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EFFECT OF ETHANOL EXTRACT OF RICE BRAN AS AN ANTIOXIDANT ON THE FLAVOUR OF STORED BUFFALOES'MILK GHEE

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ABSTRACT

Rice bran ethanol extract (RBEE) was used as a natural antioxidant at two concentrations (0.02% and 0.05% w/w) being added to buffaloes milk ghee. Butylated hydroxytoluene (BHT), the chemical antioxidant was also used at the concentration of 0.02 % w/w. Samples treated with natural (RBEE) and chemical (BHT) antioxidants were stored for 12 months at room temperature. The development of the flavour in the samples was estimated using Reichert-Meissl (RM), Polenske (PV) values, saponification number (SN) and Iodine value (IV). The fatty acid composition of the stored samples as well as thiobarbituric acid test (TBA) and peroxide value (POV) were also detected. Sensory score was carried out on all stored samples. Results revealed that the short chain fatty acid content of the control and RBEE (0.02% w/w) treated samples decreased with an associated decrease in RM, PV and SN. The IV and unsaturated fatty acid content also decreased with an increase in the saturated fatty acids. The TBA and POV were in agreement with the sensory score. The flavour deterioration was more evident as the values of TBA and POV increased. Concerning the samples treated with RBEE (0.05% w/w) and BHT (0.02% w/w), the addition of the RBEE succeeded to give similar results obtained by the addition of BHT at 0.02% w/w.
Keywords: Flavour, stored ghee, rice bran, antioxidants.

INTRODUCTION

There has been a growing demand for natural antioxidants due to reports that butylated hydroxy anisole (BHA) and butylated hydroxytoluene (BHT) have toxic and carcinogenic effects in animals *(Johnson, 1971 ; Branen, 1975 and Ito et al., 1985)*. Studies on the natural antioxidants showed that the incorporation of cooked wild rice into beef patties could retard the development of rancidity during frozen storage and improve sensory scores *(Minerich et al., 1991)*. It was postulated that wild rice may contain some natural antioxidant components. Other study indecated that methanol extract of rice hull seeds exhibited antioxidative activity stronger than that of α-tocopherol *(Ramarathnam et al., 1988)*.

Soybeans, defatted soy flour, soy protein concentrates, and soy isolates possess appreciable antioxidant activity in lipid-aqueous systems. The antioxidant properties of soybeans, defatted soy flour, and soy protein concentrates are due to polyphenolic compounds (Pratt, 1972, 1976, Pratt and Birac,1979 ; Hammerschmidt and Pratt, 1978). Wheat bran was extracted using hexane, chloroform and ethanol. The ethanol extract showed the highest activity when casued 90% inhibition on the oxidative degradation

of linoleic acid. The ethanol extract of wheat bran has significant effect in reducing rancidity in the stored beef samples in addition to decreasing the decomposition rate of the unsaturated fatty acids of the beef fat (Abd El-Mageed and Fadel, 1999). Ghee samples treated with 1.0% of fresh leaves of curry (Murraya Koenigi) or betel (piper betel) showed higher resistance to oxidation and higher sensory scores at the end of the storage period than those treated with BHT + BHA (Patel and Rajorhia, 1979 ; Thakar *et al.,* 1984). Ghee prepared from goats and buffaloes'milk in the ratios 3:1 and 1:1. Buffaloes'ghee and its blends tended to have slightly better shelf life than goats ghee (Arora and Singh, 1987).

Commercial butter oil from 5 different batches were stored at room temperature, 15 ^{o}C and 4 ^{o}C. It was reported that the flavour of butter oil were normal up to 4 months at 15 ^{o}C and 4 ^{o}C storage, slightly oxidized after 2 months storage at room temperature and 6 months storage at 15 ^{o}C and 4 ^{o}C and oxidized after 4 months storage at room temperature and 8 months storage at 15 ^{o}C and 4 ^{o}C (Vyas and Vyas, 1976). Several natural materials and different methods were used to improve the stability of ghee, oils and butter oil during storage. High stability oils, are usually referred to as being partially hydrogenated liquid vegetable oils which are strongly resistant to the development of off-flavour and have anextended shelf life (Anon, 1993).

Incorporation of 15-20% ghee-residue in the flavoured butteroil improved the product shelf-life (Wadhwa *et al.,* 1991). The possibility of using beewax and its unsaponifiable components as antioxidant agents against butteroil and cottonseed oil rancidity during storage were studied It could be revealed that the addition of whole beewax at 0.5 and 1% (w/w) had no effect on peroxide and TBA values of butteroil (Farag *et al.,* 1993). Carob which used as antioxidant and its antioxidant activity were compared with butylated hydroxyanisole (BHA). The addition of carob had the same effect as BHA in decreasing rate of peroxide formation in oil during storage (Abd El-Rahman *et al.,* 1977). Mastich, a resin from (Pistacia lentiscus) has been used by Egyptian farmers as a preservative for butteroil. It was reported that mastich concentration of 0.05 and 0.10% showed good keeping quality for oils at 25 and 35 ^{o}C, but at 45 ^{o}C it was better to use 0.10% mastich. 0.05% mastich contains approximately 0.02% terpenolic acid, equal to 0.02% of BHA (Abd El-Rahman and Youssef, 1975). Metalloproteins and ionic Fe and Cu salts are major catalysts of fatty acid peroxidation, but chelating agents, such as ethylene-diaminetetraacetic acid, citric acid in water, can retard or reduce this catalytic effect. Chelating agents when added in water shows more effect. Depending upon the concentration, water can act as an antioxidant in peanut butter, it was also reported that the effect of moisture content on the keeping quality of ghee is dependent on both its own concentration and type of antioxidant added (St Angelo and Ory, 1975 ; Gupta *et al*., 1978).

The aim of this work is to evaluate the rice bran ether extract as an alternative of the chemical antioxidant such as butylated hydroxytoluene.

MATERIALS AND METHODS

- Rice bran and Chemical Sources :

Rice bran was supplied by a rice dehulling and whitening company. Food grade antioxidant, butylated hydroxytoluene (BHT) and thiobarbituric acid were obtained from Sigma Chemical Company.

- Solvent Extraction :

Rice bran sample (150 gm) was extracted twice with 400 ml of ethanol overnight, followed by filtration. The extract was evaporated under reduced pressure.

- Thiobarbituric Acid Test :

The thiobarbituric acid (TBA) test was used to determine the antioxidant activity of the rice bran ethanol extract (RBEE), the same procedure was carried out to determine the degree of oxidation of stored samples at room temperature for 12 month. Antioxidant activity of RBEE was compared with BHT *(AOCS, 1989)*. The TBA values were calculated from the absorbance at 532 nm.

- Reichert-Meissl (RM), Polenske value (PV), Iodine value (IV), Saponification number (SN), were evaluated for each sample during storage period as described by Williams (1950).
- Peroxid value (POV) was determined as illustrated by AOAC (1980).

- Samples Preparation :

Buffaloes'milk ghee was obtained by heating (130°C) fresh buffaloes'milk butter. Four kilograms were devided into four parts. The first part was mixed well with 0.02% w/w of RBEE, the second part was also treated with 0.05% w/w RBEE, the third part was mixed with 0.02% w/w BHT and the fourth part was left without any addition to be the control. All samples were treated with previous additions at 50°C. Each treatment was divided into six portions packed in glassy Jars and kept at room temperature (25-30°C).Samples were analysed and scored organoleptically bimonthly.

- Methylation :

Fatty acids of ghee samples were converted to the corresponding methyl esters using methanol, zinc chloride and zinc dust as a catalyst (Shahin, 1977).

- Chromatographic analysis :

The determination of the resulted fatty acids methyl ester were carried out using gas chromatography, type konic 3000 with double flame ionized detector and with multilevel temperature programmer. Column used was a 12 feet stainless steel packed with 3% OV 17 on chromosorb w (60 – 80 mesh). The conditions were as follows :

- Column internal diameter 1/8 inch.
- Programing temperature 130 - 300°C at Rate 8°C / min.
- Injection port temperature 220°C.
- Detector port temperature 260°C.
- Carrier gas flow rate : Nitrogen 30 ml/min,

- Hydrogen gas flow rate : 30 ml/min and Air flow rate was 300 ml/min.

Sensory Evaluation :

The samples of stored ghee were scored organoleptically by ten panel dairy experts from Dairy technology Department, Animal Production Research Institute.

RESULTS AND DISCUSSION

Table (1) shows the changes in the fatty acid composition of buffaloes'milk ghee treated with RBEE (0.02% and 0.05% w/w) and BHT (0.02% w/w) in addition to the untreated sample (control) during the storage period at room temperature. It is obvious that the short chain fatty acids of the control and the sample treated with 0.02% RBEE were clearly affected. The short chain fatty acids gradually decreased throughout the storage period. The values started as high as 11.4%, 11.13%, and then it decreased to 8.85%, 8.52% for the control and the treated with 0.02% w/w RBEE samples, respectively. On the other hand, from the same table, the short chain fatty acids of the other treated samples with 0.05% RBEE, and 0.02% BHT slightly changed from the initial values of 11.2%, 11.22% to 10.87%, 10.90% at the end of storage period, respectively.

Total unsaturated fatty acids of the control sample decreased gradually throughout the storage period, oleic acid ($C_{18:1}$) the major unsaturated fatty acid decreased from 29.8% to 27.82% at the end of storage period. Linoleic and lenolenic acids disappeared after 10 and 6 months of storage, respectively. Similar trend were observed for the total unsaturated fatty acids of the samples treated with antioxidant RBEE 0.02% w/w. However, the addition of the antioxidant retarded the disappearance of linoleic and linolenic acids. Saturated fatty acids of the control and antioxidant treated sample (0.20% w/w RBEE) markedly increased as the total unsaturated fatty acids decreased. The decrease in the unsaturated fatty acids might be attributed to the formation of hydroperoxide (Selke and Frankel, 1987 ; Snyder *et al.,* 1988 ; Abd El-Mageed and Fadel, 1995 ; Tannous and Merat, 1969).

The same table shows the changes occurred in the total unsaturated fatty acids of the samples treated with 0.05% w/w of RBEE and 0.02% w/w of BHT. It was observed that both samples were more stable in their unsaturated fatty acids values when compared with the unsaturated fatty acids values of the control and antioxidant treated (RBEE 0.02% w/w) samples. This result might indicate that the addition of the RBEE antioxidant at the level of 0.05% w/w could be of the same effect of the BHT antioxidant at the level of 0.02% w/w. Generally, they improved the keeping quality of the ghee, especially, the RBEE as a natural antioxidant. Table (1), includes the RM, PV, SN and IV values of the control and the samples treated with 0.02%, 0.05% w/w RBEE as well as the sample treated with 0.02% w/w BHT.

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The values of RM, PV and SN of all samples were varied according to the variation in short chain fatty acids content. In general, it was observed that the RM, PV and SN values decreased as the storage period advanced (Ibrahim *et al.,* 1989). However, RM, PV and SN were not related to flavour characteristics of stored samples (Shivashraya-Singh, 1982).

Since IV is directly related to the unsaturation content (Youness, 1991) it had the same trend of the total unsaturated fatty acids values in all samples (Hassan *et al.,* 1989).

Table (2), reported the thiobarbitioric (TBA) and peroxid (POV) values of the control and treated samples with RBEE antioxidant as well as BHT. The TBA values of the control sample considerably increased throughout the storage period from 0.02 after two storage months to 0.33 at the end of storage period. A parallel increase was observed in the POV, as it was 0.5 m.equiv/kg fat in the first 2 months and reached to 4.11 m.equive./kg fat. at the end of storage period. The POV of the control sample after two months indicates poor keeping quality leading to high oxidative rancidity (Murthi *et al*., 1984; Khalifa and Mansour, 1988 ; Basu, 1980). From the same table, the TBA values and POV of sample treated with the antioxidant RBEE (0.02% w/w) showed lower values than observed in the control sample. However, these values were higher than the values of fresh ghee (Ahmed, 1975). The sample treated with 0.05% w/w of RBEE and the sample treated with 0.02% w/w BHT exhibited the lowest values of TBA and POV, when compared with the values of control sample and treated with 0.02%w/w RBEE sample. The values obtained for the TBA and POV were within the limits of the TBA value and POV of the good quality ghee.

Table (2) : Values of TBA and POV of Buffaloes'milk samples ghee treated with RBEE and BHT, during storage period at room temperature.

Storage period/ month	Control		RBEE 0.02%		RBEE 0.05%		BHT 0.02%	
	TBA*	POV**	TBA*	POV**	TBA*	POV**	TBA*	POV**
2	0.02	0.50	0.013	0.41	0.003	0.35	0.003	0.35
4	0.06	1.15	0.02	0.53	0.005	0.39	0.005	0.37
6	0.13	1.73	0.06	0.67	0.010	0.39	0.009	0.36
8	0.22	2.33	0.09	0.73	0.021	0.47	0.013	0.40
10	0.28	2.90	0.12	0.96	0.033	0.58	0.017	0.50
12	0.33	4.11	0.14	1.11	0.080	0.65	0.020	0.55

POV = Peroxide value
TBA = Thiobarbituric acid
RBEE = Rice bran ether extract.
*** = Absorbance value.**
**** = meq/kg fat.**

These results could also be obtained due to the addition of the antioxidants RBEE (0.05% w/w) and BHT (0.02% w/w) to the samples (Rao, *et al.,* 1984). It was also observed that the addition of RBEE antioxidant at the ratio of 0.05% w/w to the ghee sample retarded the development of the TBA value and the POV of the stored sample. The RBEE at the level of 0.05% w/w in the ghee sample was almost of the same antioxidant effect of

the BHT antioxidant. Hence, it could be advisable to us the RBEE at the level of 0.05% w/w as a natural and effective antioxidant in ghee.

Tables 3, 4 and 5 shows the sensory score of buffaloes'milk ghee, the control sample and the samples treated with 0.02% w/w and 0.05% w/w of RBEE and the sample treated with 0.02% w/w of BHT during the storage period. From table (3) it could be observed that the organoleptic evaluation of the control sample revealed that the sample had a marked off-flavour after four months of storage. More marked deterioration in the ghee flavour was evident after six months of storage. The sensory score of the control sample was on the contrary to the TBA value and the POV of the sample. The sensory score of the control sample decreased as the TBA values and POV of the sample increased. From table (4), it was reported that the level of 0.02% w/w of RBEE in the sample could not protect the properties of the ghee from deterioration. The sensory score of the sample during the storage period was rather low. The sample was characterized with off-flavour after six months. The flavour score was gradually decreased until it gained (zero) after the 10 months. However, the sample was markedly deteriorated when the flavour score was lower than 25 point (Gaba and Jain, 1974 and 1975). From the same table (table 4), the sample treated with 0.05% w/w of RBEE had a high flavour score. The sensory score of the sample started as high and decreased slightly towards the end of the storage period. The same behaviour was observed in the values of sensory score for the sample treated with 0.02% w/w of BHT (table 5). Sensory evaluation of both samples indicated that they have high flavour score. The results obtained from the sample treated with 0.05% w/w of RBEE could be acceptable.

Table (3) : Sensory score of buffaloes'milk ghee (control) stored at room temperature.

Storage period/month	Appearance	Body & texture	Flavour	Total
	15	30	55	100
2	14	29	50	93
4	14	20	25	59
6	14	25	22	61
8	13	20	10	43
10	13	18	0	31
12	12	15	0	27

Table (4) : Sensory score of Buffaloes'milk ghee treated with (0.02% and 0.05% RBEE) stored at room temperature.

Storage period/ month	Appearance (15)		Body & texture (30)		Flavour (55)		Total (100)	
	0.02%	0.05%	0.02%	0.05%	0.02%	0.05%	0.02%	0.05%
2	14	14	29	29	54	54	97	97
4	14	14	29	29	52	53	95	96
6	14	14	27	28	25	45	66	87
8	13	13	25	27	18	45	56	85
10	13	13	20	27	0	30	33	70
12	12	13	15	25	0	30	27	68

RBEE = Rice bran ethanol extract.

Table (5) : Sensory score of Buffaloes'milk ghee treated with 0.02% BHT.

Storage period/month	Appearance	Body & texture	Flavour	Total
	15	30	55	100
2	14	29	54	97
4	14	29	53	96
6	14	28	50	92
8	13	28	47	88
10	13	28	43	84
12	13	27	40	80

BHT = Butylated hydroxytoluene.

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نكهة مسلى اللبن الجاموسي المخزن والمعاملة بمستخلص الإيثانول لردة الأرز كمضاد أكسدة

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استخدم الناتج من استخلاص ردة الأرز بكحول الإيثانول كمضاد أكسدة طبيعي وذلك بنسبتين (0.02% ، 0.05% وزن / وزن) أضيفت إلى مسلى اللبن الجاموسي كذلك أضيف مضاد الأكسدة الكيماوي (Butylated hydroxy toluene BHT) بنسبة (0.2% وزن / وزن) .

خزنت العينات المعاملة بمضادات الأكسدة لمدة 12 شهراً على درجة حرارة الغرفة – قدر التطور في نكهة العينات المخزنة باستخدام رقم ريخارت ، بولينسك ، رقم التصبن والرقم اليودي ، كذلك تركيب العينات من الأحماض الدهنية بالإضافة إلى اختبار (Thiobarbituric acid) ورقم البيرأكسيد كما أجري التحكيم الحسي على العينات المخزنة .

أوضحت النتائج أن محتوى عينة الكنترول من الأحماض الدهنية قصيرة السلسة وكذلك محتوى العينة المعاملة بمستخلص ردة الأرز بنسبة 0.2% (وزن / وزن) من الأحماض الدهنية قصيرة السلسلة قد انخفض مصحوباً بانخفاض في أرقام ريخارت وبولينسك ورقم التصبن والرقم اليودي ومحتوى العينات نفسها من الأحماض الغير مشبعة قد انخفض مصحوباً بزيادة في محتواها من الأحماض المشبعة .

كان تدهور نكهة العينات أكثر وضوحاً بزيادة قيم اختبار (Thiobarbituric acid) ورقم البيرأكسيد . فيما يختص بالعينة التي عوملت بمستخلص ردة الأرز بنسبة 0.05% (وزن / وزن) قد نجحت في إعطاء نتائج مماثلة لنتائج العينة التي عوملت بمضاد الأكسدة BHT بنسبة 0.02% (وزن / وزن) .

Table (1) : Fatty acid composition of buffaloes'milk ghee samples treated with 0.02% and 0.05% w/w RBEE and with 0.02% w/w BHT and stored at room temperature.

Fatty Acids*	Control						RBEE 0.02% (Storage period/month)					
	2	4	6	8	10	12	2	4	6	8	10	12
C_4	3.32	3.21	2.90	2.81	1.93	1.93	3.3	3.21	3.08	2.90	2.25	1.81
C_6	2.47	2.35	2.55	2.75	1.85	2.10	2.45	2.81	2.73	2.35	2.61	2.22
C_8	1.40	1.50	1.55	1.65	1.48	1.50	1.37	1.45	1.44	1.67	1.21	1.60
C_{10}	1.91	2.10	1.99	2.00	1.83	1.53	1.89	2.35	2.75	2.70	2.05	2.75
C_{12}	2.14	2.27	2.21	2.16	1.89	1.79	2.11	2.15	2.15	2.16	1.99	1.14
C_{14}	10.11	10.39	10.91	10.70	12.01	12.86	10.01	10.35	10.67	10.86	11.97	12.73
$C_{14:1}$	2.71	2.70	2.53	2.44	1.97	1.76	2.99	2.61	2.12	2.00	1.68	1.70
$C_{16\ iso}$	2.33	2.41	2.49	2.88	2.78	1.99	2.55	2.28	2.45	2.45	2.57	2.50
C_{16}	30.83	31.18	32.72	33.05	33.53	34.21	30.99	30.89	30.97	31.65	32.93	34.11
$C_{16:1}$	0.84	0.68	0.43	0.28	T	T	0.84	0.71	0.85	0.70	0.40	0.21
C_{18}	10.56	10.83	10.78	10.95	12.73	12.51	10.49	10.29	10.32	10.73	11.85	12.20
$C_{18:1}$	29.80	29.19	28.63	28.13	28.00	27.82	29.71	29.62	29.39	29.35	28.49	28.01
$C_{18:2}$	1.00	0.89	0.31	0.20	-	-	0.89	0.81	0.63	0.41	-	-
$C_{18:3}$	0.49	0.30	T	T	-	-	0.51	0.47	0.45	T	-	-
Riechert-Meissl (RM)	30.12	30	30.04	29.30	28.21	28.30	30	31	31.5	31.5	31	28.5
Polenske value (PV)	2.15	2.0	2.0	2.0	1.80	1.80	2.17	2.21	2.38	2.56	2.98	1.99
Saponification number (SN)	228	229	228	228	220	219	228.5	230	234	231	229	219
Iodine value (IV)	41.22	40.20	39.50	39.47	36.50	35	41.83	40.10	38.31	37.79	35.81	35.37

***Values expressed as area percentages.**
Reported values are the mean of two determinations.
RBEE = Rice bran ethanol extract. BHT = Butylated hydroxytoluene. T = Trace

Continued :

Fatty Acids	RBEE 0.05%						BHT 0.02 %					
	2	4	6	8	10	12	2	4	6	8	10	12
C_4	3.32	3.30	3.29	3.30	2.99	2.80	3.29	3.30	3.25	3.18	3.0	3.10
C_6	2.46	2.46	2.45	2.47	2.50	2.60	2.45	2.45	2.41	2.39	2.4	2.40
C_8	1.41	1.40	1.37	1.45	1.47	1.51	1.45	1.41	1.39	1.41	1.46	1.44
C_{10}	1.90	1.72	1.91	1.97	1.81	1.79	1.93	1.92	1.88	1.83	1.79	1.81
C_{12}	2.11	2.17	2.08	2.11	2.20	2.17	2.10	2.27	2.16	2.18	2.25	2.15
C_{14}	10.11	10.25	10.15	10.20	10.24	10.55	10.09	10.15	10.29	10.90	10.66	10.91
$C_{14:1}$	2.75	2.80	2.73	2.69	2.66	2.71	2.81	2.70	2.66	2.66	2.52	2.53
$C_{16\ iso}$	2.37	2.28	2.33	2.35	2.35	2.33	2.40	2.35	2.37	2.33	2.37	2.35
C_{16}	30.91	30.88	31.08	31.02	31.51	31.97	30.90	30.90	30.99	31.11	31.78	31.97
$C_{16:1}$	0.82	0.79	0.81	0.81	0.71	0.81	0.80	0.80	0.83	0.73	0.75	0.76
C_{18}	10.52	10.49	10.60	10.67	10.90	11.30	10.55	10.55	10.63	10.86	10.97	10.97
$C_{18:1}$	29.83	29.85	29.80	29.77	29.67	29.07	29.79	29.80	29.71	29.15	29.04	29.11
$C_{18:2}$	1.04	1.11	1.00	0.83	0.74	0.35	0.99	1.00	0.98	0.83	0.71	0.50
$C_{18:3}$	0.44	0.50	0.40	0.36	0.25	T	0.45	0.40	0.45	0.39	0.30	T
Riechert-Meissl (RM)	30.1	30	30	30.15	29.73	29.85	30	30.50	30	29.70	29.70	29.70
Polenske value (PV)	2.30	2.15	2.15	2.11	2.01	2.06	2.21	2.35	2.17	2.00	2.00	2.08
Saponification number (SN)	228	228	228	228	227	225	228	228	227	223	223	223
Iodine value (IV)	40.29	40.45	40.61	39.41	39.16	37.27	40.21	40.31	39.75	39.11	38.14	38