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ORIGINAL ARTICLE

Some Chemical and Microbiological Characteristics of Gouda Cheese

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ABSTRACT
The present study was aimed to produce Gouda cheese at laboratory level and to evaluate the chemical and microbiological quality of the resultant cheese. The results showed that Gouda cheese contained high fat content which was 41±0.4% and 38±0.4% in the laboratory made Goudacheese (LMGC) and in the Dutch Gouda cheese (DGC), respectively.However, the protein contents of LMGC and DGC were 25±0.6% and 23.8±0.6%, respectively. Non-significant differences were noticed between the manufactured and Dutch Gouda cheese in fats and protein contents. The contents of Caproic acid (C_6), Caprylic acid (C_8) and Capric acid (C_{10}) were 0.24%, 0.51% and 1.88% in LMGC respectively. The essential amino acids of LMGC constituted 41% of total amino acids. The essential amino acid found in LMGCwere : isoleucine (4.79%), histidine (2.27%), leucine (9.25%), lysine (8.11%), methionine (2.41%), phenylalanine (3.73%), threonine (6.82%) and valine (4.62%). The non-essential amino acids included: alanine (3.26%), arginine (2.26%), aspartic acid (0.19%), cysteine (0.49%), glutamic acid (6.45%), glycine (17.40%), serine (4.07%) and tyrosine (3.39%). The microbiological analysis revealed that the total viable count of LMG was $2X10^5$ cfu/g, while the counts for streptococcus, yeast and mould were$1.4X10^7$cfu/g and $2X10^5$cfu/g, respectively. However, cheese samples were devoid from harmful microorganisms. The study recommends encouraging the local dairy industries to produce more Gouda cheese to meet the local needs.
***Key words**: Cheese, fat, protein, total viable counts, salmonella, yeast and moulds*

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INTRODUCTION

The existence of seasonally surplus milk at low prices in many parts of Sudan has promoted a few entrepreneurial merchants to set up small-scale rural cheese-making plants which offer women higher prices for whole milk than they could otherwise obtain for buttermilk [1].

Sudanese traditional fermented foods represent the main source of nourishment for country and urban groups. Dairy products took part in improvement of the economy, finance and business of local societies. Although, numerous dairy product studies have been conducted in Sudan, information on the microbiology and technology is still sparse. The greater part of the research conducted has relevance to organisms associated with fermentation and those considered spoilage.

Gouda cheese, however, originate in South Holland and is like Edam cheese. At first the essential goal of cheese manufacturing was to broaden the time span of usability as and to conserve the wholesome of milk through fermentation [2]. Presently, around 33% of the world's milk production is utilized as a part of cheese manufacturing [3].

Gouda cheese must contain a minimum of 28% fat (49% fat on dry matter basis) and a maximum of 43% moisture. Gouda is made in round or block forms and the cheese vary in weight from 600 g to 20 kg. A gas-forming culture is used to induce eye formation[4].

As per FAO [5] Gouda is a matured firm/semi-hard cheese. The body has a close white or ivory through to light yellow or yellow colour and a firm-textured (when squeezed by thumb) texture, appropriate for cutting, with few to copious, more or less round pin's head to pea sized (or generally up to 10 mm in diameter) gas holes, dispersed in a sensible consistent way all through the interior or the cheese, however, few openings and splits are acceptable. The shape is of a flattened cylinder with convex sides, a flat block, or a roll. The cheese is made and sold with a dry skin, which might be covered. Gouda of flat block or loaf shape is also sold without rind. The objectives of the present study was to evaluate the microbiological and chemical characteristics of the manufactured Gouda cheese and to compare the chemical components between the Gouda cheese at laboratory level and Dutch Gouda cheese.

MATERIALS AND METHODS

Preparations of Gouda cheese at laboratory level

Gouda cheese was prepared at the laboratory level according to the procedure followed at KenanaDariyFriezland Company ((KFD). Firstly, 300 liters of cow's milk were pasteurized in a pasteurizer for 30 minutes at 72° C, and then cooled to 30°C. 75 ml of calcium chloride and 150 ml of sodium nitrate were then added to the cooled milk. After that 3 liter of starter culture were added (1% of milk), and the mixture was blended. After 15 minutes, 4 grams rennet and 3.8 ml of annatto coloring matter were added to mixture. The mixture was blended for 15 minutes and was let for 3 hours until the curds separated from the whey. The curd was then washed by water and pressed into circular moulds for 2 hours. Next, the cheese was soaked in a 20% brine solution. After immersion in the brine for 3 hours, the cheese was then dried for 2 days before being coated. Then cheese was placed in a cheese curing room for 6 weeks at a temperature of 10°C.

Determination of fats and fatty acids contents

Gerber tube was used to determine fat content of different samples according to the method described by DE Vleeschauwer et al. [6]. The sample was homogenized by placing it in water bath at 40°C and cooled to 20°C. The butyrometer was filled with 10 ml H_2SO_4. 11 ml sample was pipetted in to the butyrometer. Then 2 ml amyl alcohols were added and the butyrometer was closed with a stopper. The contents were shaken vigorously until all protein particles were dissolved. The butyrometer was then placed in a water bath at 65°C for 5 minutes (fat column under the water surface). Moving the stopper regulated the fat column. The butyrometer was then placed in a centrifuge for 5 minutes, and then placed in a water bath at 65°C for 5 minutes. The fat content was read directly on the butyrometer scale in g/100 g sample.

Fatty acid composition of different samples from different sources was analyzed by gas liquid chromatography (GLC) using capillary columns.Lipid samples were converted to their constituent fatty acid methyl esters by the method of Timms and Watts [7]. The analysis of the fatty acid methyl ester was performed on a Hewlett-Packard GC (model HP 6890 series) with a SGE bpx 5 column (50.0 m x 0.22 mmi.d) and quantified by flame ionization detector (FID) . The split ratio was 20:1. The GC conditions were as follows: injection port temperature was 250°C; flame ionization detector temperature was 260°C. Oven temperature program was set at an initial temperature of 50°C for 1 min, then raised to 140°C at 5°C/min and held at 140°C for 5 min, and again the temperature was raised to 250°C at 5°C/min. The carrier gas was helium. The column flow rate was 1.9 ml/min. In the detector, helium gas flow was 30 ml/min. The sample size injected for each analysis was 1 ml. Samples were manually injected into the GC port. Compounds were identified by comparison with the retention times of known standards (Supelco™ 37 component FAME mix and also two pure FAMEs).

Determination of protein and free amino acids contents

Protein content was determined by Kjeldahl procedure according to AOAC [8]. One ml of sample (milk) was placed in a digestion flask; copper catalyst and antibumping granules were added. Then 25ml of concentrated sulfuric acid were added and the content was digested within two-three hours. After digestion, the contents were cooled and diluted to 100ml by addition of distilled water. Five ml of diluted digest were poured into the distillation unit and 10ml of 40% sodium hydroxide were added. Twenty-five ml of boric acid with 2-3drops of methyl red were added to the digest and placed in a conical flask. Distillation of the reaction mixture liberated ammonia and reacted with boric acid, changing the colour from red to light greenish-blue. Excess alkali was then titrated using (0.1N) hydrochloric acid, until color changed to light purple. The titration reading was recorded. The protein content was determined by multiplying the percentage of nitrogen by the empirical factor 6.36, as follow:

$$\% N = \frac{\text{Volume of HCL} \times T \times 14 \times \text{dilution factor} \times 100}{1000 \times \text{volume of sample}}$$

Then, % protein = % N ×6.36
Where:
14 = The molecular weight of nitrogen.
T = Normality of acid, HCL
N = Nitrogen content

The free amino acid contents were determined according to the method described by Alder-Nissen[9]. One gram of sample (cheese) was placed in a digestion flask; copper catalyst and antibumping granules were added. Then 25ml of concentrated sulfuric acid were added and the content was digested within two-three hours. After digestion, phosphotungstic acid (5%) and trichloroacetic acid (12%) and soluble nitrogen assays were performed on sample prepared by blending with a 2.0% sodium citrate diluents (pH 6.5). Amino acid quantification was performed by separation with HPLC, post column digitization with ninhydrin and UV detection. The concentration of free amino acids in the sample matrix was determined from averages of duplicate injections of the extract preparations. The results were reported as amino acid per 100 grams sample.

Microbial analysis

The viable count was enumerated by culturing them on Plate Count Agar (PCA) medium and incubating for 24 to 48 hours at 25^{o}C. For determination of total coliform, the most probable number (MPN) test was carried out according to APHA [10]. The multiple tube fermentation technique was performed as a presumptive test for total coliform using tubes containing Mac Conkey Broth and inverted Durham tubes. Inoculation was carried out as follows: (i) To each of 3 double-strength MacConkey broth tubes. All tubes were incubated at 370C for 48 hours for the observation of gas production. First reading was taken after 24 hours to record positive tubes, and the negative ones were incubated for another 24 hours

For *E. coli* test, plates showing positive coliform were subjected to the confirmation test using Brilliant green bile lactose broth in test tubes with Durham tubes. The test tubes were then incubated at 44^{o}C for 48 hours. Each confirmed positive tube was sub cultured into E.C broth media and then incubated at 44.5^{o}C for 24 hours. Tubes showing any amount of gas production were considered to be positive.

For determination of Staphylococcus count**,** from suitable dilutions, 0.1 ml was plated onto Baird Parker Agar media and the inoculums were distributed evenly using sterile glass rod. The plates were then incubated at 37^{o}C for 24-48 hours and the counts were presented as colony forming units per gram (cfu/g).

Salmonella detection was carried out by inoculating 100ml of cheese at 37^{o}C for 24 hours. Then 10 ml were drawn aseptically and added to 100 ml selenite broth. The broth was incubated at 37^{o}C for 24 hours. Then with a loopful streaking was done on dried Bismuth sulphite agar plates. The plates were then incubated at 37^{o}C for 72 hours.

Black metallic sheen discrete colonies indicated the presence of salmonella. A confirmatory test was carried out by taking a discrete black sheen colony and sub culturing it in a Triple sugar iron agar tubes. Production of a black colour at the bottom of the tube confirms the presence of salmonella.

Edward agar medium (Oxoid, CM27) was used for isolation of streptococcus from cheese sample. From sample 0.2 ml decimal dilutions 10^5 and 10^6 were streaked on the dried duplicate plates of the medium. The plates were incubated at 37^{o} C for 48 hours. Colonies of streptococcus were counted by colony counter and recorded. They were further characterized by sub-culturing on thin blood aesculin agar plates and by catalase test performed according to Barrow and Feltham[11]. Cultural and morphological characteristics were studied by Gram's Method.

The yeast and mould strains were enumerated by culturing them on Potato Dextrose Agar (PDA) medium and incubating for 72 hours at 25^{o}C.

RESULTS AND DISCUSSION

Fat and protein contents

The fat content (Table 1) was 41$\pm$0.4% and 38$\pm$0.4% in the LMGC and the DGC, respectively. This value of fat in the LMGC was lower than that of Gouda cheese determined by Wasseela[12], who determined a value of 54.0$\pm$3.67%. The LMGC contained a high content of fat than Sudanese white cheese determined by Osman and Omer [13] who reported a value of 22.8%, and higher than those reported by Alyand Galal [14] and Khalid and El Owni[15] who reported that the fats values were 12.80% and 11.70%, respectively in white cheese. The high average of fat content in the LMGC could be caused by the high pressure during the manufacturing process.

Table 1. Fat and protein contents (%) of Gouda cheese samples

Cheese type	Fat (%)	Protein (%)
LMGC	41 a	25 a
DGC	38 a	23.8 a
SE±	0.41	0.59

a: Means within the same raw bearing the same superscript were non-significantly deferent (P≤ 0.05).
LMGC: Laboratory-made Gouda cheese.
DGC: Dutch Gouda cheese

The protein level in the LMGC sample and DGC were 25±0.6% and 23.8±0.6%, respectively. The value of protein in LMGC was higher than that in Gouda cheese determined by Wasseela[12], who reported a value of 20.3±0.80%. The protein in LMGC was higher than that determined by Osman and Omer [13] in Sudanese white cheese which was 22.5%.

Fatty acids composition of LMGC

Fatty acids constitute over 90% of total edible fats and oils[16]. There are many naturally occurring fatty acids with very different chemical and physical characteristics. Unsaturated fatty acids, particularly those with more than one double bond, are susceptible to changes and alterations caused by chemical and physical factors. Free fatty acids (FFA) are likely to contribute to the flavor and odor of Gouda cheese[17][18]. Table(2) shows mean value of fatty acids composition of laboratory-made Gouda cheese (LMGC) expressed as g/100g of the total fatty acids. The contents of Caproic acid (C_6), Caprylic acid (C_8) and Capric acid (C_{10}) were 0.24%, 0.51% and 1.88% in LMGC, respectively. The obtained values of those fatty acids in LMGC were lower than those determined by Suleiman [19]; Sulieman[20] in the Sudanese traditionally fermented milk product (*Rob*); he found that the contents of Caproic acid (C_6), Caprylic acid (C_8) and Capric acid (C_{10}) were 2.02%, 1.9% and 2.18%, respectively. However, LMGC contained low values of some fatty acids like Lauric (C_{12}), Myristic (C_{14}), Palmitic (C_{16}) and Linoleic (C_{18}) which were 0.08%, 0.02%, 16.60% and 0.14%, respectively. These levels were too low compared to those of Koman and Stibilj (2000) who found that the contents of Lauric, Myristic Palmitic, and Linoleic in Dutch Gouda cheese were 2.24%, 9.41%, 28.42%, and 2.34%, respectively. The contents of other fatty acids like Margaric (C_{17}), Stearic (C_{18}), Oleic ($C_{18:1}$), Linolenic ($C_{18:3}$) and Arachidic (C_{20}) were 0.6%, 17.65%, 33.86%, 0.10% and 0.58%, respectively. The contents of these fatty acids, however, was in close agreement to Koman and Stibilj[21], who reported values of 1.02%, 14.54%, 30.32%, 0.08%, and 0.41% of the same mentioned fatty acids, respectively in their sample.

Table 2.Fatty acids content of Gouda cheese

No	Systematic Name	Common Name	Simpot	L.M.G.C %
1	Hexanoic	Caproic	6:0	0.24
2	Octanoic	Caprylic	8:0	0.51
3	Decanoic	Capric	10:0	1.88
4	Dodecanoic	Lauric	12:0	0.08
5	Tetradecanoic	Myristic	14:0	0.02
6	Pentadecanoic		15:0	0.24
7	Hexadecanoic	Palmitic	16:0	16.60
8	Heptadecanoic	Margaric	17:0	0.60
9	Octadecanoic	Stearic	18:0	17.65
10	Octadecenoic	Oleic	18:1	33.86
11	Octadecadienoic	Linoleic	18:2	0.14
12	Octadecatrienoic	Linolenic	18:3	0.10
13	Eicosanoic	Arachidic	20:0	0.58

L.M.G.C = laboratory-made Gouda cheese.

Total amino acids content of Gouda cheese

The amino acids derived from protein breakdown are precursors that are absolutely key to cheese flavor [22]. Amino acids are organic acids that are used by the body as basic building blocks to assemble thousands of complicated proteins. By definition, each amino acid has an amino (amine, NH_2) group and a carboxylic acid (carboxyl, COOH) group. The human body uses only about twenty of these amino acids in constructing its proteins. They are characterized as either essential or non-essential. Essential amino

acids are not manufactured by the body and must be obtained through diet. Non-essential amino acids can be manufactured by the body from other compounds (James, 2001). Table (3) shows the content of the amino acids in LMGC as g/100g total amino acids. The essential amino acids of LMGC constituted 41% of total amino acids. The essential amino acid found in included: isoleucine (4.79%), histidine (2.27%), leucine (9.25%), lysine (8.11%), methionine (2.41%), phenylalanine (3.73%), threonine (6.82%) and valine (4.62%). The non-essential amino acids included: alanine (3.26%), arginine (2.26%), aspartic acid (0.19%), cysteine (0.49%), glutamic acid (6.45%), glycine (17.40%), serine (4.07%) and tyrosine (3.39%).

Table 3.Total amino acids content of Gouda cheese

Amino Acid	mg/100g
Alanine (Aln)	3.26
Arginine (Arg)	2.26
Aspartic acid (Asp)	0.19
Cysteine (Cys)	0.49
Glutamic acid (Glu)	6.45
Glycine (Gly)	17.40
Histidine (His)	2.27
Isoleucine (Ile)	4.79
Leucine (Leu)	9.25
Lysine (Lys)	8.11
Methionine (Met)	2.41
Phenylalanine (Phe)	3.73
Serine (Ser)	4.07
Theronine (Thr)	6.82
Tyrosine (Tyr)	3.39
Valine (Val)	4.62

LMGC = laboratory-made Gouda cheese.

Microbiological characteristics of Gouda cheese

The count of the different microbial groups investigated in the laboratory-made Gouda cheese (LMGC) is shown in Table (4). The total viable count was $2X10^5$cfu/g. The *streptococcus* in LMGC was $1.4X10^7$cfu/g. Streptococcus bacteria is a genus of coccus, or sphere like, Gram-positive, chained bacteria belonging to the lactic acid bacteria (LAB) group. According to Mou et al [23] all species of lactic *streptococci* contain the peptidases necessary for complete hydrolysis of casein to amino acids.

E.coli, *staphylococcus* and *salmonella* were not found in the various samples. The absence of these pathogenic bacteria gives Gouda cheese the opportunity for consumption without fear of causing food borne diseases. *Escherichia coli* (abbreviated as *E. coli*) are a large and diverse group of bacteria. Although most strains of ***E. coli*** are harmless, others can cause disease. Some kinds of ***E. coli*** can cause diarrhea, while others cause urinary tract infections, respiratory illness and pneumonia, and other illnesses[24]. Staphylococci can cause a wide variety of diseases in humans either through toxin production or invasion. For example the most common cause of food poisoning is staphylococci toxins. The bacteria grow in in-properly stored food, the cooking process kills them but the toxins they produce are heat resistant. Staphylococci can grow in foods with relatively low water activity (such as cheese and salami [25]. *Sa*lmonella refers to a genus of rod shaped bacterium. Some of these salmonella bacterium are responsible for many illnesses in humans and other animals. Most commonly, salmonella is the cause of food poisoning and typhoid fever. The freeness of Gouda cheese from contamination with the previously mentioned pathogens could be due to use of pasteurized milk in the process, as well as following hygienic practices during its processing at the laboratory.

Total yeast and mould in LMGC was $2.5X10^4$cfu/g. It is known that yeasts imparts flavor for dairy products, so the level of yeast found in this study was good for giving the cheese a good flavour.

Table 5.Microbiological characteristics of Gouda cheese

Cheese sample	Total viable counts cfu/g	Streptococcus cfu/g	E.coli cfu/g	Staphylococcus cfu/g	Salmonella cfu/g	Yeast and Mould Cfu/g
LMGC	$2X10^5$	$1.4X10^7$	0	0	0	$2.5X10^4$

LMGC: laboratory-made Gouda cheese; DGC: Dutch cheese

CONCLUSION

Microbial analyses showed the levels in the analyzed cheeses were within acceptable range and free from contamination. The high contents of fat made its taste unacceptable to the some, but generally it is accepted. No significant differences were noticed between the manufactured and Dutch Gouda cheese with regard to chemical components. From the result of this study we can draw the following recommendations: Encouraging the local industry of Gouda cheese and the processed cheeses to meet the local needs, and decreasing the percentage of fat in the Gouda cheese to be more acceptable, by using milk containing a lower level of fat.

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