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Reasons of Bitterness in Ultrafiltrated White Cheese

Sukru Karatas, Zeynep Hazal Tekin, Ebru Kiran

Department of Food Engineering, Istanbul Aydin University, Istanbul, TURKEY

Abstract: *Bitterness is an important flavour defect and generally occurred in cheese. In this study, the reasons of bitter flavour occur in white cheese produced by ultrafiltration method were investigated. For this purpose, white cheese samples were collected from markets randomly and "lily-white" cheese samples produced by ultrafiltration were used as research materials. Bitterness occurred product and new products were subjected to a variety of chemical and microbiological analyses. In this context, titratable acidity, fatty acid analyses with gas chromatography as chemical analyses were subjected to bitterness occurred product and new products while psychrophilic Pseudomonas, Bacillus cereus, Lactic streptococci and lipolytic bacteria as microbiological analysis was subjected to bitterness occurred in products were investigated. Ca amount and bitterness were related and the bitterness was increased as the amount of calcium was increased. As the acidity results were examined, the titratable acidity values of all cheese samples were lower than the limit value (3%), so bitterness showed no relation with increasing acidity. The amounts of caproic acid, caprylic acid, lauric acid, myristic acid and margaric acid were reduced in bitter white cheeses as compared to fresh white cheeses. The amount of long chain free fatty acids (C11 to C20:1) were increased as bitterness were increased. According to API 20 Strep test, there was a 90% chance that it was Streptococcus. Bacillus cereus didn't present on MYP agar; however, Lactic streptococcus presence were coccus shaped and Gram (+), Lipolytic bacteria presence were rod shaped and Gram (-) and 99% chance of Pseudomonas presence result was obtained by CFC selective agar.*

Keywords: *Bitterness, White Cheese, Ultrafiltration, Bacillus cereus, Lactic streptococcus, Lipolytic bacteria, Pseudomonas.*

1. INTRODUCTION

Cheese is diversified according to flavor and texture characteristics which are directly related on types and quality of milk, cheese producing and ripening conditions and the actions of lactic acid bacteria and fungi [1].

During cheese ripening which is a period to be a delicate balance for supplying between various reactions in cheese production for good taste improving, compounds which are occurred by microbiological, biochemical, and chemical reactions and formed by cleavage of protein and lipids found in cheese composition affect taste of cheese [2,3]. In this process, pH, the functions and amount of rennet, milk proteinase, type of starter culture, seconder microorganisms, non-starter lactic acid bacteria are important [3,4,5]. In addition, the enzymatic hydrolysis of casein in various ways and at different places provides to obtain sub-products which take part in specific features of cheese types [6,7].

The taste which involves sweet, sour, bitter, salty and umami is more important than appearance, flavor, color, and smell for consumer choice and acceptance. Generally, consumer doesn't prefer bitter products except for beer, wine and coffee [8,9,10].

Bitterness, which is one of the flavor defects is occurred as a result of bitter peptide accumulation related with casein proteolysis occurred by the effects of proteolytic enzymes produced from milk coagulating agents, starter and non-starter lactic acid bacteria and from the endogenous milk proteinase system [11,12,13,14]. Lowrie and Lawrence [15] asserted that the bitter flavor in cheese was developed when microbial numbers or microbial activity increased. Leumiex and Simard [11] reported that bitter flavor was not produced by the effect of lipase on milk fat but excessive activity of proteases which affected caseins accelerated bitterness. Extensive lipolysis is considered undesirable because free fatty acids cause bitterness.

The ultrafiltration has been used as a membrane process for cheese production since 1959 in France [16]. This process provides some advantages such as increasing cheese yield owing to incorporation of whey proteins, increasing product uniformity and preferable weight adjustment on product. In addition to these advantages, ultrafiltration optimizes the resources for cheese production. Compared to traditional methods, ultrafiltration process requires fewer amounts of rennet, starter culture, salt and color. Moreover, logistic

attributes such as space and handling reduced and biological oxygen requirement is lower in permeate compared to whey [17]. On the other hand, ultrafiltration may cause some disadvantages such as bitterness. In the present study, Ca determination, titratable acidity, fatty acid compositions were analyzed with gas chromatography Addition to that chemical and microbiological analyses were made in order to determine the reasons of bitter flavor in white cheese product which were produced by ultrafiltration method.

2. MATERIAL AND METHODS

2.1. Material

Bitter white cheeses produced by using ultrafiltration method (within the scope of the raw material project) and fresh white cheeses were collected from markets randomly and transferred to Istanbul Aydın University by unbroken cold chain and stored at deep-freezer. In this study, 4 fresh UF white cheeses (500-gram package) and 4 bitter UF white cheeses (500-gram package) were chosen according to sampling squares method. The all samples were hold at 4 °C until further analyses and all same conditions were provided for all samples. Each analysis was conducted in triplicate.

2.2. Methods

2.2.1. Ca Determination on Brine of Cheese

Calcium could be determined by titration with a chelating agent (EDTA) at a pH 13 [18]. After 20 ml bitter white cheese brine and 20 ml fresh white cheese brine were replaced into flasks, 5 ml buffer solution were added to flask and the mixture was titrated with 10 drops 0.01 M EDTA (Eriochrome black T indicator) until blue color according to AOAC Method 991.25 [18]. The amount of Ca (mg/ml) was calculated by using following equation (Eqn.1) [19].

$$Ca\ \left(\frac{mg}{ml}\right) = \frac{V * 0.4008 * 1000}{m} \qquad \text{Eqn.1}$$

V: volume of 0.01 M EDTA (ml)

m: amount of sample (ml)

2.2.2. Total Titratable Acidity Determination

Titratable acidity method which considers the concentration of disassociated and un-disassociated hydrogen molecules was used. 10 gr homogenized sample were taken into flask and mixed with 100 ml distilled water at 40 °C and then the mixture was filtered by using filter paper. The acidity in cheese was measured by titrating filtered sample (25 ml) until obtaining pink color that had 2-3 drops phenolphthalein indicator solution added to it with the 0.1 N NaOH solution. % titratable acidity (TA) was calculated by using Eqn. 2 [20,21] and % lactic acid was calculated by using following equation [22].

Eqn.2

V = total volume of 0.1 N NaOH (ml)

f = factor of NaOH

SF = dilution factor

m = sample amount (g)

Molecular weight of lactic acid = 90.08

2.2.3. The Examination of Fatty Acid Composition in Cheese by Using Gas Chromatography

Free fatty acid extraction in cheese was conducted by using adopted procedures by Deeth et al. [23]. Bitter and fresh white cheese samples should be completely dry before extraction of fat. For this reason, 10 gr samples were spread uniformly in the petri dishes and they were dried at 105 °C for 2 hours. After drying, the petri dishes were placed into desiccator in order to cool to the room temperature (about 30 min). The dried samples were removed from petri dishes and insert in a Soxhlet unit and mixed with 200 ml petroleum spirit and extracted for 4 hours. After extraction, fat flasks were placed in rotary evaporator under vacuum for removing the solvent and solvent were evaporated. The solvent was placed into oven dryer at 105 °C for 1 hour in case having any solvent [24]. The obtained fat samples were analyzed by using Gas Chromatography (Agilent technologies, 7890A). Each 0.1 gr fat sample was added into centrifuge tube and mixed with 10 ml GC grade hexane. Methanol (100 µL, 2N) and dissolved KOH were added to centrifuge tube for saponification and the cover of the centrifuge tube was closed. After centrifugation at 4000 rpm for 5 min, 1 µL of the sample was injected to Gas Chromatography which was arranged according to conditions showed in Table 1.

Table 1: *Gas Chromatography Conditions*

Instrumentation Chromatographic system	
Inlet Detector	Agilent 6890 GC Split/Splitless FID or Agilent 5973 MSD
Linear Column A	Split liner (p/n 5183-4647) 100m x 0.25 mm ID. 0.2 µm HP-88 (j% 112-88A7)

Experimental Conditions GC-FID	
Inlet temperature	250°C
Injection Volume	1 µL
Split Ratio	1/50
Carrier gas A	Hydrogen
Carrier gas B	Helium
Head pressure	2 mL/min constant flow
Oven temperature A	120°C, 1 min, 100°C /min to 175°C, 10 min, 5°C min to 210°C, 5min 5°C/min to 230°C, 5 min
Oven temperature B	175°C, 10 min, 3°C/min, 220°C, 5 min
Detector temperature	280°C
Detector gases	Hydrogen: 40 mL/min: Air450 mL/min: Helium make-up gas:30 mL/min

2.2.4. Microbiological Analysis

Each of bitter white cheese samples (5 gr) were delivered in sterile containers, mixed with 45 ml peptone-water and then the mix was homogenized in stomacher (Easy mix). Different dilutions were preferred for different inoculations. Pseudomonas species were determined on Pseudomonas CFC Selective Agar (Merck 1.07620). The bitter white cheese samples were diluted up to 10-5 and introduced into sterile plates and added by pour plate method. After the plates were incubated at 25 °C for 48 h, colonies were counted. Bacillus cereus was enumerated on MYP (Mannitol Egg Yolk Polymyxin) Agar (Oxoid CM0617). The bitter white cheese samples (10-5 dilutions) was introduced into sterile plates and added by pour plate method. After the plates were incubated at 37 °C for 24 h, colonies were observed. M17 Agar Base (Merck 1.15108) was used to differentiate Lactic streptococci. The bitter white cheese samples (10-8 dilutions) was introduced into sterile plates and added by pour plate method. After the plates were incubated at 37 °C for 24 h, colonies were observed. Tributyrin Agar Base (Oxoid PM0004) was preferred for determination of existence of Lipolytic bacteria. The bitter white cheese samples (10-7 dilutions) was introduced into sterile plates and added by pour plate method. After the plates were incubated at 30 °C for 72 h, colonies were observed. API test and gram staining method had been made for identification of colonies.

3. RESULTS & DISCUSSION

3.1. Ca and Bitterness Determination in Cheese

Calcium was calculated as 205 mg/L in fresh white cheese and 357.7 mg/L in bitter white cheese according to AOAC Method 991.25 [18]. The amount of calcium was increased as the bitterness was increased.

3.2. Total Titratable Acidity Determination

The cheese acidity is important because the growth of microorganism and enzymatic activity depends on acidity during the maturation process also the taste and flavor is affected from acidity [25,26]. After applying titratable acidity, acidity in fresh white cheese was 0.998% and 1.099% in bitter white cheese (both as lactic acid). In TS-591 White Cheese Standards, titratable acidity in white cheese was limited with 3% and both results were lower than this limit value, so bitterness showed no relation with growing acidity [27].

3.3. Studying Fatty Acid Composition in Cheese Using Gas Chromatography

According to gas chromatography results shown in Figure. 1&2, butyric acid which was short chain fatty acid found in fresh white cheese; however, wasn't present in rancid white cheese. Table. 2 reveals that amounts of caproic acid, caprylic acid, lauric acid, myristic acid and margaric acid were decreased in bitter white cheese as compared to fresh white cheese. The other free fatty acids (C11 to C20:1) were increased as bitterness were increased. Free fatty acids cause bitter flavor in cheese because of lipolysis; however, the bitterness in cheese and short-chain free fatty acid aren't clearly related in the literature [28]. Woo and Lindsay [29] stated that excess amount of free fatty acids (C10 and C12) cause bitter flavor.

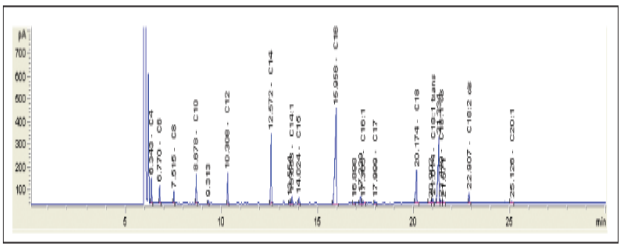

Figure 1: *Gas Chromatography of fresh cheeses*

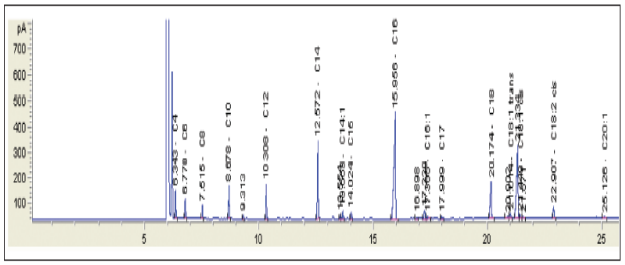

Figure 2: *Gas Chromatography of bitter cheeses*

Table 2: *Organic fatty acid composition of fresh and bitter cheeses*

Peak %		Fatty acid type
New cheese	Bitter cheese	
3.981	0.000	C4
2.551	2.071	C6
1.566	1.247	C8
3.471	3.471	C10
0.069	2.706	C11
3.888	3.197	C12
0.131	0.129	C13
11.777	11.188	C14
0.046	0.036	C14:1
1.108	1.198	C15
0.000	0.000	C15:1
31.763	32.764	C16
0.395	0.411	C16:1
0.502	0.513	C17
8.32479	8.92917	C18
1.35851	2.30404	C18:1 trans
18.18780	20.89523	C18:1 cis
0.16141	0.21634	C18:2 trans
0.09473	0.12181	C18:2 cis
0.13488	0.14245	C18:3 n6-C20
0.09079	0.09813	C18:3n3
0.41932	0.55765	C20:1
0.06769	0.05488	C22
0.11118	0.10068	C20:3n3
0.10198	0.05533	C22:2

3.4. Microbiological Analysis

For determining Bacillus cereus presence, microorganisms planted on MYP agar gram coloring images and API 50 CHB test had result been taken and showed no Bacillus cereus presence. It had been found out that microorganisms isolated from M17 agar that planted for Lactic streptococcus presence, were coccus shaped and Gram (+) after running gram coloring test and microscope examination. In addition, according to API 20 Strep test, there was a 90% chance that it was Streptococcus (Figure. 3).

Microorganisms isolated from Tributyrin agar to determine Lipolytic bacteria presence were rod shaped and Gram (-). After that, API 20E test was applied and results showed that 99% chance of Pseudomonas presence (Figure. 3). Same procedure applied on microorganisms that isolated from Pseudomonas CFC selective agar and 99% chance of Pseudomonas presence result was obtained again.

Figure 3: *General view of API test*

Microorganisms that contaminate milk from various sources reproduce fast. Some of these microorganisms are saprophytic and causes bad smell and aroma in cheese by producing metabolites by using resources in cheese such as protein, fat and carbohydrates. As a result of that, rancidity, bad smell and acidification takes place in cheese [30]. In this study, this result was confirmed by microbiological analysis. Two bacteria were found for reason of this situation. One of these bacteria was Streptococcus and some strains of Streptococcus were able to ferment lactose. Faulty disintegration of proteins by Streptococcus and micrococcus causes bitter taste by producing specific peptides. Some other microorganism activity causes caustic taste because of growing fat acid, monoglyceride and diglyceride concentration because of fat hydrolysis [30]. Based on this information, it was believed that Streptococcus was responsible for rancidity in white cheese. But a psychrophilic pseudomonas bacterium has higher profile for rancidity compared to Streptococcus. Along with Streptococcus, a second bacterium that's been found was Pseudomonas [31]. Morul and Isleyici [31] stated that growing numbers of psychrophilic microorganisms causes bitterness, rancidity and color changes in cheese, half of the research samples showed significant amount of such microorganisms and enzymes such as lipase and protease is another cause for these taste defects.

4. CONCLUSIONS

In this study, it was determined that Ca amount and bitterness were related. The bitterness was increased as the amount of calcium was increased. In the gas chromatography, butiric acid, one of the small chained fat acids, wasn't present but, caproic acid, caprylic acid, lauric acid, myristic acid and margaric acid amount

were decreased in bitter white cheese, compared to fresh white cheese. It was believed that Lipase enzyme produced by psychotropic Pseudomonas disintegrates fat acid into smaller pieces which causes undesired bitter taste in cheese. Our results and researches done by other studies support this idea [30].

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