and compare the effect of the manufacturing temperature on the shelf life by performing microbiological analysis, following the pH value, and observing the physical changes of the product.

The temperatures examined in the production of Ziger were chosen extremities within the recipe range found in the literature which were 88 ^{o}C and 93 ^{o}C. Because the manufacturing temperature was the parameter that was researched, the circumstances otherwise had to be exact. This meant that the holding time and the amount of acid and salt added had to be stable parameters. For statistical relevance, each batch was repeated three times which resulted in altogether six production series which can be seen in Table 1. The preliminary hypothesis was that the higher manufacturing temperature results in a higher yield and a

Table 1. The experimental design of the Ziger manufacturing temperatures. The production was repeated three times for both temperatures which resulted in six repetition in total.

Temperature	Repetition
93 °C	3
88 °C	3
Total number of series = 6	

lower moisture content. Contrarily, the lower manufacturing temperature results in a lower yield and a higher moisture content of the product.

2.2 Materials and methods

2.2.1 Production

During the preliminary testing of the production, it was tested whether old whey *i.e.,* 24 hour-old whey could be used in the production of Ziger whey cheese. The whey was not heat-treated, and the lactic acid bacteria of the cheese starter caused the pH value of the whey to decrease to 4,4 and the manufacturing process of Ziger did not result in any yield. The whey proteins were denatured but it did not result in insoluble protein and could not be collected from the remaining whey. The low yield of Ziger from the old whey could be explained by further proteolysis of the whey proteins due to the lactic acid bacteria. As a result, only fresh whey was used in the production.

The Ziger whey cheeses were produced at the University of Helsinki in the facility of the pilot dairy plant (Helsinki, Finland). The milk was delivered by Valio Oy and the whey originated from the production of a Raclette-type cheese. The milk underwent two different kinds of heat-treatments in which the milk was heated at 63 °C and at 77 °C for 15 seconds. The pasteurizer was an automatic milk treatment unit (Pro Ruchti, Switzerland) and the principle was a plate-heat exchanger E 5 FHG (Fischer Maschinen und Apparatebau AG, Austria). However, given the cheese making process and the high temperatures of the whey cheese production, it was assumed that the differences in the heat-treatments had little or no effect on the outcome of the Ziger. The cheese starter which was added to the milk included *Lactococcus lactis* subsp. *lactis/cremoris/lactis* biovar. *diacetylactis* and *Streptococcus thermophilus* (Danisco, 2020). Also, calcium chloride (Tetrachemicals Europe, Kokkola, Finland) was added to the milk to improve the rennetability.

After the Raclette-type cheese production, the whey was immediately processed to whey cheese. Both pure whey and washed whey (33 % water) were collected to a steam kettle (BAMINOX AG, Switzerland) where the inner volume was 190 liters and the steam and water isolation around the kettle was 90 liters. The steam was directly injected around the kettle with a hose, and it started heating immediately. The whey was mixed with a horizontal rotor. The whey was then heated according to the investigated parameters to 88 °C and 93 °C. When the temperatures were achieved the mixing was stopped and changed to a vertical manual mixing with a milk can mixer. The lactic acid 80 % (IP Ingredients GmbH, Süderlügum Germany) and NaCl (Nouryon Suprasel, Denmark) were added. The whey was then allowed to rest, and the proteins were allowed to precipitate for 10 minutes. The Ziger was then collected with a sieve and molded. The Ziger was allowed to drain in the molds for 20 hours and was then weighed and packaged in cheese paper and stored at 4 °C. The production steps can be seen in Table 2. A manufacturing protocol was held from each batch which can be found in Appendix 1. Samples from each batch were stored in a freezer until protein and fat content analyses were performed, within 10 months.

Table 2. The Ziger whey cheese manufacturing process.

Process step	Process parameter	Description
Heating fresh whey	88 °C / 93 °C	Fresh whey, pH range 6,5, Mixing equally and slowly
Acid addition	30 mL/100 L	Lactic acid 80 %, sudden decrease in the pH value
Salt addition	240 g/ 100 L	NaCl decreases thermal stability of whey proteins
Holding time	10 minutes	Allows denatured whey proteins to gather onto the surface, remaining liquid is clear, no mixing
Molding		Filling the cheese forms
Draining	20 hours	Allowing the product to drain and lose liquid
Packaging		Cheese paper
Storage	4 °C	

2.2.2 Microbiological analysis

The laboratory work took place in the laboratory facility of the food science students at the University of Helsinki. The microbiological analysis included the investigation of the growth of the following micro-organisms: total viable count, yeasts and molds, coliforms, lactic acid bacteria, and psychrophiles. The sample and agar preparations and cultivation were carried out by hand and individually using dish planting in Petri dishes. The investigated micro-organisms and specific agars can be seen in Table 3.

The agars were prepared according to the instructions from Valio Oy (1993). The Agars were weighed accordingly to 250- and 500-mL bottles and filled with Milli-Q water and sterilized in an autoclave. The agars were then aseptically pored to Petri dishes and allowed to set and stored at 4 °C. For the Ziger whey cheese samples, a saline solution was prepared by weighing 9 g of NaCl (Riedel-de Haen GmbH, Germany) and filling to a volume of 1 L Milli-Q water giving a 0,9 % NaCl solution and sterilizing it in an autoclave. For the dilution series, 4,5 mL of saline solution was measured to test tubes which were then sterilized in an autoclave as well.

Table 3. The investigated micro-organisms and the agars used.

Sample	Agar
Total viable count	PCA (Plate count agar)
Coliforms	VRB (Violet red bile agar)
Lactic acid bacteria	MRS (De Man, Rogosa and Sharpe agar)
Yeasts and molds	YGC (Yeast Extract Glucose Chloramphenicol Agar)
Psychrophiles	PCA (Plate count agar)

The samples from Ziger whey cheeses for the microbiological analysis were taken at 0 days and 21 days of storage time. 10 g of Ziger samples were aseptically weighed into Stomacher bags and filled with 90 mL saline solution to achieve a 10^{-1} dilution. The samples were mixed with a bag mixer (BagMixer, Interscience, France) for 30 seconds. A sample from each batch was taken with duplicate cultivation for dilutions which were 10^{-6} and 10^{-7} for statistical relevance. The dilution series were done in test tubes with 4,5 mL saline solution and the dilution factor was held constant by pipetting 0,5 mL to the subsequent dilution. During the diluting, the pipettes were changed after each pipetting, and the test tubes were mixed. The entire process was carried out in a fume hood. The principle of the dilution series can be seen in Figure 1.

The total viable count, psychrophile, and lactic acid bacteria samples were inoculated using a pour plate method in which 1 mL of the sample in its right dilution was inoculated onto a Petri dish followed by adding liquid agar and mixing it thoroughly. It was important for the agar to be touchable by hands so that the temperature did not damage the bacteria investigated. The plate was then allowed to set and placed in the temperature cabinet. Yeasts and molds were inoculated using a spread plate method. The sample was inoculated onto the surface of a set agar and spread using a sterile spatula and then transferred to the temperature cabinet. Coliforms were inoculated using a mixed method between pour plate and spread plate method. The sample was inoculated on a set agar and spread with a spatula and then a layer of liquid agar was poured on top of it, and it was allowed to set. This method decreased the

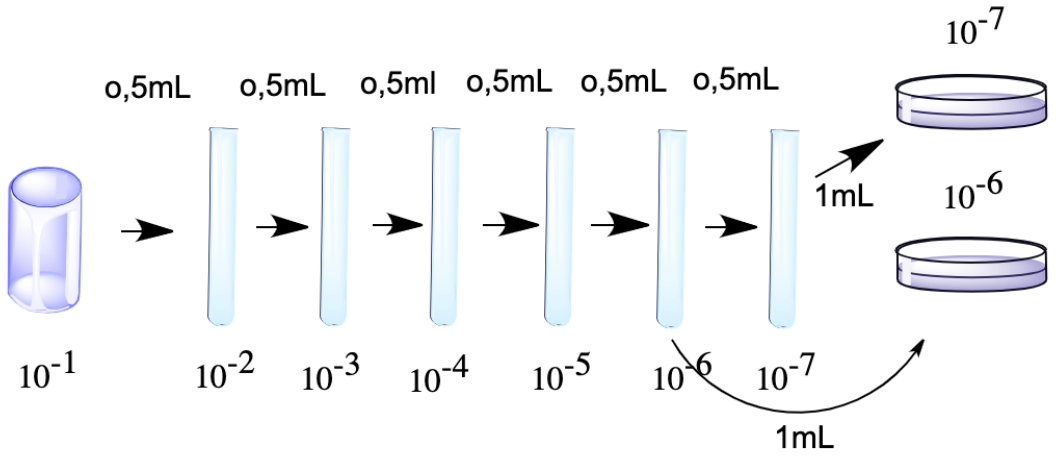

Figure 1. Illustration of the dilution series used for PCA and MRS agar dishes.

amount of oxygen in the environment of the sample. The samples were then allowed to cultivate in the temperature cabinets according to the times mentioned in Table 4.

The method of calculating the results was adapted from Aarnio et al. (2019). The results were calculated with a weighted average by dividing the number of colony-forming units by the sample volume which can be seen in Equation 1. The results were reported as CFU/mL and converted into a logarithmic scale for convenience and normality.

$$N = \frac{(C_1 + C_2 + \ldots + C_n)}{(n_1 x\, V_1 + n_2\, x\, V_2 \ldots + n_n x\, V_n)} \quad (1)$$

Where:

$C_1 + C_2 + \ldots + C_n$ = the colony-forming units in each plate used in the calculation
n_1 = the number of duplicates in the first dilution series
n_2 = the number of duplicates in the second dilution series
V_1 = the sample volume of the first sample
V_2 = the sample volume of the second sample

Table 4. Agars and their manufacturers, incubation time and temperature of the samples.

Agar:	**Incubation time:**	**Incubation Temperature:**
PCA (Lab M Limited, Lancashire, UK)	72 h	30 °C
PCA (psychrophiles) (Lab M Limited, Lancashire, UK)	10 days	6,5 °C
VRB (Lab M Limited, Lancashire, UK)	24 h	30 °C
MRS (Lab M Limited, Lancashire, UK)	72 h	37 °C (anaerobic)
YGC (Merck KGAA, Germany)	5 days	25 °C

2.2.3 Physicochemical properties

The physicochemical properties included the analysis of the moisture content, the yield, the pH value, and the rate of syneresis.

The moisture content was determined using a gravimetric method by evaporation (Mattila, et al., 2001). A muffle furnace (Nabertherm, Germany) and ceramic crucibles were used in the determination. The ceramic crucibles were first calibrated in the muffle furnace by placing them in for 24 hours at 105 ^{o}C for the moisture to evaporate. The crucibles were then allowed to cool in a desiccator for 30 minutes and then weighed. Approximately 3 g from each batch with a duplicate were weighed to ceramic crucibles. The samples were then placed in the muffle furnace for 24 hours at 105 ^{o}C and were then allowed to cool in a desiccator for 30 minutes and then weighed again. The moisture content was calculated according to Equation 2.

$$W = \frac{(\mathrm{W}_2 - W_3)}{(W_3 - W_1)}\, x\, 100 \qquad (2)$$

Where:

W_1 = Weight of empty container
W_2 = Weight of container + wet sample
W_3 = Weight of container + dry sample
Weight of moisture = $W_2 - W_3$
Weight of dry sample = $W_3 - W_1$

The yield was measured by weighing the Ziger whey cheeses obtained from each batch and calculated in relation to the amount of whey which is illustrated in Equation 3. This is the most common definition of the yield (Walstra et al., 2006), and it is chosen to indicate the success rate of the outcome and the relative amount of the insoluble whey protein.

$$Yield - \% = \frac{Ziger\ whey\ cheese\ (g)}{Amount\ of\ whey\ (g)}\ x\ 100 \quad (3)$$

The pH value was measured with a Testo 206-PH1 (Testo, Germany) pH meter. Buffer solutions of pH 4 and 7 were used to calibrate the pH meter. The aim was to create a pH profile for the production steps and the storage period. The pH was measured from the fresh whey, heated whey before the addition of acid, after the addition of acid, after 20 hours, and after 21 days.

There were significant physical changes in the product during the storage. Ziger whey cheese is a fresh dairy product and therefore consumers are not likely to tolerate any changes in the physical appearance. During the preliminary testing during the storage, the whey cheese samples appeared to show syneresis and release whey (inside the paper). A similar phenomenon was observed by Borba et al., (2014) for creamy Ricotta cheese, and their method was adopted for this thesis. The syneresis was calculated as the weight of whey in grams released from whey cheese samples inside the package divided by the weight of cheese of the same package in grams and multiplied by 100. The whey cheeses were packaged in cheese paper which could absorb liquid and hence the papers were weighed, too. By subtracting the original weight of the paper, the amount of whey absorbed by the paper could be determined. The syneresis of the samples was measured after 21 days of storage.

2.2.4 Chemical composition

The determination of the chemical composition included the lactose, crude protein, and total fat content. The lactose content of Ziger was analyzed in Metropolilab Oy (Helsinki, Finland) with the presumption that Ziger contains more than 0,2 g/100 g lactose. For there was a chance to send one sample only, a mixture of two batches was sent for the analysis. These were the 3rd batch of 88 °C and the 1st batch of 93 °C. The method used was NMKL 148/1993 which is a quantitative liquid chromatographic (LC) determination of fructose, glucose, lactose, maltose, and saccharose (Metropolilab Oy, 2020).

The fat content was determined as the total fat content by solvent extraction method (Mattila, et al., 2001). The Ziger whey cheese samples were thoroughly mixed in a mixer to get a homogenous matrix and weighed. The samples and their duplicates first underwent acid hydrolysis (Soxtec 2047 Soxcap System, Foss Tecator, Denmark) in order to extract the lipids completely from the samples. The samples were cooked in a 4 N HCL solution for ca. 1 hour and were then rinsed with water until the pH value was neutral. Meanwhile, the crucibles were calibrated in a temperature cabinet. The samples were then dried in a microwave in defrost mode for ca. 1 hour. Soxtec Avanti 2050 Extraction Unit (Foss Tecator, Denmark) was used in the lipid extraction. The samples were attached to the extraction socks, and 80 mL petroleum ether (which was used as the extraction solvent) was measured to the calibrated crucibles. After the extraction, the crucibles were weighed again, and the fat content was calculated as seen in Equation 4. W_1 and W_2 were the weights in grams of the calibrated crucible and the crucible with the extracted lipids, respectively.

$$Total\ fat - \% = \frac{(W_2 - W_1)}{fresh\ weight\ (g)}\ x\ 100 \quad (4)$$

The protein content was determined as the raw protein content using the Kjeldahl method (Mattila, et al., 2001). Ca. 400 mg of the homogenous and mixed samples and their duplicates were weighed into Tecator tubes using a filter paper to help with the accuracy. A Kjeltab (Thompson & Capper Ltd, UK) and 18 mL of strong (95–97 %) sulfuric acid were added into

each tube. The samples underwent wet combustion (Digestor 2020, Foss Tecator, Denmark) for 1 hour at 400 ^{o}C which caused the organic nitrogen to be bound by ammonium sulfate. The samples were then cooled, and 75 mL of Milli-Q water was added to each sample. The samples were distilled (Kjeltec 2300 Analyzer Unit, Foss Tecator, Denmark) by injecting steam during which NaOH was added to the samples in order to convert the ammonium sulfate to ammonia. Finally, the samples were titrated using 0,05 M HCL (Kjeltec 2300 Analyzer Unit, Foss Tecator, Denmark). During the distillation and titration, a blank sample was first tested which result was automatically subtracted from the actual results. The crude protein content was calculated from the nitrogen (N) concentration. The key to the determination of the nitrogen concentration was the amount of HCl used in the titration, which can be seen in Equation 5. The coefficient selected for the presentation of protein content was 6,25.

$$N(mg) = V_{HCL}\ x\ c_{HCL}\ x\ M_N \quad (5)$$

2.2.5 Sensory properties

The Ziger whey cheeses were evaluated according to their preferability by a panel of 3 untrained individuals. They were asked to select the attributes that best described each batch of Ziger whey cheese as well as choose their favorite.

2.2.6 Statistical analysis

The relationship between the manufacturing temperature and the chemical and physicochemical properties was investigated by Pearson's correlation. The coefficients were calculated using the SPSS software (IBM SPSS Statistic, version 27, IBM, New York, US). Probability value $p < 0,05$ was considered statistically significant. Linear regression was used to interpret the microbiological quality and was done using the Analysis ToolPak (Microsoft, US). The premise was the assumption that the results for the CFU/mL of the micro-organisms are normally distributed over time.

2.3 Results

2.3.1 Microbiological quality

The results of the microbiological analysis are presented in Table 5. A linear regression analysis was used to test whether the manufacturing temperature affected the microbiological quality. The temperature was used as the independent variable and the logarithmic count of the CFU/mL as the dependent variable. Separately, for each micro-organism, the result showed no significant ($p > 0,05$) correlation between the manufacturing temperature and the number of micro-organisms in the samples.

Coliforms, yeasts, and molds resulted in no growth in every sample during the sampling at 0 days. The absence of these micro-organisms was to be expected due to the high manufacturing temperatures and good hygienic practices. The presence of lactic acid bacteria (LAB) could be explained by the environment of the dairy plant where LAB are present in the surroundings at the time of drainage and packaging. The LAB, by producing lactic acid, could explain why the pH value (Figure 3) of the samples decreased during the storage. A linear regression analysis was made with the pH value and the logarithmic count of the LAB in CFU/mL and there was a significant correlation between the growth of LAB and the decrease of the pH value, $r(8) = 0,86$ and $p < 0,05$. This test did not include the manufacturing temperature as a variable.

The psychrophiles showed prevalence in the samples taken at 0 days and counted after 10 days. This is a significant factor to be taken into account for they can overgrow the other microflora and cause spoilage of the product (Scatassa, et al., 2018). Although the hygienic practices in the dairy plant met the requirements, there was no guarantee of preventing cross-contamination and post-contamination of the finished products.

The accuracy of the results should however be questioned. The total viable count is widely used as a method to determine the shelf life, and the spoilage of a fresh cheese like Ziger whey cheese starts at 10^7 CFU/mL of total viable count (ICMSF, 1984), and the failure criteria of total viable count for whey cheese can be set at $>10^5$ CFU/mL (Hough, et al., 1999). The

Table 5. Microbiological growth in Ziger whey cheese samples. The initial mean counts are presented in (log_{10} CFU/mL). Samples were made from each production series resulting in 6 samples in total. The time stamps are at 0 days and 21 days.

	93a		93b		93c		88a		88b		88c	
	0'	*21'*	*0'*	*21'*	*0'*	*21'*	*0'*	*21'*	*0'*	*21'*	*0'*	*21'*
Total viable count	6,86[a]	8,48	0	-[b]	6,57	8,45	0	8,30	7,72	8,48	5,88	-
Coliforms	0[c]	-	0	-	0	-	0	-	0	-	0	-
LAB	0	7,03	7,53	-	0	6,89	0	7,25	0	8,32	0	-
Yeasts and molds	0	-	0	-	0	-	0	-	0	-	0	-
Psychrophiles	6,10	-	6,57	-	0	-	6	-	7,02	-	5,40	-

[a]Each value represents mean value of duplicate samples in duplicate dilutions
[b]Sample was not taken
[C]No growth

samples showed either zero growth or substantial growth which was above the failure criteria at 0 days. At 21 days of storage, every sample crossed the threshold of spoilage (at 10^7 CFU/mL). The logarithmic scale of the CFU/mL for total viable count was plotted against the time stamps of 0 days and 21 days, and trendlines were generated (in the form of y = mx + b) for samples from both manufacturing temperatures. By setting the value for the total viable count at 10^7 CFU/mL the equivalent time stamps were 13,3 and 13,4 days for the Ziger whey cheese manufactured at 93 ^oC and 88 ^oC, respectively. Both of the time stamps rounded to 14 days and had therefore equally long storage times to reach the spoilage point. Ziger whey cheese is supposed to be consumed rapidly after production but the shelf life is strictly related to the initial microbial load and therefore, the total viable count values at first were alarming. Because of the high manufacturing temperature, probable causes were post-contamination during the packaging and storage. During the cultivation, an error in the dilutions or contamination during sampling was also possible.

2.3.2 Chemical composition

The protein, fat, and lactose contents are presented in Figure 2. All of the Ziger whey cheeses had a higher fat than protein content. The lactose content of the analyzed sample was 2,3 ± 0,58 g/100 g which was more than 1 g/100 g which is the limit for products that can be called low lactose (Ruokavirasto, 2020). Therefore, the Ziger whey cheese could not be called low lactose. The protein and fat contents were similar to the ones found in the literature. Sieber (1998) reported mean protein content of 11,1 g/100 g for Swiss Ziger whey cheese and in the present study, the mean protein contents were 9,2 and 10,04 g/100 g for the Ziger whey cheese manufactured at 93 °C and 88 °C, respectively. Sieber (1998) reported mean fat content of 7,2 g/100 g for Swiss Ziger whey cheese and in the present study, the fat contents were 12,44 and 13,27 g/100 g for the Ziger whey cheese manufactured at 93 °C and 88 °C, respectively.

Pearson's correlation was used to test whether the manufacturing temperature affected the protein and fat contents of the Ziger whey cheese. The result showed no significant ($p > 0,05$)

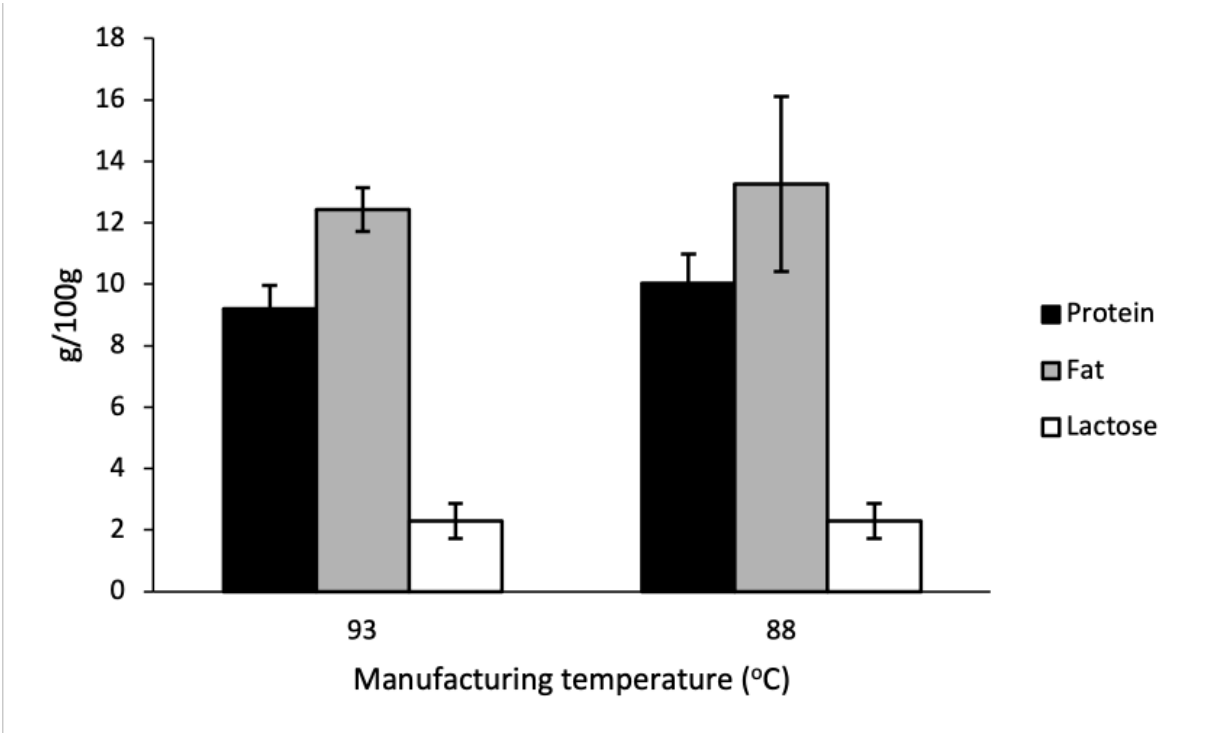

Figure 2. The mean protein, fat and lactose contents and their standard deviations presented as g/100 g. The contents are presented as total weight (dry matter + moisture content).

correlation between the manufacturing temperature and protein and fat contents. The Pearson's correlation table of coefficients can be found in Appendix 2.

There are several ways to express fat content and according to the CODEX standard (284-1971) for whey cheeses, the fat content should be expressed as fat content on a dry basis (FDM). For skimmed whey cheese the FDM is less than 10 %, for whey cheese 10–33 %, and for creamed whey cheese at least 33 %. The Ziger whey cheeses in this study (n=6) had an FDM average of 45 % ± 0,04 which was above the limit of 33 %. The Ziger whey cheese could therefore be categorized as creamy whey cheese. The EU legislation divides cheeses into categories according to their moisture content on a fat-free basis (MFFB) (Regulation [EU] No. 96/16 of the European Parliament and the Council). The categories describe the level of hardness of the cheeses which are soft cheese, semi-soft cheese, semi-hard cheese, hard cheese, and very hard cheese. The Ziger whey cheeses in this study (n=6) had an MFFB average of 82,2 % ± 1,38 which was above the limit of 68 % which was the limit for soft cheeses. Based on these categorizations, the Ziger whey cheese in this study could be referred to as creamy and soft whey cheese.

During the fat content analysis, an interesting phenomenon emerged. The 3rd batch from both of the series showed an increase in the fat content. During the manufacture, the milk was manually transferred to the cheese making vat from the milk tank where the valve was located at the bottom of the tank. The milk was heat-treated on the previous day and stored in the milk tank which could lead to creaming due to cold agglutination (Walstra, et al., 2006). Logically, milk for the last batch would contain more fat for the batch of milk would be from the top of the milk tank. Pearson's correlation coefficient between the batch order and the fat content was 0,691. Although the correlation was not significant ($p > 0,05$), a positive correlation with these variables suggested that the order of the batches did play a role in the resulting fat content. This could explain why the manufacturing temperature produced no change in the fat content.

2.3.3 Yield

The results of the yield can be seen in Table 6. Pearson's correlation indicated that there was a significant positive correlation between the temperature and the yield, $r(4) = 0{,}856$ and $p < 0{,}05$. The positive correlation between these variables concluded, that the higher the temperature during the manufacture, the higher the yield was, and it concurred with the hypothesis.

Table 6. The mean yield (n=3 per sample) of the samples and their standard deviations

Manufacturing temperature of the samples	Yield (%)	Standard deviation
93 °C	3,96	0,12
88 °C	2,87	0,56

2.3.4 Moisture content

Opposed to the original hypothesis, the moisture content of the Ziger whey cheese manufactured at the temperature of 93 °C was higher than at the temperature of 88 °C as seen in Table 7. In the production of whey cheeses, a general method of removing a fracture of the moisture content is by cooking (Pintado, et al., 2001). In the past studies, the differences in the moisture content of whey cheeses have been consistently related to the precipitation temperature (Pintado, et al., 1996). The fundamental importance of the moisture content in whey cheeses is its correlation with textural characteristics such as firmness. In Table 7 the moisture contents of similar whey cheeses were listed as a reference for comparison. In light of the scrutiny of the different moisture contents of different whey cheeses, the Ziger whey cheese produced in the present study had a relatively low moisture content. For future research, it would be interesting to examine, how the relatively low moisture content affects the textural characteristics of the product.

Table 7. Moisture contents of some whey cheeses as a reference and the moisture content of the Ziger whey cheese produced at the precipitation temperature of 93 °C and 88 °C. The moisture content is presented as an average with the standard deviation.

Cheese/Location	Raw Material/ Manufacture Technique	Moisture (%, w/w)	Reference
Ricotta/Sassari, Italy	Cow whey/traditional	76.0 ± 4.2	Cossedu et al., (1997)
Ricotone/USA	Cow: 100 % Whey	82.5	Kosikowski, (1982)
Ziger/Switzerland	Cow: 100 % Whey	76.3 ± 4.0	Sieber, (1998)
Ziger/Finland	Cow: 100 % Whey (93 °C)	72.6 ± 1.25	
Ziger/Finland	Cow: 100 % Whey (88 °C)	70.7 ± 2.53	

The moisture content had a significant negative correlation with the fat content, $r(4) = -0{,}832$ and $p < 0{,}05$. The negative correlation concluded that the higher the moisture content was, the lower the fat content of the product was. The moisture and the protein content did not show a significant correlation. Lower moisture content (as seen in Table 7) and higher fat content of traditional whey cheeses have been observed in the literature earlier, too. Pintado et al. (1996) discussed the same phenomenon as they stated that traditional ways of whey cheese production result in lower moisture content and higher fat content very likely due to the high manufacturing temperatures. When heated, the denatured whey proteins become associated with fat globules (Walstra, et al., 1999). The fat globules cause the curd to have wider pores which possibly accelerates the drainage of whey which therefore leads to lower moisture content.

2.3.5 pH value

Figure 3 suggests that the pH value of the whey decreased by mere heating. The heat-induced change of the pH value could refer to a buffering effect of intrinsic lactate and phosphate (Shon & Haque, 2007). Moreover, the decrease in the pH value could also be caused by lactose degradation (Salaün, et al., 2005). Several biochemical changes were occurring simultaneously, and the intensity of the heat-treatment affected the resulting pH value. It can be seen in Figure 3, that the pH value was lower at 93 °C than it was at 88 °C which concurs with this theory.

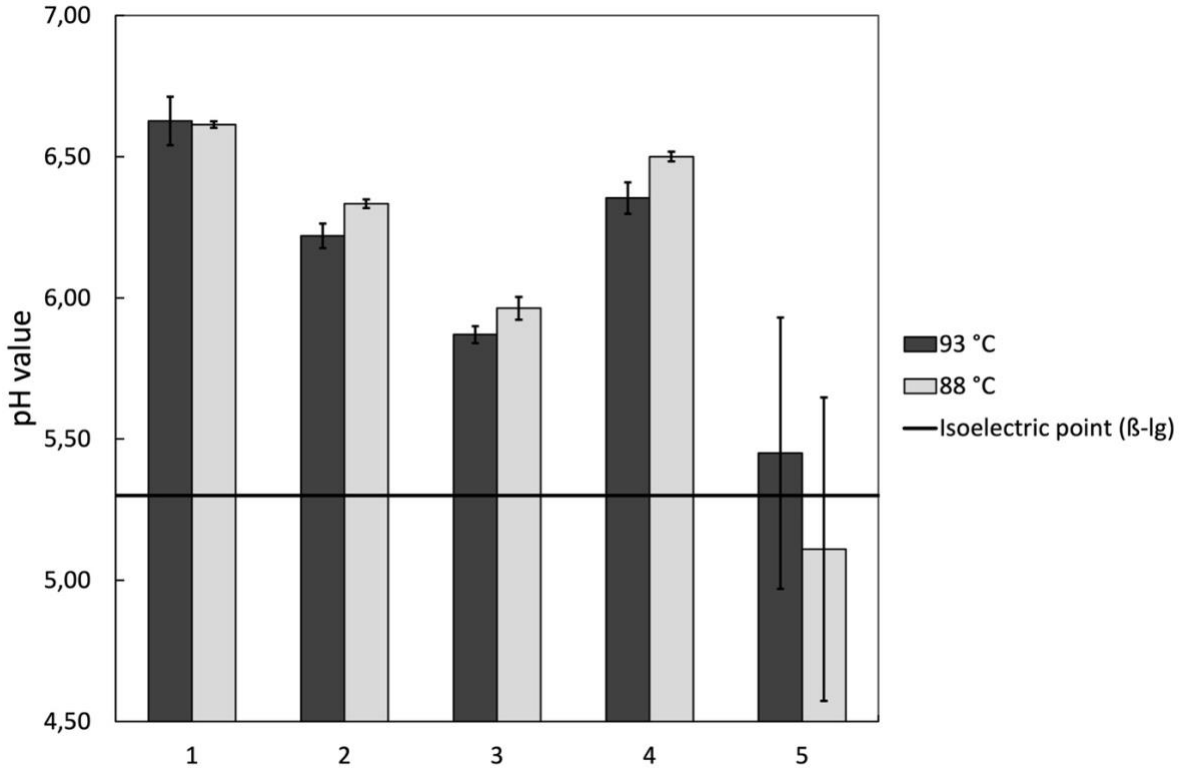

Figure 3. The pH value measured during the manufacture and storage. On the X axis 1 = whey prior to heating, 2 = whey at the set temperature, 3 = Whey after the addition of acid and NaCl, 4 = Ziger whey cheese after 20 hours of manufacture, and 5 = Ziger whey cheese after 21 days of manufacture. The horizontal line illustrates the isoelectric point of β-lactoglobulin.

A further decrease in the pH value is seen in Figure 3 as the lactic acid was added to the whey. The pH value of the final product (after 20 hours) increased in relation to the previous pH value during the manufacture. After 21 days the pH value of Ziger whey cheese dropped significantly. High moisture content and an almost neutral pH value were the reasons for the limited shelf life and the degradation which first signs included the decrease of the initial pH value. Similar results have been noticed before in the production of Ricotta regarding the pH value by Hough et al. (1999) as they measured the pH value of the Ricotta whey cheese throughout its storage time.

2.3.6 Syneresis

Syneresis after 21 days of storage for the samples manufactured at 93 °C (n=2) was 1,04 ± 0,52 g 100 g^{-1} and for the sample manufactured at 88 °C (n=1) was 0,51 g 100 g^{-1}. Similar

studies are available and *e.g.,* Meira et al., (2015) measured the syneresis of goat Ricotta cheese during the storage and after 7 days the syneresis was 2,70 ± 0,02 g 100 g^{-1}.

2.3.7 Sensory analysis and appearance

The appearance of all the Ziger whey cheeses was of the same type, which is creamy and white. The smell did not differ among the batches either and was plain and milky. During the storage, the cheeses exhibited drying and syneresis which caused them to appear dryer as can be seen in Figure 4. The panelists described the Ziger whey cheeses manufactured at 93 °C to be mild in flavor and light and the Ziger whey cheeses manufactured at 88 °C to be creamy and yogurt-like. The 3rd batch from both series was described as creamier compared to the other samples. This could be explained by the increased fat content due to the creaming by cold agglutination in the milk tank which was discussed in section 2.3.2 Chemical composition 67 % of the panelists preferred the Ziger whey cheese manufactured at 88 °C compared to the samples manufactured at 93 °C. However, given the number of panelists, the sensory evaluation could not be considered statistically relevant and was intended to indicate how the Ziger whey cheeses were perceived.

Figure 4. The Ziger whey cheese manufactured at 93 °C fresh 1 day after the production (left) and after 21 days (right).

2.4. Discussion

2.4.1 Production

During the manufacturing process (2.2.1 Production), the behavior of the whey proteins followed the fundamental kinetics of the formation of thermally induced β-lactoglobulin aggregates. The main parameters that influenced the whey protein aggregation were the temperature, the pH value, and the addition of lactic acid and NaCl. The high manufacturing temperature caused the whey proteins to denature and unfold. As presented in the literature, in sweet whey the formation of calcium bridges occurs between the proteins due to the presence of calcium which promotes the formation of large aggregates (Havea, et al., 2002).

The addition of lactic acid during the manufacture shifted the pH value towards the isoelectric point of the whey proteins (Figure 3). Lactic acid was added because whey proteins' solubility is at its lowest at the isoelectric point where the proteins have the most interaction with each other and are more likely to aggregate and precipitate (Pelegrine & Gasparetto, 2005). The addition of NaCl in the Ziger whey cheese processing can be explained by the following observations. The addition of NaCl causes charge neutralization of the unfolded whey proteins which can then further continue to aggregate to larger sizes (Schmitt, et al., 2007). High ionic strengths due to the addition of NaCl promote protein aggregation significantly, and high concentrations of NaCl can modify the conformation of the β-lg as well as decrease their thermal stability (Xiong, et al., 1993).

The manufacturing process was adapted from a process that is used in the Alpine region during the transhumance of dairy cows in the highland pastures. The temporary farms enjoy the benefits of pasture-based feed and dairy processing on the spot which results in less storage time. The circumstances during this study were therefore vastly different including silage-based feed and long storage times of the milk. Despite the previous, the adaption was manageable and quite successful. In the highland pastures, the products, however, have an added value linked to terroir and historical production traditions (Bergamaschi, et al., 2016), which the Finnish conditions lack. The notion of a market gap in the whey cheese branch in Finland is a better approach than appealing to the tradition of the product.

The traditional way of manufacture results in an enjoyable fresh whey cheese delicacy, and since the fundamental recipe has now been tested it is time to focus on product development which includes the efficiency of manufacture. One alternative for improving the efficiency of the manufacture is a continuous processing model. The Ricotta whey cheese is produced thereby industrially which has several pros; It improves the product yield and saves energy costs (Modler & Emmons, 2001). Energy costs are decreased by recirculating the heat in a plate heater and by making the heating process faster instead of using a batch process. It also saves labor costs and improves the shelf life up to 10 weeks. The continuous production of Ricotta cheese starts with the heat-treatment of a mixture of whey and whole milk in a plate heater at 70 °C and advances to a section of the plate heater in which the whey reaches temperatures of 88–90 °C. The entire travel time of the holding and denaturation tubes in the plate heater is about 15 minutes. Next, acid is injected into the coagulation line causing the curd to separate from the liquid. In the holding tubes, the flow is rather turbulent, but the velocity is reduced to laminar flow in the coagulation line. The product is directed to a moving belt with a mesh from where the product is collected to cheese forms for draining. The speed of the moving belt can be adjusted to the time of drainage. From the moving belt, the product is directed to the filling machine in which the product needs to maintain a temperature of above 75 °C to ensure long shelf life. In addition to Ricotta, the continuous process could be an alternative for the production of Ziger whey cheese as well.

2.4.2 Shelf life

When determining the shelf life of Ziger whey cheese (2.3.1 Microbiological quality), it was clear that the traditional recipe had no contribution to the preserving qualities of the product other than the high manufacturing temperature. The guidelines for the traditional Ziger whey cheese manufacture recommend a shelf life of six days starting from the moment of packaging (Ernst & Gonzalez, 2015). The possible reason for this is that the traditional Ziger whey cheese is manufactured on the spot and marketed through direct sales of agritourism where freshness is the primary quality attribute and not the shelf life.

The growth of any bacteria in any matrix is depended on certain parameters such as water activity, pH value, salt, and temperature. Ziger whey cheese is a fresh cheese with high water content, pH value close to neutral, and low salt content which makes it a relatively good matrix for bacterial growth and especially molds, yeasts, and *Enterobacteriaceae* (Pintado, et al., 2001). In the traditional Ziger whey cheese manufacturing process, which was also applied in this study, the time for drainage in the molds is 12–24 hours (Ernst & Gonzalez, 2015). This poses a risk of post-contamination and growth of mesophilic bacteria since the temperature drops during the draining to near room temperature. On a related note, good microbiological quality of whey cheese can be linked to a high packaging temperature, say above 65 ^{o}C (Hough, et al., 1999). By shortening the drainage time before packaging and increasing the packaging temperature, the microbiological quality could be enhanced. The drainage could be enhanced mechanically *e.g.,* by pressing, too.

In this study, the cheese paper as a package did not protect the product. Ziger whey cheese is an easily perishable dairy product and therefore its shelf life is strongly dependent on the packaging method. Hough et al. (1999) studied the growth of aerobic mesophiles in commercial Ricotta cheese with aerobic packaging conditions in a similar way to this study. In this study, 67 % of the samples had reached the failure criteria of 10^5 CFU/mL during the first sampling (Table 5) while the commercial Ricotta in the study by Hough et al. (1999) reached the same failure criteria after nearly 30 days of storage at 6 ^{o}C. Several studies have been made on the shelf life extension of several different kinds of whey cheese.

The shelf life of Turkish whey cheese *Lor* could be extended by using modified atmosphere packaging (MAP) and vacuum package (Irkin, 2011). Especially modified atmosphere conditions with 80 % CO_2 and 20 % N_2, or with the most CO_2, were effective against total viable count and *Enterobacteriaceae.* The vacuum package inhibited the growth of lactic acid bacteria the most. However, the absence of oxygen cannot be the only solution because anaerobic conditions could be optimal for anaerobic pathogens. Also, the shelf life of Greek whey cheese *Myzithra Kalathaki* could be extended due to MAP by 14–20 days based on microbiological quality, chemical composition alterations, and sensory attributes (Dermiki, et al., 2008). Based on these findings, the modified atmosphere and vacuum packaging could be experimented on Ziger whey cheese as well to extend the shelf life.

A possible direction of product development could be the addition of probiotic strains to Ziger whey cheese. The probiotic bacteria can improve the microbial quality and promote nutritive benefits in terms of the therapeutic impact of the probiotic strains. (Gomes & Malcata, 1999). Madureira et al. (2011) studied the antimicrobial activity of two probiotic strains (*Lactobacillus casei* and *Bifidobacterium animalis*) which were inoculated to whey cheese. The probiotic strains produced organic acids through their metabolism which have antimicrobial effects. Especially *L. casei* inhibited the growth of contaminant bacteria which included *Listeria innocua, Salmonella* Enteritidis, *Staphylococcus aureus, Pseudomonas aeruginosa* and *E. coli.* It has also been suggested that especially the probiotic strains of *L. brevis* and *B. animalis* can survive in the gastrointestinal tract in vitro (Madureira, et al., 2005).

It was concluded in section 2.3.7 Sensory analysis and appearance that the taste of the Ziger whey cheese is rather mild in flavor. The mild flavor could be altered by *e.g.,* adding spices into the cheese matrix, and apart from only giving flavor, spices could prolong the shelf life. El-Batawy et al. (2017) studied the effects of different spices on the shelf life of Ricotta whey cheese. The different spices included hot green pepper, garlic, and onion paste. During the cold storage period of 25 days, they noticed a decrease in total bacterial count, yeasts, and molds in spicy Ricotta samples compared to the control samples without spices. The most effective spices against the growth of the beforementioned micro-organisms were hot green pepper and garlic, and Ricotta cheese fortified with hot green pepper gained the best scores regarding flavor. The addition of spices could improve the flavor and the shelf life of Ziger whey cheese as well and should be taken into consideration regarding future research.

2.4.3 Chemical composition

The composition of Ziger whey cheese is dependent on the composition of whey. The dairy industry produces different kinds of whey currents of which the most prevalent in the manufacture of whey cheeses is rennet-induced sweet whey (Kammerlehner, 2009). Some factors affect the fat and protein contents of sweet whey. For example, in the cheese making process, a smaller cheese curd causes the whey to have a higher fat and protein content. In addition, a higher heat-treatment of the cheese milk causes denaturation of the whey proteins which eventually become part of the cheese curd. Various cheeses require a washing

phase of the curd during the manufacture which means that the whey is diluted in water. In this study, the whey was diluted with 33 % water which meant that the concentration of the whey constituents was lower in comparison to pure whey. The protein recovery could therefore be enhanced by using non-diluted whey. Furthermore, Salvatore et al. (2014) suggested that further concentrating whey could increase the protein recovery and yield in ovine Ricotta whey cheese production, and this could be considered in the bovine based Ziger whey cheese production, too.

As mentioned in Figure 2, in this study the mean protein contents were lower and the mean fat contents were higher compared to Swiss Ziger whey cheese studied by Sieber (1998). However, it has been observed that there is a lack of uniformity regarding the composition of various whey cheeses. Madalozzo et al. (2015) discovered that among Brazilian Ricotta cheeses the coefficients of variation for fat and protein contents were 48,35 % and 23,61 %, respectively. They argued that the large coefficient of variation for fat content is caused by insufficient standardization of milk fat levels in the cheese milk. It was concluded in section 2.3.2 Chemical composition that the cause of the fat content variation in this study was partly due to the creaming and cold agglutination of fat during the storage in the milk tank. It should also be considered that the breed of the cows and the variations in milk due to the seasons and the animal feed also play a role in the composition of whey (Pintado, et al., 2001). Whey is complex and has a large variety in nature, which makes it difficult for whey cheeses to achieve the same composition (Paskaš, et al., 2019).

Since the Ziger whey cheese can be considered creamy whey cheese, it does not meet the consumers' demand for healthier alternatives. The fat content could however be reduced by means of the manufacture. Kaminarides et al. (2015) compared the chemical compositions of the traditional Greek whey cheese Myzithra produced from 100 % whey and from a mixture with a 9:1 ratio of whey to skimmed ovine milk. The fat contents of the Myzithra whey cheese were 12,17 ± 0,16 % and 9,24 ± 0,59 % for the 100 % whey and the mixture of whey and skimmed ovine milk, respectively. The yield was also higher with the addition of skimmed milk. By increasing the non-fat dry matter could therefore decrease the fat content and improve the yield and could be considered in the production of Ziger whey cheese, too.

According to the FACEnetwork (Farmhouse and Artisan Cheese & Dairy Producers European Network), Finland has the 3rd least small-scale dairies out of the 15 member states. The neighboring countries Sweden and Norway have almost 6 times more small-scale dairies in comparison to Finland. The adaption of traditional hand-made products would be in advocacy of the Finnish small-scale dairy industry and Ziger whey cheese would ever so fit in this scheme.

3. Conclusions

In this study, the production of Ziger whey cheese with the manufacturing temperature as the changing parameter was carried out successfully. There was a significant positive correlation between the temperature and the whey cheese yield. The microbiological analysis resulted in no correlation between the manufacturing temperature and the number of micro-organisms in the whey cheese. However, there was a significant correlation between the growth of lactic acid bacteria and the pH value where the bacterial count increased as the pH value decreased. The accuracy of the microbiological analysis should be questioned for the total viable counts of the whey cheese samples were very close to the failure criteria of > 10^5 CFU/mL during the first sampling at 0 days and each whey cheese sample crossed the spoilage threshold of 10^7 CFU/mL after 21 days of storage.

The manufacturing temperature had no significant correlation with the protein and fat contents. Based on the CODEX standard and the EU legislation regarding the declaration of the fat content, the Ziger whey cheese can be categorized as creamy and soft whey cheese. Opposed to the original hypothesis, the moisture content of the Ziger whey cheese at the manufacturing temperature of 93 ^oC was higher than at the temperature of 88 ^oC. Instead of the manufacturing temperature, the moisture content was correlated with the fat content, for the fat globules caused the curd to have wider pores which possibly accelerated the drainage of whey which therefore led to lower moisture content. However, the results are similar to the ones found in the literature for Ricotta, Ricottone, and Swiss Ziger whey cheeses. After 21 days of storage, the pH value of Ziger whey cheese dropped significantly.

High moisture content and an almost neutral pH value were the reasons for the limited shelf life and the degradation which first signs included the decrease of the initial pH value.

Based on the findings in this study and the existing literature, the manufacturing temperature of 93 ^{o}C resulted in a greater yield of Ziger whey cheese and therefore was favorable compared to the manufacturing temperature of 88 ^{o}C. That was because there were no other significant effects of the temperature on the product's characteristics examined in this thesis. For future reference of research, the quality and shelf life of Ziger whey cheese could be improved by modified atmosphere or vacuum packaging, high packaging temperatures, and shorter drainage time. Moreover, the production could be made more efficient by a continuous process in comparison to batch production.

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Appendices

Appendix 1. Manufacturing protocols

	Setpoint	Unit	Ziger Production from:					
Production series	–	–	93a	93b	93c	88a	88b	88c
Whey	–	L	70	74	65	70	70	55
Precipitation enhancers								
Lactic acid 80 %	**30 mL**	**/100L**	21	22,2	19,5	21	21	16
NaCl	**240 g**	**/100L**	168	177,8	156	168	168	132
Precipitation temperature	**88-93**	**°C**	93	93	93	88	88	88
Heating time	–	**Min.**	30	33	25	18	21	24
Holding time	**10**	**Min.**	10	10	10	10	10	10
Draining time	**20**	**h**	20	20	20	20	20	20
pH value								
Fresh whey	–	–	6,72	6,55	6,61	6,6	6,62	6,62
Whey after heating	–	–	6,19	6,2	6,27	6,35	6,33	6,32
Whey after acid addition	–	–	5,84	5,87	5,9	5,92	6	5,97
Ziger after 20 hours	–	–	6,41	6,35	6,3	6,51	6,48	6,51
Amount of Ziger	–	**g**	2684,9	2952,4	2640,6	2237,8	2232	1223,5
Storage temperature	**Max. 4**	**°C**	4	4	4	4	4	4

Appendix 2. Pearson correlation coefficients

Correlations between the manufacturing temperature, yield, fat, protein, and moisture content and the batch order calculated as Pearson correlation coefficients (n=6)

	Temperature	Yield	Fat (%)	Protein (%)	Moisture (%)	B.O.
Temperature	1					
Yield	**0,856***	1				
Fat (%)	-0,222	-0,669	1			
Protein (%)	-0,457	-0,105	-0,532	1		
Moisture (%)	0,460	0,758	**-0,832***	0,307	1	
B.O.[a]	0,00	-0,238	0,691	-0,545	-0,602	1

[a]Batch order

* Significance level $p < 0,05$