RESEARCH ARTICLE

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Effect of tomato lycopene, turmeric and beetroot extract on microbial and chemical properties of cow's milk butter

ABSTRACT

The aim of this study was to determine the effects of different natural sources of bio coloring agents including pure lycopene, turmeric extract and red beetroot extract powder on the chemical (acidity and peroxide values) and microbiological (coliform, mold and total microbial counts) properties of cow's milk butter. For this purpose, different dosages of the coloring agents (at three levels) were used and analysis was done after 8 months. Results showed the peroxide index in all the treatments was in the permitted range allowed by the national standard (0.5-1.5 meq/kg). The efficacy of additives to control peroxide and acidity values was in the following order: red beetroot > lycopene > turmeric > control and turmeric > red beetroot > lycopene> control respectively. The best peroxide (0.65 meq/kg) and acidity (0.113% w/w) indexes were observed in treatments containing the highest amounts of red beetroot and turmeric powders. Turmeric extract powder induced significant effect on mold, coliform, and total microbial count in all dosages ($p \leq 0.05$) and the count reached respectively to 17, 18, and 960 CFU/mL at the highest dosage level. According to the findings, turmeric was a suitable treatment in view of chemical and microbiological properties and might be considered for future studies.

Key words: butter, tomato lycopene, turmeric, beetroot

Introduction

It has been determined that the color, constitutes the main visual predicates concerning sensory properties, as taste and flavor of food and beverages (Delwiche, 2012). We know that the consumer is strongly influenced by the color of the foods. Color is the first notable property of a beverage or food, which often predetermines our expectations for taste and flavor (Griffiths, 2005). In other words, in the case of unacceptable color, the other factors important for consumers such as flavor and texture, are unlikely to be judged at all. However, during food processing, not only color is lost substantially, but also other properties like organoleptic and nutritional parameters are adversely affected, therefore natural or synthetic colors and other additives are added (Rymbai et al., 2011) to allow desirable appearance as well as to increase nutritional properties. Among different additives, there are some natural coloring sources, which are used to emulate colors and the nutritional properties too, such as pepper, saffron, red beet, tomato and turmeric (Lakshmi, 2014). Also, some examples of natural colors permitted in food and drinks in the United States by FDA, are annatto extract, β-carotene, beet powder, caramel, grape color extract, grape skin extract, paprika, saffron, and turmeric (Code of Federal Regulations, 2016).

For centuries, synthetic colors have been used extremely due to their lasting and low costs and also more stability to environmental conditions including light, moisture, oxygen, and variations in pH and temperature (Mendi et al., 2000). Nevertheless, some researches have indicated to allergenic and intolerance reactions of synthetic coloring agents (Rymbai et al., 2011; Sardarodiyan & Mohamadi Sani 2016). But nowadays, due to consumer awareness on the health problems of synthetic additives including the colorant, the demand for natural coloring agents is increasing (Murphy, 2009) so that Carocho et al. (2014) reported increasing the use of naturally derived colors in food systems.

Dairy products like cream cheese, fermented milk, milkshakes, and butter have been among different food systems in which natural colorants have been used (Pineda-Vadillo et al., 2017; Vinha et al., 2018;). Turmeric, tomato, and beetroot are excellent sources for natural colorant (Vinha et al., 2018) which even can be used directly to food systems. Amr (1991) used turmeric powder as an antioxidant source to sheep's anhydrous butterfat. Foda et al. (2007) used turmeric powder in yogurt due to its antioxidative and anti-inflammatory properties and as a cancer chemoprevenfive agent. Buch et al. (2014) evaluated the efficacy of turmeric as a preservative in paneer (a type of cheese). Bandyopadhyay et al. (2007) added turmeric to Sandesh, (an Indian sweet dairy product) as an antioxidant agent. Siwach et al. (2016) and Kaur et al. (2011) studied the application of lycopene as an extending shelf life agent in butter. In addition, beetroot as a source of betalains has been used in dairy products in different researches (Pasch et al. 1975; Chhikara et al. 2019).

So the aim of this study was to use three natural coloring agents including turmeric extract, tomato lycopene, and red beetroot extract, in powdered form in cow's butter and determine the microbial and chemical properties after storage.

Materials and Methods

Raw milk supplied by an industrial farm near Mashhad-Iran. Lycopene (≥ 90%, from tomato) purchased from Merck (Germany), and turmeric powder and red beetroot supplied from retail stores. All the solvents, chemicals, reagents, and medium cultures ware analytical grade (Merck-Germany).

Turmeric extract powder

The turmeric powder (250 g) was extracted with distilled water (100°C, 6 hours). The extract was filtered and concentrated to remove the water under spray drying. The powder was stored in laboratory and dark containers (Sadashiva et al., 2019).

Red beetroot extract powder

The powder was produced according to the method by Seremet et al. (2020). The whole red beetroots were washed, peeled and crushed, and then spread evenly on baking paper in a circular glass tray and dried using a home microwave. The dried red beetroot was milled and then extracted in water (6 hrs at $40^{\circ C}$) and consequently spray dried (Pilotech YC-015, China).

Butter production

Butter was produced by the churning method according to the method explained by Soundous et al. (2019) on pilot scale. The coloring agents were added during the kneading stage until a uniform texture in which the colorant dispersed thoroughly was obtained.

Chemical analysis tests

Acid Value

The acid value (AV) is a qualitative parameter analyzed in fats and oils. AV is defined as the weight of potassium hydroxide (mg) for neutralizing the organic acids existing in 1g of fat. Increasing the amount of free fatty acids in fat or oil shows the hydrolysis of triglycerides. Five grams of the samples were dissolved in 40 mL of ethanol: diethyl ether mixture (1:1, v/v), and consequently 5 drops of phenolphthalein were added into the mixture, stirred and titrated with 0.1 N potassium hydroxide. Analyses were determined on the basis of the following formula:

$$AV= (a-B)*N*56.1/W$$

Where a is the volume of KOH solution used in butter sample; B is the volume of KOH solution used in a blank sample; N is the normality of KOH solution; W is the weight of butter sample, and 56.1 is potassium hydroxide molar mass (g/mol) (ISO 1740:2004).

Peroxide Value

Five grams of the butter sample were dissolved in 30 mL of chloroform: acetic acid (2:3, v/v) mixture and subsequently a saturated potassium iodine solution were added. The mixture was agitated and kept in dark condition for 5 minutes. Then distilled water was added and the released iodine was titrated with 0.001 molar sodium thiosulphate, using 1% w/v starch solution as an indicator. The peroxide content was expressed as mmol of active oxygen/1 kg of butter sample and calculated according to the following formula:

$$PV= (S-B)*N*1000/W$$

Where, S and B are the volumes of $Na_2S_2O_3$ solution (mL) used for butter and blank sample titration. N is the normality of $Na_2S_2O_3$ solution and W is the butter mass (g) (ISO 3976:2006).

Microbiological analysis

Total Plate Count, coliform and mold count and Coliform were done following the SLS 516: Part 1: 1991; SLS 516: Part 3: 1991; and SLS 516: Part 2: 1991 microbial test methods respectively (Nuwanthi et al., 2016).

Statistical analysis

The research was set by a simple randomized design and the treatments (shown in Table 1) were done in triplicate. Data were processed by SPSS software version 16 using the ANOVA test at 0.95 confidence level.

Table 1. *Butter formulations according to the colorant type and dosage.*

Treatment	Coloring agent type and dosage		
	Lycopene (g/kg)	Turmeric extract powder (g/kg)	Red beetroot extract powder (g/kg)
L1-L	0.5	0	0
L2-L	1	0	0
L3-L	1.5	0	0
L1-T	0	1	0
L2-T	0	1.5	0
L3-T	0	2	0
L1-R	0	0	0.2
L2-R	0	0	0.5
L3-R	0	0	1
Control	0	0	0

Abbreviations: L1-L3: level of colorant dosage; L: Lycopene; T: Turmeric; R: Red beetroot

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Results and Discussion

In the current research, we studied the effect of different natural sources of coloring agents including pure lycopene, turmeric extract, and red beetroot extract, in the powdered form on chemical and microbiological properties of cow's milk butter. As the native color of butter is yellow (weak to heavy yellow), we tried to use natural sources which give us a yellowish color or colors near the choice of the consumers, so we selected lycopene (ranging from yellow thru to red), turmeric (yellow) and red beetroot (red) colors to make and evaluate butter samples near the choice of consumers. For this purpose, we tested primarily different dosages of the colorant and selected a range for concentrations of the colors, which was acceptable by the sensory evaluation analysis, and then we limited the concentrations according to Table1. Figures 1-3 show different treatments.

Figure 1. *Butter samples produced using turmeric extract powder. L1-L3 is the levels of the additive according to Table 1.*

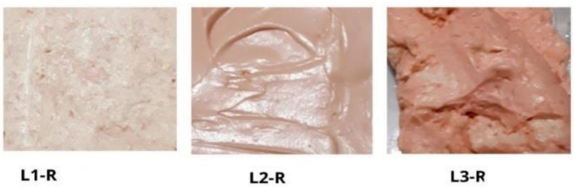

Figure 2. *Butter samples produced using red beetroot extract powder. L1-L3 is the levels of the additive according to Table 1.*

Figure 3. *Butter samples produced using lycopene. L1-L3 is the levels of the additive according to Table 1.*

Chemical properties

The effect of type and dose of different coloring agents used in this research on chemical properties of cow's milk butter, including peroxide and acid values are represented in Figures 4-5. According to Figure 4, the peroxide index in all the treatments was in the permitted range allowed by the national standard: 0.5-1.5 meq/kg (ISIRI, 2016). The efficacy of additives to control peroxide value was in the following order: L3R > L3L > L3T > control for the highest dosage. Figure 5 shows the effect of additives on the acidity of butter samples, which indicates significant differences ($p \leq 0.05$) in all the levels, and additives in the following order: Turmeric > Red beetroot > Lycopene > control.

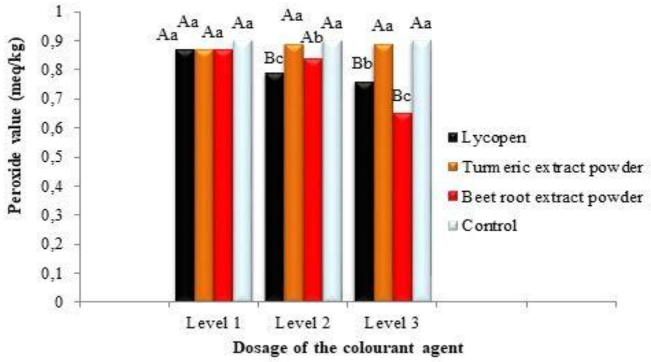

Figure 4. *Effect of type and dose of coloring agent on peroxide value of cow's milk butter. The amount of levels for different treatments is indicated in Table 1. Different letters represent significant difference at 95 % ($p \leq 0.05$). Means followed by the same lower case letters in each levels and capital letters between the levels, do not differ significantly.*

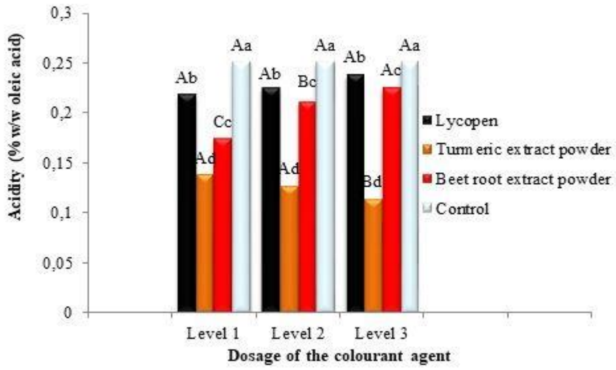

Figure 5. *Effect of type and dose of coloring agent on peroxide value of cow's milk butter. The amount of levels for different treatments is indicated in Table 1. Different letters represent significant difference at 95 % ($p \leq 0.05$). Means followed by the same lower case letters in each levels and capital letters between the levels, do not differ significantly.*

Different researches are done about the application of natural colorant in fat-based products. Amr (1991) found that turmeric was as effective as BHA-BHT to control oxidative rancidity of sheep's anhydrous butterfat during the first four months of storage. Siwach et al. (2016) used lycopene (30, 60, 90, 120 ppm) in extending the shelf-life of anhydrous cow milk fat and found significant prevention in development of oxidative rancidity in all treatments compared to control samples (positive control: 200 ppm BHA and negative control: without any additive). Lycopene containing samples scored significantly higher in terms of sensory attributes as compared to controls. Kaur et al. (2011) studied the shelf life enhancement of butter by the addition of lycopene (20 ppm) during storage for 4 months. They found that the peroxide value generally increased with the storage time. Higher peroxides and free fatty acids were observed in control samples compared to lycopene-treated products. Sensory analysis showed that lycopene slowed the development of off-flavor, off-odors, and color changes in butter samples during the 4 months of storage. Bandyopadhyay et al. (2007) who used different herbs into sandesh (an Indian sweet dairy product) revealed that the antioxidative effect of the herbs decreased in the order turmeric > curry leaf > aonla > spinach > coriander leaf. Total antioxidative statuses of the mentioned herbs were lower than samples with TBHQ but similar to those with 200 mg/kg BHA: BHT (1:1). Alshehry (2019) used beetroot powder for the antioxidant and antimicrobial activity in a cupcake and found good total phenolics content in the final product with acceptable antioxidant activity.

All of the above researches are in agreement with our findings. All the treatments had an acceptable effect on the chemical properties of the butter samples and the peroxide and acidity values were improved compared to control.

Microbiological properties

Microbial contamination is one of the major challenges faced by the food processing industry. In the current study, the effect of type and dose of different bio coloring agents on microbiological properties of cow's milk butter, including total microbial count, coliform and mold count was investigated and the results are represented in Figures 6-8. Figure 6 indicates to the significant effect of turmeric extract on the total microbial count in all dosages ($p \leq 0.05$). Also according to Figure 7, the butter samples containing turmeric extract powder had lower coliform count compared to other treatments and control ($p \leq 0.05$).

The effect of coloring agents on the mold count of butter samples is shown in figure8, which indicates to the significant effect of the treatments as follows: turmeric > red beetroot extract > lycopene > control ($p \leq 0.05$). Turmeric powder extract had an excellent effect to control mold count.

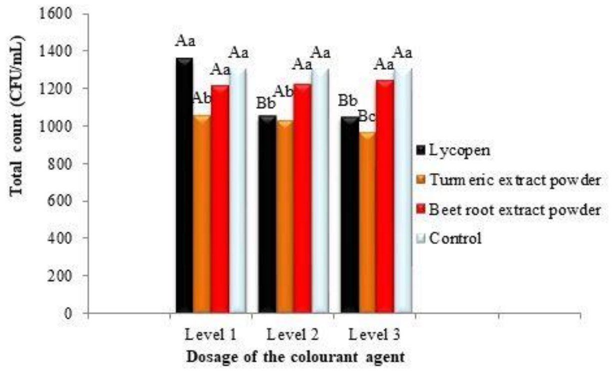

Figure 6. *Effect of type and dose of coloring agent on total microbial count of cow's milk butter. The amount of levels for different treatments is indicated in Table 1. Different letters represent significant difference at 95 % ($p \leq 0.05$). Means followed by the same lower case letters in each levels and capital letters between the levels, do not differ significantly.*

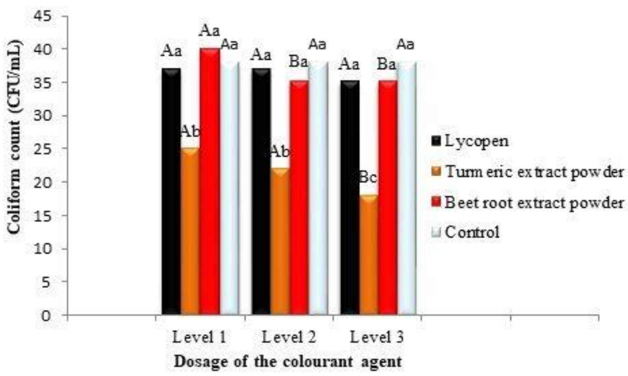

Figure 7. *Effect of type and dose of coloring agent on coliform count of cow's milk butter. The amount of levels for different treatments is indicated in Table 1. Different letters represent significant difference at 95 % ($p \leq 0.05$). Means followed by the same lower case letters in each levels and capital letters between the levels, do not differ significantly.*

The use of natural antimicrobial compounds to extend the shelf life of foods is an interesting strategy. Other scientists have investigated the effect of the bio colorant studied in the current research in different systems. Vulić et al. (2013) studied the antimicrobial activity of commercial beetroot pomace extract and indicated the high susceptibility of a Gram (-) and Gram (+) bacteria, while yeasts and molds were resistant. In another research, Alshehry (2019) found that the total count of bacteria, fungi and molds were slightly decreased in cupcake fortification with beetroot than cupcake control during the storage period. The betalain and phenolic compounds in beets possess antimicrobial properties (Cho et al., 2017). Overall, betalains have been shown to exhibit a wide range of antimicrobial activity (Gengatharan et al., 2015) and our findings approve this for cow's milk butter.

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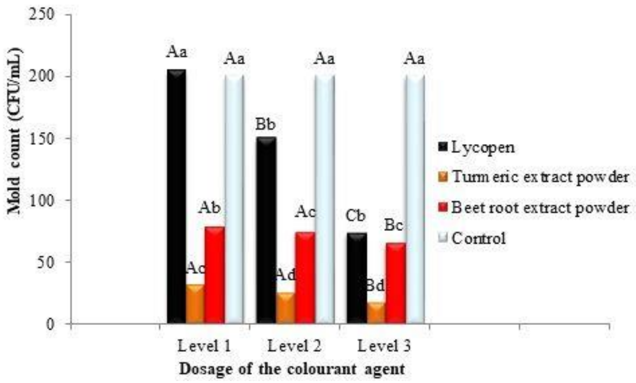

Figure 8. *Effect of type and dose of coloring agent on mold count of cow's milk butter. The amount of levels for different treatments is indicated in Table 1. Different letters represent significant difference at 95 % (p ≤ 0.05). Means followed by the same lowercase letters in each levels and capital letters between the levels, do not differ significantly*.

Curcumin has been widely used as a traditional antimicrobial agent. Several studies have reported the broad-spectrum antimicrobial activity for curcumin including antibacterial, antiviral, antifungal, and antimalarial activities, which almost have been done in in-vitro conditions (Zorofchian Moghadamtousi et al., 2014). While most of the research works are done on the antibacterial activity of curcumin, Apisariyakul et al. (1995) studied the activity against pathogenic molds and found fungistatic effect. Dovigo et al. (2011) indicated to susceptibility of clinical isolates of *Candida* to curcumin. Arulkumar et al. (2017) used turmeric extract (0.5%) for shelf life extension and biogenic amine control of cuttlefish and found antimicrobial activity on aerobic mesophilic and psychrophilic bacteria.

The antimicrobial activity of lycopene has been analyzed in some studies; however, more focus has been on the antioxidant activity. Ranjbar and Ranjbar (2016) studied the antimicrobial activity of tomato lycopene oleoresin against Gram (-) and Gram (+) bacteria and found acceptable antibacterial activity. Desai et al. (2018) studied the antioxidant and antifungal properties of lycopene from tomato and papaya and found that none of them showed inhibiting activity against the growth of the fungus *Candida albicans*. According to our findings, turmeric extract powder had more antimicrobial activity compared to red beetroot extract powder and lycopene.

Conclusion

The application of bio coloring agents to improve the functional properties of food systems is widely extended. Butter is one of the main food products, which needs a coloring agent to have stable vision quality. Turmeric, red beetroot and lycopene are bio colorant sources that can improve the butter appearance quality because their color is near the yellow, in the case a suitable concentration is used. In the current study, we used the mentioned bio-coloring agents in dosages, which led to color near the consumer's choice, and then the chemical and microbiological properties were assayed. According to the results, adding the coloring agents improved the chemical and microbiological properties and the acidity and peroxide values decreased and coliform, mold and total microbial count decreased in the treatments compared to the control sample. The samples containing turmeric was better than the other samples with acceptable appearance. Turmeric is traditionally used in butter production in villages of Iran and we think this subject might be interesting for more researches.

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