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The Combined Effect of Dried Rosemary,Thyme and Basil with Fresh Garlic on Quality Characteristics of Ricotta Cheese During Storage

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THIS study was aimed to investigate the combined effect ofrosemary, thyme, basil individually as powder herbs at the rate of 0.5% with 1% fresh garlic on the quality of Ricotta cheese stored for 21 days at 5±2°C.The chemical composition, total phenolic compounds, antioxidant activity, microbiological and sensory characteristics of Ricotta cheeses were evaluated during storage.No significant effect ($P < 0.05$) of adding herbs on cheese moisture, fat and protein contents. A significant effect ($P < 0.05$) was observed on pH value and ash content between control and treated samples.Thymegarlic-added cheeses exhibited significantly higher ($P< 0.05$) total phenolic compounds and antioxidant properties compared with the other samples.During storage, the antioxidant activity was slightly decreased in the following order: thyme with garlic <rosemary with garlic < basil with garlic-added Ricotta cheeses. All tested herbs with fresh garlic had a significant effect ($P < 0.05$) on the microbial load, but the effect of dried thyme with fresh garlic was significantly higher ($P < 0.05$) than dried rosemary or basil with fresh garlic.Thyme garlic-added Ricotta cheese gained the highest scores for flavor and texture, which was superior to that of the other treatments. Ricotta cheese containing 0.5% thyme and 1% fresh garlic could be produced to obtain highly acceptable cheese with more antioxidant properties.

Keywords: Ricotta, Antioxidant activity, Rosemary, Thyme, Basil, Garlic.

Introduction

Ricotta cheese, a traditional Italian whey protein cheese,is widely consumed worldwide and is appreciated for its taste, its nutritional benefits as well as for its therapeutic valuesthat can be increase further by adding health-promoting ingredients such as the aromatic plants.Plantsare potential sources of natural antioxidants which are progressively applied in dairy products to produce functional foods exhibiting highernutritional and therapeutic properties(Alenisan et al., 2017). The consumption of cheese with health-promoting ingredients may improve human health and prevent certain diseases (Ceylan and Fung, 2004).

Among, plants, herbs (rosemary, basil, thyme, sage, oregano) and vegetables (garlic, onion, pepper, chili, dried tomatoes) are the most interesting and promising aromatic plants due to their flavoring, antimicrobial effects and antioxidant functional properties (Embuscado, 2015 and Pisoschi et al., 2018). Herbs addition has been tested in different cheeses not only because of their antioxidant, antimicrobial effects but also to attract consumer's attention and to propel the sales of these cheeses, consequently to improve its marketing place and its storability (Hayaloglu and Farkye, 2011).For those reasons, several attempts to produce cheese with aromatic plants (leaves, extracts, essential oils) have been undertakento increase their functionality and antioxidant capacity (Hala et al., 2010 , Olmedo et al., 2013 and Khorshidian et al., 2018). Numerous studies have been published and focused on using the aromatic plants extracts to extend shelf-life of foods through their antioxidant and antimicrobial activities (Mahgoub et al., 2013 , Bor et al., 2016 and Zhang et al., 2016). However, most of the published reports showed that the aromatic plants in high concentration may cause negative organoleptic effects and therefore their use is limited in dairy products (Hsieh et al., 2001 and Asensio et al., 2013). For example, Asensio et al. (2014) concluded that the addition of oregano essential oil into Ricotta cheese improves quality parameters but affects its sensory attributes.

Herein, rosemary (*Rosmarinus ofcinalis*), thyme (*Tymus vulgaris*), basil (*Ocimum basilicum*) and garlic (*Allium sativum*) are aromatic plants

commonly used in dairy products to improve sensory properties and extend their shelf life due to their antimicrobial and antioxidant activity (Ceylan & Fung, 2004 ; Oraon et al., 2017 and Kaptan & Sivri, 2018). Experiments on their bioactive compounds and therapeutic effects have been well documented (Nieto et al., 2018).

Therefore, this study was planned (1) to enhance the functional value of Ricotta cheese with the combined use of 0.5% dried rosemary, thyme, basil individually with 1% fresh garlic and (2) to determine its sensorial characteristics in order to determine the adequate combination of herbs with fresh garlic for a more appreciated product.

Materials and Methods

Materials

The whey (0.8% fat, 6.0% dry matter, 0.7% protein and 0.5% ash)and whole cow´s milk (3.50% fat, 12.40% dry matter and 3.21% protein) were supplied by the pilot dairy plant of the Faculty of Agriculture, Fayoum University, Egypt. Dried rosemary (*Rosemarinus officinalis*), thyme (*Thymus vulgaris*), basil (*Ocimum basilicum*) and fresh garlic (*Allium sativum*) were purchased from local market, Fayoum, Egypt and ground in an electric blender to a fine powder before use. Salt was purchased from EMSEIIAL Company, Fayoum, Egypt.

Methods

Cheese making

Four Ricotta cheese making trials were performed in Pilot Plant of Dairy Department, Fac. of Agric.,Fayoum Univ.,Egypt as described by Niro et al. (2013). Briefly, the whey (100L) collected after Ras cheese making was fortified with 5% whole cow´s milk (5L) and 1% salt was added. The mixture was heated to 87ºC and appropriate amount of diluted citric acid was added (pH ~5.7) for coagulation and curd formation. The coagulated curds float to the surface of the whey, and the curds were left in the hot whey for about an hour to enhance whey drainage. The curds were scooped off, placed in the cheese cloth and pressed by hand to drain and cool.

The resultant Ricotta cheese (4kg) was divided into four parts. Three parts were mixed well with ground rosemary, thyme or bail at the rate of 0.5% for each and combine with 1.0% ground fresh garlic. The latest part served as a control. Cheese treatments were packed in plastic containers (100g) and stored in arefrigerator at 5±2°C for 21 days. Cheese samples were analyzed for basic chemical composition, total phenolic compounds, antioxidant activity, microbiological and sensory qualities at 0, 10 and 21 days of storage.

Gross chemical analyses

All cheese samples were analyzed in triplicate for moisture, protein, fat, and ash contents by standard techniques (A.O.A.C., 2010). The pH measurement was carried out on a sample (10 g) of cheese dispersed in 20 mL of distilled water using a pH meter with a glass electrode Model pH (Kent EIL 7020). Total phenolic content was determined spectrophotometrically at 765 nm using Folin-Ciocalteu method (Ozsoy et al., 2008) and the results expressed as mg of Gallic acid equivalents (GAE) per gram of sample (mg/g).The antioxidant activity was evaluated by DPPH (2,2-diphenyl-1-picrylhydrazyl) method as described by Karaaslan et al. (2011) and the results were expressed as percentage inhibition of the DPPH radical.

Microbiological analyses

All cheese samples were examined for total bacterial count, psychrophilic count, coliform count and yeast & mould count according to APHA (2015).

Sensory evaluation

The samples were evaluated organoleptically at 0, 10, and 21 days of refrigerated storage by ten panelistsin terms of their texture, flavor, appearance and overall evaluation. Evaluation was scored on a 9-point scale (0-4 very bad; 5-9 acceptable-excellent) according to Karaaslan et al. (2011).

Statistical analysis

The presented data are the mean values±SD (Standard Deviation) of the three replicates. The statistical significance of differences among the samples'means was determined by one-way analysis of variance and LSD (Least Significant Difference) at $P< 0.05$ using XLSTAT software (version 2007).

Results and Discussion

Chemical composition

Effects of rosemary, thyme or basil addition with fresh garlic on the chemical composition of Ricotta cheeses during storage for 21 days are shown in Table 1. As shown in this table, the compositional characteristics (moisture, protein and fat contents)of experimental Ricotta cheeses were nearly similar to those of control

cheese, indicating no significant differences ($P< 0.05$) between the control and treated samples in chemical composition. During storage, the moisture content decreased slightly in a similar manner in the control and experimental Ricotta cheeses, probably due to an evaporative loss of some amount of moisture during storage (Hala et al., 2010).Only significant differences were found in pH value and ash content between control and the treated samples. Herbs-added Ricotta cheeses showed significantly ($P< 0.05$) lower pH values in comparison to control on almost all days of storage which might be attributed to the acidic and phenolic compounds present in the tested herbs (Hala et al., 2010). The pH values of Ricotta cheese were gradually decreased in all treatments either in the control or experimental cheeses during 21 days of storage which might also be due to the formation of acidic degradation products (Foda et al., 2009 and Hala et al., 2010). The treated samples possessed the highest contents of ash which might be attributed to the higher ratios of ash in the tested herbs compared to milk retentate, as demonstrated by Hayaloglu and Farkye (2011).According to these results, the main differences observed in the compositional characteristics of Ricotta cheese was related to pH value and ash content in treated samples. The results obtained in this study showed similarities with those of Ayar (2002) ; Foda et al. (2006) ; Hayaloglu and Farkye (2011) ; Tarakci et al. (2011) ; Hamid and Abdelrahman (2012) and Regu et al. (2016).

TABLE 1. The chemical composition of Ricotta cheese samples containing rosemary, thyme or bail powder with fresh garlic during refrigerated storage for 21 days.

Parameters	Storage time (days)	Treatments			
		Control	Ricotta cheese containing 1% fresh garlic and 0.5%		
			Rosemary powder	Thyme powder	Basil powder
pH	Fresh	6.44±0.01[a]	6.27±0.03[b]	6.19±0.25[c]	6.21±0.02[c]
	10	6.26±0.05[a]	5.99±0.03[b]	5.76±0.01[c]	5.73±0.02[c]
	21	6.12±0.03[a]	5.58±0.06[b]	5.52±0.02[b]	5.54±0.01[b]
Moisture (%)	Fresh	69.32±0.13[a]	69.29±0.10[a]	69.11±0.06[a]	69.16±0.07[a]
	10	69.21±0.44[a]	69.11±0.06[a]	69.01±0.07[a]	69.10±0.02[a]
	21	69.11±0.11[a]	69.00±0.45[a]	68.99±0.10[a]	69.00±0.19[a]
Fat (%)	Fresh	15.00±0.10[a]	15.18±0.58[a]	15.15±0.29[a]	15.12±0.20[a]
	10	15.10±0.12[a]	15.25±0.29[a]	15.22±0.22[a]	15.15±0.27[a]
	21	15.15±0.29[a]	15.27±0.29[a]	15.25±0.32[a]	15.23±0.29[a]
Protein (%)	Fresh	11.48±0.26[a]	11.51±0.26[a]	11.57±0.26[a]	11.51±0.26[a]
	10	11.51±0.31a	11.81±0.26[a]	11.67±0.26[a]	11.65±0.89[a]
	21	11.76±0.91[a]	11.87.±0.34[a]	11.76±0.45[a]	11.79±0.65[a]
Ash (%)	Fresh	1.58±0.46[b]	2.48±0.02[a]	2.49±0.02[a]	2.43±0.32[a]
	10	1.65±0.25[b]	2.59±0.11[a]	2.55±0.13[a]	2.54±0.16[a]
	21	1.96±0.08[b]	2.87±0.15[a]	2.85±0.15[a]	2.90±0.16[a]

Means with the different letters in the same row are significantly different at $P < 0.05$.

Total phenolic compounds

The total phenolic contents of the control and experimental Ricotta cheeses are presented in Table 2. The total phenolic content was significantly higher (P<0.05) in experimental cheese samples compared to that of the control, providing additional evidence of supplementation. On day 1, the highest value of total phenolic compounds was detected in Ricotta cheeses containing dried thyme with fresh garlic (8.30 ± 0.08 mg GAE/g); followed by Ricotta cheese containing dried rosemary with fresh garlic (6.87 ± 0.80 mg GAE/g), Ricotta cheese containing dried basil with fresh garlic (6.15 ± 0.23 mg Gallic acid equivalents/g), and control cheese (2.29 ± 0.05 mg GAE/g). A gradual decrease was observed in the total phenolic content of cheese samples over the storage period, probably due to the transformation of phenolic compounds during storage as reported by Legrand (2005). After 21 days, rosemary garlic-added cheese exhibited the highest remarkable decrease (37.55%) in total phenolic content among the treated cheeses and followed by basilgarlic-added cheese (34.90%) and then thymegarlic-added cheese (22.16%). The addition of herbs with fresh garlic to Ricotta cheese is responsible for 60% variation of the total phenolic content, while the storage period is responsible for 20% variation.

TABLE 2. Total phenolics and antioxidant activity of Ricotta cheese samples containing rosemary, thyme or bail powder with fresh garlic during refrigerated storage for 21 days.

property	Storage time (days)	Treatments			
		Control	Ricotta cheese containing 1% fresh garlic and 0.5%		
			Rosemary powder	Thyme powder	Basil powder
Total phenols (asmg GAE/g)	Fresh	2.29±0.05^{d}	6.87±0.08^{b}	8.30 ±0.80^{a}	6.15±0.23^{c}
	10	2.01±0.03^{d}	5.51±0.07^{b}	7.52±0.67^{a}	5.03±0.54^{c}
	21	1.55±1.54^{d}	4.29±0.21^{b}	6.46±0.٥٦^{a}	4.00±0.33^{c}
Antioxidant activity (%)	Fresh	26.16±1.2^{d}	65.65±1.4^{b}	81.16±1.7^{a}	59.83±1.2^{c}
	10	21.05±0.4^{d}	57.48±3.3^{b}	72.35±0.3^{a}	45.25±0.7^{c}
	21	18.23±0.2^{d}	45.85±0.4^{b}	63.25±0.2^{a}	39.35±0.6^{c}

Means with the different letters in the same row are significantly different at P ≤ 0.05; GAE, Gallic acid equivalent

Total antioxidant activity

As shown in Table 2, the antioxidant activity as measured by DPPH radical scavenging activity method was significantly higher (P < 0.05) in the thyme garlic-added Ricotta samples than that in the other treatments or the respective control. This might be attributed to the higher phenolic content in thyme garlic-added samples than in the other samples. The highest antioxidant activity of the experimental cheeses was detected in the following order: thyme garlic< rosemary garlic < basil garlic-added Ricotta cheeses toward the DPPH radical.During storage, the antioxidant activity of control and experimental Ricotta cheeses presented the same pattern as observed for the total phenolic content,decreased as the time of storage increased.The loss of antioxidant activity ranged from 30.31% in the control cheese to 22.01% in the thyme garlic-added cheese.

The results obtained in this study are consistent with those of many other studies (El-Din et al., 2012 ; Josipović et al., 2015 and El-Khalek et al., 2016). From the previous data, adding herbs with fresh garlic into Ricotta cheese curd could be considered as health promoting ingredients due to their potential antioxidant activity.

Microbiological counts

The viable counts of the microbiological groups (log_{10}cfu/g) in control and experimental Ricotta cheeses during 21 days of refrigerated storage are presented in Table 3. Generally, all treated cheeses had slightly lower microbiological counts than the corresponding control. During refrigerated storage, the numbers of different microbiological groups increased in all treatments. During the whole storage, Ricotta cheese containing thyme with fresh garlic had the lowest log cfu/g,whereas rosemary garlic and basil garlic-added cheeses

had the highestin microbial groups, owing to the antimicrobial effect of thyme with fresh garlic compared with the other treatments considered. The coliforms and psychrophilic were not detected over the storage period in both control and treated samples.This may be due to the heat treatments (80-85°C) and the high hygienic conditions during cheese processing.

Overall, based on the results of microbial examination, the dried thyme with fresh garliccheese had a higher antimicrobial effect on microbial groups than the other cheeses.Similar findings were also observed by Foda et al. (2009); Kavas et al. (2015) ; Sağdıç et al. (2017).

TABLE 3. Microbiological counts (log_{10}cfu/ g) total bacterial, yeasts and moulds of Ricotta cheese samples containingrosemary, thyme or bailpowder with fresh garlic during refrigerated storage for 21 days.

Storage time (days)	Treatments			
	Control	Ricotta cheese containing 1% fresh garlic and 0.5%		
		Rosemary	Thyme	Basil
		Total bacterial count		
Fresh	6.69±0.40[a]	6.60±0.04[a]	6.00±0.03[c]	6.60±0.06[a]
10	6.77±0.45[a]	6.69±0.08[b]	6.30±0.27[c]	6.74±0.25[b]
21	7.69±0.28[a]	7.30±0.14[b]	6.69±0.25[c]	7.37±0.30[b]
		Yeasts and moulds count		
Fresh	6.30±0.02[a]	5.47±0.10[c]	5.30±0.11[d]	5.78±0.10[b]
10	6.47±0..08[a]	6.00±0.58[c]	5.86±0.41[d]	6.47±0.48[b]
21	6.84±0.64[a]	6.00±0.38[c]	5.90±0.47[d]	6.64±0.16[b]

Means with the different letters in the same row are significantly different at P <0.05.

Sensory evaluations

The sensorial evaluation scores of control and experimental Ricotta cheeses during the refrigerated storage are recorded in Table 4. In general, all cheeses made with different types of herbs and garlic had acceptable sensory attributes. The sensorial attributes of the herbs-added Ricotta cheese samples generally had significantly higher (P< 0.05) scores than the control cheese either fresh or throughout storage period. The flavor and appearance of herbs-added Ricotta cheese became more preferable to all the panelists than the control cheese.However, thyme garlic-added Ricotta cheese had the highest score (P< 0.05) followed by rosemary garlic cheese, while the lowest one was for basil garlic-added Ricotta cheeses.Thus, the addition of thyme powder with fresh garlic as sources of natural antioxidant and flavoring agents may develop better sensory properties in Ricotta cheeses. The decrease in the overall preference score reflects the score of appearance, flavor, and texture decline along with the time of storage. Similar observations were reported by Mervat I. Foda et al. (2008) and also by Hala et al. (2010)_ENREF_12 in low fat UF-soft cheese supplemented with rosemary during refrigerated storage.

Conclusions

The present study has demonstrated that the incorporated of 0.5% thyme powder with 1% fresh garlic in Ricotta cheese curd enhances its antioxidant properties besides, it produces a highly acceptable cheese. Over storage, thyme garlic-added Ricotta cheese exhibited the most effective antimicrobial effect and the highest antioxidant activity. The loss of antioxidant activity ranged from 22.01% in thyme with garlic to 34% in basil with garlic-added cheeses.Further studies using herbal powders or extracts herbs powder together with its extracts and with fresh garlic are necessary and may give better antimicrobial effect.

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TABLE 4. Sensory evaluation scores of Ricotta cheese samples containing rosemary, thyme or bail powder with fresh garlic during refrigerated storage for 21 days.

Sensory attributes	Storage time (days)	Treatments			
		Control	Ricotta cheese containing 1%fresh garlic and 0.5%		
			Rosemary powder	Thyme powder	Basil powder
Appearance	Fresh	7.16±0.73^{d}	8.72±1.30^{b}	9.14±0.71^{a}	7.60±0.44^{c}
	10	6.58±0.71^{d}	8.60±1.20^{b}	8.91±0.62^{a}	7.50±0.50^{c}
	21	6.18±0.44^{d}	8.53±0.93^{b}	8.78±0.71^{a}	7.45±0.53^{c}
Flavor	Fresh	6.60±0.21^{d}	7.88±0.25^{b}	8.81±0.10^{a}	7.62±0.12^{c}
	10	6.41±0.34^{d}	7.75±0.24^{b}	8.72±0.15^{a}	7.50±0.14^{c}
	21	5.78±0.56^{d}	7.65±0.08^{b}	8.55±0.04^{a}	7.34±0.18^{c}
Texture	Fresh	6.51±0.34^{d}	8.50±0.11^{b}	8.93±0.13^{a}	7.62±0.05^{c}
	10	6.35.±0.54^{d}	8.32±0.14^{b}	8.62±0.15^{a}	7.52±0.06^{c}
	21	6.00±0.23^{d}	8.22±0.08^{b}	8.51±0.12^{a}	7.39±0.09^{c}
Overall preference	Fresh	6.22±0.20^{d}	9.00±0.34^{b}	9.22±0.18^{a}	7.90±0.11^{c}
	10	6.10±0.34^{d}	8.95±0.54^{b}	9.20±0.05^{a}	7.81±0.14^{c}
	21	6.01±0.32^{d}	8.90±0.23^{b}	9.15±0.11^{a}	7.61±0.12^{c}

Means withthe different letters in the same row are significantly different at P <0.05 .

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التأثير المشترك لمسحوق الروزماري، الزعتر والريحان مع الثوم الطازج على خصائص جودة الجبن الريكوتا خلال التخزين

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الخلفية والهدف: تستهلك الجبن الريكوتا)وهي جبن بروتين مصل اللبن) على نطاق واسع في جميع أنحاء العالم تقديرا لمذاقها، وفوائدها الغذائية وقيمتها الوظيفية التي يمكن تعزيزها بشكل أكبر بدون التأثير علي خواصها الحسية وذلك بإضافة بعض النباتات العطرية (في صورتها الجافة أو الطازجه) بهدف تعزيز صحة المستهلك، وعلي الجانب الآخر المساهمة في تحسين طعمها ومكانتها التسويقية وقابليتها للتخزين.

تصميم التجربة وطرق الدراسة:في هذا البحث تم تصنيع أربعة معاملات من الجبن الريكوتا (الناتج من خلط ١٠٠ كجم شرش مع ٥ كجم لبن بقري كامل) مضاف إليه ٠,٥٪ مسحوق الروزماري أو الزعتر أو الريحان مع ١٪ ثوم طازج، باالاضافة إلي جبن الريكوتا بدون أي أضافات (جبن الكنترول).وتم تخزين الجبن الناتج في الثلاجة علي ٥±٢°م لمدة ٢١ يوم لدراسة تأثير إضافة هذه الأعشاب في صورتها الجافةمع الثوم الطازج علي التركيب الكيميائي، والمركبات الفينولية الكلية، والنشاط المضاد للأكسدة، والمجاميع الميكروبيولوجية والخصائص الحسية للجبن الريكوتا وذلك علي فترات ٠، ١٠، ٢١ يوم. وكانت اهم النتائج ما يلي:
كيميائيا:تبين من النتائج عدم وجود أي تأثير معنوي (P <٠,٠٥) لمسحوق الأعشاب التي تم اختبارها مع الثوم الطازج على محتويات الجبن من الرطوبة والدهون والبروتين.بينما لوحظ أنها لها تأثير معنوي (P <٠,٠٥) على قيم الـ pH ومحتوى الرماد بالمقارنة مع الجبن الكنترول. وأظهرت جبنالزعتر مع الثوم الطازج (P <٠,٠٥) أحتوائها علي المركبات الفينولية الكلية أعلي بشكل ملحوظ ومن ثم أرتفاع خاصية النشاط المضاد للأكسدة مقارنة بالكنترول والمعاملات الأخرى. وكانت جبن الزعتر مع الثوم الطازج الأعلى في نشاط مضاد للأكسدة، يليها الثوم والروزماري، ثم جبن الريكوتا بالريحان مع الثوم. وخلال فترة التخزين أنخفض النشاط المضاد للأكسدة وكان الانخفاض تنازليا بالترتيب التالي: الريحان مع الثوم، روزماري مع الثوم والزعتر مع الثوم.
ميكروبيولوجيًا: جميع الأعشاب المختبرة مع الثوم الطازج كان لها تأثير معنوي (P <٠,٠٥) على الحمل الميكروبي للجبن الريكوتا، لكن تأثير الزعتر مع الثوم الطازج كان أعلى بشكل ملحوظ (P <٠,٠٥) بالمقارنة بباقي المعاملات.وتبين أيضا زيادة العد الكلي للبكتريا، وعدد الخمائر والفطريات في جميع المعاملات أثناء التخزين.

حسياً: حصل جبن الزعتر مع الثوم الطازج علي أعلى الدرجات للنكهة والقوام والمظهر وكانت إجماليا الأكثر قبولا لدي المحكمين من المعاملات الأخرى، يليها جبن الروزماري مع الثوم ثم جبن الريحان مع الثوم يأتي في الأخير.

الخلاصة والاستنتاج:جبن الريكوتا المصنع باضافة مسحوق الزعترمع الثوم الطازج امتلك أقوى نشاط مضاد للأكسدة ومضاد للميكروبات مقارنة بالأعشاب الأخرى، يليها الروزماري مع الثوم ثم الريحان مع الثوم، ومن ثم يوصي إضافة الزعتر مع الثوم لإنتاج جبن ريكوتا وظيفي دون التاثير علي جودتها الحسية.