RESEARCH ARTICLE

Chemical changes of *kradi* cheese stored at refrigeration temperature under vacuum and normal conditions

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Abstract: This study investigated changes in chemical properties of *kradi* cheese stored under vacuum and normal conditions at 5°C at weekly intervals for a period of fourteen weeks. The initial moisture content of 49.09 % in normal packed samples decreased to 48.34 % while in vacuum packed samples it decreased to 48.31 % at 14th week of storage. The drop in initial pH was small in both vacuum packed and normal packed samples. The decrease in acidity was small in vacuum packed samples than normal packed samples. Fat leakage was less under vacuum packaging. The initial free fatty acid content of 0.93 meq/g in normal packed samples increased to 17.9 meq/g while in vacuum packed samples it increased to 21.31 meq/g on 14th week of storage. The initial tyrosine value of 0.013 µg 5ml⁻¹ in normal packed samples increased to 5.25 µg 5ml⁻¹ while in vacuum packed samples it increased to 4.93 µg 5ml⁻¹ on 14th week of storage. The vacuum packaging retarded increase in acidity in comparison to normal packaging.

Keywords: Free fatty acids, *Kradi* cheese, Moisture loss, Refrigerated storage, Tyrosine value, Vacuum packaging

Introduction

Kradi, semisoft, white, unripened fresh cheese (Punoo et al. 2018a, 2018b and 2018c) is a famous traditional milk product of Jammu and Kashmir, India manufactured primarily by the tribal population

of Gujjar and Bakarwal community. It is sold unpackaged, undergoes moisture loss and increase in acidity. Despite this, due to increased demand for this cheese, the demand for modern packaging is increasing at production units. The annual production is 21 thousand kilogram during 2017 and sales of product has increased by 15% in 2016-17. Although the sale of *kradi* cheese is increasing annually but no studies has been reported with respect to chemical changes during storage. The study of chemical changes in this product is necessary from food safety point of view for clearing the concerns of consumers.

During storage of cheese biochemical changes involve proteolysis and lipolysis mainly by rennet enzymes or bacteria (Fox and Mc Sweeney, 2004). The chemical degradation of cheese is not desired during storage. Lipolysis involves accumulation of free fatty acids (FFA) with most of the FFA being released from triglycerides.

Vacuum Packaging has developed much attention in production of dairy products for keeping safe from harm, prolonging the shelf life, retarding the activity of bacteria and reducing the use of preservatives. Descriptive sensory analysis, physico chemical, microbiological, textural and microstructural properties of *kradi* cheese have been reported (Punoo, et al. 2018c, Punoo, et al. 2018a; Punoo, et al. 2018b).

As compared to normal packaging, vacuum packaging can reduce chemical deterioration. The alteration in packaging condition by vacuum can either accelerate or inhibit the biochemical changes during storage of *kradi* cheese. Thus vacuum packaging can be a way of preserving the chemical quality of *kradi* cheese. Therefore present study was aimed at assessment of chemical changes of *kradi* cheese throughout its storage at different periods at refrigeration temperature under vacuum and normal packaging conditions.

Materials and Methods

Preparation of *Kradi* cheese

Kradi cheese was made as per the method described (Punoo et al. 2018a). The fresh product was packed in multilayer laminates (5-layer natural PFP, 20x20 cm size and 105-175 micron thickness

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of film) under vacuum and normal packaging conditions and stored at refrigeration temperature ($5\pm 1^\circ\text{C}$). The products stored were evaluated at weekly intervals to monitor changes in various chemical properties for a period of fourteen weeks.

Chemical analysis

Moisture

The moisture content in *kradi* cheese was analyzed by the method as described in IS: SP: 18 (Part XI) 1981.

Titrateable acidity

Titrateable acidity of *kradi* cheese was determined by the method recommended by the Association of Official Analytical Chemists (AOAC, 1984).

pH

Ten g of the grated sample was mixed with 10 ml of glass distilled water and slurry was prepared thereof in a mortar. The pH of slurry was determined by using double junction electrode pH Scan 2+, Eutech Instrument Pvt. Ltd., Singapore.

Fat leakage

Fat leakage of *kradi* cheese samples was determined by the method of Nilson and La Clair (1976).

Free fatty acids (FFA)

The total free fatty acids content of the *kradi* cheese was estimated by the method delineated by Deeth and Fitz-Gerald (1976).

Tyrosine value

The tyrosine content as a measure of proteolysis in *Kradi* was calorimetrically determined according to the method of Hull (1947). Five grams of sample were weighed and taken in a clean dry test tube, added with 10 ml of distilled water followed by addition of 10 ml of 0.72 N TCA. The test tubes were then stoppered shaken vigorously and incubated at 27°C for 10 min. The precipitated proteins were filtered through Whatman No. 42 filter paper and the tyrosine content in the filtrate was determined calorimetrically. To 5.0 ml of this TCA soluble filtrate, 2.0 ml of distilled water, 10 ml of sodium carbonate reagent (75.0 g anhydrous Na_2CO_3 and 10.0 g sodium tetra phosphate dissolved in glass distilled water and diluted to 500 ml) followed by 2.0 ml of the Folin's phenol reagent (Folin's phenol reagent diluted 1: 2 with distilled water) was added, mixed well and incubated again at 27°C for 10 min for colour development. This solution was used as a blank. The intensity of blue colour so developed was measured at 650 nm using UV visible spectrophotometer. A tyrosine standard curve was prepared using concentrations of 0, 10, up to 100 $\mu\text{g}/5\text{ml}$ in

TCA filtrate. A regression equation was obtained using the absorbance of the standard solutions against their tyrosine concentration ($\mu\text{g } 5 \text{ ml}^{-1}$). The tyrosine equivalent in *Kradi* based on its concentration in the final 5 ml TCA soluble filtrate obtained from 5 g sample used for producing blue colour was expressed in terms of tyrosine equivalent ($\text{mg } 100 \text{ ml}^{-1}$).

Statistical analysis

The data obtained during the present investigation was compared by one-way analysis of variance (ANOVA) with the application of SYSTAT software, version 6.0.1 copyright © 1996, SPSS INC. Significant difference ($p < 0.05$) among treatments were detected using Duncan's multiple range tests.

Results and Discussion

The loss of moisture during storage is a common observation for cheeses. Level of moisture in the product plays a significant role on quality of the product during storage as far as bacterial activity, browning reaction and the acceptability of *kradi* is concerned. Therefore, packaging materials and packaging techniques are carefully selected to check the moisture loss during storage. The initial moisture content of 49.09 % in air packed *kradi* samples (Table 1) decreased to 48.34 % while in vacuum packed samples it decreased to 48.31 % on 14th week of storage. There was more loss of moisture under normal packaging conditions compared to vacuum conditions in *saloio* cheese stored at 8°C for 60 days (Pantaleao et al. 2007). Both packaging conditions and storage periods had no significant effect on the moisture content of *kradi* samples stored at 5°C (Table 2). The initial pH of 5.4 in normal packed *kradi* samples dropped to 4.82 while in vacuum packed samples it dropped to 4.80 on 14th week at 5°C of storage temperature. The effect of packaging conditions and the effect of storage periods was non significant on pH of samples (Table 2). Fuentes, L. et al. 2015, reported significant decrease in pH of pasta filata mexican cheese during refrigerated vacuum storage for 24 days. The initial acidity of 0.54 % lactic acid (LA) in normal packed samples increased to 1.14 % LA on 14th week of storage while in vacuum packed samples it increased to 1.05 % LA. The higher development of acidity under normal packaging conditions could be attributed to increased activity of lactic acid bacteria under normal compared to vacuum conditions. The effect of packaging conditions and storage periods was highly significant ($p < 0.01$) on the acidity of samples (Table 2). Since vacuum packaging did not favoured increase in acidity of *Kradi* cheese, therefore vacuum packaging can be more desirable from consumers point of view as the sourness in *kradi* cheese because of acidity development will be less. A slower increase in acidity for white cheese under vacuum compared to normal conditions stored at $4\text{--}5^\circ\text{C}$ for 90 days was reported (Cinbas and Kilic, 2006). Free-oil formation, also called 'oiling off' or 'fat leakage' is the tendency of liquid fat to separate from melted cheese and accumulate in pockets or pools, particularly at the surface of

cheese. The excessive free oil and limited free oil are considered serious defects in cheese. Table 1, depict the trend and values for fat leakage of *kradi* packed in two types of atmospheres (vacuum and normal) and stored at 5°C for fourteen week period. At 5°C of storage temperature, the initial fat leakage of 0.16 (cm²), in normal packed samples increased to 0.81 (cm²) after 14th week of storage while in vacuum packed samples it increased to 0.71 (cm²). Similar observations were also recorded by Kindstedt and Rippe (1988) who observed that refrigerated storage of mozzarella cheese increased the amount of free oil. Gobetti et al. 2002, reported an increase in flowability during 60 days ripening of *caciocavillo pugilese* cheese. ANOVA (Table 2) revealed that both packages as well as storage periods had no significant effect on the fat leakage of samples stored at 5°C. Slower fat leakage under vacuum packaging is good for storage of product. The initial FFA of 0.93 meq/g in air packed *kradi* samples increased to 17.9

meq/g on 14th week of storage while in vacuum packed samples it increased to 21.31 meq/g. The slower rate of lipolysis at 5°C indicates that rate of lipolysis was slower under refrigeration conditions. A slower rate of lipolysis for white cheese under vacuum compared to normal conditions stored at 4-5°C for 90 days was reported (Cinbas & Kilic., 2006). Ahuja and Goyal (2013) reported that increase in concentrations of free fatty acids (FFA) of vacuum packed paneer tikka samples during refrigerated vacuum storage for a period of 30 days and observed influence of storage intervals on the FFA values was more significant ($\bar{n}<0.01$) than types of packages ($\bar{n}<0.05$) during storage. Fuentes et al. 2015, reported that concentrations of free fatty acids (FFA) did not change ($P > 0.05$) in pasta filata mexican cheese during refrigerated vacuum storage for 24 days. The camembert cheese manufactured using tibetan kefir co-culture underwent lipolysis (Mei et al. 2015). The packages and storage periods had no

Table 1 Effect of vacuum packaging on chemical characteristics¹ of *kradi* cheese stored at 5±1°C

Period of Storage (weeks)	Chemical characteristics											
	Moisture %		pH		Acidity (LA %)		Fat Leakage (cm ²)		Free fatty acids (µeq/g)		Tyrosine value (µg 5ml ⁻¹)	
	VP	NP	VP	NP	VP	NP	VP	NP	VP	NP	VP	NP
0	49.09	49.09	5.40	5.40	0.54	0.54	0.16	0.16	0.93	0.93	0.01	0.01
1	49.00	48.95	5.33	5.36	0.56	0.57	0.16	0.16	2.00	2.66	0.14	0.29
2	48.90	48.85	5.23	5.30	0.58	0.62	0.2	0.22	2.60	4.00	0.44	0.73
3	48.85	48.78	5.18	5.24	0.62	0.65	0.24	0.28	3.33	4.66	0.55	1.45
4	48.76	48.73	5.13	5.18	0.65	0.68	0.28	0.34	4.06	7.06	0.86	2.15
5	48.74	48.69	5.08	5.14	0.67	0.77	0.32	0.4	4.73	8.60	1.56	2.71
6	48.71	48.65	5.05	5.10	0.69	0.77	0.36	0.46	5.66	9.66	1.95	2.99
7	48.68	48.65	5.02	5.06	0.71	0.84	0.4	0.52	6.66	11.33	2.43	3.50
8	48.60	48.62	5.00	5.02	0.73	0.91	0.44	0.58	7.66	12.33	2.71	3.82
9	48.58	48.56	4.99	4.98	0.75	0.97	0.48	0.62	8.22	13.00	2.98	4.05
10	48.56	48.51	4.98	4.96	0.76	1.02	0.52	0.66	9.00	13.66	3.38	4.36
11	48.52	48.47	4.96	4.88	0.78	1.07	0.54	0.71	11.66	15.12	3.70	4.59
12	48.47	48.42	4.94	4.85	0.81	1.11	0.56	0.74	14.33	16.49	3.81	4.79
13	48.43	48.39	4.92	4.82	0.84	1.14	0.58	0.77	16.21	17.9	4.21	5.11
14	48.39	48.34	4.88	4.80	0.87	1.17	0.62	0.81	17.92	19.11	4.43	5.25

¹ Values are mean of three trials NP=Normal packaging VP=Vacuum pack

Table 2 Analysis of variance for chemical characteristics of *kradi* cheese stored at 5±1°C

Attributes	df (between packaging system)	Mean sum of squares		F-value
		Packaging System	Time interval	
Moisture %	1	0.01 ^{ns}	0.04 ^{ns}	0.25
pH	1	-1.11x10 ⁻¹⁶ ^{ns}	0.03 ^{ns}	-3.6x10 ⁻¹⁵
Acidity %	1	0.16 ^{**}	0.02 ^{**}	5.59
Fat leakage	1	0.08 ^{ns}	0.03 ^{ns}	2.16
Free fatty acids	1	57.51 ^{ns}	30.03 ^{ns}	1.91
Tyrosine value	1	5.32 ^{ns}	2.75 ^{ns}	1.93

** Significant at 1%, * Significant at 5%, ns Non-significant

significant effect on the FFA of *kradi* samples (Table 2). The initial tyrosine value of $0.013 \mu\text{g } 5\text{ml}^{-1}$ in air packed *kradi* samples increased to $5.25 \mu\text{g } 5\text{ml}^{-1}$ while in vacuum packed samples it increased to $4.93 \mu\text{g } 5\text{ml}^{-1}$ on 14th week of storage. The slower rate of proteolysis at 5°C indicates that proteolytic enzymes were less active under refrigeration conditions. Slower rate of proteolysis was reported for white cheese (Turkish cheese) under vacuum compared to non-vacuum conditions stored at 4-5°C for 90 days (Cinbas and Kilic, 2006). Ahuja and Goyal (2013) reported that increase in concentrations of tyrosine content of vacuum packed paneer tikka samples during refrigerated vacuum storage for a period of 30 days and observed duration of storage as well as type of package significantly affected tyrosine content. Fuentes et al. 2015, reported that proteolysis did not showed significant changes during storage in pasta filata mexican cheese during refrigerated vacuum storage for 24 days. The camembert cheese manufactured using tibetan kefir co-culture underwent proteolysis (Mei et al. 2015). The effect of packaging conditions and storage periods was non significant on the tyrosine value of *kradi* samples (Table 2). The chemical analysis was discontinued after 14 weeks of storage under normal packaging and vacuum packaging as the samples expired due to off flavor development.

Conclusions

This study revealed that vacuum packaging of *kradi* cheese prevented loss of moisture, drop in pH was low and increase in acidity was also slow besides a lesser fat leakage. Proteolysis and lipolysis were slowed down by vacuum packaging. The increase in acidity of product was retarded under vacuum packaging which is a very good characteristic from consumer's point of view. Chemical quality of product was better maintained at refrigeration temperature of 5°C. This study also reveals that although chemical changes occurred both under normal and vacuum packaging, but the magnitude of changes under vacuum packaging was less. This study supports that both normal and vacuum packaging are desirable for packaging of *kradi* cheese. However from a technological point of view, vacuum packaging could be an alternative to conventional packaging for fresh *kradi* cheese production designed for long storage. Therefore vacuum packaging of *kradi* cheese can extend the shelf life of this traditional regional product and can guarantee the consumers a quality product.

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