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Effect of Type of Colorant on the Physicochemical Properties of Ras Cheese and its Whey

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Abstract:

Ras cheese was manufactured utilizing different cheese colorants (annatto, Curcumin, beta carotene based, and cheese without color), and coagulants (recombinant bovine). This study was conducted to understand their effect on the chemical, microbiological and rheological properties of Ras cheese. The highest yield was observed in the cheese treated with curcumin compared to other treatments of Ras cheese. The highest fat content was detected in β-carotene cheese after 180 days of ripening. The highest value of Water-soluble nitrogen (WSN) was recorded in β-carotene cheese (0.46) while the lowest value in Curcumin cheese (0.39). Total bacteria count and proteolytic bacteria were gradually decreased reaching its minimum at the end of the ripening period (180) days. The count of mold and yeast at 180 days old cheese were 0.17×10^5, 0.11×10^5, 0.07×10^5, and 0.09×10^5 CFU/g for treatments of cheese without color added, Annatto cheese, Curcumin cheese, and β-carotene cheese, respectively. The addition of colorants improved slightly the composition and sensory properties of Ras Cheese.

INTRODUCTION

Ras (Kafalotyri) cheese is the most popular hard cheese in Egypt. It is made with full cream milk and ripened throughout two to three months. During the past 10 to 15 years, the American Heart Association, and other Health Organizations have called for a reduction in total dietary fat to 30% of calories for most people (AHA, 1986; DHHS, 1988). They have also recommended that no more than 10% of total calories should come from saturated fats. A high intake of total dietary fat is associated with an increased risk of obesity, some types of cancers, gallbladder disease, high blood cholesterol, and coronary heart disease. Removal of fat from cheese results in undesirable texture and appearance, altered rheological parameters, lack of flavor, poor keeping quality, and poor meltability and stretching properties (Rodriguez, 1998; Sipahioglu *et al*., 1999; Koca and Metin, 2004*)*. Low-fat cheeses are usually characterized by a rubbery body and lack of flavor (Mistry, 2001).

The presence of active constituents, having different pharmacological effects signifies the potential use of plants (Ashraf *et al.,* 2020; Iqbal and Ashraf, 2018, 2019; Shahzad *et al.,* 2017). The use of herbal medicine recognizing its effectiveness has been documented in various studies (Ullah *et al.,* 2018; Zaynab *et al.,* 2018). Curcumin is a major isolated polyphenol from the rhizome of turmeric (*Curcuma longa*) (Ansari *et al.,* 2020). A few clinical studies (Aronson, 2009) as well as the Food and Drug Administration (FDA) established curcumin to be safe (Prasad *et al.,* 2014). β-carotene is an important ingredient in many pharmaceutical and food products as a precursor of vitamin A (Baker and Günther, 2004). Among all vegetables, the highest β-carotene content is found in carrots (Otálora-Orrego and Martin-G, 2020).

This study aimed to study the possibility of extracting some dyes of *Curcumin* from rhizomes of *C. longa* and *β-carotene* from carrot peels. Applying individually both extracted colorants in Ras cheese making, following their effects on the chemical, microbiological and rheological properties of Ras cheese as compared with Ras cheese dyed by Annatto colorant.

MATERIALS AND METHODS

Herbal Material

Turmeric Rhizomes

C. longa was purchased from a herbal market in Damietta, Egypt.

Carrot peels

The carrot was obtained from a local market, Damietta, Egypt.

Pure *Curcumin*

Curcumin crystalline extra pure (assay 98%) was purchased from Alpha Chemika, India.

Pure β-*Carotene*

Beta carotene powder with a minimum purity of 99% was purchased from Alpha Chemika, India.

Commercial *Annatto*

Liquid annatto (assay 4%) was purchased from MIFAD Company, Badr City Egypt.

Salt

Dry fine commercial food-grade salt was obtained from El-Nasr Company, Alexandria, Egypt.

Other Chemicals

All chemicals and solvents were purchased from El-Gomhoria for the chemicals company, Mansoura, Egypt.

Cheese milk

Fresh cow and buffalo milk were obtained from the farm of Damietta, faculty of Agriculture. The chemical composition of milk used in Ras's

cheese manufacturing was fat 5.1%, protein 3.8%, and TS 14.7%.

Methods

Ras cheese manufacture

Ras's cheese was manufactured according to the protocol as described by Hofi et al. (1970) as mentioned in Figure (1). A mixture of raw cows and buffalo milk (80:20) having 14.7% T.S, 3.8% protein, and 5.1% fat was warmed to 32°C, and rennet was added to the milk sample (3.0g/100kg milk). Milk was then stirred quickly for 3 min and set to coagulate at 30 – 40 minutes. The curd was cut into cubes about the size of chickpea grains in size of 1*1*1 cm and then vigorously stirred. The curd-whey mixture was stirred and scalded to 45°C for 45 min. Whey was drained when its acidity reached 0.14% as lactic acid. The curd was salted (3%, w/w, NaCl). The curds were put into wheel-shaped stainless-steel molds for prepress. The curds were pressed to expel some of the remaining whey. Light mechanical pressure follows over the next 4 hours at which point the cheese was reversed in the press and repressed for 12 more hours. Following the removal of whey, fresh cheese was salted with dry salt on the surface of cheese during the first month of storage, the cheese was ripened at 14°C for 6 months with a relative humidity of 85%.

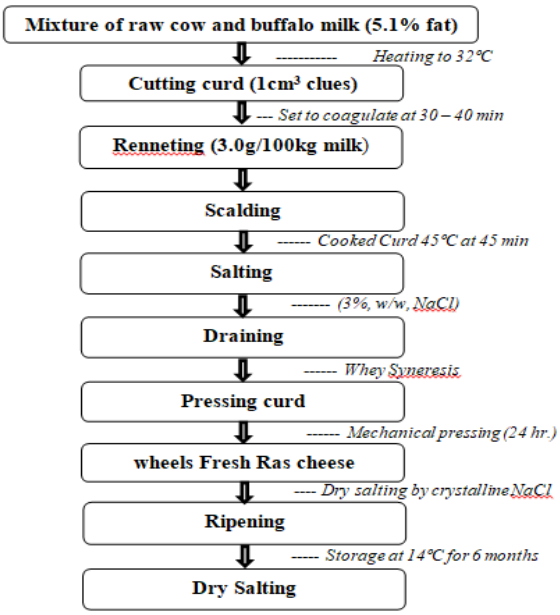

Fig. 1. The flow chart for manufacturing Ras cheese**.**

Experimental design

Ras cheese as control and samples were manufactured in El-faiomy laboratory, Damietta for making Ras cheese.

Four treatments were performed for the manufacture of Ras cheese. A total of 120 L of raw Standardized milk was used and divided into batches by changing cheese colour, the treatments were as follows:

1. (T_0) NRC: (Noncolorant Ras cheese)

Ras's cheese was made without adding any colorant.

2. (T_1) ARC: Annatto Ras cheese (commercial Ras's cheese)

Ras's cheese was made with Annatto (0.03% (v/v)).

3. (T_2) CRC: Curcumin Ras cheese

Ras's cheese was made with Curcumin extract (0.03% (v/v)).

4. (T_3) BRC: Beta-carotene Ras cheese

Ras's cheese was made with beta-carotene extract (0.03% (v/v)).

Samples for analysis were periodically taken at 0, 15, 30, 60, 90, 120,150, and 180 days of ripening.

Calculation of cheese yield

The yield of fresh and ripened Ras cheese was calculated according to Hattem et al. (2012) using the following equation:

Yield (%) = Weight of cheese (kg) / Weight of milk used (kg) × 100

Physicochemical Analysis of Ras Cheese

To make them uniform, we took 2 quarts from the center of the cheese (2 mm under the crust) and about 10 g was grated to yield particles of 1 mm as described by Guinee (2002). Mixed the crushed stored in an airtight container. The sample was kept at -18°C until analysis. All determinations were carried out in triplicate.

Total Solid Content

Total solid content of milk and cheese was determined gravimetrically according to AOAC (2012) by drying a sample to constant weight in an oven at 105ºC for 4-5 hours. The difference in weight before and after drying showed total solid content.

Total solid content (%) = [(Total solid content of cheese (g) / cheese (g))] ×100

Ash Content

Ash determination was carried out by the method of AOAC (2012). Samples were dried in the oven for 1 h and burned in an ash oven at 550°C until the all-black color was disappeared. After cooling in the desiccator, the crucibles were weighed. The difference in weight before and after burning process demonstrated the ash content.

pH Value

The pH values for the milk, whey, and cheese were determined by using a standard digital electronic pH meter.

Protein Content

The Protein content of cheese was determined according to AOAC (2012) by measuring the total nitrogen content of samples by the Kjeldahl method and converted into protein using the conversion factor (6.38).

Soluble Nitrogen (SN)

The water-soluble nitrogen was determined by the Kjeldahl method according to AOAC (2012).

Non-Protein-Nitrogen (NPN)

The Non-protein-nitrogen was determined by the Kjeldahl method according to AOAC (2012).

Total Volatile Fatty Acids (TVFA)

Total volatile fatty acids were determined according to AOAC (2012).

Salt Content

The salt content of samples was estimated using Volhard's method according to Richardson (1985).

Procedure: 5 g of cheese sample was dissolved in hot distilled water. The same process was repeated 5-6 times to enable all salt transferred to the water. The final volume reaches 500 ml, 25 ml of this solution taken from it, 1-2 drops of K_2CrO_4 (5%) was added as an indicator and titrated cheese extract solution against 0.1N $AgNO_3$ until tile red color appeared.

% Salt = ((G* 0.00585)* 100)/P

Where;

G = Consumed 0.1 N $AgNO_3$ amount (ml)

P = Cheese amount included in titration (0.25 g)

Salt in Dry Matter (SDM) content was calculated by the formula

SDM % = S % x 100 / DM

where S is the percentage of salt of cheese.

Titratable Acidity (TA)

For determination of titratable acidity (% lactic acid) 3 g cheese was weighed and crushed with 10 ml water in a porcelain mortar. This solution was transferred into a conical flask, 5 drops of phenolphthalein were added and titrated with 0.1 N NaOH to the first permanent colour change to faint pink (AOAC, 2012).

Fat Content

Fat content was determined using the Gerber's method was followed using the special butyrometer tubes for milk and cheese as described by AOAC (2012).

Fat in dry matter (FDM) was calculated as follows:

FDM % = F % x 100 / DM

where F is the percentage of fat of cheese and DM is the percentage of dry matter of cheese.

Microbiological Analysis

Nutrient agar medium was used for counting total bacteria according to Ronald (2010). Plates were incubated at 30°C for 3 days.

Skim Milk Agar was used for counting Proteolytic bacteria according to Ronald (2010). Plates were incubated at 25°C for 3 days.

Potato Dextrose Agar (PDA) was used for yeasts and fungi (molds) cultivation and enumeration (Ronald, 2010). Plates were incubated at 25°C for 5 days.

Sensory Evaluation

The sensory evaluation was carried out at the Department of Dairy Science and Technology, Damietta University, including staff members and assistants. Cheese samples were graded at 0, 15, 30, 60, 90, 120, 150, and 180 of the storage period. The score points were 15 for colour and appearance, 35 for body and texture, and 50 for flavour, which gives a total score of 100 points as described by Scott (1981).

RESULTS AND DISCUSSION

Effect of type of colorant on the yield of Ras cheese

Ras Cheese yield is affected by many factors such as type of milk, seasonal variations of milk composition, milk quality, heat treatments of milk, type of coagulant used, curd firmness at cutting, salt, and loss of moisture during ripening (Abd El-Gawad and Ahmed, 2011). Data shown in Table (1) illustrated the effect of type of colorants on the yield of the cheese, the yield of fresh cheese ranged between 9.66% and 9.83%, the highest was for *Curcumin* treatment compared to other treatments, which can be explained as *Curcumin* can hold moisture in the cheese. Whereas the lowest average value was found to be in without any colourant cheese added (9.66% and 6.96%) before and after ripening, respectively. The difference in yield is not highly marked, especially for fresh cheese.

Table 1. Yield (%) of Ras cheese as affected by the type of colorant.

Cheese Yield	Ras cheese without colour	Ras cheese with Annatto	Ras cheese with Curcumin extract	Ras cheese with β-carotene extract
When Fresh	9.66	9.76	9.83	9.76
After Salting	8.38	8.74	8.86	8.57
180 days	6.96	7.05	7.10	7.00

Effect of type of colorant on Physical properties of Ras cheese during 180 days of storage

Loss of weight

From Table (2) the weight of all cheeses decreased gradually as the ripening period advanced, because of the evaporation of moisture from the wheels of Ras cheese, *Curcumin* treatment had the highest weight was for fresh and 180 days old.

Table 2. Physical examination of Ras cheese samples during ripening period as affected by the type of colorant.

Treatments	**Storage periods**	**Parameter**					
		Weight (Kg)	**Height (Cm)**	**Diameter (Cm)**	**Volume (Cm^3)**	**Shrinkage of volume%**	**Loss of weight %**
Ras cheese without colour	0	2.900	12.00	16.00	2411.52	0.00	0.00
	15	2.516	11.50	15.50	2168.86	10.06	13.24
	30	2.323	11.30	15.20	2049.44	15.01	19.90
	60	2.239	11.00	15.00	1942.88	19.43	22.79
	90	2.190	10.80	14.60	1807.17	25.06	24.48
	120	2.124	10.70	14.40	1741.72	27.78	26.76
	150	2.156	10.50	14.30	1685.51	30.11	25.66
	180	2.090	10.50	14.00	1615.53	33.00	27.93
Ras cheese with Annatto	0	2.930	12.00	16.00	2411.52	0.00	0.00
	15	2.624	11.50	15.50	2168.86	10.06	10.44
	30	2.433	11.20	15.00	1978.20	17.97	16.96
	60	2.324	11.00	14.70	1865.94	22.62	20.68
	90	2.211	10.80	14.50	1782.50	26.08	24.54
	120	2.190	10.70	14.30	1717.61	28.77	25.26
	150	2.156	10.60	14.20	1677.85	30.42	26.42
	180	2.115	10.50	14.10	1638.69	32.05	27.82
Ras cheese with Curcumin extract	0	2.950	12.00	16.00	2411.52	0.00	0.00
	15	2.658	11.20	15.50	2112.28	12.41	9.90
	30	2.450	11.00	15.30	2021.37	16.18	16.95
	60	2.360	10.90	15.00	1925.21	20.17	20.00
	90	2.295	10.70	14.90	1864.77	22.67	22.20
	120	2.284	10.60	14.70	1798.08	25.44	22.58
	150	2.250	10.50	14.60	1756.97	27.14	23.73
	180	2.131	10.50	14.50	1732.99	28.14	27.76
Ras cheese with β-carotene extract	0	2.930	12.00	16.00	2411.52	0.00	0.00
	15	2.572	11.20	15.00	1978.20	17.97	12.22
	30	2.433	11.00	14.80	1891.41	21.57	16.96
	60	2.303	10.80	14.60	1807.17	25.06	20.48
	90	2.219	10.70	14.50	1765.99	26.77	24.27
	120	2.142	10.60	14.20	1677.85	30.42	26.89
	150	2.135	10.50	14.20	1662.02	31.07	27.13
	180	2.100	10.50	14.20	1662.02	31.07	28.33

Cheese for *Curcumin* treatment, while the lowest was for cheese without any colourant. Also, from Table (2) *Curcumin* treatment had the highest moisture content. Foda et al. (2007) found that the addition of *Curcumin* to buffalo's milk increased the holding capacity of water in yogurt. On the other hand, during ripening, all cheeses gradually decreased their weights. A similar finding was reported by El-Sissi (2003).

These losses of weight may be due to the biochemical changes and lactic acid development which result in curd contraction and expulsion of the aqueous phase of cheese. The percentage of losses ranged between 27.76% and 28.33% by the end of ripening, the lowest was for *Curcumin* cheese and the highest was for β-carotene cheese. These results agreed with El-Shazly et al. (2011), who reported the moisture percentage of Ras cheese treatment with turmeric increased by increasing the turmeric concentration.

Shrinkage of volume

Little change was observed for the diameter of cylinders during ripening for the four treatments. The highest was 16 cm for all cheeses, by the end of ripening *Curcumin* cylinder had 14.50 cm while the other three treatments recorded 14.00 cm, 14.10 cm, and 14.10 cm for Ras cheese without colour, Annatto cheese, and β-*carotene* cheese respectively. No change in height was found during ripening started by 12.0 cm and ended by 10.5 cm for all treatments.

The volume of fresh cheese was 2411.52 cm3, the gradual decrease was observed for all treatments, the highest was for *Curcumin* 180 days old cheese. Percentage of shrinkage of volume during 180 days of ripening was 33.00%, 32.05%, 28.14%, and 31.07% for treatments of cheese without colour added, *Annatto* cheese, *Curcumin* cheese, and β-*carotene* cheese, respectively.

Physicochemical properties of Ras cheese whey as affected by the type of cheese colorant

The chemical composition of Ras cheese whey is summarized in Table (3). Acidity values in whey were 0.73, 0.72, 0.79, and 0.70 for treatments of cheese without colour added, Annatto cheese, Curcumin cheese, and β-carotene cheese respectively. The value of fat content was recorded as 0.45%, 0.44%, 0.47%, and 0.41% for treatments of cheese without color added, Annatto cheese, Curcumin cheese, and β-carotene cheese, respectively. The total solid content in whey ranged between 7.12% and 7.45%. Protein content was 0.80%, 0.78%, 0.85%, and 0.76% for treatments of cheese without color added, Annatto cheese, Curcumin cheese, and β-carotene cheese, respectively.

Table 3. Physicochemical properties of Ras cheese whey as affected by the type of cheese colorant

Treatment	pH	Acidity	Total solid (TS)	Fat (%)	Protein (%)	Ash (%)
No colour	5.12	0.73	7.25	0.45	0.80	0.41
Annatto	5.11	0.72	7.20	0.44	0.78	0.43
Curcumin	5.14	0.79	7.45	0.47	0.85	0.45
β-carotene	5.16	0.70	7.12	0.41	0.76	0.39

Chemical composition of Ras cheese treatments during the ripening period

Acidity and pH values

From Table (4) for all treatments acidity values gradually increased as the ripening time processed probably as a result of fermentation of residual lactose to lactic acid continuously and degradation of protein and fat **(**Hofi *et al*., 1970). An increasing trend in titratable acidity during cheese ripening was also expounded by many previous findings (Shehata *et al*., 2004; Hattem *et al*., 2012). Acidity values for fresh, 120, and 180 days were (0.79/1.71/1.49), (0.85/1.74/1.58), (0.92/1.80/1.62) and

(0.81/1.72/1.53) for treatments of cheese without colour added, Annatto cheese, Curcumin cheese, and β-carotene cheese respectively. These results are in agreement with El-Shazly et al. (2011), who found the higher values of titratable acidity in Ras cheese as a result of higher turmeric concentration added. Similar trends were reported by Foda et al. (2007) who observed the addition of turmeric in yogurt increased the titratable acidity values during cold storage.

While pH values gradually decreased as the ripening period advanced. A slight decrease in pH was observed in all cheese treatments at the early stages of ripening. A similar effect has also been reported in other studies (Awad, 2006). The pH values at Fresh / 180 days old cheese were (5.93/5.30), (5.85/5.23), (5.80/5.19) and (5.90/5.25) for treatments of cheese without colour added, *Annatto* cheese, *Curcumin* cheese, and β-*carotene* cheese, respectively. The highest either for fresh and 180 days old cheese was for cheese without dye. The lowest was *Curcumin* treatment. Similar results were found by El-Shazly et al. (2011). From the previous results, it is clear that the addition of *Curcumin* extract to cheese milk raised the acidity and reduced pH values of Ras cheese.

Cheese moisture

As shown in Table (4), moisture content of the cheeses during 180 days of storage. All treatments show a gradual decrease of moisture during the ripening period. This might be due to some of the water gradually evaporating during ripening or becoming bound with the protein as about water, as ripening progressed cheese samples during the ripening periods due to water evaporation from cheese surface. These results agree with those obtained by Awad (2006). Fresh/180 day's old cheese was (44.55/34.52), (43.45/33.87), (45.95/34.79) and (43.36/33.80) for treatments of cheese without colour added, *Annatto* cheese, Curcumin cheese, and β-*carotene* cheese, respectively.

The highest value either for fresh and 180 day's old cheese was for *Curcumin* treatment. The lowest was cheese without dye. On the other hand, the Ras cheese made with *Curcumin* extract till the end of the ripening period had higher moisture content compared to the other cheese treatments. Similar results were obtained by Foda et al. (2007). This may be due to that the add agents help cheeses to hold more water or retard the water losses Compared to the other cheeses.

Cheese fat content

From Table (4), fat content increased in all samples till the end of the storage period. The slight increase in fat content during the ripening period may be attributed to the decline in moisture content and the increase in the TS during storage. Similar trends were reported by Shehata et al. (2004) and Mehanna et al. (2009). The higher fat content was detected in β-carotene cheese than the other cheese treatments at the end of ripening storage followed by Annatto cheese, Curcumin cheese, and cheese without any colour added, respectively. The values of fat content were recorded as 33.80%, 33.57%, 33.00%, and 32.79% respectively at 180 days of ripening.

Cheese protein content

And similarly, protein content in Table (4) increased in all samples till the end of the storage period. The values of protein content were recorded as (22/29.87), (22.25/30), (21.92/29.55), and (23/30.82) of fresh/180 days old cheese for treatments of cheese without colour added, *Annatto* cheese, Curcumin cheese, and β-carotene cheese, respectively.

Salt content

From Table (4), salt content affects cheese structure by increasing the level of protein hydration and results in a swelling of the protein matrix (Enab *et al*., 2012). Concerning the salt % gradually increased in all samples till the end of the storage period. Salt contents of Fresh / 180 days old cheese were (1.87/4.43), (2.10/4.35), (1.97/4.31) and (1.76/4.29) for treatments of cheese without colour added, *Annatto* cheese,

Curcumin cheese, and β-*carotene* cheese, respectively. There is a gradual increase in salt content to the second month, and then it remains almost constant until the end of the ripening period dry salting of Ras cheese is a slow process. These results are in agreement with those obtained by Hofi et al. (1970). The values of Salt/moisture contents were 12.83, 12.84, 12.39, and 12.81 for treatments of cheese without colour added, *Annatto* cheese, *Curcumin* cheese, and β-*carotene* cheese, respectively at the end of the storage period.

Table 4. Effect of type of colorant in cheese milk on chemical properties during ripening of Ras cheese.

Formulations	Ripening Period (day)	Physicochemical Parameters						
		pH	Acidity (%)	Moisture (%)	Fat (%)	Protein (%)	Salt (%)	Salt/Moisture (%)
Ras cheese without colour	0	5.93	0.79	44.55	24.50	22.00	1.87	4.20
	15	5.80	0.93	41.60	26.23	23.83	3.00	7.21
	30	5.62	1.13	40.11	27.18	24.85	3.38	8.43
	60	5.47	1.30	38.35	28.80	26.35	3.72	9.70
	90	5.30	1.51	37.21	30.35	27.00	3.90	10.48
	120	5.07	1.71	36.78	31.12	28.35	4.11	11.17
	150	5.21	1.60	35.50	32.25	29.00	4.36	12.28
	180	5.30	1.49	34.52	33.00	29.87	4.43	12.83
Ras cheese with Annatto	0	5.85	0.85	43.45	25.14	22.25	2.10	4.83
	15	5.67	1.03	41.00	26.75	24.15	3.33	8.12
	30	5.54	1.21	38.26	28.49	25.39	3.65	9.54
	60	5.41	1.39	37.71	29.83	26.50	3.80	10.08
	90	5.20	1.57	36.57	30.98	27.63	3.95	10.80
	120	5.02	1.74	35.81	32.00	28.49	4.09	11.42
	150	5.12	1.63	34.19	32.86	29.38	4.23	12.37
	180	5.23	1.58	33.87	33.57	30.00	4.35	12.84
Ras cheese with Curcumin extract	0	5.80	0.92	45.95	24.25	21.92	1.97	4.29
	15	5.60	1.06	41.68	26.50	23.75	3.29	7.89
	30	5.52	1.29	40.46	27.00	24.59	3.40	8.40
	60	5.35	1.47	38.50	28.00	25.47	3.78	9.82
	90	5.03	1.65	37.31	29.57	26.85	3.95	10.59
	120	4.94	1.80	36.86	30.86	28.12	4.15	11.26
	150	5.06	1.75	35.65	31.33	28.80	4.20	11.78
	180	5.19	1.62	34.79	32.79	29.55	4.31	12.39
Ras cheese with β-carotene extract	0	5.90	0.81	43.36	25.65	23.00	1.76	4.06
	15	5.75	0.96	40.57	27.30	24.55	2.93	7.22
	30	5.60	1.18	38.11	28.61	26.05	3.23	8.48
	60	5.43	1.35	37.13	30.15	27.58	3.40	9.16
	90	5.25	1.55	36.35	31.00	28.03	3.55	9.77
	120	5.04	1.72	35.70	32.13	29.33	3.85	10.78
	150	5.19	1.61	34.00	33.00	29.65	3.94	11.59
	180	5.25	1.53	33.50	33.80	30.82	4.29	12.81

The values of dry matter, Protein/ dry matter, and Fat/dry matter of Ras cheese as affected by the type of cheese colorant

Dry Matter of cheese

From Table (5), all treatments showing gradually increase of dry matter (%) during ripening period this increase of dry matter as a result of moisture evaporation. DM values for fresh, 120 and 180 days were (55.45% / 63.22% / 65.48%), (56.55% / 64.19% / 66.13%), (55.05% / 63.14% / 65.21%) and (57.64% / 64.30% / 66.50%) for treatments of cheese without colour added, *Annatto* cheese, *Curcumin* cheese and β-*carotene* cheese, respectively.

Protein/ Dry Matter

The values of Protein/Dry Matter for fresh, 120 and 180 days were (39.68% / 44.84% / 45.62%), (39.35% / 44.38% / 45.37%), (39.82% / 44.54% / 45.32%) and (39.90% / 45.61% / 46.35%) for treatments of cheese without colour added, *Annatto* cheese, *Curcumin* cheese and β-*carotene* cheese respectively (Table 5).

Fat/Dry Matter

Fat/Dry Matter content in Table (5) in the cheese of more than 50% is required for the development of true Cheddar flavor (Ohren and Tucky, 1969).

Table 5. Effect of type of colorant on dry matter, Protein/ dry matter, and Fat/dry matter during ripening of Ras cheese.

Formulations	Ripening Period (day)	Physicochemical Parameters				
		Dry Matter (%)	Protein (%)	Protein/ Dry Matter (%)	Fat (%)	Fat/Dry Matter (%)
Ras cheese without colour	0	55.45	22.00	39.68	24.50	44.18
	15	58.40	23.83	40.80	26.23	44.91
	30	59.89	24.85	41.49	27.18	45.38
	60	61.55	26.35	42.81	28.80	46.79
	90	62.79	27.00	43.00	30.35	48.34
	120	63.22	28.35	44.84	31.12	49.22
	150	64.50	29.00	44.96	32.25	50.00
	180	65.48	29.87	45.62	33.00	50.61
	Mean	61.41	26.41	42.90	29.18	47.43
Ras cheese with Annatto	0	56.55	22.25	39.35	25.14	44.45
	15	59.00	24.15	40.93	26.75	45.34
	30	61.74	25.39	41.12	28.49	46.14
	60	62.29	26.50	42.54	29.83	47.89
	90	63.43	27.63	43.56	30.98	48.84
	120	64.19	28.49	44.38	32.00	49.85
	150	65.81	29.38	44.64	32.86	49.93
	180	66.13	30.00	45.37	33.57	50.76
	Mean	62.39	26.72	42.86	29.95	47.90
Ras cheese with Curcumin extract	0	55.05	21.92	39.82	24.25	44.05
	15	58.32	23.75	40.72	26.00	44.58
	30	59.54	24.59	41.30	27.00	45.52
	60	61.50	25.47	41.41	28.00	45.83
	90	62.69	26.85	42.83	29.57	47.17
	120	63.14	28.12	44.54	30.86	48.88
	150	64.35	28.80	44.76	31.33	48.69
	180	65.21	29.55	45.32	32.79	50.29
	Mean	61.23	26.13	42.59	28.73	46.88
Ras cheese with β-carotene extract	0	57.64	23.00	39.90	25.65	44.50
	15	59.43	24.55	41.31	27.30	45.93
	30	61.89	26.05	42.10	28.61	46.24
	60	62.87	27.58	43.86	30.15	47.96
	90	63.65	28.03	44.03	31.00	48.70
	120	64.30	29.33	45.61	32.13	59.98
	150	66.00	29.65	44.92	33.00	50.00
	180	66.50	30.82	46.35	33.80	50.83
	Mean	62.79	27.38	45.76	30.21	49.27

The average fat contents on dry weight basis were 47.43%, 47.90%, 46.88%, and 49.2% after 180 days of ripening period for treatments of cheese without colour added, *Annatto* cheese, Curcumin cheese, and β-*carotene* cheese, respectively. This average agreed with Egyptian Standards (ES: 1007-5/2005), where Fat/DM is not less than 45%.

Effect of type of colorant in cheese milk on the ripening indices of Ras cheese during the storage period

Table (6) showed the change in TN, WSN, WSN/TN, NPN, NPN/TN, and TVFA contents of Ras cheeses as a result of adding some colorant extracts.

The total nitrogen (TN) content

The total nitrogen (TN) content increased slightly between the beginning and the end of ripening. The total nitrogen contents of Fresh / 180 days old cheese were (1.87/4.43), (2.10/4.35), (1.97/4.31) and (1.76/4.29) for treatments of cheese without colour added, *Annatto* cheese, *Curcumin* cheese, and β-*carotene* cheese, respectively.

Water-soluble nitrogen (WSN)/total nitrogen (TN) ratio

Water-soluble nitrogen (WSN) values indicate the intensity degree of proteolysis in cheese samples (Table 6) in agreement with Andronoiu et al. (2015). The values of WSN were recorded 0.41, 0.45, 0.39, and 0.46 at the end of ripening time for treatments of cheese without colour added, *Annatto* cheese, *Curcumin* cheese, and β-*carotene* cheese, respectively. On the other hand, the ratio between WSN and TN gradually increased to reach 8.76, 9.57, 8.42, and 9.52 at the end of ripening time for treatments of cheese without colourarnt added, *Annatto* cheese, *Curcumin* cheese, and β-*carotene* cheese, respectively.

Non-protein nitrogen (NPN) / total nitrogen (TN) ratio

All treatments showed a gradual increase in NPN values during the ripening period. The higher (NPN) content was found in β-*carotene* cheese than the other cheese treatments followed by *Annatto* cheese and cheese without colourant added and *Curcumin* cheese, respectively. The value of the end of storage recorded as 0.144 %, 0.139 %, 0.133 % and 0.127 %, respectively (Table 6). An increase in NPN is reported by many other authors that studied Ras cheese ripening (El-Desoci, 2013). Non-protein nitrogen (NPN) is an indicator of secondary proteolysis (Andronoiu *et al*., 2015). NPN is associated with casein-derived peptides which are either tasteless or bitter and do not contribute directly to the typical taste or flavour of cheese, but their production, particularly in the initial weeks of cheese maturation, is essential to the softening of the rubbery, elastic cheese curd texture (Law, 2010).

Changes in microbial composition of Ras cheese during storage period as affected by the type of colorant

During the ripening period, TBC (total bacteria count) and proteolytic bacteria in all Ras cheese treatments gradually decreased reaching its minimum at the end of ripening period (Table 7). It could be attributed to the decrease in the water activity and the increase in salt content and acidity in cheese. This conclusion was in agreement which Ismail et al. (2004). The total bacteria counts of fresh / 180 days old cheese were (46/17), (36/12), (35/9) and (41/11) (cfu×x10^5) for treatments of cheese without colour added, *Annatto* cheese, Curcumin cheese, and β-carotene cheese respectively. On the other hand, the counts of proteolytic bacteria of fresh / 180 days old cheese were (0.17/0.05), (0.15/0.04), (0.13/0.03), and (0.16/0.03) (cfu×x10^5) for treatments of cheese without colour added, *Annatto* cheese, *Curcumin* cheese, and β-*carotene* cheese, respectively.

Table 6. Effect of type of colorant in cheese milk on the ripening indices of Ras cheese during storage period.

Formulations	Ripening Period (day)	Chemical Parameters					
		TN%	WSN %	WSN/TN (%)	NPN%	NPN/TN (%)	TVFA*
T0	0	3.45	0.13	3.77	0.054	1.57	8.75
None	15	3.74	0.17	4.55	0.061	1.63	10.00
	30	3.90	0.19	4.87	0.069	1.77	11.36
	60	4.13	0.26	6.25	0.082	1.99	12.87
	90	4.23	0.31	7.33	0.095	2.25	14.25
	120	4.44	0.35	7.96	0.103	2.32	15.00
	150	4.55	0.37	8.27	0.117	2.57	16.22
	180	4.68	0.41	8.76	0.133	2.84	17.65
Annatto	0	3.49	0.15	4.78	0.058	1.66	9.00
	15	3.79	0.20	5.28	0.065	1.72	10.45
	30	3.98	0.26	6.53	0.079	1.98	11.84
	60	4.15	0.30	7.23	0.091	2.19	13.50
	90	4.33	0.34	7.85	0.113	2.61	14.72
	120	4.47	0.39	8.72	0.120	2.68	15.43
	150	4.61	0.42	9.11	0.124	2.69	16.50
	180	4.70	0.45	9.57	0.139	2.97	18.00
Curcumin	0	3.44	0.11	3.20	0.040	1.16	8.38
	15	3.72	0.15	4.03	0.046	1.24	9.56
	30	3.85	0.18	4.68	0.059	1.53	10.32
	60	3.99	0.23	5.76	0.062	1.55	12.05
	90	4.21	0.27	6.41	0.079	1.88	13.45
	120	4.41	0.32	7.26	0.085	1.93	14.97
	150	4.51	0.35	7.76	0.093	2.06	16.04
	180	4.63	0.39	8.42	0.127	2.74	17.35
Beta-carotene	0	3.61	0.18	4.99	0.062	1.72	9.65
	15	3.85	0.23	5.97	0.075	1.95	11.36
	30	4.08	0.29	7.11	0.082	2.01	12.45
	60	4.32	0.32	7.41	0.094	2.18	13.68
	90	4.39	0.38	8.66	0.118	2.69	15.53
	120	4.60	0.41	8.91	0.124	2.70	16.00
	150	4.65	0.43	9.25	0.136	2.92	17.50
	180	4.83	0.46	9.52	0.144	2.98	18.25

TN: total nitrogen, WSN: Water-soluble nitrogen, NPN: Non-protein nitrogen, TVFA: Total volatile fatty acids expressed as ml NaOH 0.1N/ 100g cheese.

The initial mold and yeast counts were not detected in all treatments of Ras cheese. The wheel of Ras cheese without colorant added exhibited heavy mold growth on its surface but surface mold growth was less apparent in cheese with *annatto* and beta *carotene* and no mold growth was found on the surface of *Curcumin* cheese. These results indicate that the *Curcumin* extract had an inhibitory effect on surface mold growth. The count of mold and yeast at 180 days old cheese were 0.17, 0.11, 0.07, and 0.09 (CFU$\times 10^5$/g) for treatments of cheese without colour added, *Annatto* cheese, *Curcumin* cheese, and β-*carotene* cheese, respectively.

Effect of type of colorant on organoleptic evaluation of Ras cheese during 180 days of storage

The sensory evaluation of Ras cheese is mainly based manufacturing process of Cheese, type of cheese milk used, the salt amount added, and conditions of the ripening period (Abou-Donia, 2002). The scoring points and remarks of the ten judges are summarized in Table (8).

Table 7. Changes in microbial composition of Ras cheese during storage time (CFU log/g).

Storage period (days)	None	Annatto	Curcumin	Beta-carotene
Total bacterial count (TBC) (CFU × 10^5/g)				
0	46	36	35	41
15	39	33	30	38
30	35	29	28	33
60	31	25	24	29
90	28	22	20	21
120	25	19	15	17
150	20	16	13	14
180	17	12	9	11
Yeast & Mold (CFU × 10^5/g)				
0	0	0	0	0
15	0	0	0	0
30	0	0	0	0
60	0	0	0	0
90	0.02	0	0	0
120	0.09	0.03	0	0.02
150	0.13	0.08	0.04	0.05
180	0.17	0.11	0.07	0.09
Proteolytic bacteria (CFU × 10^5/g)				
0	0.17	0.15	0.13	0.16
15	0.14	0.11	0.10	0.13
30	0.12	0.09	0.09	0.11
60	0.10	0.08	0.08	0.09
90	0.08	0.06	0.07	0.07
120	0.07	0.05	0.04	0.05
150	0.07	0.05	0.03	0.05
180	0.05	0.04	0.03	0.03

Colour and Appearance

The Colour of cheese is affected by the type of milk used and also by the quality of colour added if any (Gupta *et al*., 2009). On the other hand, cheese without dye was not acceptable by the ten judges; they were accustomed to the yellow colour of the cheese, after three months, a heavy layer of fungi appeared on the surface of the cheese. The decrease in colour of *Annatto* Ras cheese, *Curcumin* Ras cheese, and β-*carotene* Ras cheese during ripening resulted in marked improvement of *Curcumin* cheese. The judge observation was the colour of beta-carotene was to the same extent nears to the colour of *Annatto*. The colour of cheese with *Curcumin* was improved by the development of ripening. All cheese decreased in ways for the four treatments. Colour and appearance scoring points for fresh and six-month-old cheese were (12/8), (13/9.5), (12.5/9), and (13/9.5) of 15 for treatments of cheese without colour added, *Annatto* cheese, *Curcumin* cheese, and β-*carotene* cheese, respectively.

Body and texture

The texture is an important characteristic used to differentiate many cheese varieties (Foegeding *et al*., 2003). For all treatments on the ripening time advanced the body and texture markedly improved. This could be due to the improvement of the final body and texture because of the chemical and structural changes with cheese ripening. On the other hand, after three months of ripening the texture is moderate to the same extent. Body and texture of cheese without colour added, Annatto cheese and *Curcumin* cheese were nears to each other while β-carotene cheese gave smoother. Body and texture scoring points for fresh and six-month-old cheese were (20/31), (22/33), (21/32), and (21/33) of 35 for treatments of cheese without colour added, Annatto cheese, *Curcumin* cheese, and β-carotene cheese, respectively.

Flavour

In Table (8), all treatments showed gradual improvement in flavour as a result of casein breakdown for all, the maximum scoring point was for *Curcumin* cheese. The lowest flavour point for cheese without dye because of the growth of microorganisms, the judges gave cheese with *Curcumin* dye the highest scoring points of flavour science it is a known spice well known most Egyptian people. Where *Curcumin* is one of the primary ingredients in turmeric and curry powders that are used as spices in Middle Eastern. Thanks to its colour and taste, turmeric was named “Indian saffron” (Rajashree *et al*., 2013). In contrast, although annatto has been used in foods as a spice, there is little information about how volatile compounds of annatto contribute to flavor (Kang *et al*., 2010). Annatto cheese and β-carotene cheese were the highest total scoring points (87.5/100), while the lowest total scoring points (80/100) were for cheese without dye.

Table 8. Organoleptic evaluation of Ras cheese, as affected by the type of colorant.

Scoring point	Ripening Period (Day)	Organoleptic properties				Remarks
		Colour appearance (15)	Body texture (35)	Flavour (50)	Total Scores (100)	
Ras cheese without colour	0	12.0	20	27	59.0	Pale yellow colour, slightly bitter, white mold surface growth
	15	12.0	22	30	64.0	
	30	11.0	25	32	68.0	
	60	11.0	27	34	72.0	
	90	10.0	27	35	72.0	
	120	10.0	28	37	75.0	
	150	9.0	30	39	78.0	
	180	8.0	31	41	80.0	
Ras cheese with Annatto	0	0	13.0	22	30	Yellowish colour, clean taste (not bitter), slightly salt taste
	15	15	13.0	24	32	
	30	30	12.0	26	35	
	60	60	12.0	27	39	
	90	90	11.0	29	41	
	120	120	10.5	30	43	
	150	150	10.0	32	44	
	180	180	9.5	33	45	
Ras cheese with Curcumin extract	0	0	12.5	21	31	Shiny yellow colour, spicey flavour, slightly salty, clean appearance without mold growth
	15	15	12.0	22	34	
	30	30	12.0	26	36	
	60	60	11.0	28	40	
	90	90	10.0	29	42	
	120	120	10.0	30	44	
	150	150	9.5	31	45	
	180	180	9.0	32	46	
Ras cheese with β-carotene extract	0	0	13.0	21	31	Yellowish colour, slightly salty smooth
	15	15	12.5	23	33	
	30	30	12.0	27	35	
	60	60	11.0	29	39	
	90	90	11.0	31	42	
	120	120	10.5	32	43	
	150	150	10.0	32	44	
	180	180	9.5	33	45	

Total scoring points for fresh and six-month-old cheese were (59/80), (65/87.5), (64.5/87) and (65/87.5) of 100 for treatments of cheese without colour added, *Annatto* cheese, Curcumin cheese, and β-*carotene* cheese respectively. These results are in agreement with Manoharan

et al. (2012), who found that the addition of *Curcumin* powder at the maximum of 0.5% for butterscotch flavored ice cream had the maximum organoleptic scores?

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CONFLICT OF INTEREST

The authors declare that they have no competing interests.

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