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Effect of Oregano and Sage Extracts on Microbiological Quality of Molten Butter

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Effects of oregano (*Origanum vulgare* L.) and sage (*Salvia fruticosa* L.) extracts on microbiological quality of butter were investigated. The extracts and their combinations were individually added into butter at 0.3% (w/w) concentrations. For comparison, 0.2% (w/w) sorbic acid was used. The close-up samples were stored at 20°C for 4 weeks. Microbiological qualities of butters treated with spices extracts were better than that of control. Spices extracts and their combinations exhibited antimicrobial effects in butter; however their antimicrobial activities were lower than that of sorbic acid. Oregano among the spices extracts showed highest inhibition effect on moulds and yeasts, lipolytic, proteolytic microorganisms and coliform group, and sage was effective on total bacteria, lactobacilli.

Keywords: butter, oregano, origanum vulgare, sage, salvia fruticosa, storage, microbiological quality

Butter consists primarily of milk fat, a small amount of milk solids non fat and water. Serum and water in butter affect its storage period because they are an appropriate medium for spoilage microorganisms. Microorganisms have ample opportunity to gain access to butter during its manufacture. The growth of undesirable organisms in butter is a major cause of its deterioration during storage (Rosenthal, 1991; Cassava, 1993). The main defects, which develop in butter during storage, are oxidative and hydrolytic rancidity and putrefactive taint. The first two of those defects are due to the breakdown of fat. The breakdown of protein by putrefactive organisms gives rise to so-called putrefactive taint. The presence of this defect in butter indicates poor sanitation and manufacturing conditions (Siezen & Van den Berg, 1992; Reineccius, 1994). *Lactobacillus*, yeasts and *Pseudomonas* spp. are the microbial groups having significant proteolytic activity in butter. Rancidity is a major problem, which occurs due to hydrolysis of milk fat by various milk and microbial enzymes, arising in butter during storage. The extent of lipolysis is mainly governed by the growth and lipolytic activity of molds and yeasts, depending on the strain used, storage and amount of the residual lipolytic activity of milk (Casberg, 1992). Particularly, unsalted or very minimally-salted butter has some sensory defects due to fast growth of molds. The total bacterial count of butter rises at the beginning of the storage period because of low thermal conductivity, especially for unsalted butters (Rosenthal, 1991; Cassava, 1993).

For increasing shelf life of food products and improving stability of their lipids, antibiotic, such as sorbic acid, benzoic acid, sodium chloride and nitrate have traditionally been used. There is a demand from the consumers for replacing synthetic preservatives with natural alternatives. Research on the antimicrobial effects of spices and spices showed that natural preservatives like thyme, sage, mustard, oregano, savory, cinnamon and clove had high antimicrobial properties in foods (Farag *et al.*, 1990; Erkmen & Özcan, 2001; Sağdıç & Özcan, 2003). The composition of spices extracts is very complex. It seems possible that the extent of the inhibitory effect of oregano and sage on microbial growth may be due to aspects of their chemical structures. It is well known that the phonolic components of spices or their essential oils show the strongest antimicrobial activity. Extracts or essential oils of herbs and spices exhibit antimicrobial effects (Farag *et al.*, 1990; Paster *et al.*, 1994; Çon *et al.*, 1998; Özcan, 1998). However, there are just studies on the antimicrobial effects of spices added directly to foods (Zaika *et al.*, 1983; Akgül, 1993).

In the present study, the antimicrobial effects of oregano and sage extracts in butter samples during storage were evaluated using harmonious amounts with organoleptic properties of butter.

Experimental or Methods

Methods Pure crystalline sorbic acid was obtained from Sigma Chemical Company (St. Louis, MO). Turkish Oregano (*Origanum vulgare* L.) and sage (*Salvia fruticosa* L.) were collected from plants growing wild in Turkey. Spices were extracted with pure methanol (at 65°C) using a Soxhlet apparatus. This solvent was preferred, having been reported as the most effective in other works. The crude extracts were filtered and then evaporated in a rotary evaporator at 45°C. Residues were kept in a hermetically closed glass vessel at 4°C until use.

Milk was separated and the cream, having 39% fat, was pasteurised (72°C for 15 s). The cream was cooled to 6°C for at least 2 h to initiate fat crystallisation followed by warming to 20°C. It was then inoculated with the starter culture containing *Lactococcus lactis* subsp. *lactis* and *Lactococcus lactis* subsp. *cremoris* (Wiesby, Germany) (2%) but not added to control group. The cream was ripened for 6 h at 20°C until a pH 4.9 was achieved and then chilled again to stop the fermentation and complete crystallisation of fat. The ripened cream was

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churned and then worked into a fine homogeneous mass.

Spice extracts at 0.3% or their binary combinations in equal amounts of each (1.5 g extract in 500 g butter) and 0.2% sorbic acid (1 g in 500 g butter) were individually added into the samples of melted butter at 40°C and were stirred. On the case of starter culture added positive control, the whole process of butter manufacture described above was carried out in the absence of additives but the starter culture. Open flasks were stored in an incubator at 20°C for 4 weeks. Butter samples were stored at 20°C because this temperature is optimal for spoilage microorganisms. Butter samples were periodically evaluated for microbial growth. A total of 40 samples were prepared (2 replicates ×5 treatments×4 storage intervals).

Analyses Coliform, Lactobacilli, mold and yeast counts were determined as described by Marshall (1992). Total bacterial counts, proteolytic and lipolytic microorganisms were determined as indicated by Vanderzant & Splittstoesser (1992). The sample (10 g) of butter was taken in a 250 ml sterile beaker, heated in warm water (at 40°C), until the butter melts enough to be pipetted. One ml of liquefied butter was diluted appropriately in 0.5% peptone water. Lactobacilli were determined by plating appropriate dilutions of the butter samples on to MRS agar. Colony development was facilitated by using an agar overlay. Plates were incubated for 48 h at 37°C. Lipolytic microorganisms on tributyrin agar without Victoria Blue B were detected by transparent zone surrounding the colony on an opaque background. Plates were incubated for 72 h at 30°C. Proteolytic microorganisms were detected on agar agar added skim milk agar after incubated for 72 h at 21°C. Flooded the incubated plates for 1 min with a solution of 1% HCl. Poured off the excess acid solution, then counted the colonies that were surrounded by clear zones produced by proteolysis.

The growth inhibition level caused by each the spice extracts on test microorganisms were determined according to the following equation (Ozcan, 1998):

$$\text{Growth inhibition level} = [(\text{control population} - \text{treated population})/\text{control population}] \times 100.$$

Statistical analysis Analysis of variance was performed on data obtained at different stages of experiment. The experimental data for the spice extract and microorganisms were subjected to analyses of variance using ANOVA (Minitab, 1991).

Results and Discussion

The initial counts of total bacteria, molds and yeasts, *Lactobacili*, proteolytic microorganisms, lipolytics microorganisms and coliforms in butter samples were determined as 3.1, 2.3, 2.0, 2.5, 2.3 and 0.5 log cfu/ml determined, respectively.

Total bacterial count the most increased in the control sample during storage. The sorbic acid added-butter sample was the lowest total bacterial count. The counts in extract-added butter samples were lower than that of the control sample but higher than that of the sorbic acid–added butter. Sage extract (0.3%) among the spices extracts showed highest inhibition ratio for total bacteria. Generally, a total bacterial count varied throughout the storage period among butter samples and was the highest at the end of the storage period.

Mold and yeast counts increased during the second week and then gradually decreased up to the final stage of storage period. Molds and yeasts were more inhibited by 0.3% oregano than that of other extracts (Table 1). In consistent with this study Kıvanç & Akgül (1989) determined that oregano exhibited inhibitory effect on yeasts.

The lipolytic microorganism count of the butter samples, in general, increased up to the week 3 and then decreased. 0.3% oregano exhibited the highest inhibition on lipolytic microorganisms among the extracts and combinations ($p<0.01$) (Table 1). The increase in the number of lipolytic microorganism in control sample was maximum. The sorbic acid–added butters had lower counts. The lipolytic microorganism counts in the

Table 1. Inhibitory effects of oregano and sage extracts and sorbic acid on microbial counts of butters (% inhibition).

	Time (weeks)	Positive control	Sorbic acid 0.2%	Oregano 0.3%	Sage 0.3%	Combination 0.3%
Total bacteria	1	39.16*	78.62	52.14	61.98	61.10
	2	53.41	91.09	43.77	71.82	71.82
	3	58.60	86.51	61.10	73.37	55,33
	4	56.27	98.14	71.82	76.56	71,16
Molds and yeasts	1	51.23	93.97	74.30	69.10	74.88
	2	32.18	88.52	48.71	46.30	46.30
	3	63.07	96.90	80.05	80.50	71.82
	4	41.66	99.83	62.85	42.46	71.16
Lipolytic microorganisms	1	30.35	78.63	61.99	48.85	44.53
	2	23.90	68.38	46.32	46.89	46.32
	3	54.35	70.49	65.33	66.89	65.33
	4	49.64	94.11	78.62	62.85	58.32
Proteolytic microorganisms	1	39.51	85.87	53.23	57.34	43.76
	2	55.38	98.38	71.11	69.10	67.65
	3	68.43	99.75	74.88	61.97	63.70
	4	81.04	100	98.22	97.96	94.75
Lactobacilli	1	0**	49.84	30.79	28.56	25.86
	2	0	93.39	33.93	39.35	86.82
	3	0	80.50	22.73	45.86	24.14
	4	0	99.00	39.74	40.82	33.93
Coliform	1	49.17	100	100	50	70
	2	52.43	100	100	100	100
	3	Not determined	Not determined	Not determined	Not determined	Not determined
	4	Not determined	Not determined	Not determined	Not determined	Not determined

* Inhibition was calculated according to control. ** Inhibition was calculated according to positive control.

extract-added butter samples were lower than that of the control but higher than that of the sorbic acid added butters.

Proteolytic counts were increased during the first week. Then, proteolytic microorganism counts in extract-added butters not showed significant difference. The proteolytic counts at the end of storage were higher than that of the initial counts. 0.3% oregano and sage extracts showed the high inhibition on protelytic microorganisms.

Lactobacilli counts, in general, increased as has already been observed in the case of total bacteria counts. Oregano and sage extracts didn't show important inhibition effect on lactobacilli. It can be said that lactic acid bacteria are resistant to extracts of oregano and sage. A similar result was reported by Zaika *et al.* (1983). Kıvanç *et al.* (1991) reported that oregano and its essential oils inhibited growth of lactic acid bacteria.

Coliform counts were increased for first week of storage but for the first week in sorbic acid and 0.3% oregano added samples. Then, they were completely inactived.

Microorganism counts in sorbic acid–added butter samples were generally the lowest. 0.3% oregano showed the highest inhibition on coliform among the extracts. Growth of microrganisms in spice extract–added butters samples and lactic culture added sample were lower than that of the control sample. Akgül & Kıvanç (1990) observed that oregano had antimicrobial effect in cabbage pickle. The effectiveness of oregano and sage on the growth of the microorganisms are probably due to major substances such as thymol and carvacrol showing antimicrobial effects (Akgül, 1993).

In conclusion, 0.3% oregano and sage extracts or their combinations did not have preservative effects as 0.2% sorbic acid. However, extract-added butter samples exhibited antimicrobial effects in comparison to the control sample. Growth of microorganisms in lactic culture added sample was lower than that of the control sample ($p<0.01$). The inhibitory effect of spice extracts were increased by lactic culture. In considering with, butter storage at the lower temperatures, for this effect of spices extracts on butter may be of importance with respect to protection against microorganisms types considerable. Finally, further research is needed on the antimicrobial activity of different varieties or higher concentrations, and the combinations of spice extracts and their derivates.

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