Characterization of Enzyme Modified Ras Cheese

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Abstract

Enzyme Modified Cheese (EMC) as a concentrated cheese flavor was used as a food additive for low fat Ras cheese in order to improve its acceptability, functionality, and reduction of ripening period. Hence, chemical and sensorial characteristics of EMC produced using pepsin at the levels of 500, 1500, and 3000 U.g-1 cheese, while lipase (5000 U.g-1 cheese); as well as Ras cheese during the ripening period were investigated. The results indicated that no significant ($p \leq 0.05$) differences in EMC components treated with pepsin and lipase enzymes compared to control sample except in the highest level of pepsin (3000 U.g-1). Ras cheese results indicated that significant ($p \leq 0.05$) differences in protein, fat, and ash contents of Ras cheese with EMC compared to control cheese with the opposite trend for moisture content except in the 1st level of EMC (1.25%). Also, the ripening indices of EMC-Ras cheese showed that significant ($p \leq 0.05$) increasing of both water-soluble nitrogen (WSN) (0.4-3.3%) and total volatile fatty acids (TVFA) (5-15%) at all levels compared to control cheese (0.3-0.7% for WSN), and (5-12 % for TVFA). EMC-Ras cheese recorded the highest essential amino acids (EAA) including (Arg, Val, and Phe) compared to control cheese. Moreover, EMC addition to Ras cheese decreased biogenic amines including cadaverine (0.4 mg.kg-1), and putrescine (0.2 mg/kg) compared to control cheese (3.44 and 0.23 mg.kg-1, respectively). Also, serotonin, β-phenyl ethyl amine, and spermidine are detected in EMC-Ras cheese with trace concentrations. However, the sensorial evaluation of low fat Ras cheese indicated that EMC-Ras cheese flavor is higher than control cheese especially after 1st month until the end of ripening period without any bitterness was pronounced; texture evaluation showed that the addition of EMC to low fat Ras cheese significantly improved the body and texture scores in comparison of control cheese especially after 1st month until the end of ripening period. Thus, EMC could be used as a function ingredient for low-fat Ras cheese with good chemical, and nutritional characteristics, without any defects appeared during the ripening period.

Keywords

Enzyme Modified cheese; Low fat; Ras Cheese; Cheese flavor; Functional.

INTRODUCTION

One of the most innovative uses of enzymes in food technology during the last decade is the modification of food proteins due to their ability to change the functionality of food proteins. The application of exogenous enzymes to immature or semi-ripened cheese produces a cost-effective intense flavor of that cheese, which is known as Enzyme Modified Cheese (EMC) and is suitable to be incorporated in many formulations and in a variety of foods, such as bakery, confectionery, processed cheese, and cheese sauce, as a partial or whole replacement of natural cheese. Also, EMC is used in low-fat products in order to replace fat functionality due to health-related concerns of saturated fat and cholesterol in traditional dairy products [1]. However, low-fat dairy products were considered to be less acceptable to consumers than full-fat products due to texture defects lack of flavor [2].

EMC technology is based on the incorporation of certain enzymes in order to produce the final flavor of cheese from a suitable substrate within 72 to 96 h under optimum conditions. These enzymes are a mixture of proteases, peptidases, lipases, and esterases. Currently, a wide range of these enzymes is commercially available from microbial and animal sources, which could be used in the production of different varieties of EMC [3, 4].

Cheddar EMC is probably the most widely used cheese flavor ingredient and is available as a range of products, differing in purported sensory properties [5]. Habibi-Najafi and Lee [6] produced Cheddar EMC without bitterness using Neutrase and crude enzyme extract from Lactobacillus casei ssp. casei LLG. EMC supplemented with Neutrase tended to induce the accumulation of intense bitter peptide. Azarnia et al. [7] investigated the effect of natural crude enzyme or recombinant aminopeptidase, both derived from Lactobacillus rhamnosus S93, in the presence of a commercial proteinase (Neutrase) on the proteolysis of Cheddar EMC. They reported that the EMC containing the recombinant aminopeptidase alone resulted in the complete disappearance of proline after 1 day of maturation time. Mousavi-Nasab et al. [8] produced EMC by Aspergillus oryzae and Aspergillus niger. The EMC produced using both A. oryzae and A. niger had the best score of flavor and odor after 3 days of storage. Najafi and Miri [9] used Neutrase and Flavourzyme as commercial enzymes to produce Lighvan EMC. They reported that the level of these enzymes and the incubation period do not significantly affect bitterness due to the high salt content of the initial substrate. Also, the sensorial evaluation indicated that the Flavourzyme enzyme significantly affected texture and general acceptance during the 24, 72, and 96 h incubation periods. Moreover, the Neutrase enzyme had a significant effect on the texture during the 96-h incubation period. However, there is no significant difference between the samples in terms of other sensory properties.

There is an increasing demand in the world to produce dairy-based or non-dairy products with specific cheese flavor. Hence, the present work will be focused on EMC production as a concentrated cheese flavor which could be used in the addition of cheese flavor for dairy product in order to improve its acceptability and functionality, as well as it could be used for acceleration of hard cheese ripening as an attempt to reduce the final cost of cheese and stabilize their characteristics. Thus, the objective of the present study was EMC production as a food ingredient in order to use it in the production of low-fat Ras cheese. Also, chemical and sensorial characteristics of the resulted EMC and their product during the ripening period were investigated.

Materials and methods

Chemicals and raw materials

Fresh cow's and buffalo's milk were obtained from the Animal Production Research Institute, Ministry of Agriculture, Giza, Egypt. Microbial rennet powder was purchased from Gaglio Star, Spain. Cheese starter cultures (Lactococcus lactis subsp. lactis and Lactococcus lactis subsp. cremoris) were obtained from the Egyptian Microbial Culture Collection, Ain Shams University, Egypt. Standard amino acids were purchased from Sigma-Aldrich (St. Louis, Missouri, USA). All other reagents and chemicals were used in the analytical grade.

Methods

Preparation of EMC

Ras cheese was made by the conventional method according to Hofi et al. [10] using a mixture of cow's and buffalo's milk. EMC was prepared as described by Mousavi-Nasab et al. [8] using fresh Ras cheese which mixed with distilled water, NaCl, trisodium citrate, sodium orthophosphate, and nisin. The resulting mixture was heated for 30 min at 70 °C and then was cooled to 40 °C. Also, the mixture was treated with pepsin at the levels of 500, 1500, and 3000 U.g-1 cheese, while lipase (5000 U.g-1 cheese) was added and mixed well. The control EMC sample was prepared in the same condition without the addition of pepsin and lipase enzymes. All treatments were incubated at 40 °C for 4 days then EMC paste samples were heated to 85 °C for 10 min to inactivate the enzymes. All EMC treatments were incubated for 4 days and analyzed daily for both chemical and sensorial properties.

Preparation of Ras cheese with EMC

Low-fat Ras cheese (prepared using low fat milk) was made using EMC in the levels of 1.25, 2.5, and 5% for cheese curd before molding. Ras cheese samples were analyzed monthly for both chemical and sensorial properties during their ripening period for 4 months.

Chemical characterization

Chemical analysis of the resulting EMC and Ras cheese samples for moisture, protein, and fat were performed according to AOAC [11]. Also, water-soluble nitrogen (WSN), and ash of the same samples were determined according to Ling [12], while total volatile fatty acids (TVFA) were determined as described by Kosikowski [13].

Acidity and pH

Titratable acidity of EMC and Ras cheese samples were determined according to Ling [12]. Also, the pH of the same samples was measured using a digital pH-meter (Hanna Instruments, Woonsocket, Rhode Island, USA).

Amino acids profile

The amino acid profile of Ras cheese samples were detected at 254 nm (2489 UV/Vis Detector) using high-performance liquid chromatography (HPLC) Waters associates equipped with the 600 E Multi Solvent Delivery System and Pico-Tag Workstation amino acid analyzer (Waters, USA) with a column (150 x 3.9 mm) at 38 °C, flow rate 1 ml/min according to Cohen et al. [14].

Biogenic amines content

Biogenic amines content of Ras cheese samples were determined according to Fu et al. [15] including Agmatin, Treptamine, β-phenyl ethyl amine, Putrescine, Cadaverine, Histamine, Serotonin, Tyramine, Spermidine and Spermine. Biogenic amines were performed with HPLC using Agilent Technologies 1260 Infinity II liquid chromatograph equipped (USA) with an auto sampler and a diode-array detector. The analytical column was an Eclipse XDB-C18 (150 X 4.6 µm; 5 µm) with a C18 guard column (Phenomenex, Torrance, CA) was used for biogenic amines separation. Biogenic amines were detected at 254 nm using UV detector. Data were integrated and recorded using Chromele on Software program.

Sensory evaluation

Sensory evaluation of both EMC and Ras cheese samples were performed using a five-point hedonic scale (ranked from extremely bad or 1 to extremely good or 5) as described by Ghazizadeh and Raseghi [16]. Tested samples were assessed by panelists from the staff members of Dairy Science Department (National Research Centre); as well as Nutrition and Food Sciences Department (Faculty of Home Economics), for flavor, bitterness, texture, and general acceptability.

Statistical analysis

All statistical analysis of results was performed by SAS statistical software (SAS Institute, Cary, North Carolina, USA) using the ANOVA procedure for analysis of variance. The results were expressed as mean ±

standard error and the differences between means were tested for significance using Duncan’s multiple ranges at p≤0.05.

Results and discussion

EMC characteristics

Chemical characterization of EMC

The chemical changes of EMC during their incubation period, which was treated with pepsin at different levels, including 500, 1500, and 3000 U.g-1 and lipase (5000 U.g-1) were shown in (Table 1).

The results indicated that no significant (p≤0.05) differences in moisture, protein, fat, and ash contents of EMC treated with such enzymes compared to control except in the highest level of pepsin (3000 U.g-1). However, Ali et al. [17], and Abdella et al. [18] observed a slight increase in the moisture content of EMC compared to the control. Also, no significant (p≤0.05) differences were observed in such parameters during the incubation period for 4 days (Table 1).

However, (Table 1) shows the influence of EMC enzymatic treatment of EMC on WSN and TVFA as ripening indices. The results showed that significant (p≤0.05) increases of both WSN (0.8-1.2%) and TVFA (25-45%) at all levels compared to control EMC (0.8-1.0% for WSN), and (11-32 % for TVFA). After 4 days, such ripening indices of EMC samples were increased as the incubation period progressed (Table 1), which is mainly attributed to the proteolytic and lipolytic activities of pepsin and lipase. Also, WSN and TVFA are increased during the incubation period due to moisture loss of EMC. These findings align with previous studies by Kilcawley et al. [19], Noronha et al. [20], and Abdella et al. [18], who reported higher levels of WSN and TVFA in EMC in comparison to control.

It could be noted that the EMC acidity, with opposite trends in their pH values, treated with pepsin and lipase was close to control EMC (1.1-1.3% and 5.2-5.8 pH) with significantly (p≤0.05) increasing (1.4-1.7% for acidity and pH of 5.0-5.2) after a 4-day of incubation period (Table 1). It could probably be due to the proteolytic and lipolytic activities of such enzymes, combined with other acidic components resulting from the fermentation of residual lactose and degradation of cheese fat [21-22]. Similar findings were reported by Ali et al. [17], and Abdella et al. [18], who observed lower pH values of EMC products compared to control.

Sensorial attributes of EMC

The sensorial evaluation of EMC during the incubation time for 4 days as presented in Fig. 1 indicated that the EMC flavor is higher than the control except the highest level of enzyme addition. It could be mainly attributed to the excessive degradation of EMC proteins resulting from the pepsin enzyme which tends to stimulate the accumulation of intense hydrophobic bitter peptides during the 4th day of incubation period [18-20]. After 4 days, the first level of enzymes (pepsin 500 U.g-1 and lipase 5000 U.g-1) had the highest flavor score among all samples; while the other levels of enzymes decreased with the incubation time progressed. Similarly, Mousavi-Nasab et al. [8] reported that EMC produced by both A. oryzae and A. niger after 3 days of storage had the best score of flavor and odor. However, high salt content of the initial substrate is one factor that may contribute to flavor enhancement [23].

Also, Fig. 1 shows that body and texture scores of EMC in the first level are close to control, while such scores were decreased with the enzyme levels increased. No bitterness was pronounced in EMC treated with enzymes (pepsin 500 U.g-1 and lipase 5000 U.g-1), and control; while in the higher levels of enzymes the bitterness was observed and increased with the enzymes increased. However in EMC products, bitterness is one of the very common defects and the best EMC has a minimum [6]. The commercial proteases such as Neutrase and Flavourzyme significantly contributed to the bitterness due to high levels of amino- and carboxy-peptidase activities [23].

However, overall acceptability confirmed the other sensorial attributes finding which indicated that the first level of enzymes (pepsin 500 U.g-1 and lipase 5000 U.g-1) used for EMC products had the highest score of overall acceptability during the incubation time for 4 days in comparison to the control as well as other treatments (Fig. 1).

Cheese characteristics

Cheese chemical characteristics

The chemical changes of low-fat Ras cheese with EMC during their ripening period for 4 months at different levels including 1.25, 2.5, and 5% into cheese curd before molding were shown in Table 2.

The results indicated significant ($p \leq 0.05$) differences in protein, fat, and ash contents of Ras cheese with EMC compared to control cheese with the opposite trend for moisture content especially in in the 3rd level of EMC (5%). Also, significant ($p \leq 0.05$) differences were recorded in such parameters during the ripening period progressed for 4 months (Table 2). However, protein, fat, and ash contents increased in all resulted Ras cheese treatments throughout their ripening period progressing mainly due to the loss of the moisture content of cheese during ripening [24].

Acidity and pH of cheese

It could be noticed that Fig. 2 shows Ras cheese acidity, with opposite trends in their pH values, treated with EMC (1.2-1.8% and 5.2-4.6 pH) were close to control cheese (1.3-1.8% and 5.2-4.6 pH). After the 2nd month of ripening, the acidity of EMC-Ras cheese was slightly higher than that of control cheese with opposite trends in their pH values. It could probably be due to EMC components resulting from enzymatic modification with pepsin and lipase enzymes [25], combined with other acidic components resulting from the fermentation of residual lactose and degradation of cheese fat [21, 22].

Ripening indices of cheese

It could be noted that Fig. 3 shows the influence of EMC addition during Ras cheese production on WSN and TVFA as ripening indices [26]. The results showed that significant ($p \leq 0.05$) increases of both WSN (0.4-3.3%) and TVFA (5-15%) at all levels compared to control cheese (0.3-0.7% for WSN and 5-12% for TVFA). Such ripening indices of EMC-Ras cheese samples were increased as the ripening period progressed (Fig. 3), which is mainly attributed to the proteolytic and lipolytic activities of pepsin and lipase, which affect both the flavor and texture of the final cheese characteristics [27]. Also, WSN and TVFA are increased during the incubation period due to moisture loss of cheese (Table 5). However, during the early stages of proteolysis, WSN production occurs, which serves as an indicator of both the rate and extent of proteolysis. This process is crucial for the development of flavor and texture in cheese during its storage period [27, 28].

Cheese sensorial attributes

Since the origins of humankind, odor and taste have been sensorial attributes used instinctively for helping in the selection and increasing the palatability of foods [29]. Also, there has been substantial interest in developing some dairy products with reduced fat contents to avoid some health problems associated with high fat intake [30]; while such products were less acceptable to consumers than full-fat products due to texture defects and lack of flavor [2]. In the present study, EMC was used during the production of low-fat Ras cheese in order to overcome the defects of such products. Hence, the sensorial evaluation of Ras cheese during the ripening period for 4 months, as presented in Fig. 4, indicated that EMC-Ras cheese flavor is higher than control cheese, especially after the 1st month until the end of the ripening period. It could be mainly due to the addition of EMC, which contains the resulting degraded proteins and fats by enzyme addition, including pepsin and lipase. This finding confirmed the sensorial attributes of EMC (Fig. 1), which affect the low-fat cheese flavor as well as improve their flavor due to their content of peptides with small and medium molecular weight and free amino acids (FAA) contributing to the intense flavor [25]. Also, FAA served as precursors to other catabolic reactions that produced amines, ammonia, keto acids, aldehydes, alcohols and acids which are essential to the aroma and taste of cheese [31]. On the other side, lipolysis is also an important component in the production of medium-and short-chain free fatty acids, which contributes to flavor and aroma intensity [25].

The results of texture evaluation (Fig. 4) showed that the addition of EMC to low-fat Ras cheese significantly improved the body and texture scores in comparison to control cheese, especially after the 1st month until the end of the ripening period. No bitterness was pronounced in EMC-Ras cheese, which could be mainly due to using EMC treated with pepsin 500 U.g-1 and lipase 5000 U.g-1 without any bitterness (Fig. 1).

However, overall acceptability confirmed the other sensorial attributes findings, which indicated that the second level of EMC used for low-fat Ras cheese had the highest score of overall acceptability, especially after the 1st month until the end of the ripening period.

Hence, low-fat Ras cheese sensorial attributes confirmed their chemical characteristics, including WSN and TVFA (Fig. 3). It means the addition of EMC prepared with pepsin and lipase enzymes to low-fat Ras cheese improved their sensorial attributes compared to control cheese.

Amino acids profile of cheese

The amino acids profile of the accepted Ras cheese from the sensorial evaluation (Fig. 4) compared to control cheese are presented in Table 3. The results indicated that Ras cheese recorded the highest essential amino acids (EAA), including (Arg, Val, and Phe), and EAA/total amino acids ratio (0.5). It could be due to the proteolytic activity of the pepsin enzyme [18], as well as the recorded balance of amino acid profile, which reflects no bitterness appeared in Ras cheese during the ripening period for 4 months. However, the proteolytic degradation of the cheese proteins into peptides with small and medium molecular weight, and free amino acids (FAA) significantly contributes to the intense flavor and perception of cheese [25]. Hence, Ras cheese amino acids profiles confirmed the ripening indices investigation, especially for WSN (Fig. 3), and the sensorial attributes evaluation (Fig. 4) of such low-fat Ras cheese.

Biogenic amines content of cheese

The biogenic amines content of the accepted Ras cheese from the sensorial evaluation (Fig. 4) compared to control cheese are illustrated in Table 4. The results indicated that agmatin, and spermine are not detected in both Ras cheese treated with EMC and control cheese. EMC addition to low fat Ras cheese decreased cadaverine (0.4 mg.kg-1), and putrescine (0.2 mg.kg-1) compared to control cheese (3.4 and 0.2 mg.kg-1, respectively); whereas notable increase in treptamine (26 mg.kg-1), and histamine (2.5 mg.kg-1) treated with EMC compared with control cheese (16.9, and 0.4 mg.kg-1, respectively). Also, serotonin, β-phenyl ethyl amine, and spermidine are dectected in EMC-Ras cheese with traces concentrations (Table 4). It could be mainly due to the proteolytic degradation of proteins by pepsin into small peptides and free amino acids which served as precursors to other catabolic reactions that produced amines, ammonia, keto acids, aldehydes, alcohols and acids which are essential to the aroma and taste of cheese [25, 31]. Proteolysis is a main component that is helpful in the biogenic amines accumulation of foods. Protein hydrolysis produces nitrogen compounds like free amino acids, peptides, and ammonia, which are considered the precursors of biogenic amines accumulation [32, 33]. Also, Manca et al. [34] indicated that both proteolysis activity and time of ripening were the main sources for biogenic amines formation in cheese. Proteases contents were correlated with tryptamine, tyramine and phenethylamine, while peptides were correlated with spermidine and histamine. Also, amino acid decarboxylase has positively correlated with tyramine and histamine [35]. These results can be discussed: whey tryptamine and histamine concentration in treated cheese with EMC was higher than in the control cheese.

Table 1. Chemical characterization of EMC treated with pepsin and lipase.

Parameter	Incubation period (day)	EMC treatments			
		EMC0	**EMCI**	**EMCII**	**EMCIII**
Moisture [%]	1	43.9 ± 0.01^{c}	42.5 ± 0.02^{d}	46.0 ± 0.03^{b}	46.5 ± 0.03^{a}
	4	38.4 ± 0.01^{f}	37.2 ± 0.12^{g}	38.2 ± 0.17^{f}	39.5 ± 0.02^{e}
Protein [%]	1	18.9 ± 0.11^{b}	15.7 ± 0.23^{d}	17.2 ± 0.17^{c}	17.5 ± 0.29^{c}
	4	19.6 ± 0.12^{ab}	17.0 ± 0.01^{c}	19.0 ± 0.58^{b}	20.0 ± 0.29^{a}
Fat [%]	1	35.0 ± 0.58^{c}	35.0 ± 0.01^{c}	35.0 ± 0.58^{c}	38.0 ± 0.58^{b}
	4	37.0 ± 0.01^{b}	35.0 ± 0.01^{c}	37.0 ± 0.58^{b}	40.0 ± 0.58^{a}
Ash [%]	1	6.4 ± 0.12^{e}	8.6 ± 0.17^{d}	9.7 ± 0.12^{b}	10.9 ± 0.06^{a}
	4	9.1 ± 0.12^{c}	8.9 ± 0.02^{cd}	9.9 ± 0.03^{b}	11.2 ± 0.01^{a}
WSN [%]	1	0.8 ± 0.12^{f}	0.8 ± 0.06^{ef}	0.9 ± 0.06^{def}	1.0 ± 0.01^{bcd}
	4	1.0 ± 0.02^{cde}	1.1 ± 0.01^{abc}	1.1 ± 0.01^{ab}	1.2 ± 0.06^{a}
TVFA [%]	1	11.0 ± 0.58^{f}	25.0 ± 0.01^{e}	32.0 ± 0.58^{d}	37.0 ± 0.58^{c}
	4	32.0 ± 0.58^{d}	37.0 ± 0.01^{c}	39.0 ± 0.58^{b}	45.0 ± 0.58^{a}
Acidity [%]	1	1.1 ± 0.12^{cd}	0.9 ± 0.06^{d}	1.0 ± 0.12^{cd}	1.0 ± 0.12^{cd}
	4	1.3 ± 0.06^{bc}	1.4 ± 0.06^{b}	1.7 ± 0.03^{a}	1.7 ± 0.06^{a}
pH	1	5.8 ± 0.12^{a}	5.3 ± 0.17^{b}	5.3 ± 0.17^{b}	5.3 ± 0.12^{b}
	4	5.2 ± 0.06^{b}	5.2 ± 0.06^{b}	5.1 ± 0.17^{b}	5.0 ± 0.01^{b}

All values represent the mean values ± standard error obtained from two repetitions. Means with different small superscript letters in the same row and different capital superscript letters in the same column are significantly different at $p \leq 0.05$.

EMC0: EMC from fresh Ras cheese curd without enzymes addition; EMCI: EMC from fresh Ras cheese curd treated with 500 U of pepsin and 5000 U of lipase enzymes per gram cheese; EMCII: EMC from fresh Ras cheese curd treated with 1500 U of pepsin and 5000 U of lipase enzymes per gram cheese; EMCIII: EMC from fresh Ras cheese curd treated with 3000 U of pepsin and 5000 U of lipase enzymes per gram cheese.

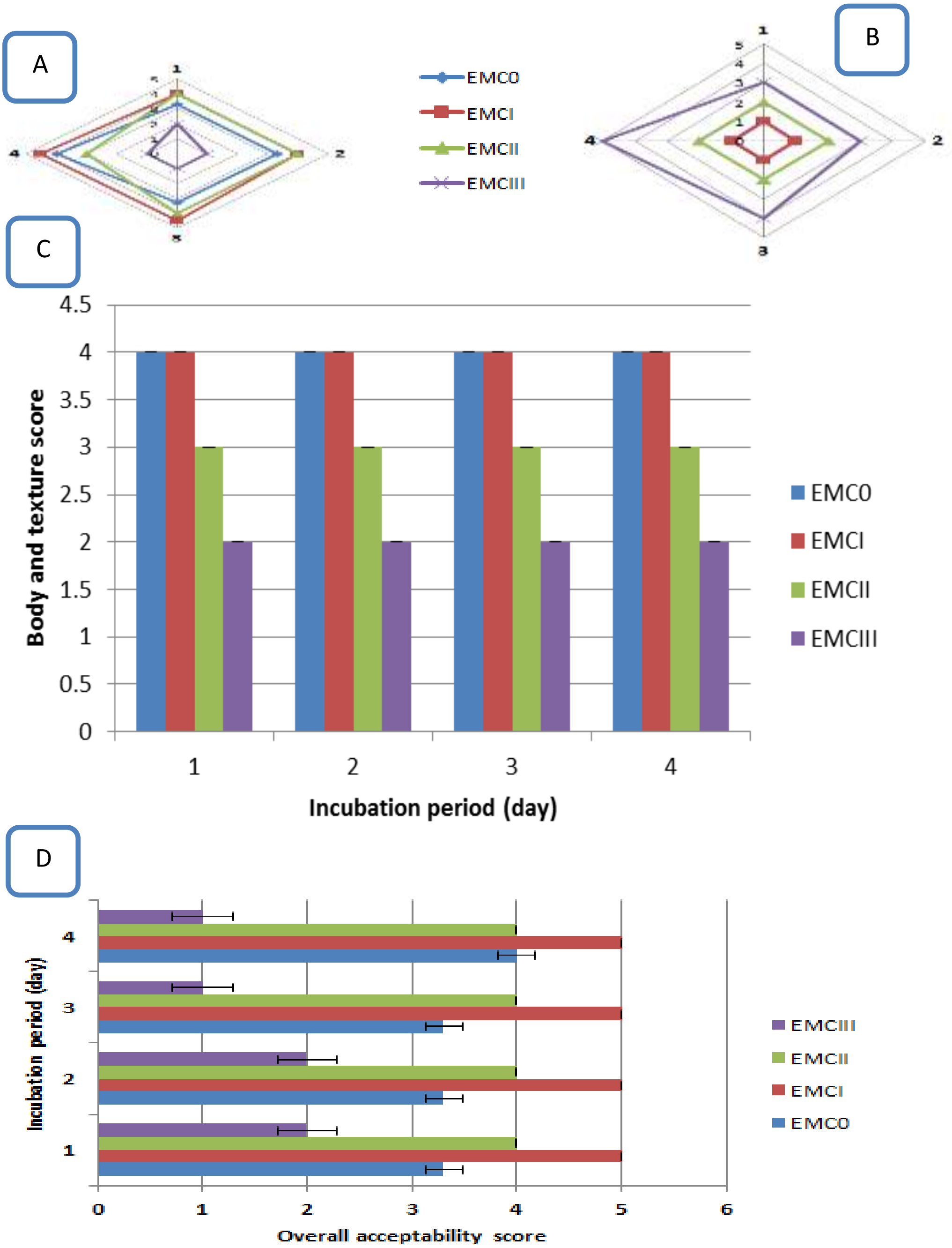

Fig. 1. Sensorial attributes of EMC treated with pepsin and lipase during 4 days.
.Explanation of abbreviations is the same as in the caption of Table 1
A: flavor; B: body and texture; C: bitterness, and D: overall acceptability

Table 2. Chemical characteristics of Ras cheese with EMC.

Parameter [%]	Ripening period (month)	Ras cheese treatments			
		Control	**RI**	**RII**	**RIII**
Moisture	1	42.2 ± 0.29^{a}	41.4 ± 0.23^{a}	32.8 ± 0.29^{e}	32.1 ± 0.40^{ef}
	2	39.7 ± 0.40^{b}	38.1 ± 0.23^{c}	31.1 ± 0.40^{f}	31.7 ± 0.35^{ef}
	4	38.6 ± 0.23^{c}	36.7 ± 0.40^{d}	31.0 ± 0.58^{f}	31.5 ± 0.29^{f}
Protein	1	17.2 ± 0.35^{e}	12.9 ± 0.29^{f}	20.6 ± 0.35^{cd}	18.0 ± 0.35^{e}
	2	21.4 ± 0.40^{bc}	18.0 ± 0.23^{e}	21.4 ± 0.35^{bc}	20.3 ± 0.29^{d}
	4	21.6 ± 0.29^{bc}	20.1 ± 0.35^{d}	24.0 ± 0.35^{a}	22.3 ± 0.29^{b}
Fat	1	25.0 ± 0.58^{d}	25.0 ± 0.01^{d}	25.0 ± 0.58^{d}	27.0 ± 0.01^{c}
	2	27.0 ± 0.01^{c}	25.0 ± 0.58^{d}	27.0 ± 0.58^{c}	30.0 ± 0.01^{b}
	4	30.0 ± 0.58^{b}	30.0 ± 0.58^{b}	31.0 ± 0.01^{b}	33.0 ± 0.01^{a}
Ash	1	2.8 ± 0.40^{b}	3.5 ± 0.23^{ab}	3.8 ± 0.29^{ab}	3.9 ± 0.29^{a}
	2	3.8 ± 0.23^{a}	3.7 ± 0.35^{ab}	4.1 ± 0.23^{a}	4.0 ± 0.35^{a}
	4	3.9 ± 0.35^{a}	3.9 ± 0.29^{a}	4.2 ± 0.40^{a}	4.3 ± 0.35^{a}

All values represent the mean values ± standard error obtained from two repetitions. Means with different small superscript letters in the same row and different capital superscript letters in the same column are significantly different at $p \le 0.05$.
Control: Ras cheese without EMC addition; RI: Ras cheese with 1.25% EMC for cheese curd before molding; RII: Ras cheese with 2.5% EMC for cheese curd before molding; RIII: Ras cheese with 5% EMC for cheese curd before molding.

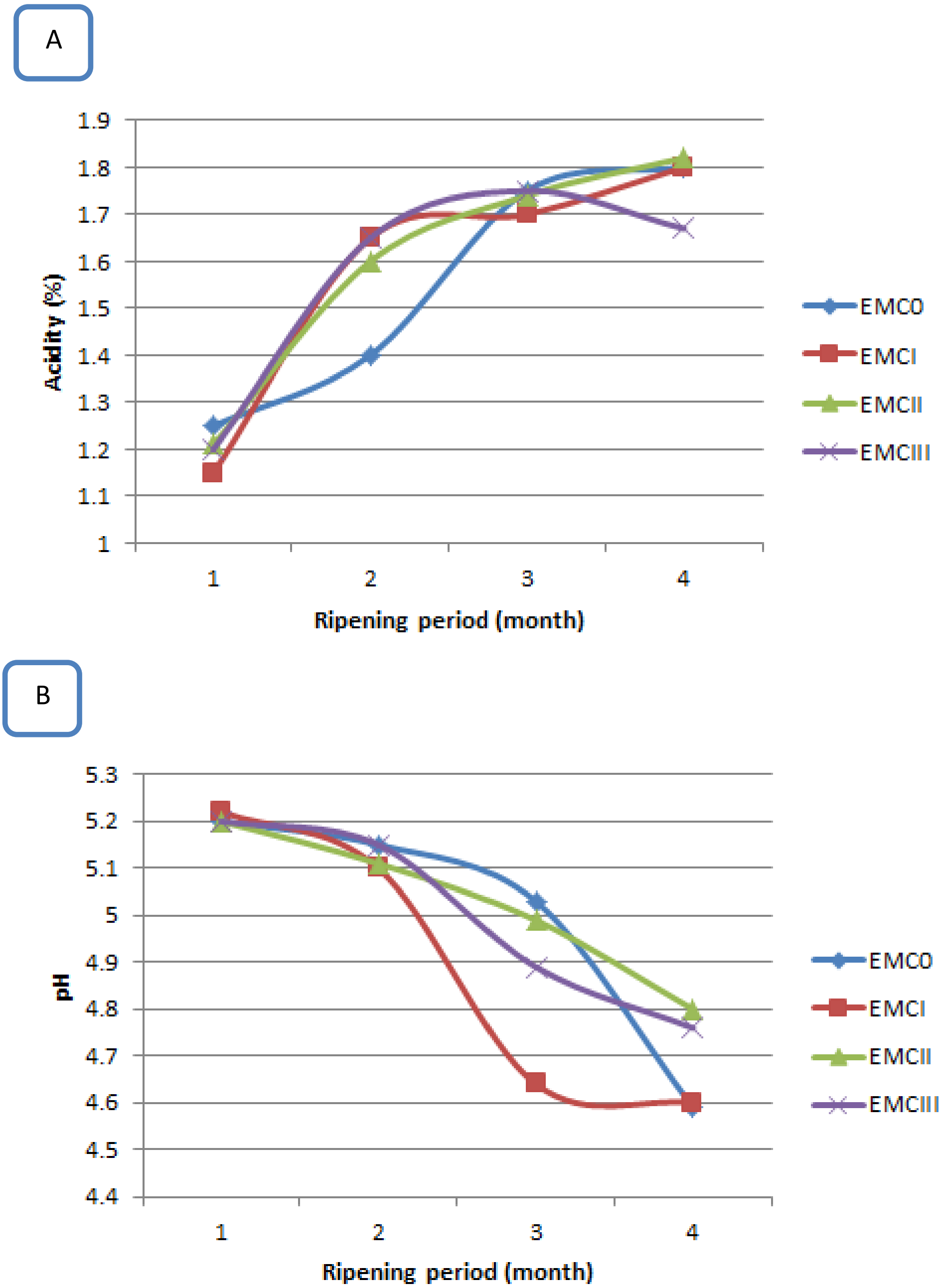

Fig. 2. Acidity and pH of Ras cheese with EMC.
Explanation of abbreviations is the same as in the caption of Table 2.
A: cheese acidity; B: cheese pH

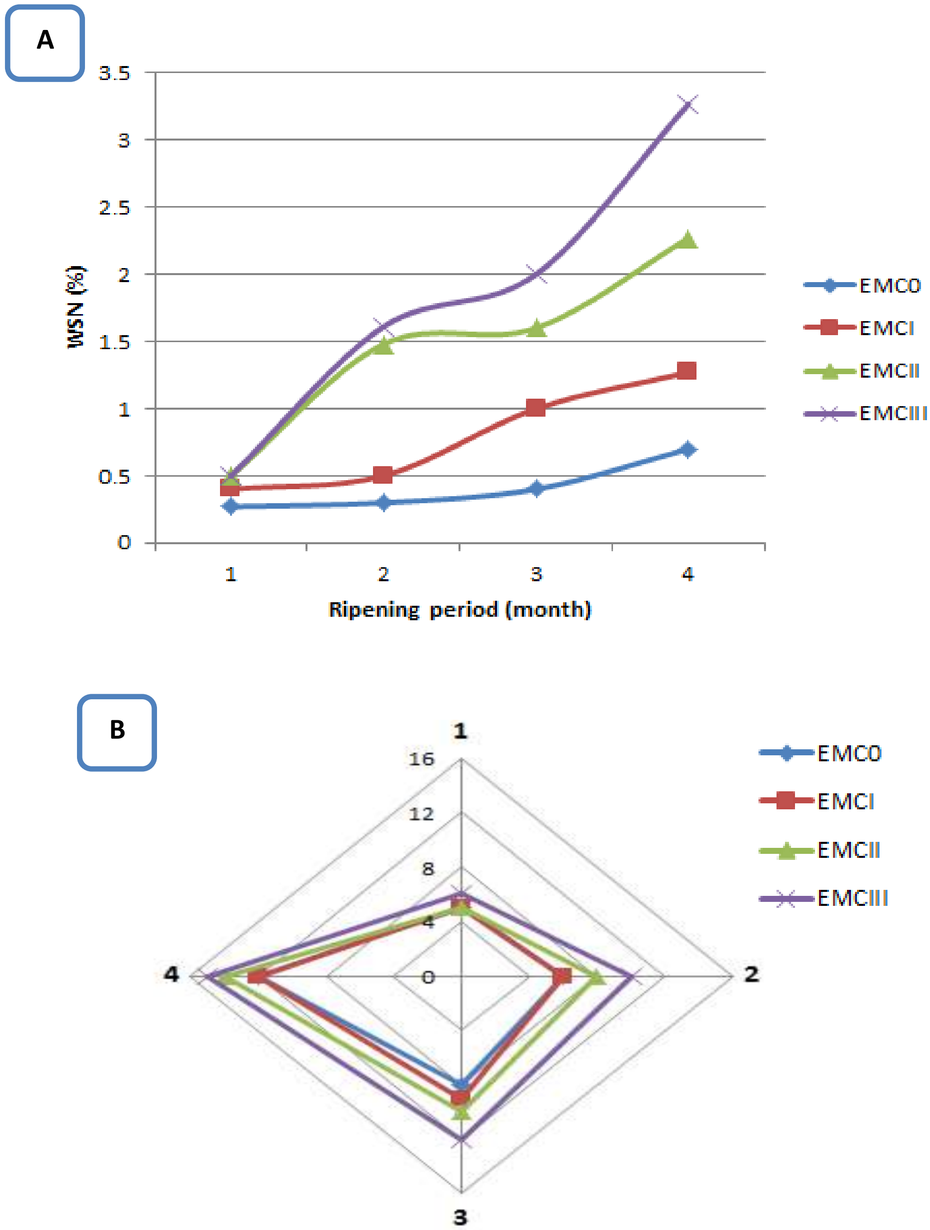

Fig. 3. Ripening indices of Ras cheese with EMC.
Explanation of abbreviations is the same as in the caption of Table 2.
A: water soluble nitrogen (WSN); B: total volatile fatty acids (TVFA).

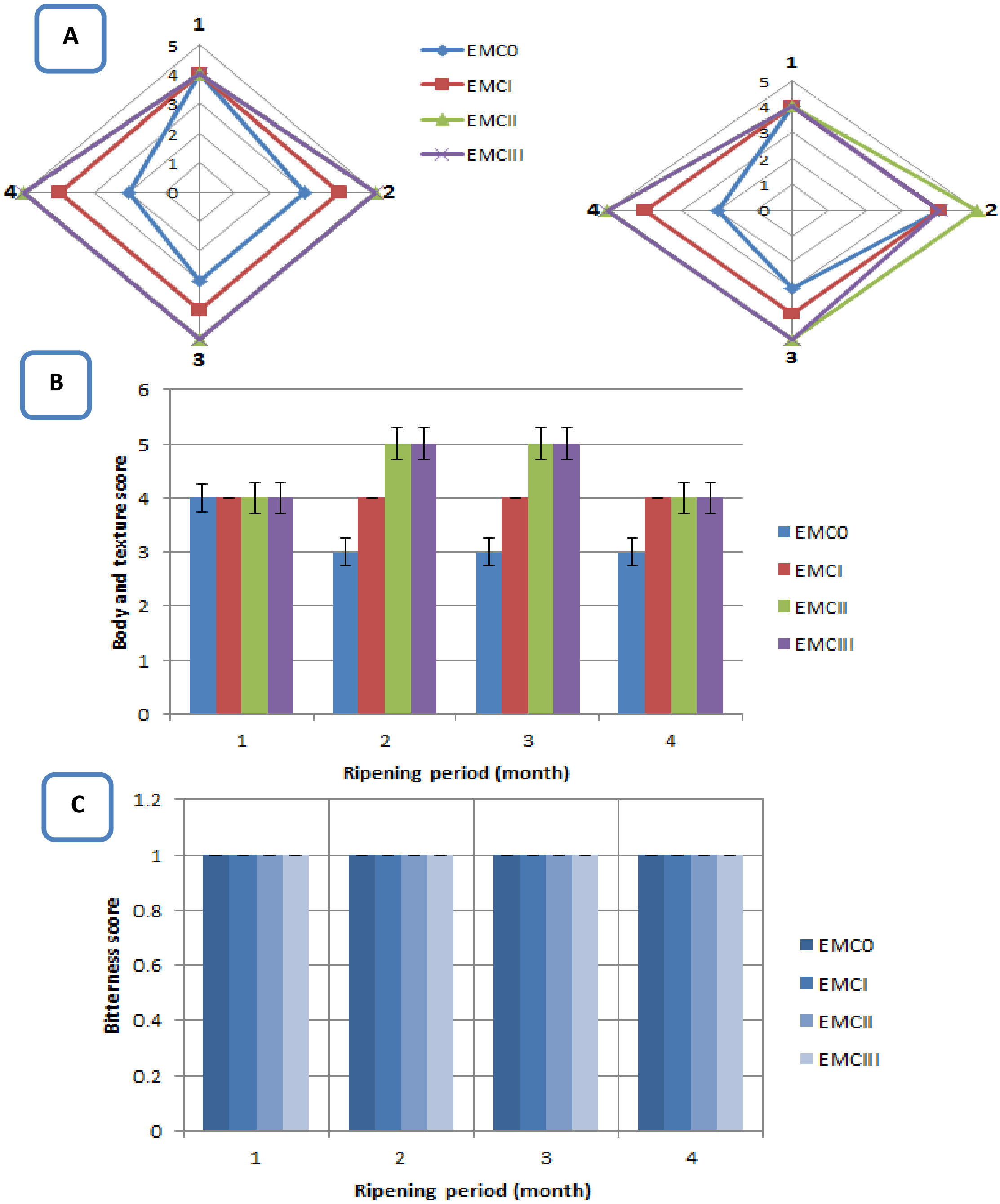

Fig. 4. Sensorial attributes of Ras cheese with EMC during 4 months.
Explanation of abbreviations is the same as in the caption of Table 2.
A: flavor; B: body and texture; C: bitterness, and D: overall acceptability.

Table 3. Amino acids profile of Ras cheese with EMC.

Amino acids (mg.g^{-1} protein)	Ras cheese treatments	
	Control	**RII**
Aspartic acid	88.5	150.6
Glutamic acid	103.2	97.3
Serine	51.2	39.2
Glycine	24.8	24.1
Histidine	44.8	35.9
Arginine	51.8	59.5
Threonine	53.9	61.7
Alanine	51.5	44.8
Proline	62.3	24.5
Tyrosine	67.2	45.2
Valine	52.5	56.8
Methionine	44.4	44.1
Cysteine	11.5	20.6
Isoleucine	53.2	47.7
Leucine	64.1	61.6
Phenylalnine	62.9	79.5
Lysine	64.1	58.2
Total EAA	437.8	443.2
Total amino acids	952.0	951.1
EAA/TAA ratio	0.5	0.5

Explanation of abbreviations is the same as in the caption of Table 2.

Table 4. Biogenic amines content of Ras cheese with EMC.

Biogenic amines (mg.Kg^{-1})	Cephalotyre (Ras) cheese treatments	
	Control	**RII**
Agmatin	ND	ND
Treptamine	16.9	26.0
β-phenyl ethyl amine	ND	0.1
Putrescine	0.2	0.2
Cadaverine	3.4	0.4
Histamine	0.4	2.5
Serotonin	ND	0.2
Tyramine	ND	ND
Spermidine	ND	0.1
Spermine	ND	ND

ND: not detected.
Explanation of abbreviations is the same as in the caption of Table 2.

Conclusion

Enzyme-modified cheese (EMC) was successfully produced by enzymatic biotransformation using pepsin and lipase enzymes. EMC as a food ingredient produced from fresh Ras cheese curd treated with such enzymes has good sensory and chemical properties without any apparent defects compared to the untreated sample.

EMC could be used as a functional ingredient during the production of low-fat Ras cheese in order to overcome the health problems associated with high fat intake, as well as avoid the texture defect and lake flavor of such products.

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الملخص العربي

توصيف الجبن الراس المعدل إنزيميًا

يستخدم الجبن المعدّل إنزيميًا (EMC) كنكهة جبن مركزة كمادة مضافة فى إنتاج الجبن الراس قليل الدسم وذلك لتحسين قبول المنتج وتقليل فترة التسوية. ومن ثم، تم دراسة الخصائص الكيميائية والحسية لكل من معجون EMC والجبن الراس المضاف اليه EMC الناتج خلال فترة التسوية. وأشارت النتائج إلى عدم وجود فروق معنوية($p\leq0.05$) في مكونات EMC المعالجة بإنزيمات الببسين والليباز مقارنةً بالمجموعة الكنترول باستثناء أعلى مستوى من البيبسين (٣٠٠٠ وحدة/جرام). وأشارت نتائج تحليل الجبن الراس إلى وجود فروق معنوية ($p\leq0.05$) في محتوى البروتين والدهون والرماد في جبن الراس المضاف إليه EMC مقارنةً بالجبن الكنترول. كما أظهرت مؤشرات تسوية جبن EMC-Ras زيادة معنوية ($p\leq0.05$) في كل من النيتروجين الذائب (٠.٤-٣.٣٪) و الاحماض الدهنية الطيارة (٥-١٥٪) على جميع المستويات مقارنةً بالجبن الكنترول (٠.٣-٠.٧٪ للنيتروجين الذائب ، و٥-١٢٪ للاحماض الدهنية الطيارة. (كما سجل جبن EMC-Ras أعلى نسبة من الأحماض الأمينية الأساسية (EAA) بما في ذلك الارجنين, الفالين, الفينيل الانين مقارنةً بالجبن الكنترول. علاوة على ذلك، أدت إضافة EMC إلى الجبن الراس إلى انخفاض الأمينات الحيوية بما في ذلك الكادافيرين (٠.٤ ملجم/كجم) والبوتريسين (٠.٢ ملجم/كجم) مقارنةً بالجبن الكنترول (٣.٤٤ و٠.٢٣ ملجم/كجم على التوالي). كما تم الكشف عن وجود السيروتونين وبيتا فينيل إيثيل أمين والسبيرميدين في جبن EMC-Ras بتركيزات ضئيلة. ومع ذلك، أشار التقييم الحسي للجبن الراس قليل الدسم خاصةً بعد الشهر الأول وحتى نهاية فترة التسوية إلى أن نكهة جبن EMC-Ras أعلى من جبن الكنترول دون أي ظهور للطعم المر, وكذلك أظهر تقييم القوام والتركيب الى أن إضافة EMC إلى الجبن الراس قليل الدسم حسّنت بشكل ملحوظ من درجات تقييم قوام الجبن مقارنةً بالجبن الكنترول. وبالتالي، يمكن استخدام EMC كمكون وظيفي فى إنتاج الجبن الراس قليل الدسم بخصائص كيميائية وغذائية جيدة دون ظهور اى عيوب خلال فترة التسوية.

الكلمات المفتاحية: جبن معدّل إنزيميًا، قليل الدسم، جبن راس، نكهة جبن، وظيفي.