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Full Length Research Paper

Effects of different adjunct starter cultures on proteolysis of reduced fat Cheddar cheese during ripening

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The evolution of non-protein-nitrogen (NPN), total and free amino acids in reduced fat Cheddar cheese, prepared by formulated milk for Cheddar cheese with optimized texture, using a single and mixture of adjunct starter cultures during storage was studied. The concentrations of NPN, free and total amino acids were significantly affected by the type of microorganism present and the storage time. The total concentration of free amino acids (TFAA) and NPN in reduced fat cheeses inoculated with adjunct starter culture were higher than full fat control cheese without any adjunct starter culture. Also, higher level of TFAA and NPN were observed in reduced fat cheeses made with single culture of *Lactobacillus helveticus*. The concentration of glutamine, methionine and leucine as major precursors and contributors to sapid taste and flavor compound in reduced fat cheeses made with single culture of *L. helveticus* were higher than reduced fat Cheddar cheeses inoculated with *Lactobacillus casei and Streptococcus thermophilus,* and even higher than full fat control cheese made with usual mesophilic starter culture.

Key words: Cheddar cheese, amino acid, non-protein-nitrogen, adjunct starter culture, xanthan gum.

INTRODUCTION

Over the past decade, dietary fat has been linked to risk of diseases such as coronary heart disease, obesity and breast cancer which are related to oxidation of lipid. This health risk has necessitated the need for improved consumer awareness and a vivid change in the supply and demand for low-fat foods, including cheese (Katsiari et al., 2002). However, due to poor consumer perception of the products, as a result of inadequate taste and texture, the consumption of reduced-fat cheeses still remains significantly low. Generally, low fat cheeses tend to have low intensity of taste, typical flavor, and rubbery, hard, dry and grainy textures (Banks, 2004). Therefore, the challenge in reduced fat cheese development is to improve both the sensory attributes and texture of the product to produce a cheese which is comparable to its full-fat counterpart. In the light of this, quite a number of methods have been investigated in an effort to improve the texture and flavor of reduced-fat cheeses, for example, using fat replacers and adjunct cultures, respectively (Banks, 2004). A number of researchers have attempted to study a combination of these methods. Fat replacers are effective components that improve the textural properties of reduced fat cheeses. Moreover, adding adjunct starter cultures have shown great promise in the manufacturing of reduced-fat cheeses with reduced defects and improved flavor (Drake et al., 1997).

Basically, an adjunct is a culture used in addition to a regular lactic starter culture. Adjunct starter cultures can perform an essential function in reduced fat cheese manufacture by increasing proteolysis, particularly amino-peptidase activity resulting in a decrease of bitterness and an increase in the levels of desirable flavor peptides and precursors of volatile flavor compounds.

Abbreviations: NPN, Non-protein-nitrogen; **TFAA,** total of free amino acids.

Lactobacillus species are the most usual adjunct cultures applied in reduced fat cheeses (Drake et al., 1997). Skeie et al. (1995) reported an in-depth study on the potential of *Lactobacillus helveticus* CNRZ 303 as an adjunct culture in improving quality of low fat Gouda cheeses and their findings concluded that such a culture have the potentials to improve flavor, accelerate proteolysis and decrease bitterness of the low fat cheeses. Previous investigation by Drake et al. (1996) on the use of *L. helveticus* WSU19 as an adjunct culture reported a tremendous improvement in flavor of full fat Cheddar cheeses based on responses from both trained and consumer panelists. In another study Drake et al. (1997) reported that reduced fat cheeses inoculated with *L. helveticus* WSU19 as an adjunct culture showed higher oaky/nutty flavor, more proteolysis and less bitterness in contrast to control reduced fat cheeses. Katsiari et al. (2002) observed that low-fat cheeses that contained *Lactobacillus casei* as adjunct cultures exhibited flavor scores that are parallel to those of the full-fat cheese, but with much lower texture and body scores. Guinee et al. (1999) studied the effect of *Streptococcus thermophilus* as adjunct starter culture on the improvement of half-fat Cheddar cheese flavor and compared it with half fat Cheddar cheeses without any adjunct starter culture as control. Their findings demonstrated that the samples that were inoculated with *S. thermophilus* contain significantly higher levels of low molecular mass peptides and free amino acids compared to the control half-fat Cheddar cheese. It is interesting to note that identification and selection of suitable adjunct cultures which produce a premium quality reduced-fat cheese will be useful to the industry and also have implications for use in accelerated ripening of reduced-fat cheeses' flavor and texture development (Tarakci and Tuncturk, 2008).

The objectives of this study were to investigate the effect of *L. helveticus*, *L. casei* and *S. thermophilus* (individually and in combination) on the quality of reduced-fat Cheddar cheese manufacture, using an optimized cheese milk formula containing 2% cheese milk fat and enriched with 0.045% xanthan gum as fat replacer; and to examine the chemical composition, biochemical changes during ripening (75 days at 12°C), and finally to find out an appropriate adjunct culture in combination with xanthan gum that can be to manufacture reduced-fat Cheddar cheese with similar quality (textural properties and flavor compounds) as that of the full-fat counterpart.

MATERIALS AND METHODS

Samples and chemicals

Fresh milk was collected in the morning from a farm located in Universiti Putra Malaysia shortly before commencement of the trials. Direct-to-vat frozen starter cultures: R-704 (*Lactococcus lactis, spp cremories,* and *Lactococcus lactis, spp lactis*), LH-$B0_2$ (*L. helveticus*), *Lb.casei*-0_1 (*L. casei*), ST-$B0_1$ (*S. thermophilus*) and standard cheese rennet (single-strength fermentation-derived chymosin) were kindly donated by Chr. Hansen (Milwaukee, WI, USA). Xanthan gum as a carbohydrate based fat replacer (food-grade) was purchased from V.I.S. Foodtech Ingredient Supplies (Kuala Lumpur, Malaysia). Sodium phosphate monobasic monohydrate, sodium hydroxide, boric acid, acetonitrile (LC grade), methanol (LC grade), and Trichloroacetic acid (TCA) were obtained from Merck KGaA (Darmstadt, Germany). Hydrochloric acid was obtained from Sigma-Aldrich (St. Louis, MO). Amino acid standard mixture, Borate buffer (0.4 N in water, pH 10.2) and *o*-phthalaldehyde (OPA) were obtained from Agilent (Agilent Technologies, Waldbronn, Germany).

Preparation of Cheddar cheese

Reduced-fat Cheddar cheeses were made by using 30 L pasteurized milk prepared based on the best optimum point and formulation from full factorial design (cheese milk fat: 2%, xanthan gum: 0.045%) with 90% textural desirability (Nateghi et al., 2012). The enriched milks for each batch were inoculated with 0.015 g/kg direct-to-vat frozen commercial mesophilic lactic cultures (R-704). In order to develop flavor compound in the reduced-fat samples, single and mixed adjunct starter cultures (*L. helveticus*, *L. casei* and *S. thermophilus*) were added alongside the commercial starter culture, as shown in Table 1. Full fat Cheddar cheese was made from whole milk (3.5% w/w fat) and inoculated with 0.015 g/kg direct-to-vat frozen commercial mesophilic lactic cultures (R-704) without adding adjunct starter cultures. Cheeses were prepared in duplicate batches in a completely randomized design (CRD) and according to the procedures of Awad et al. (2005). The fresh cheese samples were removed from the mold, packed in oxygen barrier (Cryovac bags) by vacuum packaging machine (Model vac master, Kansas, USA) and ripened at 12°C and a relative humidity of about 85% for six ripening periods. Samples from each cheese were taken for analyses at 0, 15, 30, 45, 60 and 75 days after production.

Non-protein-nitrogen (NPN) solubility in tricholoroacetic acid (TCA)

NPN solubility in TCA (12%) was determined at six ripening periods (0, 15, 30, 45, 60 and 75 day) by the method described by Barbano et al. (1991). Grated cheese samples were blended in TCA followed by centrifugation (Sartorius 3-18 K, Sigma, Germany), at 3,000×*g* for 30 min at 4°C in order to precipitate the protein compounds. The filtrate was analyzed for nitrogen content by the Kjeldahl method according to AOAC (1997) method.

Total and free amino acids measurement

In the present study, amino acids and free amino acids content were determined by using reverse-phase high performance liquid chromatography (RP-HPLC) with ultraviolet-visible (UV-Vis) detection. In order to perform extraction of total amino acids, Pico-Tag method (White et al., 1986) was used. 0.2 gram of samples were weighed in a close test tube and then hydrolyzed by adding 15 ml HCl (6 mol/L), followed by thorough mixing in a screw-cap test tube (Agilent Technologies, Waldbronn, Germany). The mixture was flushed with nitrogen gas for 1 min and then incubated for 24 h at 110°C. After cooling, the sample was poured in volumetric flask, made up to volume (50 ml) with deionized water, and then filtered with Whatman filter paper No.1 (5-10 ml of first and final portions of

Table 1. Changes in NPN of reduced-fat and full-fat Cheddar cheeses during ripening time[1]

Batch	Adjunct starter culture	Code of the adjunct starter culture	Concentration (CFU)	Storage time (day)						ANOVA[2]		
				0	15	30	45	60	75	M[3]	T[4]	M*T[5]
1	control full-fat Cheddar cheese	N	None	$0.26^{d,A}$	$1.48^{e,B}$	$1.79^{e,C}$	$1.97^{e,D}$	$2.24^{f,E}$	$2.39^{e,E}$	*	*	*
2	*L. helveticus*	H	10^8	$0.44^{a,A}$	$2.37^{a,B}$	$2.88^{a,C}$	$3.25^{a,D}$	$3.73^{a,E}$	$3.85^{a,E}$	*	*	*
	L. casei	C	10^8	$0.31^{bc,A}$	$2.20^{b,B}$	$2.28^{c,B}$	$2.91^{b,C}$	$3.10^{b,D}$	$3.38^{b,E}$	*	*	*
4	*S. thermophilus*	T	10^8	$0.25^{d,A}$	$1.55^{de,B}$	$2.30^{c,C}$	$2.59^{c,D}$	$2.88^{c,E}$	$3.15^{c,F}$	*	*	*
5	*S. thermophilus*+ *L. casei*+ *L. helveticus*	T+C+H	$10^{2.6}$+$10^{2.6}$+$10^{2.6}$	$0.29^{dc,A}$	$1.63^{d,B}$	$1.77^{e,C}$	$2.30^{d,D}$	$2.51^{e,E}$	$2.79^{d,F}$	*	*	*
6	*L. casei* + *L. helveticus*	C+H	10^4+10^4	$0.38^{b,A}$	$1.89^{c,B}$	$2.21^{c,C}$	$2.52^{c,D}$	$2.71^{d,E}$	$2.88^{d,F}$	*	*	*
7	*S. thermophilus*+ *L. helveticus*	T+H	10^4+10^4	$0.26^{d,A}$	$2.02^{c,B}$	$2.61^{b,C}$	$2.82^{b,D}$	$3.01^{bc,D}$	$3.23^{c,E}$	*	*	*
8	*S. thermophilus, L. casei*	T+C	10^4+10^4	$0.38^{b,A}$	$1.59^{d,B}$	$2.00^{d,C}$	$2.29^{d,D}$	$2.71^{d,E}$	$2.85^{d,E}$	*	*	*

All reduced-fat and full-fat cheeses were inoculated with 0.015 g/kg Direct-to-vat frozen commercial mesophilic lactic cultures (R-704). [1], Values are reported as Mean of triplicate; [2], ANOVA was performed using 2 way ANOVA. * = ($p < 0.05$); [3], Microorganism type; [4], Storage time; [5], Microorganism type* Storage time; [A-F] significant difference between columns at confidence level of $p < 0.05$; [a-f], significant difference within columns at confidence level of $p < 0.05$.

filtrate discarded). In order to remove high molecular weight proteins and lipids from samples prior to amino acid analysis, the samples were passed through a Sep-pak C18 cartridge and then refiltered through 0.2 µm regenerated cellulose syringe filter (Agilent Technologies, Waldbronn, Germany).

The extraction process of free amino acids followed the recommendations of Garcia-Palmer et al. (1997). One gram of samples was homogenized in 10 ml of 0.1 N HCl in a Teflon glass homogenizer operated by a power drill (Black and Decker) at 1600 rpm. After centrifugation (10 min at 3000×*g*), 1 ml of the supernatant was deproteinized with 1 ml of 40% TCA and centrifuged (Sartorius 3-18 K, Sigma, Germany) for 10 min at 17,800×*g*. The extracted samples (amino acids and free amino acids) were automatically derivatized with OPA by programming the robotic autosampler according to the method published by Henderson et al. (1999). After derivatization, 0.5 µL of each sample was injected into Zorbax Eclipse-amino acid analysis (AAA) column (5 µm, 150 × 4.6 mm) (Agilent Technologies, Waldbronn, Germany) at 40°C, with detection at λ = 338 nm. Mobile phase A was 40 mM NaH_2PO_4, adjusted to pH 7.8 with NaOH, while mobile phase B was acetonitrile/methanol/water (45/45/10 v/v/v). The separation was obtained at flow rate of 2 ml/min with a gradient program in a total analysis time of 26 min. Concentration of each amino acid was expressed as nanomol/g of sample.

Statistical analyses

Data analyses were based on completely randomized design (CRD). In the chromatography analysis, the peak area of each compound was considered as the response variable, while the type of starter culture and storage times were considered as the independent variables in this study. The data obtained from the measurements were subjected to univariate one way analysis of variance (ANOVA) to determine the significant differences among the sample and values were compared using the Tukey's test defined at ($p < 0.05$).

Two way analysis of variance (ANOVA) was used to determine the significant interaction effect of independent variables on response variable on each chemical compound. All measurements were carried out in triplicates and reported as the mean of independent trials. The data analyses was performed by using MINITAB statistical software, release 14.2 (MINITAB Inc., state college, PA and USA).

RESULTS AND DISCUSSION

Non-protein-nitrogen

NPN is used to evaluate medium to small size peptides, amino acids, and smaller nitrogen compounds, including ammonium, urea, and amines. Changes in NPN index throughout cheese storage are shown in Table 1. A significant ($p < 0.05$) increment in initial NPN content was observed in all samples during storage time. This is consistent with previous study by Dabour et al. (2006). Due to different proteolytic activity of adjunct starter culture, the results of Table 1 indicated significant ($p < 0.05$) differences in initial NPN levels (0 day) within some experimental groups. The results of the current study also showed that the increase in NPN was more intense during the first 15 days of the ripening period. A similar trend for NPN changes in Feta cheese was previously reported by Katsiari et al. (2000). Generally, after milk coagulation, NPN value significantly increased in coagulates as proteolytic enzymes break down casein at various bonds to yield peptide and macro peptides (Emmons et al., 1990). Results obtained showed that the levels of NPN in samples containing single culture of *L. helveticus* and then *L. casei* were higher when compared with other samples during the ripening time. This indicates that both strains possessed high proteolytic activity which might have contributed to the liberation of more peptides. The rennet and adjunct starter culture contributed to the formation of both the peptides and soluble peptides. The peptidase and proteinase enzymes from the adjuncts starter culture are capable of hydrolyzing effectively, thus releasing more intermediate and smaller size peptides. These soluble nitrogenous compounds can contribute directly to cheese flavor. This was more evident after 30 days of storage possibly due to availability of more primary proteolysis products as substrates for the subsequent proteolysis by *L. helveticus* and *L. casei*. The results obtained also indicate that NPN concentration was noticeably ($p < 0.05$) influenced by the type of microorganism, storage time and their interactions (Table 1). Towards the final stage of maturation, concentration of NPN in cheeses inoculated with *L. helveticus* followed by *L. casei* were significantly higher ($p < 0.05$) than the control cheese as a result of the production of more proteinase enzymes by adjunct starter culture.

Free amino acids

Generally, the presence of free amino acids liberated in cheese can be used as an indicator of the proteolytic activities. It has long been noted that proteolysis is one of the main biochemical events, which take place throughout cheese ripening, and its products such as peptides and free amino acids can exhibit a remarkable effect on the sensory characteristics of cheese (Poveda et al., 2004). Subsequently, free amino acids produced can play direct role in the basic taste of the cheese and indirect function to cheese flavor, since they are precursors for the other catabolic reactions, giving rise to volatile aroma compounds (Wallace and Fox, 1997). Starter cultures are known to play a major role during ripening, because of their ability to contribute to the aroma and flavor of the cheese owing to carbohydrate metabolism, proteolysis, and to a lesser degree lipolysis (Hynes et al., 2003).

In this study, the effect of individual and mixed contribution of 3 strains of adjunct starter cultures (*L. helveticus*, *L. casei*, *S. thermophilus*) to produce free amino acids in a reduced fat Cheddar cheese was assessed and compared with control full-fat Cheddar cheese (without adjunct starter culture). The results show that 16 amino acids were detected and that their amounts were significantly ($p < 0.05$) influenced by the type of adjunct starter culture and storage time. Similar observations have been reported by Vicente et al. (2001) which evaluated the effect of starter and rennet type on free amino acid release during ripening of Idiazabal cheese. Their results indicated that the release of free amino acids were significantly ($p \leq 0.001$) affected by starter added to the cheese and ripening time. In agreement with the result of the current study, Poveda et al. (2004) pointed out that the concentration of the different free amino acids in cheese are mainly influenced by the kind of manufacturing technology employed. The evolutions of free amino acids (FAA) during ripening of Cheddar cheese are presented in Table 2.

The results show that the concentration of all free amino acids in all reduced-and full-fat Cheddar cheeses increased with maturation time, as a result of proteolysis. Free amino acids are released by the proteolytic agents, mainly by the microbial enzymes through the biochemical reactions that take place during cheese ripening (Poveda et al., 2004). Cheeses produced using adjunct treated cheeses contained significantly higher concentrations of free fatty acid (FAA) ($p <0.05$) in comparison with control full-fat Cheddar cheese. Addition of adjunct starter cultures to cheeses caused a significant increase in proteolysis contributing thus to the production of small peptides and free amino acids.

The result of the current study revealed that the final concentration of total free amino acids (TFAAs) in reduced fat cheeses inoculated with single culture of *L. helveticus* were significantly higher than the other reduced fat cheeses including full fat control cheeses (Table 2). In addition, combination of *L. helveticus* with *L. casei* and *S. thermophilus* increased the ability of these samples to produce higher level of TFAAs. As shown in Table 3, the final concentration of TFAAs in H+T and H+C reduced-fat cheeses were higher than other experimental cheeses except for sample H. The highest

Table 2. Evolution of the individual free amino acids during ripening of Cheddar cheeses made without adjunct culture and with *L. helveticus*, *L. casei* and *S. thermophilus* as adjunct starter culture[y].

Amino acid (nmol/g)	Ripening Time*																							
	No adjunct starter culture (N)			*L .helveticus* (H)*			*L. casei* (C)*			*S. thermophilus* (T)*			*S. thermophilus, L. casei and L. helveticus* (T+C+H)*			*L. casei* and *L. helveticus* (C+H)*			*S. thermophilus* and *L. helveticus* (T+H)*			*S. thermophilus* and *L. casei* (T+C)*		
	0	30	75	0	30	75	0	30	75	0	30	75	0	30	75	0	30	75	0	30	75	0	30	75
Asp	Nd^{a}	Nd^{a}	Nd^{a}	Nd^{a}	0.20^{bc}	0.66^{d}	Nd^{a}	Nd^{a}	Nd^{a}	Nd^{a}	Nd^{a}	Nd^{a}	Nd^{a}	Nd^{a}	Nd^{a}	Nd^{a}	Nd^{a}	Nd^{a}	Nd^{a}	0.19^{b}	0.61^{d}	Nd^{a}	Nd^{a}	Nd^{a}
Glu	0.39^{a}	0.98^{c}	2.14^{f}	1.83^{a}	3.17^{b}	6.34^{c}	0.99^{a}	1.78^{bc}	2.94^{d}	0.70^{a}	1.57^{b}	3.81^{e}	0.54^{a}	1.30^{b}	2.66^{d}	1.61^{a}	2.32^{b}	4.59^{e}	0.91^{a}	2.05^{bc}	5.06^{d}	1.20^{a}	2.57^{b}	3.69^{d}
Ser	Nd^{a}	0.16^{bc}	0.25^{d}	Nd^{a}	0.23^{c}	0.68^{e}	Nd^{a}	Nd^{a}	0.33^{d}	Nd^{a}	0.21^{c}	0.41^{e}	Nd^{a}	0.12^{b}	0.34^{e}	Nd^{a}	0.28^{bc}	0.48^{d}	Nd^{a}	0.23^{c}	0.67^{f}	Nd^{a}	0.24^{c}	0.50^{f}
His	Nd^{a}	Nd^{a}	Nd^{a}	Nd^{a}	0.49^{b}	1.40^{d}	Nd^{a}	Nd^{a}	0.44^{c}	Nd^{a}	0.22^{b}	0.57^{e}	Nd^{a}	Nd^{a}	0.45^{c}	Nd^{a}	0.28^{b}	0.73^{d}	Nd^{a}	0.35^{b}	1.17^{d}	Nd^{a}	Nd^{a}	0.46^{c}
Gly	Nd^{a}	0.13^{b}	0.39^{d}	0.15^{a}	0.61^{b}	2.32^{e}	0.13^{a}	0.35^{b}	0.70^{c}	0.13^{a}	0.42^{b}	0.93^{d}	Nd^{a}	0.35^{c}	0.87^{f}	0.16^{a}	0.27^{a}	0.87^{c}	Nd^{a}	0.69^{c}	1.66^{e}	0.11^{a}	0.44^{c}	0.89^{e}
Thr	Nd^{a}	Nd^{a}	Nd^{a}	Nd^{a}	0.35^{c}	0.66^{e}	Nd^{a}	Nd^{a}	0.32^{c}	Nd^{a}	Nd^{a}	0.24^{d}	Nd^{a}	0.21^{b}	0.46^{d}	Nd^{a}	0.40^{b}	0.69^{d}	Nd^{a}	0.16^{b}	0.76^{c}	Nd^{a}	Nd^{a}	0.45^{d}
Arg	Nd^{a}	Nd^{a}	Nd^{a}	Nd^{a}	Nd^{a}	Nd^{a}	Nd^{a}	Nd^{a}	0.20^{c}	Nd^{a}	Nd^{a}	Nd^{a}	Nd^{a}	0.16^{b}	0.36^{d}	Nd^{a}	Nd^{a}	0.16^{b}	Nd^{a}	Nd^{a}	Nd^{a}	Nd^{a}	Nd^{a}	Nd^{a}
Ala	Nd^{a}	Nd^{a}	Nd^{a}	0.12^{a}	0.36^{b}	0.57^{c}	Nd^{a}	0.21^{b}	0.45^{d}	Nd^{a}	0.19^{b}	0.42^{d}	Nd^{a}	0.19^{c}	0.25^{e}	Nd^{a}	0.17^{b}	0.48^{e}	Nd^{a}	0.28^{c}	0.51^{d}	Nd^{a}	0.14^{b}	0.24^{d}
Tyr	Nd^{a}	Nd^{a}	Nd^{a}	0.15^{a}	0.18^{a}	0.77^{c}	Nd^{a}	Nd^{a}	0.25^{b}	Nd^{a}	Nd^{a}	0.41^{c}	Nd^{a}	Nd^{a}	Nd^{a}	Nd^{a}	Nd^{a}	0.46^{d}	Nd^{a}	0.19^{b}	0.53^{c}	Nd^{a}	Nd^{a}	Nd^{a}
Val	0.19^{a}	0.26^{bc}	0.41^{d}	0.23^{a}	0.89^{b}	1.55^{d}	0.14^{a}	0.39^{b}	0.77^{c}	0.18^{a}	0.28^{b}	0.53^{e}	0.16^{a}	0.41^{bc}	0.55^{d}	0.22^{a}	0.42^{b}	0.81^{c}	0.17^{a}	0.63^{c}	1.12^{e}	0.17^{a}	0.30^{bc}	0.50^{d}
Met	Nd^{a}	Nd^{a}	0.22^{c}	Nd^{a}	0.23^{b}	1.74^{d}	Nd^{a}	0.18^{b}	0.63^{d}	Nd^{a}	0.11^{b}	0.66^{d}	Nd^{a}	0.12^{b}	0.63^{d}	Nd^{a}	0.11^{b}	0.75^{d}	Nd^{a}	0.15^{b}	0.84^{d}	Nd^{a}	Nd^{a}	0.75^{c}
Phe	0.12^{a}	0.24^{a}	0.79^{c}	0.11^{a}	0.80^{b}	2.11^{d}	0.37^{a}	0.81^{b}	1.41^{d}	0.27^{a}	0.53^{b}	1.09^{e}	0.42^{a}	0.52^{ab}	1.08^{d}	0.39^{a}	0.56^{b}	1.61^{e}	0.43^{a}	0.85^{b}	1.96^{d}	0.31^{a}	0.61^{b}	1.17^{d}
Ile	Nd^{a}	Nd^{a}	0.12^{b}	Nd^{a}	0.53^{bc}	1.68^{d}	Nd^{a}	0.13^{b}	0.67^{d}	Nd^{a}	Nd^{a}	0.32^{c}	Nd^{a}	0.11^{b}	0.62^{d}	Nd^{a}	0.28^{c}	0.68^{e}	Nd^{a}	0.21^{b}	1.51^{d}	Nd^{a}	Nd^{a}	0.35^{c}
Leu	0.13^{a}	0.37^{b}	1.46^{d}	0.23^{a}	1.69^{c}	4.95^{f}	0.15^{a}	1.18^{b}	2.64^{d}	0.13^{a}	0.86^{c}	2.05^{f}	0.13^{a}	1.18^{bc}	1.87^{d}	0.18^{a}	1.18^{c}	2.93^{f}	0.13^{a}	1.78^{c}	3.98^{e}	0.10^{a}	0.70^{b}	2.89^{d}
Lys	Nd^{a}	0.45^{b}	1.07^{d}	0.63^{a}	1.19^{a}	3.74^{c}	Nd^{a}	0.87^{c}	2.06^{f}	Nd^{a}	0.59^{b}	1.35^{e}	Nd^{a}	0.45^{b}	1.34^{e}	Nd^{a}	0.66^{b}	2.57^{e}	Nd^{a}	0.74^{c}	2.81^{e}	0.44^{a}	0.90^{b}	1.90^{d}
Pro	Nd^{a}	0.32^{b}	1.25^{d}	1.09^{a}	2.25^{b}	5.69^{c}	Nd^{a}	0.78^{b}	1.85^{c}	Nd^{a}	0.85^{b}	2.64^{e}	Nd^{a}	1.53^{b}	2.86^{d}	Nd^{a}	2.42^{b}	3.78^{d}	Nd^{a}	2.95^{c}	4.67^{e}	Nd^{a}	0.84^{b}	2.39^{e}
Total	0.83^{a}	2.91^{b}	8.1^{d}	4.54^{a}	13.17^{b}	34.86^{e}	1.78^{a}	6.68^{b}	15.66^{d}	1.41^{a}	5.83^{c}	15.43^{f}	1.25^{a}	6.65^{c}	14.34^{f}	2.56^{a}	9.35^{b}	21.59^{e}	1.64^{a}	11.45^{b}	27.86^{d}	2.33^{a}	6.74^{c}	16.18^{e}

[y], values are given as mean of triplicate; [a-f], means in the same row for each starter culture without a common letter are significantly different ($p < 0.05$); N: No adjunct starter culture, H: *L. helveticus*, C: *L. casei*, T: *S. thermophilus,* T+C+H*: S. thermophilus* + *L. casei*+ *L. helveticus,* C+H: *L. casei* + *L. helveticus*, T+H: *S. thermophilus* + *L. helveticus*, T+C: *S. thermophilus* + *L. casei*; *, Significant ($p< 0.05$).

level of major free amino acids in reduced fat cheese that contain *L. helveticus* can be explained by the higher ability of this strain to provide the aminopeptidase required to hydrolyze peptides and release peptides and free amino acids from casein compared to *L. casei* and *S. thermophilus*. Therefore, the production rate of free amino acids in reduced fat cheeses inoculated with *L. helveticus* were higher than reduced-fat cheeses made with *L. casei* and *S. thermophilus*. Moreover, the current result showed that total concentration of free amino acids in reduced fat cheeses containing single culture of *L. casei* were higher fat cheeses that contain single culture of *S. thermophilus* TH4. Therefore, single culture of *S. thermophilus* (TH4) exhibited lower proteolytic activity to produce free amino acids than others. This observation was in agreement with that reported by previous researchers. For example Hashemi et al. (2009), reported that Iranian White-brined cheese made with *L. helveticus* (LH. Bo2) exhibited significantly greater rates of free amino group formation as compared with the control cheese. Bergamini et al. (2010) found that thermophilic lactobacilli such as *L. helveticus* have much higher proteolytic and peptidolytic activity than *S. thermophilus* strains. Kebary et al. (1999) and Kenny et al. (2003) reported that *L. helveticus* was more effective in the proteolysis and liberation of free amino acids than *L. casei* and

Table 3. Evolution of the individual total amino acids during ripening of Cheddar cheeses made with *Lb. helveticus, Lb. casei and Str. thermophilus* as adjunct starter culture[y].

Amino acid (nmol/g)	Ripening time*											
	No adjunct starter culture (N)			*L. helveticus* (H)*			*L. casei* (C)*			*S. thermophilus* (T)*		
	0	30	75	0	30	75	0	30	75	0	30	75
Asp	Nd^{a}	2.47^{b}	3.63^{c}	Nd^{a}	Nd^{a}	8.73^{d}	Nd^{a}	Nd^{a}	7.44^{d}	Nd^{a}	3.37^{b}	6.02^{c}
Glu	16.78^{a}	23.85^{b}	33.08^{c}	17.56^{a}	33.59^{c}	49.66^{e}	10.81^{a}	19.91^{b}	36.89^{d}	13.06^{a}	25.92^{c}	41.77^{f}
Ser	2.97^{a}	5.45^{b}	10.14^{c}	5.06^{a}	8.323^{b}	14.84^{d}	3.73^{a}	5.96^{bc}	11.68^{e}	5.02^{a}	7.96^{b}	11.49^{d}
His	2.76^{a}	5.43^{c}	10.70^{f}	3.86^{a}	8.18^{b}	15.85^{d}	2.42^{a}	7.87^{c}	11.42^{f}	3.53^{a}	7.43^{bc}	13.20^{e}
Gly	8.15^{a}	13.97^{b}	25.01^{d}	8.06^{a}	13.92^{c}	24.08^{f}	5.73^{a}	10.40^{b}	19.26^{d}	6.98^{a}	9.58^{ab}	21.59^{d}
Thr	2.86^{a}	4.32^{bc}	5.64^{c}	2.40^{a}	3.34^{ab}	5.20^{d}	2.58^{a}	3.12^{b}	4.09^{d}	1.88^{a}	3.40^{b}	6.64^{d}
Arg	8.45^{c}	8.74^{c}	5.59^{b}	4.49^{abc}	6.56^{c}	5.88^{cb}	5.22^{bc}	7.22^{e}	6.56^{de}	7.96^{b}	7.56^{b}	6.91^{b}
Ala	0.95^{a}	2.32^{b}	5.06^{d}	1.71^{a}	2.62^{b}	4.78^{c}	1.30^{a}	2.20^{bc}	3.20^{e}	1.36^{a}	2.10^{ab}	3.93^{c}
Tyr	1.70^{a}	2.89^{bc}	4.07^{e}	2.84^{a}	3.55^{b}	5.89^{d}	2.56^{a}	3.63^{b}	4.76^{d}	2.05^{a}	2.89^{b}	4.64^{e}
Val	3.66^{a}	6.64^{b}	12.57^{d}	5.76^{a}	7.51^{b}	18.50^{e}	4.60^{a}	6.25^{b}	15.60^{d}	3.99^{a}	7.69^{b}	14.24^{d}
Met	1.60^{a}	3.61^{c}	6.59^{e}	2.66^{a}	5.89^{c}	9.39^{e}	1.94^{a}	4.27^{b}	6.99^{d}	1.81^{a}	4.42^{c}	7.05^{e}
Phe	1.51^{a}	3.79^{c}	6.49^{f}	2.44^{a}	3.61^{b}	6.18^{c}	1.53^{a}	4.38^{c}	4.87^{e}	3.64^{a}	4.23^{bc}	7.84^{e}
Ile	3.49^{a}	4.25^{b}	6.07^{d}	3.54^{a}	5.13^{bc}	7.81^{e}	2.71^{a}	4.09^{bc}	6.20^{e}	3.33^{a}	4.86^{bc}	6.85^{d}
Leu	3.50^{a}	7.14^{c}	11.90^{e}	6.06^{a}	10.90^{c}	24.69^{f}	4.50^{a}	8.52^{bc}	16.12^{e}	4.85^{a}	9.79^{c}	18.43^{f}
Lys	2.88^{a}	7.54^{c}	13.15^{e}	5.43^{a}	8.35^{b}	23.57^{d}	4.39^{a}	8.71^{c}	22.82^{f}	2.88^{a}	7.54^{c}	13.15^{e}
Total	61.26^{a}	102.41^{bc}	159.69^{e}	71.87^{a}	121.47^{bc}	225.05^{e}	54.02^{a}	96.53^{b}	177.9^{e}	62.34^{a}	108.74^{c}	183.75^{f}

Amino acid (nmol/g)	Ripening time*											
	S. thermophilus,L. casei and *L. helveticus* (T+C+H)*			*L. casei* and *L. helveticus* (C+H)*			*S. thermophilus* and *L. helveticus* (T+H)*			*S. thermophilus* and *L. casei* (T+C)*		
	0	30	75	0	30	75	0	30	75	0	30	75
Asp	3.52^{a}	6.28^{b}	5.70^{c}	2.51^{a}	4.34^{b}	7.73^{d}	Nd^{a}	Nd^{a}	3.15^{b}	Nd^{a}	Nd^{a}	2.24^{b}
Glu	17.80^{a}	17.83^{a}	46.57^{c}	15.67^{a}	21.52^{b}	42.14^{d}	15.26^{a}	26.96^{c}	45.12^{f}	11.81^{a}	16.50^{b}	37.64^{e}
Ser	4.63^{a}	9.43^{b}	13.78^{c}	4.76^{a}	8.97^{b}	14.39^{c}	5.02^{a}	9.33^{b}	12.67^{c}	4.59^{a}	7.66^{b}	11.85^{d}
His	4.35^{a}	7.82^{b}	16.15^{e}	4.09^{a}	8.06^{cb}	13.47^{e}	3.95^{a}	6.22^{b}	14.95^{e}	4.49^{a}	6.82^{cb}	13.42^{e}
Gly	6.17^{a}	11.77^{b}	24.52^{e}	6.15^{a}	11.43^{b}	22.10^{e}	7.91^{a}	11.73^{b}	22.28^{d}	6.69^{a}	11.12^{c}	18.39^{d}
Thr	3.07^{a}	4.71^{b}	7.83^{d}	2.67^{a}	3.06^{a}	5.61^{b}	3.14^{a}	3.26^{a}	7.35^{c}	2.67^{a}	4.41^{b}	7.05^{c}
Arg	7.31^{ab}	13.18^{c}	7.94^{b}	6.20^{b}	6.93^{b}	7.21^{b}	6.10^{ab}	5.71^{ab}	6.02^{ab}	5.22^{ab}	7.84^{d}	7.27^{cd}
Ala	2.81^{a}	3.89^{cb}	5.86^{e}	2.55^{a}	3.04^{b}	4.35^{d}	1.91^{a}	2.88^{abc}	4.71^{d}	1.47^{a}	1.55^{a}	3.74^{d}
Tyr	3.09^{a}	4.53^{b}	6.02^{d}	2.80^{a}	3.74^{b}	5.23^{d}	2.90^{a}	3.51^{b}	5.64^{d}	2.08^{a}	2.75^{cb}	3.84^{e}
Val	6.11^{a}	8.02^{b}	18.61^{e}	5.40^{a}	8.75^{bc}	16.21^{e}	5.63^{a}	9.11^{b}	17.21^{e}	4.26^{a}	7.59^{b}	13.26^{d}
Met	3.78^{a}	8.12^{c}	9.95^{d}	3.86^{a}	6.69^{b}	8.81^{d}	3.43^{a}	4.37^{a}	8.16^{c}	2.95^{a}	5.09^{c}	6.86^{e}
Phe	3.51^{a}	5.24^{b}	7.42^{c}	2.39^{a}	3.81^{bc}	6.72^{e}	3.11^{a}	4.32^{b}	6.13^{d}	1.37^{a}	2.70^{b}	4.56^{e}
Ile	5.30^{a}	6.24^{abc}	7.81^{d}	5.07^{a}	6.33^{bc}	8.30^{d}	3.98^{a}	5.30^{b}	7.38^{c}	3.50^{a}	5.26^{c}	7.39^{d}
Leu	5.50^{a}	10.82^{c}	24.68^{f}	7.12^{a}	12.16^{b}	20.16^{d}	5.64^{a}	10.39^{c}	22.47^{f}	4.06^{a}	8.25^{c}	15.46^{f}
Lys	6.01^{a}	11.57^{c}	22.93^{f}	6.15^{a}	7.81^{a}	18.65^{d}	6.75^{a}	9.35^{c}	16.58^{f}	5.20^{a}	9.74^{c}	20.36^{e}
Total	82.96^{a}	129.45^{b}	225.77^{d}	77.39^{a}	116.64^{b}	201.08^{d}	74.73^{a}	112.44^{b}	199.82^{e}	60.36^{a}	97.28^{b}	173.31^{e}

[y], the values are given as mean of triplicate; [a-f], Means in the same row for each starter culture without a common letter are significantly different ($p < 0.05$); N, No adjunct starter culture; H, *L. helveticus*; C, *L. casei*; T, *S. thermophilus;* T+C+H*: S. thermophilus* + *L. casei*+ *L. helveticus,* C+H; *L. casei* + *L. helveticus* , T+H; *S. thermophilus* + *L. helveticus*, T+C; *S. thermophilus* + *L. casei*; * Significant ($p< 0.05$).

L. helveticus, which contain a broad range of intracellular peptidase activities including amino-peptidase, dipeptidase, tripeptidase, endo-peptidase and proline specific peptidase. Hannon et al. (2007) reported that the strains of *L. helveticus*, added as an adjunct culture, showed a significant increase in the level of FAAs in Cheddar cheese, in comparison with control cheeses made only with lactococci. Drake et al. (1997) indicated that reduced fat Cheddar cheeses containing *L. helveticus* as an adjunct showed significantly greater

rates of proteolysis and increased oaky/nutty and sweet flavors. From the data in Table 2, it was noticed that alanine, aspartic acid, tyrosine, histidine, arginine and threonine were not detected in full-fat Cheddar cheese through the 75 days ripening time. Glycine, lysine and proline appeared to be absent from the control Cheddar cheeses up to 30 days of ripening; while methionine and isoleucine were present at low concentrations in the control full-fat Cheddar cheese after 75 days ripening. Moreover, methionine and isoleucine were detected in control cheese after 45 and 60 days ripening.

Arginine was not detected in full-fat Cheddar cheese and reduced fat cheeses in the H, T+H and T+C samples. Arginine is responsible for unpleasant or bitter taste in food products (Pappa and Sotirakoglou, 2008); therefore it is important that this amino acid concentration does not increase with ripening time. Also, arginine was detected in samples containing *L. casei*. Further-more, its concentration in reduced fat samples containing a single culture of *L. casei* and triple mix culture of H+C+T significantly ($p < 0.05$) increased during the ripening time. In addition, fluctuation in the concentration of arginine after 45 days ripening was not significant ($p < 0.05$) in samples made with mix culture of H+C. Aspartic acid was not detected during the storage time in full-fat Cheddar cheese and reduced samples in the C, H+C, T, T+C, T+C+H. Aspartic acid was only detected in reduced-fat samples containing *L. helveticus* and mixed cultures of *L. helveticus* + *S. thermophilus,* after 15 and 30 days of ripening, respectively. The main free amino acids found during the whole ripening period in all Cheddar cheeses samples were glutamic, valine, leucine, phenylalanine, proline, lysine and glycine. Histidine, isoleucine and methionine were present in the highest concentrations. These amino acids were also the main ones found in other cheese varieties during maturation, although with different levels, for example, in Cheddar cheese (Hannon et al., 2007), matured Manchego cheese (Poveda et al., 2004) and also in ewes' milk such as Idiazabal cheese (Garcia-Palmer et al., 1997). Previous findings have reported that the concentration of total free amino acids is not considered to be directly responsible for Cheddar flavor (Broome et al., 1990), but the presence of certain amino acids, principally methionine, glutamic acid, and leucine, coincides with flavor development (Wallace and Fox, 1997).

This study identified that glutamic acid is the most abundant amino acid in all Cheddar cheese samples during ripening. It is pertinent to note that glutamic acid plays a principal role in umami taste of Cheddar cheeses. Free proline, methionine and leucine concentration observed in samples inoculated with *L. helveticus* was higher than the rest of the samples. Methionine and leucine are considered to be the major contributors to cheesy flavor in the water-soluble extract of Cheddar cheese and proline is an amino acid related to sweet flavors in cheese (Wallace and Fox, 1997).

Total amino acids

Proteolysis is considered to be the most complex and the most essential biochemical event during ripening of cheese. During proteolysis, the casein initially hydrolyzed by residual coagulant activity was previously retained in the curd and by plasmin and possibly by other indigenous proteolytic enzymes to a range of intermediate-sized peptides, which are hydrolyzed by proteinase and peptidase enzymes from the starter lactic acid bacteria, non-starter lactic acid bacteria (NSLAB) and secondary microflora to shorter peptides and amino acids. It is important to note that the concentration of different amino acids in cheese are mostly associated with factors such as the manufacturing technology, addition of starters, type of curd, ripening conditions, ripening period and the degree and type of proteolysis (Pappa and Sotirakoglou, 2008).

Urbach (1995) reported that many volatile flavor compounds have structures that are consistent with their formation from amino acid precursors. Table 3 presents the evolution of total amino acids (TAA) during ripening of Cheddar cheese. The results of this study showed that the individual and total amino acids content increased in all samples over the course of ripening from 0 to 75 days because of casein degradation. In agreement with the current research, Liano et al. (1991) observed significant increase in the concentration of individual amino acids and sum of amino acids as ripening time increased. In the present findings, different increases in the relative amount of total amino acids after ripening were found, reflecting the different proteolytic activities of the starters used. The results of this study demonstrated that the highest total amino acids were produced by *L. helveticus* and triple adjunct starter cultures (*L. helveticus*, *L. casei* and *S. thermophilus*) with 225.02 and 225.778 nanomol/g, respectively at 75 days ripening. It is interesting to note that strains with high levels of peptidase activity as well as high autolytic level tend to have a significant impact on cheese ripening (Hannon et al., 2007). High concentration of total amino acids in triple mix starter culture was probably due to the stimulating role these strains have in producing higher level of amino acids. The results of this research revealed that incorporated adjunct starter cultures have positive effect in producing amino acids and free amino acids.

As shown in Table 3, the lowest activity to produce total amino acids at the end of the ripening time was associated with full-fat cheese samples containing commercial Cheddar cheese starter cultures and without any adjunct starter culture.

Hannon et al. (2007) reported that the benefits of using attenuated cells of *L. helveticus* as an adjunct in Cheddar

cheese include having significantly higher levels of amino acids, short peptides, free amino nitrogen, and flavor development without a detrimental effect on cheese texture or quality. The findings of the current study are supported by previous research by Drake et al. (1997) in which they observed significant enhancement in proteolysis when *L. helveticus* was employed as anadjunct in both full and reduced-fat Cheddar cheese. The results reveal that glutamic acid was the major amino acid in both free and total amino acid fractions. Furthermore, it was found that arginine, alanine and threonine concentrations were low in all products. However, some remarkable ($p < 0.05$) increase in glutamic acid, valine, leucine and lysine levels were observed during maturation period. The results of this study indicated that the sum of total amino acids was higher than the sum of the free amino acids in all the samples analyzed, which is indicative of the presence of peptides in the phosphotungstic acid soluble fraction (Llano et al., 1991).

A fluctuation in arginine concentrations in all samples was observed during ripening time. In agreement with the findings of current research, Llano et al. (1991) observed similar fluctuation in arginine levels for Artisan blue cheese. The results indicated that all total amino acid concen-tration was noticeably ($p < 0.05$) influenced by the type of microorganism and storage time.

This result can be explained by the fact that starter culture and ripening time are two strong factors affecting degree of proteolysis in cheese and subsequently amino acid production.

Conclusion

The present study was designed to determine the effect of adjunct starter culture on production of NPN and amino acids (Free and Total). The Level of certain FAA is extremely important in flavor and taste development. The results of this study evidently showed the concentration of amino acids and NPN in reduced-fat cheeses that contain adjunct starter culture were higher than full-fat control cheeses. Therefore, incorporated adjunct starter cultures to reduced fat cheese had positive effects on production of amino acids, FAAs and NPN. Single culture of *L. helveticus* exhibited higher proteolytic activity to produce the FAAs and NPN when compared to *L. casei* and *S. thermophilus* combined. The concentration of glutamine, methionine and leucine as major contributors to cheesy flavor and umami taste in reduced-fat cheeses made with single culture of *L. helveticus* were higher than the other experimental Cheddar cheeses. Moreover, the lowest concentration of arginine as amino acid responsible for unpleasant or bitter taste was observed in reduced fat cheeses containing single *L. helveticus*. Therefore, the findings of this study have a number of important implications for future manufacture of reduced-fat Cheddar cheese with similar and even higher quality than full-fat Cheddar cheese. By incorporating xanthan gum as carbohydrate based fat replacer and single culture of *L. helveticus* it is possible to improve textural properties, flavor and taste of reduced fat cheeses.

REFERENCES

AOAC (1997). Association of Official Analytical Chemists. Official Methods of Analysis (16th ed); Arlington.

Awad S, Hassan A, Muthukumarappan K (2005). Application of exopolysaccharide-producing cultures in reduced-fat Cheddar cheese: texture and melting properties. J. Dairy Sci. 88:4204-4213.

Banks JM (2004). The technology of low-fat cheese manufacture. Int. J. Dairy Technol. 57:199-205.

Barbano DM, Lynch JM, Fleming JR (1991). Direct and indirect determination of true protein content of milk by Kjeldahl analysis: Collaborative Study. J. Assoc. Off. Anal. Chem.74:281-288.

Bergamini CV, Wolf IV, Perotti MC, Zalazar CA (2010). Characterisation of biochemical changes during ripening in Argentinean sheep cheeses. Small Rumin. Res. 94:79-89.

Broome MC, Krause DA, Hickey MW (1990). The use of non-starter lactobacilli in Cheddar cheese manufacture. Aust. J. Dairy Technol. 45:67-73.

Dabour N, Kheadr E, Benhamou N, Fliss I, LaPointe G (2006). Improvement of texture and structure of reduced-fat Cheddar cheese by exopolysaccharide-producing lactococci. J. Dairy Sci. 89:95-110.

Drake MA, Boylston TD, Spence KD, Swanson BG (1996). Chemical and sensory effects of a lactobacillus adjunct in Cheddar cheese. Food Res. Int. 29:381-387.

Drake MA, Boylston TD, Spence NKD, Swansona BG (1997). Improvement of sensory quality of reduced fat Cheddar cheese by a Lactobacillus adjunct. Food Res. Int. 30(1):35-40.

Emmons DB, Becket DC, Binns M (1990).Milk clotting enzyme.1 proteolysis durig cheese making in relation to estimated losses of yeild. J. Dairy Sci. 73:2007-2015.

Fox PF, Wallace JM (1997). Formation of flavour compounds in cheese. Adv. Appl. Microbiol. 45:17-85.

Garcia-Palmer FJ, Serra N, Palou A, Gianotti M (1997). Free amino acids as indices of Mahon cheese ripening. J. Dairy Sci. 80:1908-1917.

Guinee TP, Fenelon M, O'Kennedy BT, Mulholland EO (1999). Improving the quality of low fat Cheddar cheese. Project Report, p. 4427.

Hannon JA, Kilcawley KN, Wilkinson MG, Delahunty CM, Beresford TP (2007). Flavour precursor development in Cheddar cheese due to lactococcal starters and the presence and lysis of *Lactobacillus helveticus*. Int. Dairy J. 17(4):316-327.

Hashemi M, Azar M, Mazlumi MT (2009). Effect of commercial adjunct lactobacilli on biochemical and sensory characteristics of Iranian white-brined cheese. Int. J. Dairy Technol. 62:48-55.

Henderson JW, Ricker RD, Bidlingmeyer BA, Woodward C (1999). Rapid, accurate, sensitive, and reproducible HPLC analysis of amino Acids. Agilent Technical Note: 5980-1193E.

Hynes E, Bergamini C, Suárez V, Zalazar C (2003). Proteolysis in ReggianitoArgentino cheeses manufactures with natural whey cultures and selected strains of Lactobacillus helveticus. J. Dairy Sci. 86:3831-3840.

Katsiari MC, Voutsinas LP, Kondyli E (2002). Improvement of sensory quality of low-fat Kefalograviera-type cheese with commercial adjunct cultures. Int. Dairy J. 12:757-764.

Kebary KMK, El-Sonbaty AH, Badawi RM (1999). Effects of heating milk and accelerating ripening of low fat Ras cheese on biogenic amines and free amino acids development. Food Chem. 64:67-75.

Kenny O, Fitz Gerald RJ, O'Cuinn G, Beresford T, Jordan K (2003). Growth phase and growth medium effects on the peptidase activities of *Lactobacillus helveticus*. Int. Dairy J. 13(7):509-516.

Llano DGD, Polo MC, Ramos M, Martin-Alvarez P (1991). Free and total amino acids in the non-protein fraction of an artisan blue cheese during ripening. Z. Lebensm. Unters. For. 193:529-532.

Nateghi L, Roohinejad S, Totosaus A, Mirhosseini H, Shuhaimi M, Meimandipour A, Omidizadeh A, Abd Manap, M.Y (2012). Optimization of textural properties and formulation of reduced fat Cheddar cheeses containing fat replacers. J. Food Agric. Environ. 10(2):46-54.

Pappa EC, Sotirakoglou K (2008). Changes of free amino acid content of Teleme cheese made with different types of milk and culture. Food Chem. 111:606-615.

Poveda JM, Cabezas L, McSweeney PLH (2004). Free amino acid content of Manchego cheese manufactured with different starter cultures and changes throughout ripening. Food Chem. 84:213-218.

Skeie S, Narvhus J, Ardo Y, Abrahamsen RK (1995). Influence of liposome-encapsulated Neutrase and heat-treated lactobacilli on the quality of low-fat Gouda-type cheese. J. Dairy Res. 62:131-139.

Tarakci Z, Tuncturk Y (2008). The effect of adjunct cultures on some chemical and biochemical properties of white-brined cheese. J. Food Biochem. 32(4):490-505.

Urbach G (1995). Contribution of lactic acid bacteria to flavour compound formation in dairy products. Int. Dairy J. 5:877-903.

Vicente MS, Ibanez FC, Barcina Y, Barron LJR (2001). Changes in the free amino acid content during ripening of Idiazabal cheese: influence of starter and rennet type. Food Chem. 72:309-317.

Wallace JM, Fox PF (1997). Effect of adding free amino acids to Cheddar cheese curd on proteolysis, flavour and texture development. Int. Dairy J. 7:157-167.

White JA, Hart RJ, Fry JC (1986). An evaluation of the Waters Pico-Tag system for the amino-acid analysis of food materials. J. Autom. Chem. 8:170-177.