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Original research

The production of cheese-flavored extruded snack exploiting anti-microbial properties of natural food colors

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A B S T R A C T

In this study, the effects of some edible pigments used in the food industry on pathogenic bacteria were investigated. For this purpose, five natural food colors were selected consisted of curcumin, β-carotene, paprika oleoresin, lycopene and turmeric oleoresin. The impacts of each color on bacterial species including *Salmonella enterica*, *Escherichia coli*, *Bacillus cereus*, and *Staphylococcus aureus* were investigated by using disc diffusion method, individually and collectively. The results have shown that, by combining these colors provided some mixtures with reasonable preventative effects against those bacteria; moreover, mixtures which contained curcumin or turmeric oleoresin demonstrated a better inhibitory effect in compare to mixtures lack of those colors. Consequently, the best synergetic inhibitory effect was related to a mixture of curcumin–beta-carotene–paprika (CBP) on *Bacillus cereus*. Furthermore, based on results, a mixture containing curcumin-lycopene was selected and used in snack coating to investigate the antimicrobial properties of cheese-flavored extruded snacks. Therefore, adding these natural colors to food not only increase their acceptability but also enhance the antimicrobial properties.

Keywords: Curcumin, Natural food colors, Antimicrobial activity, Synergistic effect, extruded snacks

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1. Introduction

Consumption of extruded food such as snacks became a part of the dietary habits of a great part of the population. Snacks can be prepared with ingredients or components that give them specific functional properties (Da costa et al., 2010). Color is one of the quality attributes of food and plays an important (sometimes decisive) role in sensory and consumers' acceptance of food. Based on their origin, there are three main categories of food pigments: natural, synthetic but identical with natural and synthetic (Stahl & Sies, 2003). The role of color, as an important factor in convincing the consumers in their purchase selection, is undeniable. This is the main argument in the use of pigments in the modern food industry (Boo et al., 2012). One of the major elements is the increasing demand for food dyes from natural sources, which can serve as substitutes for synthetic dyes due to both legislative action and consumer concerns about the detrimental effects of chemicals. During 1936 -1960 period, several studies on the safety of synthetic colorants were conducted and the results showed that some were considered unsafe for consumption (Negi et al., 1999). Natural pigments, obtained from plants and insects are renewable and sustainable bioproducts with minimal environmental impact and have been used since ancient times (Moure et al., 2001). Therefore, an interest in natural pigments, which can replace synthetic ingredients, inducing many side effects, is increasing (Gyawali & Ibrahim, 2014). Furthermore, many synthetic pigments are blamed to be the main reason for health problems in children who are generally considered a very vulnerable group (Hashem et al., 2010). In spite of lower costs and greater stability of synthetic pigments, the number of synthetic additives allowed in developed countries is declining every year (Selvam et al., 1995).

Nowadays, there is an increasing awareness among people towards the use of natural pigments as a substitute for synthetic dyes. Due to their non-toxicity and fewer side-effects, natural pigments are used more often in food products. Consumers are now

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increasingly aware of diet-related health problems; therefore, requesting natural ingredients which are considered to be safe and health-promoting is increased considerably. Safety, non-toxicity and biodegradability are required for antimicrobial agents, and the active ingredients used for antimicrobial purposes need to be registered after they have been demonstrated effective and safe to be used. Due to the fact that natural pigments can often prevent the growth of micro-organisms without toxic effect on consumer, the study and application of these pigments have gained attention and they can be used to increase the shelf-life of food and maintain their safety and nutritional quality (Han & Yang, 2005; Jayaprakasha et al., 2006).

Moreover, other biological properties such as anti-carcinogenicity and anti-mutagenicity have been reported for natural food pigments (Munawar & Jamil, 2014). Not only many natural pigments are used as food coloring, but also they are used as a substance that promotes health by preventing or even curing diseases (Mascio et al., 1989; Pennathur et al., 2013). Several studies on some of these pigments have offered biological evidence of activities such as anti-inflammatory, antibacterial and anti-carcinogenic properties (Kulpapangkorn & Mai-leang, 2012; Lal et al., 2012; Zimmer et al., 2012). The effect of carrot extract on the bactericidal activity of bovine lactoperoxidase system (LPOS) was evaluated using *Salmonella enteritidis* (10^6 CFU/ml). The LPOS antimicrobial activity increased from 1.4 to 3.8 log units by the addition of 20-fold diluted carrot extract. So, it was indicated that β-carotene is one of the major enhancers for increasing the LPOS antimicrobial activity (Hayashi et al., 2012). Maheshwari et al. (2006) studied curcumin for its biological effects. They concluded that curcumin had multiple biological effects, particularly the most important inhibitory effect on pathogenic bacteria, fungi and molds. AL-Oqaili and Salman (2014) evaluated the aqueous extract of tomatoes for activity against medically important bacteria, *Escherichia coli*, *Klebsiella* sp., *Pseudomonas* sp. and *Acinetobacter*, and showed that lycopene can inhibit the growth of some isolated bacteria. Some studies indicated that when the pigments are mixed, their biological activities improve synergistically. The result of a combination is greater than it is expected because the combined elements strengthen one another (Paulucci, 2013).

In the present study, we investigated the synergic impacts of food natural colors such as curcumin, β-carotene, lycopene, and paprika oleoresin as well as turmeric oleoresin on their antimicrobial properties using agar diffusion method. The aim of this work was introducing a mixture of food natural colors with anti-microbial properties instead of synthetic dyes in cheese-flavored extruded snacks in order to develop new functional food.

2. Material and Methods

2.1. Raw materials

The materials used in this work were: i) Corn grits (Gandomkob, Iran); ii) White cheese powder (Golshad Mashhad, Iran); iii) Vegetable oil (Nazgol, Iran); iv) Salt (Doris, Iran); v) Monoglyceride (Dorshimi, Iran) and vi) five natural colors were selected: curcumin, β-carotene (30% Oil Suspension (OS)) and lycopene (10% Cold Water Dispersible (CWD)) were collected from Performance of Nature (Diana), Holbeach Technology Park, Lincolnshire, UK; paprika oleoresin (120000 (CU)) and turmeric oleoresin (8.5% (WS)) were collected from Plant Lipids, Cochin, Kerala, India. We prepared the mixtures of pigments as indicated in Table 1. It should be noted that colors used without dilution.

2.2. The manufacture of the extruded snacks

Before extrusion, to standardize moisture content of corn grits, water was added. For extruding, a double-screw extruder (Saixin machinery, China) was used. After that, the product was dried for five hours in the oven at 70 °C. Hence, the product moisture content was reduced approximately to 1.5%. Then, a combination of cheese powder, oil, salt as well as suggested mixture of food natural colors sprayed on raw snacks in order to create the optimal taste and appearance.

2.3. Test organisms

For this experiment, two gram-negative [*Escherichia coli* (PTCC No. 1399), *Salmonella enterica* sp. (PTCC No. 1709)] and two gram-positive bacteria [*Staphylococcus aureus* (PTCC No. 1431) and *Bacillus cereus* (PTCC No. 1247)] were used. All test micro-organisms were purchased from the Persian Type Culture Collection (PTCC), Tehran, Iran.

2.4. Determination of anti-microbial activity

The synergistic effects of natural dyes on the anti-microbial activities were evaluated using the Kirby-Bauer disk diffusion method with some modification (Hudzicki, 2013). Initially, Brain Heart Infusion (BHI) agar medium containing: brain-heart infusion, peptone, glucose, sodium chloride, disodium phosphate, agar, pH 7.4 ± 0.2, was prepared and autoclaved at 121 °C for 15 min. Then, sterilized petri dishes were prepared with an equal thickness of BHI agar. Afterwards, test organisms were grown in 10 ml BHI broth in sterilized tubes overnight at 37 °C. Finally, cell concentrations were standardized until 10^7–10^8 CFU/ml, next, this inoculum was added to each plate containing BHI agar. After that, three sterile paper discs (6 mm diameter) impregnated with 10 µl of sample extracts were placed on plates. All of plates were incubated at 37 °C for 24 h after allowing 30 min at room temperature for the extracts to facilitate diffusion across the surface. Finally, the anti-microbial activity was measured as the size of the clear zone of growth inhibition. Cefazolin was used as control.

2.5. Data analysis

The statistical analysis was performed using the procedures of the statistical analysis system. ANOVA procedure followed by the Duncan test was used to determine the significant difference ($p < 0.05$) between treatment means.

3. Results

3.1. The synergistic effect of combination colors on microorganism

In the present study, five natural food pigments (Table 1) were selected to screen their synergistic effect on antimicrobial activity using the agar diffusion method and the comparative analysis results are shown in Figs. 1 to 4.

Table 1. Details of the combination of the pigments.

Pigments	Combination 1 1:1 (mL)	Combination 2 1:1:1 (mL)	Combination 3 1:1:1:1 (mL)
Curcumin	Curcumin + Lycopene	Curcumin + Lycopene + β-carotene	Curcumin + Lycopene + β-carotene + Paprika oleoresin
β-carotene (30% OS)	Curcumin + β-carotene	Curcumin + Lycopene + Paprika oleoresin	Turmeric oleoresin + Lycopene + β-carotene + Paprika oleoresin
Lycopene (10% CWD)	Curcumin + Paprika oleoresin	Curcumin + β-carotene + Paprika oleoresin	
Paprika oleoresin (120000 CU)	Turmeric oleoresin + Lycopene	Turmeric oleoresin + Lycopene + β-carotene	
Turmeric oleoresin (8.5% WS)	Turmeric oleoresin + β-carotene	Turmeric oleoresin + Lycopene + Paprika oleoresin	
	Turmeric oleoresin + Paprika oleoresin	Turmeric oleoresin + β-carotene + Paprika oleoresin	
	Lycopene + β-carotene	Lycopene + β-carotene + Paprika oleoresin	
	Lycopene + Paprika oleoresin		
	β-carotene + Paprika oleoresin		

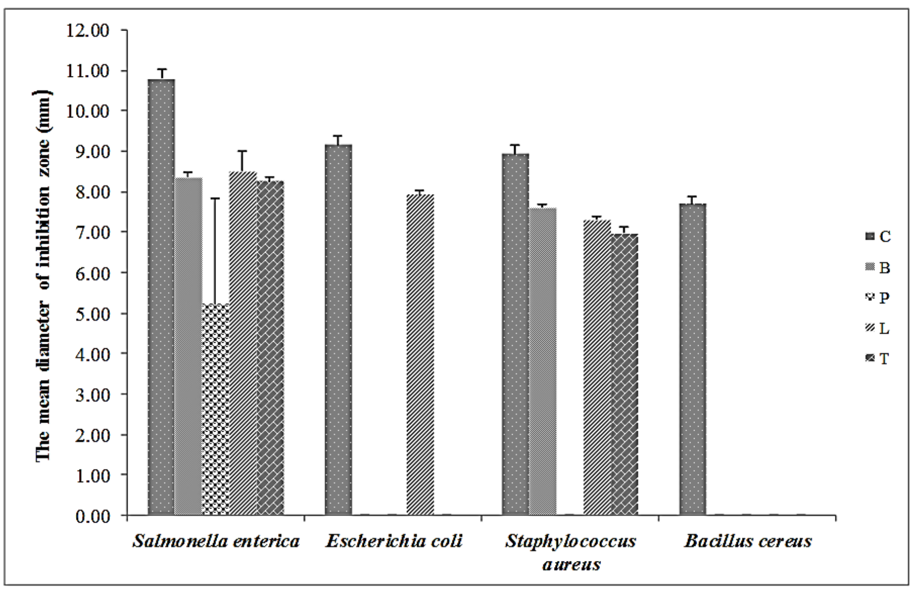

Fig. 1. Antimicrobial activities of selected pigments. (C) Curcumin, (B) β-carotene, (P) Paprika oleoresin, (L) Lycopene, (T) Turmeric oleoresin.

PTCC cultures of four pathogenic bacteria were used, of which two were gram-positive and two were gram-negative. All of the compounds were screened for their biological activity against selected human pathogenic bacteria. The zone of inhibition diameter was recorded in each case.

Data reported in Figs. 1 to 4 showed that the best inhibitory effect was produced by curcumin on *Salmonella enterica* (Fig. 1). The best synergistic effect was observed in mixtures that contained curcumin and this dye improved the antimicrobial properties of the other pigments. For example, paprika oleoresin inhibited *Salmonella enterica* itself, but when it was combined with curcumin, its inhibitory effect on the selected micro-organisms improved (Fig. 2). Among the prepared mixtures, curcumin–beta-carotene–paprika (CBP) showed the best inhibitory effect on *Bacillus cereus*, and followed by a mixture of turmeric oleoresin–lycopene–beta-carotene (TLB) on *Salmonella enterica* (Fig. 3). The mixtures turmeric oleoresin–lycopene–paprika oleoresin (TLP) and lycopene–beta-carotene–paprika oleoresin–curcumin (LBPC) inhibited *Escherichia coli* and *Staphylococcus aureus*, respectively (Figs. 3 and 4).

3.2. The use of suggested mixture in the product

In another study, we investigated the synergistic effect of studied the color mixtures on anti-oxidant properties, as well (Alhooei et al., 2017). Accordingly, among combinations of natural food colors, that we had made, the mixture of curcumin- lycopene (CL) was selected, since it had sufficient antioxidant property; moreover, it benefited from a reasonable impact on all of four pathogenic bacteria (Fig. 2). Furthermore, color was established by CL was the one that we expected. Therefore, we replaced this mixture in the coating of the product with standard density (0.5%) with an artificial dye (Sun Set Yellow) which in use extensively nowadays. We investigated shelf life, organoleptic properties, anti-oxidant and anti-microbial effects in the final product. Subsequently, we compared our product containing CL with the other one having artificial dye. Results demonstrated that this product has a desirable taste and appearance. Likewise, it has more stability against spoilage. In the end, based on surveys that were conducted, consumers accepted this product.

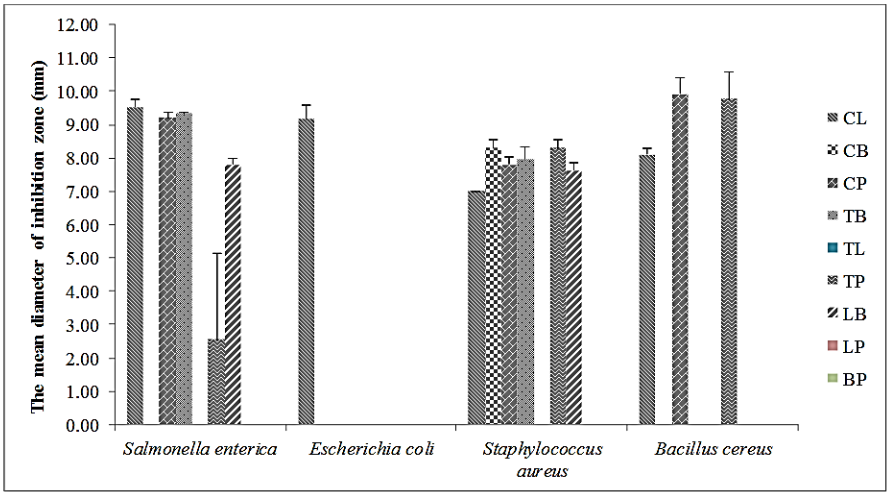

Fig. 2. Antimicrobial activities of mixtures of two pigments. (CL) Curcumin + Lycopene, (CB) Curcumin + β-carotene, (CP) Curcumin + Paprika oleoresin, (TB) Turmeric oleoresin + β-carotene, (TL) Turmeric oleoresin + Lycopene, (TP) Turmeric oleoresin + Paprika oleoresin, (LB) Lycopene + β-carotene, (LP) Lycopene + Paprika oleoresin, (BP) β-carotene + Paprika oleoresin.

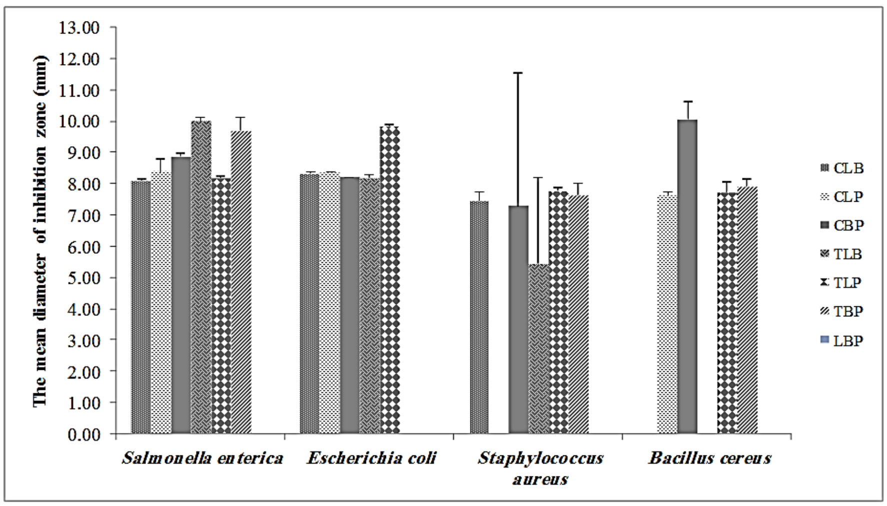

Fig. 3. Antimicrobial activities of mixtures of three pigments. (CLB) Curcumin+ Lycopene + β-carotene, (CLP) Curcumin + Lycopene + Paprika oleoresin, (CBP) Curcumin + β-carotene + Paprika oleoresin, (TLB) Turmeric oleoresin + Lycopene + β-carotene, (TLP) Turmeric oleoresin + Lycopene + Paprika oleoresin, (TBP) Turmeric oleoresin + β-carotene + Paprika oleoresin, (LBP) Lycopene + β-carotene + Paprika oleoresin.

4. Discussion

Generally, gram-positive bacteria compared to gram-negative bacteria exhibited a higher resistance against pigments which is due to differences in the structure of their cell walls. Antimicrobial activities of these pigments depend on chemical structure and functional groups present in dye molecules. That the mechanism of antimicrobial effects involves the inhibition of various cellular processes has been proposed, followed by an increase in plasma membrane permeability and finally ion leakage from the cells (Singh et al., 2010).

The results of this study were compatible with those of Parvathy et al. (2009), Khalil et al. (2012) and Ferreira et al. (2013). Parseh and Shahablavasani (2019) and Naseri and Rahati (2018) also showed that natural components could have anti-microbial impact on pathogenic bacteria. Therefore, not only can natural pigments enhance the acceptance of such foods by improving their nutrition value, but also it can address the issue of food supplementations with substances that are valuable antibiotics while adding mixtures of natural pigments to food. Although nowadays natural colors are widely being used in food processing, they have been shown to exhibit many bioactivities such as antibacterial and anticancer (Martins et al., 2013). Using appropriate ingredients with antibacterial activity in order to improve quality; moreover, to avoid economic losses can be necessary and useful. Nowadays, because of the potential adverse effects of synthetic ingredients on human body and health such as hyperactivity in children, allergic reactions, DNA damage, to name a few, using natural ingredients as an alternative in food processing are extremely recommended in order to minimize negative impacts and prevent bacterial spoilage (Shahid et al., 2013). This is an interesting finding to which requires more investigation on the role of dye structure.

Food pigments as members of food additives are widely used as colorants in food and drug industries (Zhou et al., 2014). Natural pigments are generally supposed to be non-toxic and sustainable resources with minimal environmental issues. In fact, they have attracted the attention of the scientific community to use them in a variety of traditional and newly discovered application disciplines. This study clearly shows that the studied pigments exert antimicrobial activities. Their combination sometimes has a synergic effect (Santos et al., 2011).

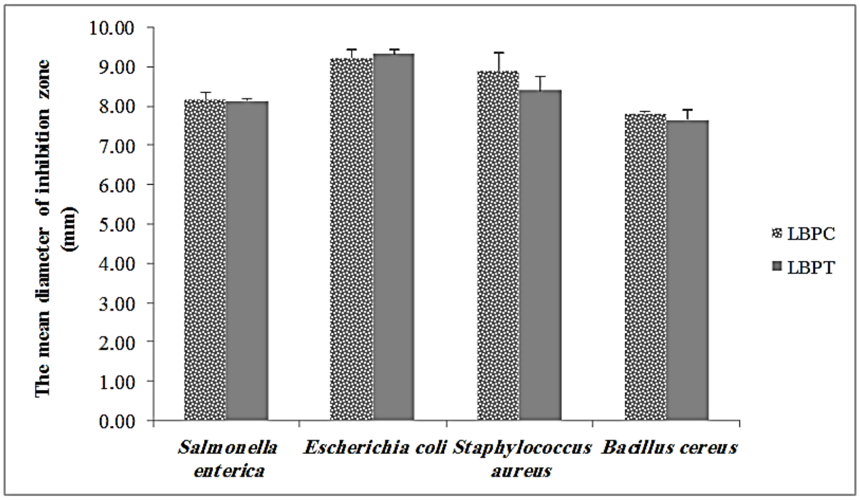

Fig. 4. Antimicrobial activities of mixtures of four pigments. (LBPC) Curcumin + Lycopene + β-carotene + Paprika oleoresin, (LBPT) Turmeric oleoresin + Lycopene + β-carotene + Paprika oleoresin.

5. Conclusion

Obtained results confirm that selected natural pigments can exhibit good antimicrobial activities. Among them, curcumin and mixtures that contain it, showed the strongest effect on the inhibition of bacteria. Curcumin individually inhibited the growth of both gram-negative and gram-positive bacteria. In other words, there is a linear relationship between mentioned natural pigments with or without mixing, and their antimicrobial properties. So that by adding these pigments into micro-organisms environment, inhibitory effects can be observed. In this study, synergistic effect of selected natural pigments of food on anti-microbial properties was investigated, and a natural color combination was proposed to replace synthetic dyes in order to increase the functional properties of food. Today, adding ingredients with functional attributes to snacks as alternative nutrients, which is too difficult to take by individuals especially children who suffering from malnutrition, is crucial. Furthermore, pigments with stronger antimicrobial effect should be added to weaker ones, their results in a mixture having considerably higher anti-microbial features. This, in turn, demonstrates that combining pigments can consider a better control method for rising food shelf-life through preventing micro-organisms growth in food systems.

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