

Cheddar Cheese Characterization and Its Biochemical Change during Ripening

Asfawosen Mamo¹

¹Department of Chemical Engineering, Adama Science & Technology University, PO BOX 1888, Adama, Ethiopia

Abstract

Cheddar cheese is classified as a hard cheese, with a long shelf life and without a surface flora. The objective of this work was to characterize and analyze the biochemical change of cheddar cheese during ripening. Cheddar cheese ripening was evaluated for 45 day of storage period at an interval of 15 days. The analyses made for biochemical change were glycolysis, lipolysis, proteolysis and ripening index. The proximate composition analysis was conducted during 0 day and after 45 day of ripening.

Glycolysis, lipolysis, proteolysis and ripening index increased significantly ($P < 0.05$) during this ripening period. But the PH decreased from 5.2 to 4.73. Regarding the proximate composition, the green cheese contained considerable amount of protein (27.68%), moisture content (36.59%) crude fat (29.45%) and ash (2.45%). After the ripening period of 45 days, it showed significant change ($P < 0.05$) in the content of protein (24.13%), moisture content (34.09%), crude fat (26.44%) and ash (2.30%). The sensory panels also rated the cheddar cheese acceptable with respect to flavor, color, mouth feel, odor, taste and appearance..

Keywords: cheddar cheese, glycolysis, lipolysis, proteolysis, ripening index

1. Introduction

Milk can be consumed in different forms depending on the nature of production. Dairy products (cheese, yoghurt, butter...) are found to be important in nutritional content. Among these different types of dairy products, cheese is source of different nutrient used for human health development. For the production of cheese varieties, different kinds of raw materials (milk, starter culture, rennet...) are vital. Along with the different raw materials needed,

rennet plays a great role in production of cheese (Walstra et al., 2005).

Cheese ripening is a very complex biochemical process by which the rubbery or elastic curd is converted into a smooth-bodied and fully flavoured cheese. Flavour and texture are considered as the two main criteria in determining the acceptability of aged cheese. The time required developing characteristic flavour and texture varies from a few weeks for soft cheeses up to three years for very hard varieties. During this period, cheeses attain their own characteristics through a multitude of chemical, microbiological and biochemical changes whereby protein, fat and residual lactose are broken down to primary products which are further degraded to secondary products (Kheadr et al., 2003).

Cheese ripening is a complex process involving a range of microbiological and biochemical reactions. Microorganisms present in cheese throughout ripening, play a significant role in the ripening process (Cogan and Beresford, 2002). Cheese manufacture and ripening involves the action of enzymes (from rennet and milk) and selected microorganisms, both directly, while growing, and indirectly, through their enzymes after death and lysis (McSweeney, 2004).

The biochemical changes occurring during ripening may be grouped into primary events that include the metabolism of residual lactose (glycolysis), lipolysis and proteolysis. Following these primary events, secondary biochemical events are very important for the development of many volatile flavour compounds and include the metabolism of fatty acids and of amino acids (McSweeney, 2004). A fine equilibrium between primary and secondary products has been shown to be responsible for typical cheese flavour and texture (Kheadr et al., 2003).

Glycolysis

Glycolysis is the conversion of lactose to lactic acid and is due to the growth of starter bacteria and the

lactate produced gives the freshly made cheese its overall acidic taste. They can also produce diacetyl, acetate and acetaldehyde, which are important compounds in flavour formation in fresh cheeses; diacetyl is also an important flavor compound in hard cheeses (Cogan and Beresford, 2002).

Proteolysis

Proteolysis is the most important event and the most complex (Cogan and Beresford, 2002; de Wit *et al.*, 2005). Proteolysis is very important for cheese texture by hydrolyzing the *para*-casein matrix which gives cheese its structure and by increasing the water binding capacity of the curd (i.e. to the new α – carboxylic and α – amino groups produced on cleavage of peptide bonds). However, the major role of proteolysis in cheese flavour is in the production of amino acids which act as precursors for a range of catabolic reactions which produce many important volatile flavour compounds (McSweeney, 2004). A high correlation exists between the intensity of Cheddar cheese flavour and the concentration of free amino acids (Fox, 1989). Due to features such as high proteolytic and lipolytic activities, some yeast species play an important role in the formation of aroma precursors such as amino acids, fatty acids and esters (Ferreira and Viljoen, 2003).

Proteolysis contributes to textural changes of the cheese matrix, due to breakdown of the protein network, decrease in water activity through water binding by liberated carboxyl and amino groups and increase in p^H , which facilitates the release of sapid compounds during mastication. It contributes directly to flavour and to off-flavour (e.g. bitterness) of cheese through the formation of peptides and free amino acids as well as liberation of substrates (amino acids) for secondary catabolic changes, i.e. transamination, deamination, decarboxylation, desulphuration, catabolism of aromatic amino acids and reactions of amino acids with other compounds (Sousa *et al.*, 2001).

Lipolysis

Lipolysis makes an important contribution to overall flavor development in cheese during ripening, especially in varieties such as Blue and hard Italian types and is mediated by lipases originating from milk, starter, non-starter and secondary starter bacteria (Anderson *et al.* 1991). While milk contains a very potent indigenous lipoprotein lipase (LPL), it normally never reaches its full activity in milk (Fox & Stepaniak, 1993; Fox *et al.* 1993) and high-temperature short-time (HTST) treatment (72.8°C for 15 s), very extensively inactivates the enzyme (Deeth & Fitz-Gerald, 1983). However, it is still thought to

contribute to lipolysis in pasteurized-milk cheese as a time and temperature combination of 78°C for 10 sec. is required for its complete inactivation (Driessen, 1989).

Lipolytic degradation of triglycerides of milk fat during cheese ripening results in the release of free fatty acids (FFA) which are further catabolized to highly flavoured compounds including methyl ketones, thioesters and lactones. FFA, particularly short chain fatty acids, are highly flavoured and, at high levels, butyric acid and other short chain fatty acids have been associated with the lipolysed flavour defect or rancidity in Cheddar cheese (Deeth & Touch, 2000).

2. Materials and methods

Cheddar Cheese was produced from the extracted rennet using the following procedure.

2.1 Proximate analysis of cheddar cheese

Fat content determination

The crude fat content in the cheddar cheese sample was determined by taking 3g dried sample and running through Soxhlet apparatus for 2-3 hours using petroleum ether as a solvent following the procedure described in AOAC (2000) method No.30-10.

$$\text{Crude fat (\%)} = \frac{\text{Wt. of fat}}{\text{Wt. of cheese sample}} * 100 \dots (1)$$

Crude protein determination

The crude protein content in raw material was estimated according to the Kjeldahl's method as described in AOAC (920) method No. 87. Two grams sample was weighed and put into the digestion tube. Twenty milliliters of concentrated sulphuric acid (98%) and 2 tablets of digestion mixture as catalyst were added into the digestion tube. The digestion was carried out for 3-4 hours (till the digested contents attained transparent color). The digested material was allowed to cool at room temperature and diluted to a final volume of 50 mL. The ammonia trapped in H₂SO₄ was liberated by adding 40% NaOH solution through distillation and collected in a flask containing 4% boric acid solution, possessing methyl indicator and titrated against standard 0.1N H₂SO₄ solution. The factor 6.38 was used for the conversion of percent nitrogen into crude protein contents of the cheddar cheese.

$$\text{Total Nitrogen} = \frac{14.007 \times (T - B) \times N \times 100}{W} \dots (2)$$

Where: T: volume in mL of the standard sulphuric acid solution used in the

titration for the test material.
 B: volume in mL of the standard sulphuric acid solution used in the titration for the blank determination.
 N: Normality of standard sulphuric acid.
 W: Weight in grams of the test material.

Percent nitrogen was multiplied by factor 6.38 for the cheddar cheese to calculate percent protein. The calculated protein to be on a total nitrogen basis.

Ash content

The raw materials (cheddar cheese) was tested for total ash content by taking 3g sample in tarred crucibles and charred on a flame until it turned black and put into a muffle furnace maintained at a temperature of 550 °C for 5 hours or till a grey color of ash was obtained. The details described in AOAC (2000) method No.08-01 were followed for the estimation of total ash contents. The ash content was calculated according to the formula given below:

$$\text{Ash (\%)} = \frac{\text{Wt. of ash}}{\text{Wt. of sample}} * 100 \dots\dots(3)$$

Moisture content

The moisture content in the raw material (cheddar cheese) and sample was determined according to AOAC (2000) method No. 44-15A by taking 5 gram sample and drying it in an air forced draft oven at a temperature of 105±5°C till a constant weight of the dried material is attained. The moisture content was calculated according to the following formula:

$$\text{Moisture (\%)} = \frac{\text{Wt.o} - \text{Wt.d}}{\text{Wt.o}} * 100 \dots\dots(4)$$

Where: *Wt.o* weight of original sample.
 : *Wt.d* weight of dried sample

Mineral contents

The mineral contents like K, Ca Na, P and Fe in the cheddar cheese was determined by the method described in AOAC (1990). One gram of sample was digested with 10 ml of nitric acid: perchloric acid (7:3) mixtures at temperature up to 180-200°C till transparent contents were obtained. The contents were diluted to a volume of 100 ml with double distilled water. Concentration of mineral contents was determined by running the diluted samples through Atomic Absorption Spectrophotometer (Model: Varian, AA-240, Victoria, Australia) using air Acetylene flame.

2.2 Biochemical change analysis

Glycolysis

Titrate acidity of the cheddar cheese samples was determined within the interval of 15 days by the

method recommended by AOAC (1975) and was expressed as percent lactic acid.

Proteolysis

Soluble nitrogen in the cheese slurry samples during ripening was measured by the method of Kosikowski (1997). In this procedure, the soluble nitrogen fraction is extracted in a suitable solvent (Sharpe’s Solution) and the nitrogen content estimated as for total nitrogen.

An accurately weighed sample of 3.0 g was taken in a mortar and a small amount of Sharpe’s extraction solution, tempered to 40°C was added to it. The contents were ground into a paste. More solution was added to make a dilute suspension. The contents were transferred into a 100 ml volumetric flask and the volume was made up using Sharpe’s extraction solution. The flask was kept in a water bath maintained at 50°C for 1 hr with occasional shaking. It was then filtered through Whatman No. 42 filter paper. Twenty milliliters of the filtrate was then transferred to a Kjeldhal flask. It was digested, distilled and titrated against a standard acid as in the determination of the nitrogen. Simultaneously, a blank was also run. The soluble nitrogen was calculated by the following formula:

$$\text{Percent soluble nitrogen} = \frac{S-B \times N \times 14.007 \times 100 \times D}{W \times 1000} \dots\dots(5)$$

Where,
 S= Titre value of sample
 B= Titre value of Blank
 W= Weight of sample (g)
 N= Normality of HCl used

$$D = \text{Dilution factor} = \frac{\text{Volume made up}}{\text{Volume of aliquot taken}}$$

Lipolysis

Total free fatty acids (FFA) were analyzed by the method of Deeth and Fitz-Gerald (1983). The method is based on the extraction of fat from a known quantity of cheese and estimating the FFA in the extracted fat by titrating against a standard alkali. Three grams cheese sample was taken in a 50 ml stoppered test tube and made into a paste, using 5 ml distilled water. To this, 10 ml extraction mixture followed by 6 ml petroleum ether was added. The test tubes were stoppered and tempered at 40°C for 10 minutes. The contents were vigorously shaken for 20 second. The two layers were allowed to separate (5-10 minute) and a 5-ml aliquot of the upper layer was withdrawn and transferred to a 50-ml conical flask. After addition of 6 drops of methanolic-phenolphthalein indicator, the contents were titrated

against 0.02N methanolic KOH. The reagent blank was used to obtain background. The FFA content of cheese was determined using the following formula :

$$FFA (\% \text{ oleic acid}) = 1.8048 \times V/W \dots \dots (6)$$

Where,

V= volume of 0.02N KOH used for titration

W= weight of cheese sample

Ripening Index

Ripening index (RI) was proposed by Alais (1984) estimated as the formula:

$$(\text{Water Soluble Nitrogen/Total Nitrogen}) \times 100 \dots \dots (7)$$

2.3 Sensory analysis

Cheddar Cheese samples was evaluated organoleptically by sensory panels / experienced personnel for flavor, color, appearance, taste, mouth feel and overall acceptability using 9 points hedonic score system with following individual scores: 9 = like extremely, 8 =like very much, 7 = like moderately 6 = like slightly, 5 = Neither like nor dislike, 4 = dislike slightly, 3 = dislike moderately, 2 = dislike very much, 1 = dislike extremely to find out the most suitable product (Peryam, et al 1952).

3 Results and Discussions

3.1 Proximate analyses

Table 1: Proximate composition of cheddar cheese (per 100gm)

Cheddar cheese	0 day	45 day
%Moisture content	36.59±0.88 ^a	34.09±0.99 ^b
% Crude fat	29.45±1.43 ^a	26.44±0.79 ^b
%Crude protein	27.68±1.09 ^a	24.13±1.21 ^b
%Ash	2.45±0.51 ^a	2.30±0.5 ^a
%Total carbohydrates	3.84±0.98 ^a	12.30±0.67 ^b

All values are means of triplicate ± standard deviation

^{a-b}Means with the same superscript letters within a column are not significantly different (p>0.05).

Table (1) summarizes the proximate composition of experimental cheddar cheese. The cheese composition had been studied in green cheese (0 day) and ripened cheese (45 day). The result showed that there was significant difference (P<0.05) in moisture content during ripening period. The moisture content at 0 day was 36.59% and after ripening decreased to 34.09%, indicating the significant effect of the ripening stage on cheese water binding.

The crude fat and protein content within the ripening period showed significant changes (P<0.05) from

29.45% to 26.44% and 27.68% to 24.13% respectively. This is due to the cheese being a biochemically dynamic product that undergoes significant changes during ripening (McSweeney and Sousa, 2000) and these changes were as a result of several microbiological, biochemical and metabolic processes.

The ash content of the experimental cheddar cheese didn't show significant change (P>0.05) throughout ripening as had been expected. The Cheddar cheese composition obtained in present study was in accordance with the findings of Hughes and Willenberg (1993) who proposed the average chemical composition of Cheddar cheese as moisture 37%, protein 25%, fat 33% and ash 4%.

Mineral content

From the table (2) it was observed that the mineral content of the experimental cheddar cheese was not significantly different (P>0.05). As it had been expected the calcium content of the cheese was more than any other types of mineral. It is for the reason that cow milk is higher in mineral content especially calcium (Fundora *et al.*, 2001). On acidification, similar solubilization of calcium and other minerals was observed by Ahmad *et al.* (2008) in cow milk that validates the non-significant changes in mineral content during ripening of cheese.

Table 2: Mineral content of cheddar cheese (mg / 100 gm)

Cheddar cheese	0 day	45 day
Sodium	788.54±1.88 ^a	791.41±3.05 ^a
Iron	2.11±0.02 ^a	2.06±0.07 ^a
Calcium	849.20±3.69 ^a	853.91±4.30 ^a
Phosphorus	755.02±3.91 ^a	753.17±3.98 ^a
Potassium	136.49±2.79 ^a	138.51±4.18 ^a

All values are means of triplicate ± standard deviation

^aMeans with the same superscript letters within a column are not significantly different (p>0.05).

3.2 Biochemical change analysis

Table 3: Biochemical analysis of cheddar cheese within 45 days.

Ripening day of cheese	0	15	30	45
% titrable acidity	0.053±0.008 ^c	0.094±0.004 ^b	0.127±0.007 ^a	0.141±0.02 ^a
P ^H	5.2±0.21 ^a	4.88±0.24 ^{ab}	4.82±0.25 ^a	4.73±0.27 ^b
% total free fatty acid	0.599±0.01 ^c	0.946±0.131 ^b	1.21±0.036 ^a	1.22±0.02 ^a
% soluble nitrogen	0.83±0.55 ^d	1.19±0.56 ^c	1.46±0.82 ^b	1.65±0.50 ^a
Ripening index (%)	15±1.63 ^d	21±2.08 ^c	25±1.87 ^b	28±2.39 ^a

All values are means of triplicate \pm standard deviation.

^{a-c} Means with the same superscript letters within a row are not significantly different ($p > 0.05$).

In this study Glycolysis was expressed as titrable acidity change on the cheddar cheese. From the table (3) the trend showed that the titrable acidity increases from 0 day to 15 days of ripening significantly ($P < 0.05$). Though some change is observed from day 30 to 45 the change was not significant. Cheese is a fermented dairy product and hence the controlled production of lactic acid from lactose by lactic acid bacteria is an essential step during the manufacturing and ripening.

The value of acidity found in the experiment is in accordance with (Vernam and Sutherland, 1994), who reported the mean acidity of Cheddar cheese. The increased in acidity showed the activity of starters, because the primary function of starters is the conversion of lactose and other sugars in milk to lactic and other acids (Azarnia et al., 2006). A critical factor in the control of Cheddar cheese quality is consistency in the rate and extent of acid production by the starter cultures (Banks et al., 2001). Residual lactose is metabolized rapidly to lactate during the early stages of ripening.

The P^H value of the cheddar cheese as stated in table (3) reveals that there is gradual decrease of acidity significantly ($P < 0.05$). As cheese is a fermented dairy product, the metabolism of lactose to lactate by selected cultures of lactic acid bacteria (LAB) decrease the p^H value. The p^H of cheese affects the texture of curd directly by influencing the solubility of the caseins; all else being equal, high p^H cheeses are softer than more acid cheeses. P^H also affects texture and flavour indirectly by affecting the activity of enzymes important to ripening and, in the case of the coagulant, the retention of enzyme in the curd during manufacture (Creamer et al. 1985).

Lipolysis is a process in change of lipid in to fatty acid through the action of lipase. It is expressed as a change of lipid into total free fatty acid. From the table 3 it was observed that the levels of FFA acids increased significantly ($P < 0.05$) throughout the ripening period. The most notable increase in TFFA was observed within 15 ripening period in cheddar cheese.

The flavour of mature cheese was the result of a series of biochemical changes that occur in the curd during ripening, caused by the interaction of starter bacteria, enzymes from the milk, enzymes from the rennet and accompanying lipases and secondary flora (Urbach, 1997). Lipid hydrolysis resulted in the formation of FFA, which may, directly, contribute to cheese flavour and also serve as substrates for further

reactions producing highly flavoured catabolic end products. Depending on their concentration and perception threshold, volatile fatty acids can either contribute positively to the aroma of the cheese or to a rancidity defect. The flavor effect of FFA in cheese was regulated by P^H . It is generally agreed that FFA, at appropriate concentrations, contribute to desirable flavours in Cheddar cheese (Reddy & Marth, 1993), while excessive lipolysis leads to undesirable or 'rancid' off-flavors.

Proteolysis is the hydrolysis of protein in to free amino acids. In this study the proteolysis is studied in terms of percentage of soluble nitrogen. As it can be observed from table (3) the amount of soluble nitrogen increased significantly ($P < 0.05$) throughout the 45 ripening days. In line with the lipolysis, proteolysis result also showed relatively higher increment up to 15th days of ripening.

The formation of soluble nitrogen compounds during ripening is an index of the rate and extent of proteolysis, in that it is an indicator of casein hydrolysis brought about by the action of the rennet and the milk proteases present at the start of ripening (Pirisi et al. 2007).

Ripening index (soluble nitrogen/total nitrogen (TN) x 100) values increased throughout ripening in cheddar cheese sample, however, RI levels were significantly higher ($p < 0.05$) in all ripening periods. Soluble nitrogen compounds increment during ripening shows casein hydrolysis by the action of the rennet and the milk proteases and starter cultures present at the initiation of ripening (Irigoyen et al., 2001).

Sensory evaluation

Table 4: Sensory characteristics of cheddar cheese

Cheddar cheese	Pre-ripened	ripened
Color	7.15 \pm 0.47 ^b	7.95 \pm 0.64 ^a
Appearance	7.70 \pm 0.48 ^b	8.30 \pm 0.63 ^a
Odor	5.95 \pm 0.92 ^b	7.98 \pm 0.72 ^a
Taste	5.80 \pm 0.89 ^b	8.15 \pm 0.53 ^a
Mouth feel	5.20 \pm 0.91 ^b	8.35 \pm 0.47 ^a
Flavor	4.60 \pm 0.77 ^b	8.0 \pm 1.01 ^a
Overall acceptability	6.15 \pm 1.15 ^b	8.05 \pm 0.36 ^a

All values are means of ten panelist \pm standard deviation

^{a-b} Means with the same superscript letters within a column are not significantly different ($p > 0.05$).

The mean scores for the sensory characteristics of the experimental cheeses are presented in Table (4). Results of sensory evaluations showed that significant ($P < 0.05$) differences were found within ripening

period in terms of color, appearance, odour, taste, mouth feel, flavor and overall acceptability. This is due to the fact that during cheese ripening, the biochemical and metabolic processes are responsible for the basic flavour and textural changes (Collins *et al.*, 2003; Lucey and Singh, 2003; Smit *et al.*, 2005). Singh *et al.* (2003) illustrated that the characteristic flavour, aroma, texture and appearance of cheese develop during ripening and these changes are predetermined by the composition of milk and starter culture.

4. Conclusion

The biochemical change (i.e glycolysis, lipolysis and proteolysis) of cheddar cheese had been conducted up to 45 day of ripening time with 15 day interval. During this ripening period, the result showed significant increment from 0.053% to 0.141% in titrable acidity and the P^H decreased from 5.2 to 4.73 which were indicative for activity of starter cultures. The total free fatty acid and soluble nitrogen, which indicate the lipolysis and proteolysis respectively, increased during ripening. This showed casein hydrolysis by the action of rennet, the milk protease and starter culture present at initiation of ripening. The proximate composition of cheddar cheese was analyzed on 0 day and after ripening of 45th day, and the result showed significant change (P<0.05) in moisture, fat content, protein, ash and carbohydrate. These changes were as a result of several microbiological, biochemical and metabolic processes. Besides the sensory evaluation using nine point hedonic scale showed the cheddar cheese was accepted by panelists and it had overall acceptability of 8.05. Thus, the cheese made with extracted calf rennet had good biochemical and acceptable sensory characteristics. The data illustrate that the calf rennet is promising in providing minimum coagulation time which results better strength. This rennet can be replaced by imported rennet which is being used in Ethiopia for the production of cheese.

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