RESEARCH | Egyptian Journal of Agricultural Research | Animal Production

Chemical composition and amino acids profile of Gouda cheese supplemented with denatured whey protein paste

Reham K. EL-Menawy, Magdy M. Ismail, Raid I. EL-Metwally*

Address:

Dairy Technology Research Department, Animal Production Research Institute, Agricultural Research Center, Dokki, Giza, Egypt

*Corresponding author: ***Magdy M. Ismail,*** abo-omar98@hotmail.com

Received: 25-12-2022; **Accepted**: 03-05-2023; **Published:**24-08-2023 **DOI**: 10.21608/ejar.2023.178794.1313

ABSTRACT

In this investigation, the denatured whey protein paste (DWPP) was utilized to improve the chemical composition, nutritional and healthy properties of Gouda cheese. Five treatments of Gouda cheese were made by mixing 0, 1, 2, 3 and 4% of DWPP with cheese curd. Supplementation of Gouda cheese with DWPP increased the values of yield, total nitrogen, non-protein nitrogen, total volatile fatty acids, K, Ca, Na and Fe. On contrary, DWPP cheese treatments contained less total solids and fat. Amino acids profile of cheese at the end of repining period exhibited higher essential amino acids (EAAs) and branched chain amino acids (BCAAs) for cheese fortified with DWPP. Addition of 1 and 2% DWPP to cheese increased the EAAs levels by 1.67 and 3.37% and raised the contents of BCAAs by 2.35 and 5.29% respectively. Significant improvement was observed in flavor of cheese contained 1 and 2% DWPP whereas adding 3 or 4% DWPP had negative effect on the body and texture of cheese. Subsequently, fortification of Gouda cheese with up to 2% DWPP could provide more additional nutritional and health benefits with improvements in chemical and sensory characteristics. From a manufacturing point of view, the addition of 1 or 2% DWPP increased Gouda cheese yield and consequently higher profits.

Keywords: Gouda cheese, Denatured whey protein paste, Amino acids profile, Yield

INTRODUCTION

Gouda cheese is a Dutch cheese that is majorly prepared from pasteurized cow's milk although a few artisan varieties utilize sheep's or goat's milk to make ripened cheeses that will be matured for a long time. Over the last decade, the production of Gouda cheese in various countries worldwide has increased significantly. For instance, in the Netherlands, the country of origin for this type of cheese, the amount of Gouda cheese produced in 2018 was over 555 million kilos (van Gelder, 2020).

The different types of cheese, including Gouda, have high nutritional and health values. Cheese contains most of the fat soluble vitamins, and also, it is a vital source of several nutritionally important elements including calcium, phosphorus, and magnesium (Fox *et al.,* 2000). In this trend, many studies have been carried out to raise the nutritional and health values of cheese. Regarding Gouda cheese, Indumathi *et al.* (2013) made iron fortified Gouda cheese. Choi *et al.* (2015) demonstrated that Gouda cheese fortified with fruit liquor (*Prunusmume* or *Cornus officinalis*) could give additional nutrients while keeping up flavor and quality. Kim *et al.* (2017) supplemented Gouda cheese with microcapsules of chili pepper extract. The results revealed that 0.5% pepper extract microcapsules fortification could provide further bioactive ingredients, besides maintain the quality of Gouda cheese. In a recent study by Khan *et al.* (2018), mango kernel fat (MKF) was utilized to fortress of Gouda cheese. Fortified cheese had superior antioxidant capacity, unsaturated fatty acids and oxidative stability. Ramos *et al.* (2022) stated that ripening of Gouda cheese results in a product with functional potential due to the presence of peptides with biological activity. Concerning supplementation of Gouda cheese with denatured whey proteins, no broad investigations are found.

Whey proteins represent a great source of amino acids and biologically active proteins that serve as a dietary (Khan and Selamoglu, 2019). It contains a high level of essential amino acids and branched chain amino-acids which are significant for the growth and fix of tissue. Leucine (branched chain amino acid) has imperative role in insulin and glucose metabolism (Castellanos *et al.,* 2006). The essential amino acids (EAAs) and branched chain amino acids (BCAAs) in whey are available in high level but also absorbed and utilized in the body more than other protein sources such as wheat, corn and soy (Marshall, 2005). Therefore, this study designed to increase the nutritional and health values of Gouda cheese and also to improve its chemical and sensory properties by supplementation with denatured whey protein paste.

MATERIAL AND METHODS

Materials:

Fresh cow's milk (acidity 0.17 %, pH 6.65, total solids 12.01, fat 3.92 and total protein 3.38 %) was obtained from El-Serw Animal Production Research Station, Animal Production Research Institute, Egypt. Cheese starter contained *Lactococcus lactis subsp. Lactis and Lactobacillus helveticus* was obtained from Chr. Hansen's Lab A/S Copenhagen, Denmark. As a coagulant, liquid calf rennet (107 IMCU⁄mL) was obtained from local market and was added to milk at a ratio of 30 mL⁄ 100 kg milk. Dry coarse commercial food grade salt was obtained from El-Nasr Company of Alexandria. Also, wax plastic was obtained from private company in Egypt.

Methods:

Preparation of DWPP:

Denatured whey protein paste was prepared as described by Ismail *et al.* (2011). The whey resulted from Ras cheese making (~100 kg) was skimmed and heated for 10 minutes at 95 °C, cooled and the flocculated denatured whey proteins were recovered by filtering through cheese cloth sacks overnight. The precipitate was moved to wooden frames and pressed (~1 par) for 2h. The resultant denatured whey protein paste had 0.26% acidity, 5.38 pH, 25. 27% total solids, 0.82 % fat, 15. 86% total protein and 7.34% salt.

Manufacture of Gouda cheese supplemented with denatured whey protein past:

Gouda cheese was prepared according to the method of Sulieman *et al.* (2018). Fresh cow's milk (200 kg) was pasteurized at 65ºC for 30 min, and cooled to 32ºC. After adding 0.02% $CaCl_2$, milk was inoculated with culture (Direct Vat Set) at 2 g/100 L and kept at 32ºC for 45 min. Then, rennet was added at 0.19 mL/kg of milk. After complete coagulation (~ 40 min.), curd was cut into cubes, settled for 10 min, and then stirred at 32°C for 20 min. A third of whey was drained, then an equal amount of warm water was added to raise the temperature to 39 ºC. From there on, more whey was drained, once again, warm water was added and simultaneously, stirring of the curd proceeded for 30 min. After all the whey had drained, the denatured whey protein paste (0, 1, 2, 3 and 4%) was mixed with the curd prior to molding. Subsequent to pressing (~1.5 par) overnight, the cheese was soaked in 19% NaCl solution for 8 h, dried in ripening room for two day, coated with plastic and matured in a cooling chamber (12±0.5°C, 85-90% relative humidity (RH)) for three months. Gouda cheese samples were analyzed when fresh and after 1, 2 and 3 months of repining period. Three replicates of each treatment were conducted.

Physicochemical analyses:

Titratable acidity, total solids, fat, ash, total nitrogen, water soluble nitrogen, non-protein nitrogen and mineral contents of cheese samples were determined as mentioned in AOAC (2006). The pH values of milk and cheese samples were estimated using a pH meter (Corning pH/ion analyzer 350, Corning, NY) after calibration with standard buffers (pH 4.0 and 7.0). Salt content was estimated utilizing Volhard method according to Richardson (1985). Total volatile fatty acids were determined according to Kosikowiski (1978).

Amino acid profile:

Amino acid profile of fresh Gouda cheese was performed following the protocol of Walsh and Brown (2000). Hydrochloric acid (6 M) was added to the sample vial for a final concentration of 5 mg of protein/mL of HCl. Hydrolysis vial was placed in an ultrasonic cleaner and flushed with nitrogen gas before sealing under vacuum. Sample was placed in a heating block for 4 hr. at 145°C. Afterwards, sample was removed from the heating block and allowed to cool before filtration through 0.2 µm filter. Sample was dried with nitrogen gas and dissolved in a dilution buffer. The prepared sample was analyzed for amino acid profile by running through Automated Amino Acid Analyzer (Model: L-8500 A, Hitachi, Japan). Areas of amino acid standards were used to quantify each amino acid in representative sample**.**

Microbiological analysis:

The microbial analysis of Gouda cheese samples was carried out using the method described by the American Public Health Association (APHA 2004) for total viable bacterial, proteolytic bacterial and lipolytic bacterial counts.

Sensory analysis:

Samples of Gouda cheese were sensory judged by the trained staff at El-Serw Animal Production Research Station (8 panelists). The score points were 15 for colour, 35 for body and texture and appearance and 50 for flavour which give a total score of 100 points (Ismail *et al.,* 2015). The sensory analysis test was done at room temperature.

Statistical analysis:

Analysis of variance (ANOVA) was conducted using SPSS 17.0 program. Significant differences among samples were determined using Duncan's multiple range test ($p<0.05$).

RESULTS

Data of fresh Gouda cheese yield are presented in Table 1. Fortification of Gouda cheese with whey proteins paste led to an increase in cheese yield. The yield values were increased by 4.80, 10.61, 15.13 and 20.02% for samples II, III, IV and V respectively. Results of titratable acidity and pH of Gouda cheese within repining period were classified in Table 1. Because of high acidity of DWPP (0.26%), Gouda cheese supplemented with it had higher acidity and lower pH values as compared to control ($P< 0.05$). The total solids (TS) and fat contents of denatured whey protein cheese were slightly lower than those of control cheese **Table (1)**. In the opposite trend to the TS and fat contents, the fortification of Gouda cheese with DWPP significantly ($P<0.05$) increased the ash and salt contents in comparison with the control cheese **Table (1)**. The high salt content of DWPP (7.34%) is primarily responsible for this increase.

The influence of adding DWPP to Gouda cheese on total nitrogen (TN), water soluble nitrogen (WSN), non-protein nitrogen (NPN) and total volatile fatty acids (TVFA) values was displayed in **Table (2)**. Supplementation of Gouda cheese with DWPP at all concentrations increased TN, WSN, NPN and TVFA contents. The greatest increase was noticed in treatment V (4 % DWPP). The outcomes of both **Tables (1** and **2**) indicate an increase in all chemical parameters as well as ripening indices of cheese during the repining period. The rising levels were higher in samples contained DWPP than that of control.

Concentrations of K, Ca, Na, Mg and Fe of Gouda cheese samples at the end of ripening period are shown in **Table (3**). The addition of DWPP, as previously mentioned, increased the value of ash, so it was expected to increase the levels of various mineral salts in cheese. The contents of K, Ca, Na and Fe of DWPP Gouda cheese (II, III, IV and V treatments) were significantly ($P< 0.05$) higher than those of control (I sample). Concerning Mg, there were no significant differences among all treatments.

The contents of total free amino acids, essential amino acids (EAAs), nonessential amino acids (Non-EAAs) and branched chain amino acids (BCAAs) of Gouda cheese are exhibited in **Tables (4** and **5)**. The total free amino acids concentrations of cheese were affected by fortification with DWPP due to the protein content of DWPP. Gouda cheese supplemented with whey protein had higher levels of total free amino acids as compared to control. The increasing rates were proportional to the added amount of whey protein. On an overall note, glutamic acid showed the highest concentration among the total amino acids while cysteine was the lowest. It is quite apparent from the data introduced in **Tables 4** and **5** that adding DWPP increased the amounts of EAAs of Gouda cheese. The levels of increase have considerably fluctuated between these acids. For instance, supplementation of cheese with 4% DWPP raised the cysteine, threonine, tyrosine and lysine contents by 20.0, 10.6, 6.2 and 1.6% respectively. Among all the essential amino acids, lysine had the highest concentration, while cysteine was the lowest. The contents of Non-EAAs of Gouda cheese were also increased by DWPP incorporation. The concentrations of Non EAAs were 19.53, 19.85, 20.14, 20.47 and 20.79 mg /100 g cheese for samples I, II, III, IV and V respectively. In all cheese treatments, the levels of EAAs were higher than Non-EAAs. Glutamic acid had the highest value of the Non-EAAs and aspartic acid was the lowest. The values of BCAAs were higher in Gouda cheese supplemented with DWPP **Tables (4** and **5**). Leucine content increased by 2.68, 6.10, 7.80 and 9.76% for treatments II, III, IV and V respectively. Respective values for isoleucine were 2.66, 4.25, 6.38 and 9.57% whereas increasing levels for valine were 1.59, 4.76, 5.95 and 9.52% for the same samples respectively.

Table 1. Effect of adding DWPP on yield, chemical and some physicochemical properties of Gouda cheese when fresh and during storage period

Properties	Treatments	Repining period (Month)			
		0	1	2	3
Yield %	I	10.84±0.02^{E}	-	-	-
	II	11.36±0.01^{D}	-	-	-
	III	11.99±0.01^{C}	-	-	-
	IV	12.48±0.01^{B}	-	-	-
	V	13.01±0.01^{A}	-	-	-
Titratable Acidity %	I	0.77±0.02^{Dc}	0.99±0.01^{Dc}	1.17±0.01^{Da}	1.35±0.02^{Ca}
	II	0.86±0.01^{Cc}	1.12±0.01^{Cb}	1.33±0.01^{Da}	1.56±0.01^{Ca}
	III	0.99±0.01^{Bd}	1.29±0.01^{Cc}	1.54±0.02^{CDb}	1.80±0.01^{Ba}
	IV	1.11±0.02^{ABd}	1.44±0.01^{ABc}	1.68±0.02^{Bb}	1.97±0.01^{Ba}
	V	1.20±0.01^{Ad}	1.54±0.02^{Ac}	1.81±0.01^{Ab}	2.07±0.02^{Aa}
pH values	I	5.72±0.01^{Aa}	5.57±0.02^{Aa}	5.48±0.01^{ABa}	5.35±0.01^{ABa}
	II	5.63±0.01^{Aa}	5.46±0.01^{Ba}	5.32±0.01^{Cc}	5.20±0.01^{Cb}
	III	5.54±0.02^{Ab}	5.36±0.01^{Bb}	5.22±0.01^{Cc}	5.08±0.01^{Dc}
	IV	5.43±0.01^{Ab}	5.26±0.01^{BCb}	5.14±0.01^{CDd}	4.99±0.02^{Dd}
	V	5.33±0.02^{Ab}	5.15±0.01^{BCd}	5.03±0.02^{Ce}	4.89±0.01^{Cd}
TS %	I	58.43±0.01^{Ba}	59.81±0.01^{ABa}	60.80±0.01^{Aa}	61.54±0.01^{Aa}
	II	58.11±0.01^{ABa}	59.50±0.01^{ABa}	60.45±0.01^{Aa}	61.20±0.01^{Aa}
	III	57.84±0.01^{Bb}	59.25±0.01^{Aa}	60.22±0.01^{Aa}	60.91±0.01^{Ab}
	IV	57.62±0.01^{Bb}	59.04±0.01^{Aa}	60.00±0.01^{Aa}	60.73±0.01^{Ab}
	V	57.46±0.13^{Cb}	57.88±0.01^{Cb}	58.84±0.02^{Bb}	60.52±0.01^{Ab}
Fat %	I	28.84±0.02^{Ba}	29.62±0.01^{Ba}	30.01±0.05^{Aa}	30.51±0.01^{Aa}
	II	28.79±0.01^{Ca}	29.55±0.01^{Ba}	30.05±0.01^{Aa}	30.43±0.01^{Aa}
	III	28.72±0.01^{Ba}	29.48±0.02^{Ba}	29.97±0.01^{Ba}	30.36±0.01^{Aa}
	IV	28.66±0.02^{Ba}	29.42±0.02^{ABa}	29.91±0.01^{Aa}	30.03±0.15^{Aa}
	V	28.58±0.03^{Ca}	29.35±0.02^{ABa}	29.83±0.02^{ABa}	30.24±0.02^{Aa}
Ash %	I	3.16±0.01^{Aa}	3.24±0.02^{Aa}	3.32±0.10^{Aa}	3.42±0.15^{Aa}
	II	3.24±0.02^{Aa}	3.31±0.15^{Aa}	3.39±0.15^{Aa}	3.48±0.10^{Aa}
	III	3.29±0.15^{Aa}	3.37±0.01^{Aa}	3.46±0.01^{Aa}	3.55±0.02^{Aa}
	IV	3.38±0.01^{Aa}	3.47±0.01^{Aa}	3.55±0.02^{Aa}	3.46±0.02^{Aa}
	V	3.44±0.03^{Aa}	3.51±0.15^{Aa}	3.61±0.15^{Aa}	3.72±0.15^{Aa}
Salt %	I	2.27±0.02^{Ba}	2.72±0.02^{Bb}	3.09±0.02^{Aa}	3.29±0.02^{Aa}
	II	2.35±0.02^{Ba}	2.85±0.01^{Bb}	3.22±0.02^{Aa}	3.40±0.01^{Aa}
	III	2.46±0.02^{Ba}	2.96±0.02^{Bab}	3.31±0.02^{Aa}	3.50±0.01^{Aa}
	IV	2.55±0.02^{Ba}	3.03±0.02^{Ba}	3.39±0.02^{Aa}	3.59±0.02^{Aa}
	V	2.63±0.02^{Ca}	3.12±0.02^{Ba}	3.49±0.02^{ABa}	3.69±0.02^{Aa}

^{abcd} letters indication to significant differences between the samples of Gouda cheese ±SD; ^{ABCD} letters indication to significant differences between storage period of Gouda cheese ±SD; Treatment I: Gouda cheese (control); Treatment II: Gouda cheese contained 1 % DWPP; Treatment III: Gouda cheese contained 2 % DWPP; Treatment IV: Gouda cheese contained 3 % DWPP; Treatment V: Gouda cheese contained 4 % DWPP

Data of **Table 6** clear the enumeration of total viable bacterial count (TVBC), proteolytic bacteria and lipolytic bacteria during maturation of Gouda cheese. The counts of the mentioned microorganisms showed significant difference between Gouda cheese treatments either when fresh or during ripening period. Cheese fortified with DWPP contained significantly ($P<0.05$) higher population of TVBC, proteolytic and lipolytic bacteria.

Impact of DWPP incorporation on sensory quality of Gouda cheese within repining period is given in **Table 7**. The panelists' data indicated that both cheese treatments IV and V (3 and 4% DWPP) had significantly less color and appearance scores. Control and cheese fortified with 1 and 2% DWPP (samples II and III respectively) were at par in terms of color and appearance scores which showed the highest. No significant differences ($p<0.05$) were observed in body and texture between the control and cheese samples fortified with 1 or 2% DWPP. Unfortunately, increasing the added amount of DWPP (3 or 4%) had a negative impact on the body and texture properties of the cheese. The findings cleared in **Table 7** revealed that significant differences ($P<0.05$) between the control cheese and the DWPP samples were observed in flavour scores. The supplementation with DWPP highly improved the flavour of Gouda cheese. Treatments II and III (1 and 2%) gained the highest scores of flavour when fresh and along maturation period.

DISCUSSION

High cheese yield is the principal aim for cheese producers, so improving the different properties of Gouda cheese might be pointless if not joined by an increase in its yield. The cheese yield was increased by adding DWPP which can be explained by two reasons: (a) the high water holding capacity of whey proteins and consequently increases the moisture content of cheese which strongly correlated with yield, (b) the increase in protein content of cheese as a result of adding DWPP. Hinrichs (2001) demonstrated that the yield of cheese increased as the fat and protein contents of milk increase, by re-incorporating whey proteins, and by incorporating other milk constituents such as lactose or ash, as well as water. Schenkel *et al.* (2011) cleared that introducing denatured whey protein concentrate into cheese milk generally increased cheese yield, a change that is attributed mainly to higher water retention.

Table 2. Effect of adding DWPP on nitrogen fractions and TVFA contents of Gouda Cheese

Properties	Treatments	Repining period (Month)			
		0	1	2	3
TN %	I	3.51±0.01^{Ba}	4.11±0.01^{Aa}	4.23±0.015^{Aa}	4.31±0.02^{Aa}
	II	3.64±0.02^{Ba}	4.20±0.10^{Aa}	4.34±0.010^{Aa}	4.40±0.10^{Aa}
	III	3.72±0.10^{Ba}	4.36±0.15^{ABa}	4.49±0.015^{Aa}	4.58±0.03^{Aa}
	IV	3.86±0.10^{Ca}	4.59±0.05^{ABa}	4.74±0.020^{Aa}	4.80±0.10^{Aa}
	V	3.97±0.08^{Ba}	4.65±0.03^{Aa}	4.84±0.020^{Aa}	4.94±0.35^{Aa}
WSN %	I	0.347±0.002^{Ca}	0.418±0.001^{ABb}	0.450±.0.010^{Ab}	0.502±0.010^{Ab}
	II	0.358±0.003^{Ca}	0.443±0.002^{Bb}	0.485±0.002^{Bb}	0.538±0.001^{Ab}
	III	0.366±0.002^{Ca}	0.477±0.002^{Bb}	0.508±0.001^{Abb}	0.553±0.002^{Ab}
	IV	0.385±0.002^{Ca}	0.486±0.002^{Ba}	0.559±0.002^{Aa}	0.598±0.001^{Aa}
	V	0.398±0.001^{Ca}	0.505±0.002^{Ba}	0.573±0.002^{Ba}	0.611±0.002^{Aa}
NPN %	I	0.139±0.07^{Bb}	0.172±0.001^{Bb}	0.196±0.001^{Ab}	0.213±0.002^{Ab}
	II	0.154±0.002^{Bab}	0.189±0.002^{ABb}	0.212±0.002^{Ab}	0.235±0.002^{Ab}
	III	0.166±0.002^{Ba}	0.202±0.012^{ABa}	0.228±0.001^{Aaa}	0.254±0.012^{Aa}
	IV	0.180±0.006^{Ba}	0.220±0.003^{Aa}	0.247±0.10^{Aa}	0.271±0.002^{Aa}
	V	0.189±0.002^{Ba}	0.236±0.002^{ABa}	0.250±0.002^{Aa}	0.285±0.002^{Aa}
TVFA*	I	7.49±0.015^{Db}	11.69±0.020^{Cab}	14.56±0.015^{Bb}	17.41±0.015^{Ab}
	II	7.62±0.015^{Db}	11.94±0.020^{Cab}	14.90±0.030^{Bab}	17.85±0.015^{Aa}
	III	7.76±0.067^{Db}	12.19±0.015^{Ca}	15.26±0.015^{Ba}	18.39±0.025^{Aa}
	IV	7.88±0.015^{Db}	12.37±0.020^{Ca}	15.41±0.015^{Ba}	18.50±0.011^{Aa}
	V	8.08±0.010^{Da}	12.57±0.020^{Ca}	15.63±0.031^{Ba}	18.52±0.015^{Aa}

^{abcd} letters indication to significant differences between the samples of Gouda cheese ±SD; ^{ABCD} letters indication to significant differences between storage period of Gouda cheese ±SD; Treatment I: Gouda cheese (control); Treatment II: Gouda cheese contained 1 % DWPP; Treatment III: Gouda cheese contained 2 % DWPP; Treatment IV: Gouda cheese contained 3 % DWPP; Treatment V: Gouda cheese contained 4 % DWPP

*expressed as ml 0.1 NaOH 100 g^{-1} cheese

Table3. Effect of adding DWPP on minerals content (mg/100g cheese) of Gouda Cheese at the end of storage periods

Treatments	Minerals				
	K	Ca	Na	Mg	Fe
I	116±1.0^{d}	611±1.0^{e}	802±1.0^{e}	24.00±2.0^{a}	0.19±0.01^{e}
II	119±1.0^{c}	610±2.0^{d}	810±1.53^{d}	24.42±2.0^{a}	0.22±0.01^{d}
III	121±2.0^{b}	614±1.0^{c}	812±1.0^{c}	24.94±0.04^{a}	0.24±0.02^{c}
IV	120±1.0^{ab}	615±2.0^{b}	813±1.0^{b}	25.12±0.02^{a}	0.25±0.01^{b}
V	123±1.0^{a}	620±1.0^{a}	817±1.0^{a}	25.19±0.02^{a}	0.27±0.02^{a}
Means	119.80±4.29	614±8.19	810.33±5.55	24.73±0.89	0.23±0.31

^{abcd} letters indication to significant differences between the samples of Gouda cheese ±SD; Treatment I: Gouda cheese (control); Treatment II: Gouda cheese contained 1 % DWPP; Treatment III: Gouda cheese contained 2 % DWPP; Treatment IV: Gouda cheese contained 3 % DWPP; Treatment V: Gouda cheese contained 4 % DWPP

Table 4. Effect of adding DWPP on the free amino acids contents (mg /100 g cheese) of Gouda cheese at the end of storage periods

Amino Acids	Treatments				
	I	II	III	IV	V
Cysteine	0.55	0.59	0.60	0.64	0.66
Histidine	1.58	1.59	1.58	1.60	1.62
Isoleucine	1.88	1.93	1.96	2.00	2.06
Leucine	4.10	4.21	4.35	4.42	4.50
Lysine	3.69	3.70	3.71	3.73	3.75
Methionine	1.87	1.88	1.90	1.90	1.93
Phenylalanine	2.31	2.34	2.35	2.39	2.42
Threonine	2.63	2.69	2.77	2.82	2.91
Tyrosine	1.12	1.13	1.14	1.16	1.19
Valine	2.52	2.56	2.64	2.67	2.76
Alanine	1.84	1.87	1.89	1.94	1.98
Arginine	1.64	1.67	1.68	1.70	1.69
Aspartic acid	0.82	0.87	0.95	1.02	1.09
Glutamic acid	6.11	6.23	.634	6.42	6.57
Glycine	1.86	1.87	1.87	1.90	1.92
Proline	5.53	5.57	5.62	5.69	5.71
Serine	1.73	1.77	1.79	1.80	1.83

Treatment I: Gouda cheese (control); Treatment II: Gouda cheese contained 1 % DWPP; Treatment III: Gouda cheese contained 2 % DWPP; Treatment IV: Gouda cheese contained 3 % DWPP; Treatment V: Gouda cheese contained 4 % DWPP

Table 5. Effect of adding DWPP on free amino acid indices ratios of Gouda cheese

Treatments	Total amino acids (mg/100 g)	Total EAAs (mg/100 g)	Total Non-EAAs (mg/100 g)	Total BCAAs (mg/100 g)	E/T (%)	Total BCAAs/Total (%)
I	41.78	22.25	19.53	8.50	53.25	20.43
II	42.47	22.62	19.85	8.70	53.26	20.48
III	43.14	23.00	20.14	8.95	53.31	20.75
IV	43.82	23.35	20.47	9.09	53.29	20.74
V	44.63	23.84	20.79	9.32	53.42	20.88

Treatment I: Gouda cheese (control); Treatment II: Gouda cheese contained 1 % DWPP; Treatment III: Gouda cheese contained 2 % DWPP; Treatment IV: Gouda cheese contained 3 % DWPP; Treatment V: Gouda cheese contained 4 % DWPP

Table 6. Effect of adding DWPP on some microbial groups (log cfu/ml) of Gouda Cheese during ripening period

Properties	Treatments	Repining period (Month)			
		0	1	2	3
Total Bacterial Count (x 10^7)	**I**	$7.91{\pm}0.01^{Ca}$	$9.84{\pm}0.02^{Ba}$	$10.88{\pm}0.01^{Ab}$	$9.01{\pm}0.01^{Bb}$
	II	$8.17{\pm}0.02^{Ca}$	$10.18{\pm}0.01^{ABa}$	$11.71{\pm}0.02^{Aab}$	$9.12{\pm}0.02^{Bb}$
	III	$7.97{\pm}0.01^{Ca}$	$10.25{\pm}0.01^{ABa}$	$11.93{\pm}0.02^{Aa}$	$9.11{\pm}0.02^{Bb}$
	IV	$8.39{\pm}0.02^{Ca}$	$9.82{\pm}0.02^{Ca}$	$11.96{\pm}0.02^{Aa}$	$10.28{\pm}0.03^{Ba}$
	V	$8.47{\pm}0.02^{Ca}$	$10.31{\pm}0.01^{Ba}$	$12.09{\pm}0.01^{Aa}$	$9.96{\pm}0.02^{Cb}$
Proteolytic bacteria (x 10^3)	**I**	$36.82{\pm}0.02^{Cb}$	$61.95{\pm}0.02^{Bb}$	$78.13{\pm}0.02^{Ab}$	$75.44{\pm}0.03^{Aab}$
	II	$37.33{\pm}0.03^{Da}$	$63.31{\pm}0.02^{Cb}$	$80.85{\pm}0.02^{Aa}$	$75.70{\pm}0.02^{Bab}$
	III	$37.47{\pm}0.02^{Da}$	$62.85{\pm}0.02^{Cb}$	$80.96{\pm}0.01^{Aa}$	$76.84{\pm}0.03^{Ba}$
	IV	$36.92{\pm}0.02^{Db}$	$64.54{\pm}0.03^{Ca}$	$81.08{\pm}0.04^{Aa}$	$77.95{\pm}0.04^{Ba}$
	V	$38.11{\pm}0.02^{Da}$	$64.60{\pm}0.01^{Ca}$	$81.39{\pm}0.03^{Aa}$	$77.90{\pm}0.07^{Ba}$
Lipolytic bacteria (x 10^3)	**I**	$25.95{\pm}0.02^{Cb}$	$46.65{\pm}0.02^{Bb}$	$56.74{\pm}0.02^{Ab}$	$53.55{\pm}0.02^{Aa}$
	II	$27.40{\pm}0.01^{Ca}$	$47.90{\pm}0.11^{Bb}$	$57.81{\pm}0.02^{Aab}$	$54.67{\pm}0.03^{Aa}$
	III	$26.67{\pm}0.02^{Cb}$	$48.82{\pm}0.03^{Ba}$	$58.13{\pm}0.01^{Aa}$	$56.58{\pm}0.01^{Aa}$
	IV	$27.79{\pm}0.03^{Ca}$	$47.95{\pm}0.02^{Bb}$	$58.07{\pm}0.02^{Aa}$	$55.86{\pm}0.02^{Aa}$
	V	$28.04{\pm}0.04^{Ca}$	$49.12{\pm}0.03^{Ba}$	$59.03{\pm}0.03^{Aa}$	$56.90{\pm}0.03^{Aa}$

^{abcd} letters indication to significant differences between the samples of Gouda cheese ±SD; ^{ABCD} letters indication to significant differences between storage period of Gouda cheese ±SD; Treatment I: Gouda cheese (control); Treatment II: Gouda cheese contained 1 % DWPP; Treatment III: Gouda cheese contained 2 % DWPP; Treatment IV: Gouda cheese contained 3 % DWPP; Treatment V: Gouda cheese contained 4 % DWPP

Table 7. Effect of adding DWPP on sensory attributes of Gouda Cheese during ripening period

Properties	Treatments	Repining period (Month)			
		0	1	2	3
Color & Appearance (15)	I	14±1.0^{Aa}	14±1.0^{Aa}	14±1.0^{Aa}	13±1.0^{Ba}
	II	14±1.0^{Aa}	14±1.0^{Aa}	14±1.0^{Aa}	13±1.0^{Ba}
	III	14±1.0^{Aa}	14±1.0^{Aa}	13±1.0^{Bb}	13±1.0^{Ba}
	IV	13±1.0^{Aab}	12±1.0^{Bb}	11±1.0^{Cc}	11±1.0^{Cb}
	V	12±1.0^{Ab}	11±1.0^{Bc}	11±1.0^{Bc}	10±1.0^{Cc}
Body & Texture (35)	I	32±1.0^{Ca}	33±1.0^{Ba}	34±1.0^{Aa}	34±1.0^{Aa}
	II	32±1.0^{Ca}	33±1.0^{Ba}	34±1.0^{Aa}	34±1.0^{Aa}
	III	32±1.0^{Da}	33±1.0^{Ca}	34±1.0^{Aa}	34±1.0^{Aa}
	IV	30±1.0^{Bb}	30±1.0^{Bb}	31±1.0^{Ab}	31±1.0^{Ab}
	V	29±1.0^{Ac}	29±1.0^{Ab}	28±1.0^{Bc}	28±1.0^{Bc}
Flavour (50)	I	40±1.0^{Cb}	42±1.0^{Bb}	44±2.0^{Ab}	45±1.0^{Ab}
	II	42±1.0^{Ca}	43±1.0^{Ca}	46±1.0^{Ba}	48±1.0^{Aa}
	III	42±1.0^{Ca}	44±1.0^{Ca}	46±1.0^{Ba}	48±1.0^{Aa}
	IV	42±1.0^{Ba}	44±1.0^{Ba}	45±1.0^{Aa}	46±1.0^{Aa}
	V	42±1.0^{Ca}	43±1.0^{BCa}	44±1.0^{Bb}	46±1.0^{Ab}
Total (100)	I	86±1.0^{Cb}	89±1.0^{Ba}	92±1.0^{Aa}	92±1.0^{Aa}
	II	88±1.0^{Ca}	90±1.0^{Ba}	94±1.0^{Aa}	95±1.0^{Aa}
	III	88±1.0^{Da}	91±1.0^{Ca}	93±1.0^{Ba}	95±1.0^{Aa}
	IV	85±1.0^{Cb}	86±1.0^{BCb}	87±1.0^{ABb}	88±1.0^{Ab}
	V	83±1.0^{Bc}	83±1.0^{Bc}	83±1.0^{Bc}	84±1.0^{Ab}

^{abcd} letters indication to significant differences between the samples of Gouda cheese ±SD; ^{ABCD} letters indication to significant differences between storage period of Gouda cheese ±SD; Treatment I: Gouda cheese (control); Treatment II: Gouda cheese contained 1 % DWPP; Treatment III: Gouda cheese contained 2 % DWPP; Treatment IV: Gouda cheese contained 3 % DWPP; Treatment V: Gouda cheese contained 4 % DWPP

The addition of whey protein may have activated cheese microorganisms, resulting in more acid production and thus increased cheese acidity. Similar findings were shown in whey protein chelated iron fortified Gouda cheese by Indumathi *et al.* (2013). DWPP Gouda cheese had high moisture content which might be due to the high water holding capacity of DWPP which may raise the moisture holding in cheese curd. These outcomes were reliable with those revealed by Perreault *et al.* (2017) who found that the presence of the denatured whey protein concentrate significantly reduced fat content in semihard cheese. Overall, TS and fat levels obtained in this study were within the ranges of previous chemical composition analysis of Gouda cheeses announced by Jo *et al.* (2018) and Eldeeb *et al.* (2020).

The addition of DWPP increased the levels of protein fractions (TN, WSN and NPN) and TVFA in Gouda cheese. This effect could be explained by the stimulation effect of DWPP for proteolysis and lipolysis during repining stage of Gouda cheese. In this context, Ismail *et al.* (2017) reported that whey proteins addition to Jameed caused an increase of WSN and NPN values. These results may be attributed to the high content of NPN in whey protein paste. On the other side, increasing protein content of Gouda cheese by fortification with DWPP adds more health benefits to cheese. In the previous study, increased protein levels in the diet were now seen as a safe and effective strategy to improve health by reducing blood lipid levels (Farnsworth *et al.,* 2003). Park *et al*. (2019) showed that protein and fat content of various Gouda cheese samples increased and moisture content decreased during the ripening period.

On the whole, addition of DWPP increased the minerals content and as a result increases the nutritional and health values of cheese. Casein phosphopeptides react with high levels of calcium and phosphate to form calcium phosphate complexes. These complexes lead to remineralization of the enamel (Walther *et al.,* 2008).

Adding DWPP to Gouda cheese increased the EAAs concentrations by 1.67, 3.37, 4.94 and 7.15% for treatments II, III, IV and V respectively. Numerous studies have shown the importance of amino acids for the human body. Khan and Selamoglu (2019) stated that high concentrations of EAAs are viable in synthesis and stimulating of proteins in adult muscles.

Whey proteins have high concentrations of branched chain amino acids (leucine, isoleucine, and valine). Not only BCAAs but also whey proteins are rich in the sulfur-containing amino acids (cysteine and methionine), which enhance immune functions through their intracellular transformation to glutathione (Onwulata and Huth, 2008). In accordance with these reports, the values of BCAAs were higher in Gouda cheese contained DWPP. Miller *et al.*

(2003) stated that in skeletal muscle, raising the level of the three BCAA or l-leucine alone reproduces the impacts of increasing the supply of all amino acids in stimulating protein synthesis and inhibiting protein degradation.

As it is known, the numbers of bacteria present in the cheese have a vital and positive role in the repining process, either directly through their metabolic activity or indirectly through the release of proteolytic and lipolytic enzymes into the cheese matrix as a result of the autolysis process after death. Accordingly, the increase of proteolytic and lipolytic bacteria numbers in DWPP-fortified cheese was a reason of activating the proteolysis and lipolysis processes and thus increasing the WSN, NPN, amino acids and TVFA values. High repining indices levels not only improve the chemical composition of the cheese but also can be considered as a repining acceleration. Fox *et al.* (2004) stated that through ripening, many cultures loose viability and release their intracellular enzymes due to autolysis. These enzymes play a significant part in cheese maturation and participate greatly in proteolysis and lipolysis process, in this way, raising the value of ripening indices during the repining period.

Control, II and III treatments characterized with golden yellow color, on the opposite, samples IV and V had the pale yellow color that can be due to its higher moisture content. The same observations were detailed by Indumathi *et al.* (2013) for Gouda cheese supplemented with whey protein chelated iron. Samples of control, II and III were described as smooth and creamy and not too soft. These attributes form the ideal body and texture of Gouda cheese. On the opposite side, treatments IV and V were described as weak and too soft due to its high moisture content and the high proteolytic activity which affects the protein matrix (Indumathi *et al.,* 2013). DWPP cheese was characterized by clean acid flavour. The improved action of DWPP on cheese flavour may be due to the raising amino acids content. The amino acids derived from protein breakdown are precursors that are totally keys to cheese flavor (Sulieman *et al.,* 2018). Also, one of the reasons for the high flavor scores of II-III samples is that the cheese pH after two months of repining was in the range 5.1-5.3, which is suitable for producing the ideal flavour of cheese.

As ripening progressed, the scores of color, and appearance tended to decrease whereas the scores of body, texture and flavour tended to increase. McSweeney (2004) stated that improvements of Gouda cheese flavour during repining could be attributed to the lipolytic, glycolytic and proteolytic breakdowns in cheese that lead to the development of property nutty flavor from volatile fatty acids and free amino acids which contribute to the basic cheese flavor. The profile of secondary proteolysis products found in ripening cheeses made with some specific LAB strains have a satisfactory impact on sensory characteristics, particularly flavor (Kvetoslava *et al.,* 2019).

CONCLUSION

The main objective of this study was to use an inexpensive byproduct to improve the chemical and sensory properties as well as to increase the nutritional and health values of Gouda cheese. Denatured whey protein paste (DWPP) produced from Ras cheese whey was mixed with Gouda cheese curd at 1, 2, 3 and 4%. Yield, acidity, total nitrogen, repining indices, mineral content, essential amino acids and branched chain amino acids values increased as a result of adding DWPP to cheese. Proteolytic and lipolytic bacteria have been stimulated by DWPP addition. Flavour of Gouda cheese fortified with 1 or 2% DWPP was better than other treatments. It can be concluded that fortification of Gouda cheese with 1 or 2% DWPP improved the chemical and sensory properties, increased the nutritional and healthy values and increased cheese yield.

Funding: Not applicable.

Conflict of Interest: The authors declare no conflict of interest

REFERENCES

American Public Health Association "APHA" (2004). *Compendium of Methods for Microbiological Examination of Foods,* A.P.H.A., NW, Washington, D.C.

AOAC. (2006). Official methods of analysis of AOAC International. *AOAC International, Gaithersburg*, MD, USA.

Castellanos, V., Litchford, M. & Campbell, W. (2006). Modular Protein Supplements and Their Application to Long-Term Care. *Nutrition in Clinical Practice*, 21, 485-504.

Choi, H., Yang, C., Choi, K. & Bae, I. (2015). Characteristics of Gouda cheese supplemented with fruit liquors. *Journal Of Animal Science and Technology*, 57, 2-6.

Eldeeb, A., Elwahsh, N. & Ahmed, M. (2020). Effect of using buffalo's milk with cow's milk and selected bacterial strains on the properties and safety of Gouda cheese. *Egyptian Journal of Food Science*, 48, 351-364.

Farnsworth, E., Luscombe, N., Noakes, M., Wittert, G., Argyiou, E. & Clifton, P. (2003). Effect of a high-protein, energy-restricted diet on body composition, glycemic control, and lipid concentrations in overweight and obese hyperinsulinemic men and women. *The American Journal of Clinical Nutrition*, 78, 31-39.

Fox, P. F., Guinee, T. P., Cogan, T. M. & McSweeney, P. L. (2000). Fundamentals of Cheese Science. *Aspen, Gaithersburg*, pp 145–163.

Fox, P. F., McSweeney, P. L., Cogan, T. M. & Guinee, T. P. (Eds.). (2004). *Cheese: Chemistry, Physics and Microbiology, Volume 1: General Aspects*. Elsevier, 245.

Hinrichs, J. (2001). Incorporation of whey proteins in cheese. *International Dairy Journal*, 11, 495-503.

Indumathi, K., Kaushik, R., Arora, S. & Wadhwa, B. (2013). Evaluation of iron fortified Gouda cheese for sensory and physicochemical attributes. *Journal of Food Science and Technology*, 52, 493-499.

Ismail, M. M., Hamad, M. N. F. & El-Menawy, R. K. (2017). Improvement of Chemical Properties of Jameed by Fortification with Whey Protein. *Journal of Nutritional Health & Food Science*, 5, 1-11.

Ismail, M. M., Ammar, E. T. M., El-Shazly, A. K. & Eid, M. Z. (2015). The impact of partial replacement of milk protein concentrates by acid whey proteins and adding bifidobacteria on some properties of functional analogue Feta cheese. *American Journal of Food Science and Nutrition Research*, 2, 10-20.

Ismail, M., Ammar, E. T. & El-Metwally, R. (2011). Improvement of low fat mozzarella cheese properties using denatured whey protein. *International JJournal of Dairy Technology,* 64, 207-217.

Jo, Y., Benoist, D., Ameerally, A. & Drake, M. (2018). Sensory and chemical properties of Gouda cheese. *Journal of Dairy Science*, 101, 1967-1989.

Khan, I., Nadeem, M., Imran, M., Ajmal, M. & Ali, S. (2018). Antioxidant activity, fatty acids characterization and oxidative stability of Gouda cheese fortified with mango (Mangifera indica L.) kernel fat. *Journal of Food Science and Technology*, 55, 992-1002.

Khan, U. M. & Selamoglu, Z. (2019). Nutritional and Medical Perspectives of Whey Protein: A Historical Overview. *Journal of Pharmaceutical Care*, 7, 112-117.

Kim, Y. K., Nam, M. S. & Bae, H. C. (2017). Characteristics of Gouda cheese supplemented with chili pepper extract microcapsules. *Korean Journal for Food Science of Animal Resources*, 37, 833-839.

Kosikowiski, F. V. (1978). Cheese and Fermented Milk Foods. 2nd^{ed}. Cornell Univ. Ithaca, New York.

Kvetoslava, S., Stanislav, K., Miroslav, F. & Pavla, B. (2019). Influence of starter culture to sensory quality of edam cheese during ripening. *Journal of Microbiology, Biotechnology and Food Sciences,* 9, 442–446.

Marshall, K. (2005). *User's Guide to Protein and Amino Acids*. Basic Health Publications, Inc. 33-56.

McSweeney, P. L. (2004). Biochemistry of cheese ripening. *International Journal of Dairy Technology*, 57, 127-144.

Miller, S., Tipton, K., Chinkes, D., Wolf, S. & Wolfe, R. (2003). Independent and Combined Effects of Amino Acids and Glucose after Resistance Exercise. *Medicine & Science in Sports & Exercise*, 35, 449-455.

Onwulata, C. I. & Huth, P. J. (2008). Whey processing, functionality & health benefits. IFT Press, Blackwell pub.

Park, W., Yoo, J., Oh, S., Ham, J., Jeong, S. & Kim, Y. (2019). Microbiological characteristics of Gouda cheese manufactured with pasteurized and raw milk during ripening using next generation sequencing. *Food Science of Animal Resources* 39, 585-600.

Perreault, V., Rémillard, N., Chabot, D., Morin, P., Pouliot, Y. & Britten, M. (2017). Effect of denatured whey protein concentrate and its fractions on cheese composition and rheological properties. *Journal of Dairy Science*, 100, 5139-5152.

Ramos, L. A., Baez, D. A., Ortiz, G. D., Ruiz, J. C. & López, V. M. (2022). Antioxidant and antihypertensive activity of Gouda cheese at different stages of ripening. *Food Chemistry,* 14, 100284.

Richardson, G. H. (1985): Standard Methods of the Examination of Dairy Products. 15th ed. *American Public Health Association,* Washington, DC.

Schenkel, P., Samudrala, R. & Hinrichs, J. (2011). Fat-reduced semi-hard cheese enriched with a microparticulated whey protein concentrate: impact on cheese-making properties and rheological characteristics. *Milchwissenschaft*, 66, 43-47.

Sulieman, A. E., Ohag, O. M., Hassan, H. M., Alreshidi, M. M., Abdelmageed, E. & Veettil, V. N. (2018). Some Chemical and Microbiological Characteristics of Gouda Cheese. *Advances In Bioresearch*, 9, 32-37.

van Gelder, K. (2020). Production volume of Gouda cheese in the Netherlands from 2000 to 2018. https://www.statista.com/statistics/1097857/

Walther, B., Schmid, A., Sieber, R. & Wehrmüller, K. (2008). Cheese in nutrition and health. *Dairy Science and Technology*, 88, 389-405.

Walsh, M. K. & Brown, R. J. (2000). Use of amino acid analysis for estimating the individual concentrations of proteins in mixtures. *Journal of Chromatograph A*, 891, 355- 360.