International Journal of
Dairy Science

ISSN 1811-9743

Academic
Journals Inc.

www.academicjournals.com

OPEN ACCESS International Journal of Dairy Science

ISSN 1811-9743
DOI: 10.3923/ijds.2017.275.281

Research Article
Preparation of Ras Cheese Flavour Concentrate using Lipolyzed Cream and Skim Milk Curd

[1]Shaimaa Hamdy, [2]Hamdy Shaaban, [1]H. Sh. Mahmoud, [1]Khaled Abbas and [2]Amr Farouk

[1]Department of Dairy Science, Faculty of Agriculture, Fayoum University, Fayoum, Egypt
[2]Department of Chemistry of Flavor and Aroma, Food Industries and Nutrition Division, National Research Centre, Cairo, Egypt

Abstract

Background and Objective: Concentrated cheese flavors developed enzymetically in the industry by dairy researchers from cheeses at different ages, in order to provide cost-effective alternative to the long classical process in addition to enhance the sensory and nutritional properties of the final product. The main purpose of this study was to produce Ras Cheese Flavor Concentrate (RCFC) by using Ras cheese curd that was prepared from skim milk, lipolyzed cream (35% fat) with microbial lipase from *Rhizomucor miehei*. **Methodology:** Ras cheese flavor concentrate was prepared using lipolyzed cream and skim milk curd. Microbial lipase (activity of 40.000 U g^{-1}) from *Rhizomucor miehei* was used at 12000 U kg^{-1} fat for the preparation of lipolyzed cream (35% fat). Changes in pH values, T.A (%), moisture (%) and fat/DM (%) of RCFC during the storage period (days) was calculated using one way ANOVA test. Statistical analysis was carried out using SPSS software version 16. Moreover, GC-MS analysis of volatile compounds was performed in four selected samples (treatments with 20 and 30% lipolyzed cream at 3 and 7 days of storage) based on the sensory evaluation at the end of storage period. **Results:** Results showed that there were significant differences ($\alpha = 0.05$) based on the comparison the gross chemical composition which responsible for producing flavors between the control and treated samples during the storage period. In addition, Prepared RCFC samples were analyzed chemically for changes in moisture (%), T.A (%), pH values, fat/DM (%), water soluble nitrogen WSN/TN (%), Total Free Amino Acids (TFAA) and Total Volatile Fatty Acids (TVFA). Seventeen volatile compounds that identified included 7 esters, 5 alcohols, 4 ketones and 1 fatty acid compounds responsible for the final aroma and taste of RCFC samples. The addition of 30% lipolyzed cream into skim milk curd which stored for 7 days at 37°C enhanced both chemical composition, organoleptic evaluation and the flavor profile of prepared RCFC samples. **Conclusion:** The level of added lipolyzed cream (35% fat) to the cheese curd apparently increased the acidity, fat/DM (%) levels and proteolysis rate. Additionally, volatile fatty acids and generated key aroma compounds responsible for the final aroma and taste of RCFC samples were developed.

Key words: Ras cheese, flavour concentrate, lipolyzed cream, skim milk curd, microbial lipase, *Rhizomucor miehe*, sensory and nutritional properties

Received: February 20, 2017 Accepted: May 03, 2017 Published: June 15, 2017

Citation: Shaimaa Hamdy, Hamdy Shaaban, H. Sh. Mahmoud, Khaled Abbas and Amr Farouk, 2017. Preparation of ras cheese flavour concentrate using lipolyzed cream and skim milk curd. Int. J. Dairy Sci., 12: 275-281.

Corresponding Author: Hamdy Shaaban, Department of Chemistry of Flavor and Aroma, Food Industries and Nutrition Division, National Research Centre, Cairo, Egypt Tel: +2-02-33371362 Fax: +2-02-33370931

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The biosynthesis and isolation of flavor components are considered as consuming and expensive processes, therefore accelerating and enhancing the major pathways (lipolysis, glycolysis and proteolysis) for flavor production were the most economically ways for having the desired and intense cheese flavor. The enzymatic modification of cheese curd, cheese slurry, cheese milk or cream by added lipases were used for the enhancement of specific cheese flavor and considered as natural and nutritious[1]. Different means have been used for the production of various cheese flavor concentrates (Cheddar, Camembert, Blue, Romano and enzyme modified Ras cheese) from which the use of fungal proteinases/lipases, bovine pepsin, yeast fermentation and different bacterial strains[2-8].

Ras cheese, one of the main traditional and popular hard cheese consumed and manufactured in Egypt everywhere from raw cow's milk without using starter cultures based on fermentation through wild microflora[9]. Based on the chromatographic analysis and sensory evaluation, different groups of volatile compounds have been identified as being responsible for the cheese aroma. These compounds comprise esters, alcohols, ketones, fatty acids and others[10]. For example, according to Ayad *et al.*[11], 68 volatile compounds were detected in Ras cheese samples, including 25 esters, 17 ketones, 13 alcohols, 11 aldehydes as the major components responsible for the cheese aroma with respect to ripening age. These results revealed the cheese aroma compounds generated during classical ripening processes, however, nothing was reported, neither the lipolysis were leads to Ras cheese flavor concentrate nor its effect on the volatiles and so the sensory properties of the final Ras cheese product.

In this study, Ras cheese curd was prepared from skim milk, lipolyzed cream (35% fat) with microbial lipase from *Rhizomucor miehei* were used to produce Ras Cheese Flavor Concentrate (RCFC). The effect of adding lipolyzed cream at different levels on the sensory evaluation as well as the development of Ras cheese flavor was studied.

MATERIALS AND METHODS

The study was conducted during the month of September until December 2016.

Materials: Cow's milk was supplied by a private farm in Fayoum governorate, Egypt. Rennet powder (CHY-MAX, 2280 IMCU mL^{-1}, Ch. Hansen, Denmark), yoghurt starter (DVS YC-X11) was obtained from Chr. Hansen's Laboratory (Copenhagen, Denmark). Calcium chloride and sodium chloride were obtained from a local market in Egypt. Microbial lipase (with activity of 40.000 U g^{-1}) from *Rhizomucor miehei* strain was obtained from Chr. Hansen's Laboratory (Copenhagen, Denmark). Experimental RCFC samples were produced in our pilot dairy plant of Faculty of Agriculture, at Fayoum University Egypt.

Methods

Preparation of lipolyzed cream: Lipolyzed cream was prepared by incubating cream 35% fat with the microbial lipase 12000 U kg^{-1} fat at 27°C for 48 h as described by Tomasini *et al.*[3].

Preparation of Ras cheese flavour concentrate: Ras cheese curd was prepared from skim cow's milk according to Hofi *et al.*[12]. The drained curd was used for the production of Ras cheese flavor concentrate with added lipolyzed cream at the rate of 0, 10, 20 and 30%. The prepared samples were incubated at 37°C for 7 days and analyzed periodically at 1, 3, 5 and 7 days of storage. The RCFC were sampled in triplicate and analyzed for some chemical composition, ripening indices and sensory evaluated at the 7th day of storage for the total flavor score.

For the determination of flavor/volatile compounds, 4 samples were analyzed using GC-MS spectroscopy. The four samples were, T1 (sample with 20% lipolyzed cream at 3rd day of storage), T2 (sample with 30% lipolyzed cream at 3rd day of storage), T3 (sample with 20% lypolized cream at seventh day of storage), T4 (sample with 30% lipolyzed cream at 7th day of storage).

Isolation of headspace volatiles: The volatiles in the headspace of each sample under investigation were isolated by using a dynamic headspace system. The samples were purged for 1 h with nitrogen gas (grade of N2 <99.99) at a flow rate 100 mL min^{-1}. The headspace volatiles were swept into cold traps containing diethyl ether and pentane (1:1, v/v) and held at -10°C. The solvents containing the volatiles were dried over anhydrous sodium sulfate for 1 h the volatiles were obtained by evaporation of the solvents under reduced pressure.

Gas Chromatographic Analysis (GC): The GC analysis was performed by using HP 5890 equipped with a Flame Ionization Detector (FID) (Hewlett Packard Corporation, Palo Alto,

California, USA). A fused silica capillary column DB-5 (60 m×0.32 mm id,) was used. The oven temperature was maintained initially at 50°C for 5 min and then programmed from 50-250°C at a rate of 4°C min^{-1}. Helium was used as the carrier gas at flow rate of 1.1 mL min^{-1}. The injector and detector temperatures were 220 and 250°C, respectively. The retention indices (Kovats index) of the separated volatile components were calculated using hydrocarbons (C8-C22, Aldrich Co.) as references.

Gas Chromatographic-Mass Spectrometric (GC-MS) analysis: The analysis was carried out by using a coupled gas chromatography Hewlett-Packard model (5890)/mass spectrometery Hewlett-Packard MS (5970). The ionization voltage was 70 eV, mass range m/z 39-400 a.m.u. The GC condition was carried out as mentioned above. The isolated peaks were identified by matching with data from the library of mass spectra (National Institute of Standard and Technology) and compared with those of authentic compounds and previous published data[13]. The quantitative determination was carried out based on peak area integration.

Chemical analysis: The RCFC samples were analyzed for their total acidity (%), pH, moisture (%), fat/DM (%), WSN/TN (%) according to Nateghi *et al.*[14]. The Total Volatile Fatty Acids (TVFA) as described by Novikova and Ciprovica[15]. Analysis of the total free amino acids method was conducted according to Folkertsma and Fox[16]. All analysis were performed in triplicate and results reported as means±standard deviations.

Sensory analysis: Prepared RCFC samples were evaluated for flavor (100 points) according to El-Hofi and Ismail[17] by 10 panelists of the staff members at Dairy Department, Agricultural Faculty, Fayoum University.

Statistical analysis: Statistical analyses were performed using one way ANOVA test. Statistical analysis was carried out using SPSS software version 16. The varying degree of the result is expressed as Mean±Standard Deviation (Mean±SD). The differences between the samples were determined using t-tests ($\alpha = 0.05$)[18].

RESULTS AND DISCUSSION

Addition of lipolyzed cream in different concentration to Ras cheese curd prepared from skim milk powder, in order to have RCFC showed a significant effect on the sensory, chemical and volatiles analysis based on the storage period. Based on the sensory evaluation, the volatile compounds produced in four samples, 20% cream stored for 3 days (T_1), 30% cream stored for 3 days (T_2), 20% cream stored for 7 days (T_3) and 30% cream stored for 7 days (T_4) were identified using GC-MS.

Seventeen compounds were detected included 7 esters, 5 alcohols, 4 ketones and 1 fatty acid compounds (Table 1). Samples had almost the same volatile constituents; with exception, 2-pentanol was not detected in sample (T_3). Quantitatively, esters e.g., ethyl hexanoate, ethyl octanoate and ethyl butanoate were the dominants among the identified aroma compounds in all samples, followed by alcohols especially 3-methyl butanol and 2-butanol. Ketones as 2-pentanone, diacetyl and 2-nonanone were also detected in a higher concentrations (Table 1). Butyric acid was the only short-chain fatty acid detected in all samples with concentrations ranged from 1.36-1.88%. The above results were found to be in agreement with Ayad *et al.*[11]. According to Olson[19] and McSweeney[20] the formation of aroma depend on the enzymes present, so different flavors can be obtained. Therefore, the flavor of samples under investigation in the present work results from the interaction of enzymes from the microorganisms. Alcohols detected in samples result from catabolism of amino acids and lactose, reduction of methyl ketones as well as the as degradation of linoleic and linolenic acids[21,22]. However, the concentrations of the different ketones varied between the different cheese samples and are formed by enzymic oxidative decarboxylation of fatty acids, through the action of mould[23,18]. Methyl-ketones are well known for their contribution to the flavor of blue mould-ripened cheeses[11,24]. Among the identified ketones, 2-heptanone is an important flavor compound of Emmental, natural/creamy Gorgonzola and Ras cheeses, while 2-nonanone is the predominant methyl ketone which is present in natural Gorgonzola, ripened Ragusano and matured Ras cheese [25,26,11].

Diacetyl found in high level in all samples (1.34-1.68%) and described as the most important diketones derived from pyruvate stemming by lactose and citrate metabolism that is produced principally due to the activity of lactic acid bacteria, especially *Lactococcus lactis* ssp. *lactis biovar diacetylactis* and *Leuconostoc* spp. and considered as a creamy aroma component of Camembert, Cheddar, Emmental and Ras cheese[27,10,11]. Meanwhile, aldehydes were not identified in RCFC samples as they are characterized as intermediate and unstable compounds, which can undergo in the reduction into alcohols or the oxidation into acids and appear at low concentrations in the aroma profile of most cheeses[15].

Significant differences ($\alpha = 0.05$) in the levels of ethylesters were also encountered among samples (Table 1).

Table 1: GC-MS analysis of volatile compounds in Ras Cheese Flavour Concentrate (RCFC)

		Samples of cheese area (%)			
Compounds	KI	T_1	T_2	T_3	T_4
Diacetyl	590	1.68	1.68	1.34	1.43
2-Butanol	605	3.70	3.06	2.74	3.78
2-Pentanone	700	3.89	3.40	3.59	5.31
Ethylpropionate	709	0.04	0.11	0.71	0.29
2-Pentanol	730	0.10	0.13	-	0.12
3-Methylbutanol	735	3.48	5.28	7.83	1.39
2-Methylbutanol	739	0.15	0.29	0.18	0.24
1-pentanol	760	0.79	0.91	1.19	1.22
Ethylbutanoate	803	12.84	11.03	10.08	16.72
Butyric acid	820	1.50	1.42	1.36	1.88
2-Heptanone	890	0.35	0.33	0.26	0.42
Ethylpentanoate	898	1.72	1.68	2.01	2.20
Ethylhexanoate	1001	40.42	39.92	30.92	35.84
2-Nonanone	1092	1.27	0.94	0.91	0.99
Ethylheptanoate	1095	0.66	0.67	0.50	0.65
Ethyloctanoate	1195	17.68	18.99	20.34	19.51
Ethyldecanoate	1394	0.79	1.26	0.70	0.87

T1: Sample with 20% lipolyzed cream at 3rd day of storage, T2: Sample with 30% lipolyzed cream at 3rd day of storage, T3: Sample with 20% lipolyzed cream at 7th day of storage, T4: Sample with 30% lipolyzed cream at 7th day of storage

These compounds are formed by chemical or enzymatic reactions of fatty acids with primary alcohols as reported by Le Bars and Yvon[28]. Ethyl butanoate, ethyl hexanoate, ethyl pentanoate, ethyl heptanoate, ethyl octanoate, ethyl decanoate have been identified as the most common odorants and reported to play an important role in the aroma profile of aged Cheddar, Emmental, creamy Gorgonzola, Grana Padano, Pecorino, natural Gorgonzola, Ragusano, Flor de Guia and aged Ras cheeses[11,24]. Fatty acids were considered as important and predominant constituents of the flavor of various cheese types and serve as precursors of methyl ketones, alcohols, lactones and esters formation. The short chain free fatty acids originate from lipolysis of milk fat by the indigenous milk lipases, moulds and exogenous lipases or the breakdown of lactose and amino acids[29]. They could be also produced by the oxidation of ketones, esters and aldehydes[22].

The chemical composition of the prepared lipolyzed cream that used for producing Ras cheese Flavor Concentrate (RCFC) was: 35% fat, 2.23% protein, 60.29% moistures, pH 5.29 and 1.50% as the total acidity. The acidity of the RCFC samples was obviously changed during the storage period due to the concentration of lipolyzed cream used in the preparation of RCFC. The acidity value (%) of the control sample was 0.42% at the 1st day of the storage compared to the corresponding values in the treated samples which were 0.69, 1.30 and 1.62% for T1, T2 and T3, respectively.

The increase of RCFC acidity was associated with a decrease of the pH values. This is might be associated with the percentage of used lipolyzed cream, whereas an accumulation of free fatty acids produced as a result of lipase reaction during the incubation of cream with the microbial lipase as reviewed by Ozcan and Kurdal[30]. Abd El-Hamid *et al.*[31] found that, the incubation temperature had a significant effect on the development of acidity as well as the protein degradation, fat hydrolysis and production of the flavor compounds during the preparation of Ras cheese slurry. The higher of the incubation temperature, the higher rate of acidity and flavor development was reported. Also, starter bacteria was reported to have a vital impact on the ripening process and the rate of developed acidification[8].

The moisture values were higher in treated samples over the control ones during the whole storage period and decreased in all samples by the end of storage as shown in Table 2. The added cream increased the moisture content of the prepared RCFC samples, while the reduction of moisture might be due to the evaporation of water as a result of the effect of the incubation temperature.

The level of fat/DM (%) increased in the treated RCFC samples depending on the amount of added lipolyzed creamand the change of the dry matter contents as shown in Table 2 which is in agreement with Hofi *et al.*[12].

The rate of proteolysis in RCFC samples treated and untreated with lipolyzed cream throughout storage period as conducted with water soluble nitrogen (WSN/TN) and the accumulation of total free amino acids TFAA are shown in Fig. 1 and 2. Cheese curd treated with lipolyzed cream exhibited noticeable differences in the proteolysis rate during storage compared to the control curd. Proteolysis in RCFC

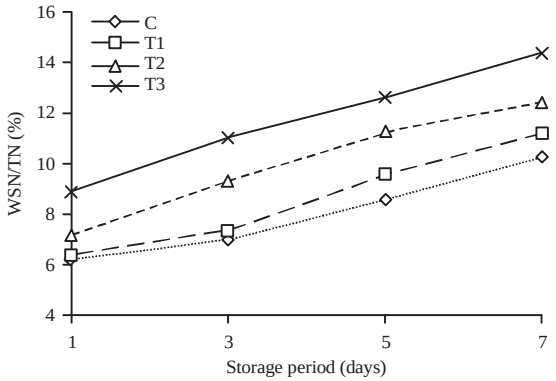

Fig. 1: Changes in water soluble nitrogen (WSN/TN) of RCFC during the storage period

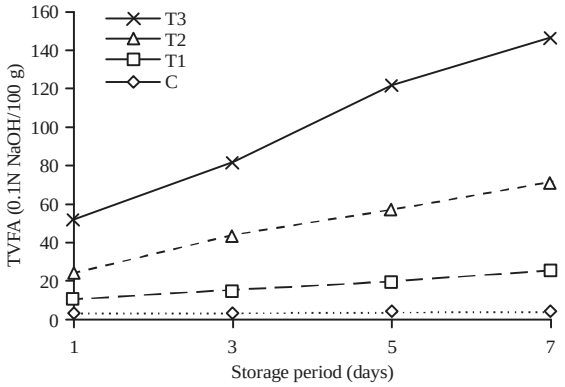

Fig. 3: Changes in TVFA contents of RCFC during the storage period

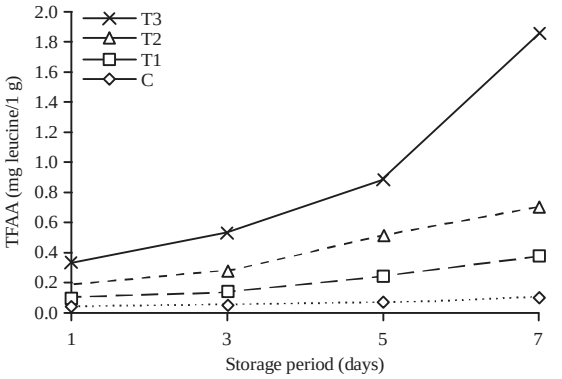

Fig. 2: Changes in TFAA contents of RCFC during the storage period

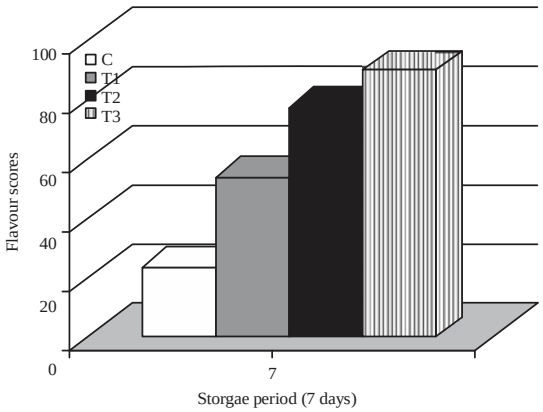

Fig. 4: Flavour scores of RCFC at the end of the storage period

Table 2: Changes in pH values, T.A (%), moisture (%) and fat/DM (%) of RCFC during the storage period (days)

		Treatments			
Properties	Storage period (days)	C	T_1	T_2	T_3
pH	1	5.93±0.05	5.87±0.02	5.43±0.03	5.38±0.02
	3	5.89±0.01	5.86±0.02	5.40±0.02	5.35±0.03
	5	5.85±0.03	5.82±0.01	5.36±0.02	5.30±0.01
	7	5.64±0.02	5.45±0.03	5.34±0.02	5.14±0.02
T.A (%)	1	0.42±0.02^{d}	0.69±0.02^{c}	1.30±0.03^{b}	1.62±0.02^{a}
	3	0.48±0.02^{d}	0.81±0.02^{c}	1.41±0.02^{b}	1.68±0.02^{a}
	5	0.72±0.02^{d}	0.89±0.02^{c}	1.61±0.02^{b}	1.72±0.02^{a}
	7	1.08±0.02^{d}	1.29±0.02^{c}	1.70±0.03^{b}	2.30±0.03^{a}
Moisture (%)	1	61.33±0.02^{d}	61.48±0.02^{c}	63.65±0.06^{b}	64.97±0.14^{a}
	3	61.31±0.01^{d}	61.43±0.03^{c}	63.61±0.04^{b}	64.94±0.00^{a}
	5	61.28±0.01^{c}	61.38±0.00^{c}	63.58±0.03^{b}	64.69±0.11^{a}
	7	61.25±0.05^{d}	61.34±0.04^{c}	63.51±0.03^{b}	64.40±0.01^{a}
Fat/DM (%)	1	1.29±0.00^{d}	7.62±0.15^{c}	16.28±0.31^{b}	25.50±0.06^{a}
	3	1.38±0.15^{d}	8.04±0.25^{c}	16.85±0.15^{b}	25.57±0.17^{a}
	5	1.46±0.15^{d}	8.55±0.26^{c}	19.93±0.16^{b}	25.87±0.14^{a}
	7	1.55±0.00^{d}	9.14±0.15^{c}	17.27±0.26^{b}	26.03±0.42^{a}

C: Ras cheese curd without lipolyzed cream, T1: Ras cheese curd treated with 10% lipolyzed cream, T2: Ras cheese curd treated with 20% lipolyzed cream, T3: Ras cheese curd treated with 30% lipolyzed cream, All values represented as Means±SD, Means in the same column with different small letter superscripts are significantly different ($\alpha = 0.05$)

control sample varied from 6.21% (WSN/TN) and 0.04 mg leucine/g at the 1st day of storage to 10.26% (WSN/TN) and 0.10 mg leucine/g at the 7th day of the storage. The same trend could be observed on the other samples, e.g., proteolysis of T1 ranged from 6.35-11.20% (WSN/TN) and from 0.06-0.27 mg leucine/g during the storage period, 7.20-12.38% (WSN/TN) and from 0.08 to 0.33 mg leucine/g for T2 and finally 8.87-14.33% (WSN/TN) and from 0.15-1.15 mg leucine/g for T3. This increase in the extent of proteolysis might be due to the action of rennin enzyme used in the curd preparation and the enzymes from the starter cultures. Moreover, the incubation temperature might have a notable effect on the rate of proteolysis as well. The results are in agreement with Abd El-Hamid *et al*.[31], who found higher levels of WSN/TN produced in Ras cheese slurry depending on the incubation temperature used and with El Neenay *et al*.[8], who produced the enzyme modified Ras cheese and found the same trend in the proteolysis development and the higher production of water soluble nitrogenous compounds.

The extent of lipolysis as assessed by the TVFA was determined for all RCFC samples during the storage periods (Fig. 3). The TVFA contents of RCFC samples increased during the progress of curd storage in comparison to the control one. This reflects continuous hydrolysis of lipid fraction in curd during storage. The obtained results suggested that lipolyzed cream affected the production of Total Volatile Fatty Acids (TVFA) which is in accordance with Abd El-Hamid *et al*.[31], Degheidi *et al*.[32], Hassan *et al*.[24] and El Neenay *et al*.[8].

The sensory evaluation of the RCFC samples are presented in Fig. 4, which indicate that the level of added lipolyzed cream affected for the flavor intensity at the end of storage time. This might be due to protein and fat hydrolysis among other enzymatic and chemical reactions during the storage time by the action of lipase enzyme used in the preparation of lipolyzed cream lead to the formation of aroma constituents responsible for flavor perception and subsequent formation of the aroma profile of resultant RCFC. It is well-known that, the higher concentrations of many aroma compounds e.g., diacetyl, 2-butanol, ethylbutanoate and hexylethanoate, may affect negatively the overall flavor profile of the cheese and so, they are reduced during cheese ripening[11]. This is in agreement to our findings and could be noted throughout the concentrations of the detected volatile constituents (Table 1) which found to be inversely proportional to the sensory grades scored for the samples under investigation.

CONCLUSION

The addition of 30% lipolyzed cream into skim milk curd which stored for 7 days at 37°C enhanced both chemical composition, organoleptic evaluation and the flavor profile of prepared RCFC samples compare with cheese aroma compounds generated during classical ripening processes. Fat/DM (%) increased in the treated RCFC samples depending on the amount of added lipolyzed cream and the change of the dry matter contents. Also, cheese curd treated with lipolyzed cream exhibited noticeable differences in the proteolysis rate during storage compared to the control curd.

SIGNIFICANCE STATEMENTS

This study discovers the effect of lipolyzed enzymes on acceleration of ripening of ras cheese and produce ras cheese flavor concentrate. Thus, Ras cheese curd was used to produce Ras Cheese Flavour Concentrate (RCFC). The effect of different levels from lipolyzed cream on the sensory evaluation of Ras cheese flavor was studied. This study will help the industry to increase the turnover of the production of ras cheese as well as the quality of the final product.

RECOMMENDATION

- It was possible to use the lipolyzed cream to speed up the ripening processes and obtain a Ras cheese similar to its sensory properties in the conventional methods
- This study is a new addition in the field of reducing the time for the ripening of cheeses and may need further studies to achieve the best results in this field

ACKNOWLEDGMENT

We are grateful to Professor Nabil Samyfrom, Dairy Science Department at National Research Centre, for his valuable discussions and opinions.

REFERENCES

1. Kilcawley, K.N., M.G. Wilkinson and P.F. Fox, 2006. A novel two-stage process for the production of enzyme-modified cheese. Food Res. Int., 39: 619-627.
2. Kwak, H.S., 1990. Implication of lipase specificity on aged Cheddar flavour development. Korean J. Dairy Sci., 12: 68-81.
3. Tomasini, A., G. Bustillo and J.M. Lebeault, 1993. Fat lipolyzed with a commercial lipase for the production of Blue cheese flavour. Int. Dairy J., 3: 117-127.

4. Garde, S., M. Avila, E. Fernandez-Garcia, M. Medina and M. Nunez, 2007. Volatile compounds and aroma of Hispanico cheese manufactured using lacticin 481-producing *Lactococcus lactis* sub sp. *lactis* INIA 639 as an adjunct culture. Int. Dairy J., 17: 717-726.
5. Tomasini, A., G. Bustillo and J.M. Lebeault, 1995. Production of blue cheese flavour concentrates from different substrates supplemented with lipolyzed cream. Int. Dairy J., 5: 247-257.
6. Liu, S.Q. and V.L. Crow, 2010. Production of dairy-based, natural sulphur flavor concentrate by yeast fermentation. Food Biotechnol., 24: 62-77.
7. Moosavi-Nasab, M., M. Radi and H.A. Jouybari, 2010. Investigation of enzyme modified cheese production by two species of *Aspergillus*. Afr. J. Biotechnol., 9: 508-511.
8. El Neenay, M., S.A. Awad, M. Abbas and I.A. Attia, 2013. Production of enzyme modified Ras cheese. Alexandria Sci. Exchange J., 34: 249-254.
9. Hofi, A.A., E.H. Youssef, M.A. Ghoneim and G.A. Tawab, 1970. Ripening changes in cephalotyre "RAS" cheese manufactured from raw and pasteurized milk with special reference to flavor. J. Dairy Sci., 53: 1207-1211.
10. Oliszewski, R., R.B. Medina, S.N. Gonzalez and A.B.P. Chaia, 2007. Esterase activities of indigenous lactic acid bacteria from Argentinean goats' milk and cheeses. Food Chem., 101: 1446-1450.
11. Ayad, E.H.E., S. Awad, A. El Attar, C. de Jong and M. El-Soda, 2004. Characterisation of Egyptian Ras cheese. 2. flavour formation. Food Chem., 86: 553-561.
12. Hofi, A.A., L.A. Hamid, N.S. Ahmed and H.M. Abbas, 1991. Acceleration of Ras cheese ripening by relevant slurry. Egypt. J. Dairy Sci., 19: 337-346.
13. Adams, R.P., 2007. Identification of Essential Oil Components by Gas Chromatography/Mass Spectorscopy. Allured Publishing Corporation, Carol Stream, IL., USA., ISBN: 9781932633214, pp: 450-460.
14. Nateghi, L., S. Roohinejad, A. Totosaus, H. Mirhosseini and M. Shuhaimi *et al*., 2012. Optimization of textural properties and formulation of reduced fat cheddar cheeses containing fat replacers. J. Food Agric. Environ., 10: 46-54.
15. Novikova, A. and I. Ciprovica, 2009. Effect of ripening conditions on Latvian semi-hard cheese quality. Chemine Technologija, 3: 93-97.
16. Folkertsma, B. and P.F. Fox, 1992. Use of the Cd-ninhydrin reagent to assess proteolysis in cheese during ripening. J. Dairy Res., 59: 217-224.
17. El-Hofi, M.A. and A.A. Ismail, 2000. Utilization of purified and characterized lipase from papaya, *Carica papaya* in acceleration of Ras cheese slurry. Egypt. J. Food Sci., 15: 255-265.
18. Nateghi, L., 2017. Identification and quantification of key volatile flavor compounds employing different adjunct starter cultures in reduced-fat cheddar cheeses by using GC and GC-MS. Applied Food Biotechnol., 4: 43-53.
19. Olson, N.F., 1990. The impact of lactic acid bacteria on cheese flavor. FEMS Microbiol. Rev., 7: 131-147.
20. McSweeney, P.L.H., 2004. Biochemistry of cheese ripening. Int. J. Dairy Technol., 57: 127-144.
21. Bergamini, C.V., I.V. Wolf, M.C. Perotti and C.A. Zalazar, 2010. Characterisation of biochemical changes during ripening in Argentinean sheep cheeses. Small Rumin. Res., 94: 79-89.
22. Vitova, E., B. Loupancova, J. Zemanova, H. Stoudkova, P. Brezina and L. Babak, 2006. Solid-phase microextraction for analysis of mould cheese aroma. Czech J. Food Sci., 24: 268-274.
23. Castillo, I., M.V. Calvo, L. Alonso, M. Juarez and J. Fontecha, 2007. Changes in lipolysis and volatile fraction of a goat cheese manufactured employing a hygienized rennet paste and a defined strain starter. Food Chem., 100: 590-599.
24. Hassan, F.A.M., M.A.M. Abd El-Gawad and A.K. Enab, 2012. Flavour compounds in cheese (review). Int. J. Acad. Res. Part A, 4: 169-181.
25. Fox, P.F. and J.M. Wallace, 1997. Formation of flavor compounds in cheese. Adv. Applied Microbiol., 45: 17-86.
26. Curioni, P.M.G. and J.O. Bosset, 2002. Key odorants in various cheese types as determined by gas chromatography-olfactometry. Int. Dairy J., 12: 959-984.
27. Welsh, F.W., W.D. Murray, R.E. Williams and I. Katz, 1989. Microbiological and enzymatic production of flavor and fragrance chemicals. Crit. Rev. Biotechnol., 9: 105-169.
28. Le Bars, D. and M. Yvon, 2008. Formation of diacetyl and acetoin by *Lactococcus lactis* via aspartate catabolism. J. Applied Microbiol., 104: 171-177.
29. Aghlara, A., S. Mustafa, Y.A. Manap and R. Mohamad, 2009. Characterization of headspace volatile flavor compounds formed during kefir production: Application of solid phase microextraction. Int. J. Food Prop., 12: 808-818.
30. Ozcan, T. and E. Kurdal, 2012. The effects of using a starter culture, lipase and protease enzymes on ripening of Mihalic cheese. Int. J. Dairy Technol., 65: 585-593.
31. Abd El-Hamid, L.B., G.A. Mahran, H. Abbas and M.A. El-Hofi, 1991. Ample production for Ras cheese slurry. Egypt. J. Dairy Sci., 19: 327-335.
32. Degheidi, M.A., N.S. Abd Rabou and A. Ismail, 1998. Improvement of Domiati cheese quality during pickling using jack fruit lipase. Egypt. J. Dairy Sci., 26: 103-116.