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Evaluation of Some Physicochemical and Sensory Properties of Beef Sausage Containing Sour Tea (*Hibiscus Sabdariffa* L.) Extract

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ABSTRACT

Although many studies have demonstrated the significance of using natural plant additives and encapsulating plant extracts in meat products to mitigate the adverse effects of chemical additives, research in this area remains limited. Hence, the current study investigated the effects of sour tea extract (Hibiscus sabdariffa L.) on enhancing the physicochemical and sensory characteristics of beef sausage. The study assesed the phenolic content of hydroalcoholic extract of sour tea using the Folin-Ciocalteu method, as well as its antioxidant activity via the DPPH assay at concentrations of 500, 1000, 1500, and 2000 ppm of the extract. The characteristics of the capsules containing this extract, including particle size, zeta potential, efficiency, solubility, mass density, morphology, and the physicochemical characteristics of the meat products containing these capsules including the amount of thiobarbituric acid, color, and sensory attributes were evaluated. Data analysis was conducted using one-way analysis of variance. The results indicated that the total phenolic content in sour tea extract was 174.6 mg of gallic acid per gram of extract, and the highest antioxidant activity was observed at concentrations above 1500 ppm of the extract. The particle size of the extract ranged from 108.517 to 646.369 micrometers, and physicochemical parameters such as zeta potential, efficiency, solubility, mass density, and capsule morphology were within appropriate ranges. During storage, the amount of thiobarbituric acid compound increased in the control sample, and this difference was significant compared to the sample containing 1500 ppm of sour tea extract. Additionally, with increasing storage time, the L color factor decreased, but sensory evaluation indicated that the treatments received acceptable scores. Overall, the results of this study demonstrate that sour tea extract, whether in free form or encapsulated, can serve as a natural additive to enhance the quality and improve the sensory properties of beef sausage, potentially replacing chemical preservatives.

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1-Introduction

Sausage is one of the oldest and most popular forms of processed meat. Meat and meat products can easily be contaminated by microorganisms and, in the absence of proper storage conditions, cause the growth of spoilage and pathogenic bacteria [1]. To prevent spoilage and reduce health risks, meat products such as sausages need to use preservatives. These preservatives can include nitrite and nitrate salts, which, in addition to improving the color and shelf life of products, are also effective in controlling microbial growth [2]. Although the use of chemical additives can be useful in maintaining the quality of meat products, their excessive use can be harmful to health and increase the risk of diseases such as cancer. Therefore, the use of natural additives instead of artificial additives is very important. Plant extracts, due to their diverse properties, can be very effective in maintaining the sensory and nutritional quality of meat products [3].

One of these herbal extracts is sour tea extract, or roselle (*Hibiscus Sabdariffa*). This plant, which belongs to the Malwasae family, does not have an exact origin and may have originated in areas such as India, tropical Africa, or Saudi Arabia. But currently, this plant is widely cultivated in tropical and subtropical climate regions of Asia, Africa, and Central and South America [4, 5]. Sour tea is consumed as a hot or cold drink in many parts of the world. This product has many therapeutic properties that are used in general medicine, including lowering blood pressure and cholesterol, improving digestion, having anti-inflammatory properties, and strengthening the immune system. In addition, sour tea has potential antibacterial, anticancer, and antimicrobial activities. [6]. This tea is a rich source of antioxidant compounds such as catechins, theines, polyphenols, and flavonoids, which can play an effective role in fighting oxidative damage and reducing inflammation [7]. Due to the antioxidant and antimicrobial properties of sour tea, it is very common to use it in meat products to prevent the oxidation and spoilage of lipids and to increase the shelf life and quality of products [8]. Plant extracts with high levels of phenolic compounds and other antioxidant substances can be effective in delaying oxidative reactions. Therefore, it is necessary to use methods to preserve the maximum antioxidant activity of the extracts. Also, to improve the chemical and biological stability of the sensitive compounds in plant extracts, microencapsulation technology can be used, which can protect them from inevitable reactions in food systems.

The microcoating of natural materials plays a very important role in increasing the stability, absorption, efficiency, and quality of products and has found wide applications in the food and pharmaceutical industries. This technology provides the possibility of controlling the release of active ingredients and improving the sensory and physical properties of products. In the food industry, microcoating can increase the shelf life of flavor, aroma, and nutrients while preventing their degradation by environmental factors such as light, heat, and oxygen. For example, vitamins, pigments, and antioxidants can be more effectively preserved in food, improving the nutritional quality and attractiveness of products [9, 10]. Recently, the applications of the microencapsulation method in the food industry have increased because the enclosed materials can be protected against moisture, heat, or other extreme conditions, and their stability and durability can be maintained [11]. Several studies have been conducted in relation to the effects of plant extracts on the shelf life of meat products,

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especially sausages. For example, a study conducted by Khanlou et al., aimed at investigating the effect of ethanol extracts of basil seeds and nettle leaves on the physical, chemical, and sensory characteristics of sausages during storage time in the refrigerator, showed that levels of 0.1% and 0.3% of nettle extract Compared with basil seed extract, it has had better results on the physicochemical characteristics of sausage [12]. Also, other research results indicated that the sausage sample containing 60% celery extract can act as a suitable substitute for nitrite, and in terms of antioxidant properties, the sausage sample containing 20% celery extract has the best antioxidant activity [13]. On the other hand, research shows that adding natural compounds with antioxidant, antimicrobial, and physicochemical properties to meat products can improve the quality and durability of these products. In this regard, the research results show that the addition of an aqueous extract of plant leaves (*Melastoma malabathricum* L.) to beef sausage improved the physicochemical, antioxidant, and antimicrobial properties of this product [14]. Also, research shows that adding sour tea extract up to 8% to chicken sausage increases moisture content and antioxidant activity [15]. Research findings show that roselle extract can be used as a suitable alternative to improve safety and food composition in some sausages [16]. In addition, microencapsulation of Zanthoxylum bungeanum essential oil has been effective in increasing flavor stability and inhibiting lipid oxidation in Chinese light sausage [17]. Also, encapsulated rosemary extract can be used as a natural preservative in beef and meat products [8]. In recent studies, it has been found that sour tea extract nanoencapsulated with carboxymethyl cellulose (CMC) can be used as a natural preservative in meat and meat products such as chicken nuggets.[18] On the other hand, the results of a study showed that the use of roselle sepal extract in the formulation of Frankfurt type sausage is not suitable because it has an adverse effect on the chemical, physicochemical, and sensory properties of this product [19]. In general, the use of chemical preservatives in meat products can have irreparable effects on the body and contribute to the development of diseases such as cancer. In this regard, the effects of plant extracts on the quality and shelf life of meat products, especially sausages, have been the focus of researchers, and numerous studies have been conducted. However, studies on the effect of free and microencapsulated extracts of sour tea sepals on the physicochemical properties of beef sausage are very limited, and the results of previous studies in this field have been contradictory to some extent. [13-16, 19] Accordingly, considering the effects of chemical preservatives on the quality and durability of meat products, especially sausages, on the one hand, and also the very useful properties of sour tea, especially from the point of view of antioxidant and antibacterial effects, on the other hand, the present research aims to use a natural additive (sour tea extract) in meat products and investigate its effect on the physicochemical and sensory properties of the final product. It has evaluated some physicochemical and sensory properties of beef sausage containing sour tea extract. The results of this research can be considered in the production of high-quality and durable meat products, especially sausages, and the use of plant extracts, especially sour tea, can be considered as a stable and healthy alternative to chemical preservatives in the meat products industry. Therefore, with phytochemical investigations, improvement of sour tea plant extract, and

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additional experiments, it can be used in industrial ways of producing high-quality sausages.

2- Materials and methods

2-1- Materials used

The beef used in this research was procured from reputable food factories located in Tehran, Iran. In addition to that, sour tea was also purchased from a reputable perfumer in Tehran. It is noteworthy that all the other raw materials used in this study were also obtained from reliable and active domestic companies in Iran. This issue indicates that in this research, an effort has been made to use materials of appropriate quality and in accordance with national standards for conducting experiments.

2-2-Preparation of the extract

Sour tea powder in a ratio of 1 to 5 with ethanol-water solvent (50%) (this is considered the solvent of choice due to its ability to effectively extract polar and non-polar compounds in sour tea) (a gram sample with 100 ml of Halal) was mixed and kept away from light for 48 hours in a shaker (Labtron, Ls-100, Iran) at a speed of 160 rpm. Then three stages of centrifugation (Hermle, z200A, Germany) were performed for 10 minutes each time at a speed of 3000 rpm, and in each step of the aqueous phase (superior phase), sediment was collected until no more sediment was seen at the bottom of the tube. Then, the collected aqueous phases were smoothed with Whatman paper No. 1. Next, by the evaporator (maximum temperature 50 degrees Celsius) (TAM 2 times, Iran), the evaporation solvent and the resulting extracts were kept at -18 degrees Celsius until the experiment [20].

2-3- Determining the amount of total phenol

The total amount of phenolic compounds in the extract was measured based on previous research experiences [21] by means of the spectroscopic method with the Folin-Ciocaltio reagent, and the results were expressed in milligrams of gallic acid per gram of extract. In this regard, 0.5 ml of 0.1% extract (a solution of 0.1 g of extract in 100 ml of solvent) was mixed with 2.5 ml of Sio Calcho reagent diluted with water at a ratio of 10:1, and 2 ml of 7.5% sodium carbonate was mixed and left at room temperature for half an hour. Their absorbance at 760 nm wavelength was read by a spectrophotometer (PG Instrument, T80, England). The standard curve was measured based on mg of gallic acid per gram of extract [21].

2-4- Evaluation of antioxidant activity

To evaluate the antioxidant activity of sour tea extract, based on previous research [22], the DPPH free radical inhibition method was used. In this method, first, 0.3 ml of the extract was added to 7.3 ml of methanolic DPPH solution (105 x 6 mol/l), and the resulting mixture was vigorously stirred. After 30 minutes of dark housing at room temperature, the light absorption of the samples was measured at 517 nm wavelength in front of the control. All these steps were carried out in the case of TBHQ as a standard and control antioxidant (DPPH solution prepared in methanol plus relevant solvents). The inhibition percentage was calculated through equation 1 below:

(1) free radical inhibition percentage DPPH = witness absorbance- sample absorbance/witness absorbance ×100

2-5- Encapsulation

According to the previous laboratory experiences of the researchers as well as the research experiences of other researchers [18], maltodextrin (DE = 16–20) and pectin (high methoxyl level) were chosen as coatings for the preparation of encapsulated sour tea extract. In fact, these materials have good protective and stabilizing properties. Maltodextrin can protect bioactive compounds from adverse conditions, and pectin provides an effective coating to protect the extract by forming a

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gel. Maltodextrin and pectin were dissolved in a chloroform/methanol solution (1:3 w/w) and then placed in a rotary evaporator (Steroglass, Strik202, Italy) to remove the solvents and form a thin layer on the wall. Also, sour tea extract was also dissolved in a dichloromethane/methanol solution (1:2 weight by weight), and the resulting mixture was combined with a ratio of 4:1 maltodextrin and pectin (maltodextrin-pectin: extract), and finally the solvents were removed under nitrogen vapor. . The thin layer was dissolved in 2 mL of phosphate buffer (10 mmol/L, pH 7.4). Then it was homogenized with a homogenizer (300VT, BioLogics, USA) for 15 minutes at a temperature of 35 °C and a pressure of 200 bar. The resulting suspension was incubated in the dark at room temperature for 2 hours. Then it was centrifuged (Hermle, z200A, Germany) at a speed of 6500 rpm at a temperature of 4 degrees Celsius. Finally, the encapsulated sour tea extract was dried using a freeze dryer (Christ, Alpha 2-4 LD Plus, Germany). A freeze dryer was used to maintain the quality and bioactive properties of sour tea extract because this drying method prevents the destruction of useful compounds at high temperatures and improves the recovery and stability of the dried extract.

2-6- Determining the optimal capsule particle size and zeta potential

Based on previous studies, in order to measure the size and specific surface of the encapsulated particles, first their dispersion was prepared in methanol, and then the size of the nanoparticles was measured with the help of an analyzer (Horiba, S2-100-SZ, Japan) [23]. Zeta potential is an important indicator for the stability of colloidal systems. This is the electric potential in the shear plane between the inner and outer ionic layers surrounding the colloidal particles. The high zeta potential causes strong electrical repulsion between particles and increases the stability of the system. This value can be measured with a dynamic light diffraction device and is used in the study of colloidal systems such as sour tea extract. In the present study, zeta potential was measured based on the surface electric charge of the particle at room temperature with a dynamic light diffraction device (Malvern, ZEN3600, England) [24].

2-7- capsule efficiency

In the current research and based on previous research [24], capsule efficiency was evaluated to determine the amount of microencapsulated extract from the amount of total phenolic compounds in the microencapsulated. In this regard, extraction of the extract from microcapsules was performed according to the method of Robert et al. [24]. For this purpose, 200 mg of microcoating was added to 2 ml of extraction solution containing methanol, acetic acid, and water in the ratio of 50–8–42 (volume/volume/volume) respectively, and stirred for one minute and then under ultrasound to The duration of 20 minutes was set in two stages with 100% intensity and 20 kHz frequency. After this step, centrifugation (Hermle, z200A, Germany) was performed at 5000 rpm for 10 minutes. Capsule efficiency was calculated according to equation 2 and expressed as a percentage.

(2) Microcoating efficiency (%) = micro-encapsulated phenolic compounds amount/phenolic substance of primary compounds amount

2-8- Measuring capsule solubility and determining capsule bulk density

The purpose of measuring solubility is to evaluate the ability to dissolve and release effective substances in water, which shows the amount and speed of their absorption in the body. On the other hand, measuring the mass density of the capsule examines the physical characteristics and uniformity of capsule filling, which affect the flowability properties and how they are filled. The

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measurement methods provided for these two parameters are simple, valid, and common. These measurements will help to better understand the physicochemical characteristics of the capsule and will be useful in evaluating and comparing the final product specifications. In this research, using the working method of previous studies [25], in order to measure the solubility of the capsule, 1 gram of the product powder was dissolved in 100 ml of water, and the product solution was dissolved for 10 minutes in a centrifuge (Hermle, z200A, Germany) at a speed of 7500 RPM to separate the insoluble parts. Then 25 ml was taken from the transparent part above the test tube and placed in an oven at 105 degrees Celsius for 5 hours. The amount of solubility was calculated based on equation 3 below and expressed as a percentage.

(3) $S=(m1-m2)/0.25\times100$

In this equation, M1, M2, and S will be the weight of the container after removing it from the oven, the weight of the empty container, and the solubility after 9 hours [25].

In order to measure the bulk density of the capsule, 5 grams of the produced powder were poured into a graduated cylinder, and then its volume was measured. The measurement was repeated three times, and the average value was recorded. The mass density of the capsule was calculated using the following equation [25].

(4) $Pb = m/vb$

In this relationship, Pb is the bulk density, m is the mass of the sample, and Vb is the bulk volume of the sample.

2-9- Capsule morphology

In order to investigate the morphology of the complex formed by scanning electron microscopy (Hitachi, SU3500, Japan), images were prepared by scanning the surface of microcapsules. The samples were coated with a thin film of gold and analyzed at different magnifications [26].

2-10- Preparation of beef sausage

Beef sausage was prepared according to the general formulation produced according to the national standard for meat products (2303) and according to the usual methods in the industry. (beef 60%, liquid edible oil 5%, water and ice mixture 22%, edible starch 5.8%, gluten 2%, soy isolate 2%, salt and spices 73.2%, ascorbic acid 0.02%, sodium nitrite 0.05%, sodium polyphosphate 0.4%). In this process, a 5-liter catheter (MADO, Germany) was used with a temperature of 0 °C. Minced red meat with salt and sodium polyphosphate was poured into the catheter with 1/3 of the ice formulation and was heated for 1.5 to 2 minutes with They were mixed at a speed of 3000 rpm. Then the remaining 1/3 of ice, soy isolate, and ascorbic acid were added to the cutter and continued for 1.5 to 2 minutes. Edible liquid was added to the catheter along with the remaining 1/3 ice, and they were mixed for a total of 3 minutes of catheterization. Then, in separate batches, in a sodium nitrite cutter and free and encapsulated extract, sour tea was prepared in different ratios (20:80, 40:60, 60:40, 80:20, 0:100, and 100:0), and these ratios were based on studies. Experimental researchers in the laboratory and conducting pilot tests were selected with the aim of achieving the best effect on the physicochemical and sensory properties of the product and were adjusted in such a way that the most optimal amount of extract could be determined to improve the properties of the product. The proportions of sour tea, along with half of the water and ice mixture, were added to the dough and filled and wrapped in special polyamide covers with a filling machine (SMART, China). Finally, the sausages were cooked in the cooking room with steam at a temperature of 80 °C for 60 minutes until their center reached a temperature of 72 °C. After cooking, the sausage samples were cooled with water at 12 °C, and after

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reaching The room temperature was transferred to the refrigerator at 4 °C, and they were kept at this temperature (4 °C ± 1 °C) until the experiment. In terms of the length of the test period, the researchers considered a 30-day storage period. In fact, in the meat products industry, such as sausages, keeping the product for up to 30 days is a common and standard approach. This period of time is enough to properly evaluate the quality changes of the product during storage. Next, on days 14, 7, 1, and 30, three sausages from each section were randomly selected and analyzed for physicochemical tests. Also, the mentioned treatments were subjected to sensory evaluation on the first day of storage. This was done because in the last days of the storage period, the quality of the product decreased to such an extent that the sensory evaluation did not provide useful information and the physicochemical analyses were considered the main indicators of the quality evaluation. All experiments were performed with three repetitions.

2-11- Evaluation of physicochemical properties

2-11-1-Measuring the amount of thiobarbituric acid

The fat oxidation rate of sausage samples was measured by the thiobarbituric acid method. 10 grams of the sample were weighed, and 35 milliliters of 4% perchloric acid and one milliliter of 0.5% BHT solution were homogenized in ethanol. The mixture was filtered with Whatman paper, and 5 ml of the filtered solution was mixed with 5 ml of 0.02 M thiobarbituric acid in a test tube with a lid and placed in a bain-marie of boiling water for 60 minutes. After cooling, absorb light with a spectrophotometer (Ningbo Mflab, GT211-NV203 Nv20, USA) was read at a wavelength of 532, and the amount of thiobarbituric acid per kilogram of sample was calculated based on milligrams of malonaldehyde (MDA). [13]

2-11-2- Colorimetry test

The color of the sausage samples was evaluated using a colorimeter (Hunterlab, Colorflex EZ, Japan). The device was calibrated with two black and white tiles, and slices of the cross section of sausages with equal diameter were prepared. Sausage slices were placed in special containers, and a black and opaque container was also placed on the special container to prevent the interference of external light. The L* factor of the samples was then evaluated [27].

2-11-3- Sensory evaluation

For the sensory evaluation of the produced sausage samples, five experienced work evaluators (3 men and 2 women in the age range of 30–40 years) were used who examined the samples based on general acceptance. The use of five tested and experienced evaluators with appropriate gender and age diversity is a suitable and usual approach for the sensory evaluation of produced sausage samples. This choice can be justified from several aspects: the appropriate number of evaluators to obtain diverse and reliable opinions, benefit from the expertise and experience of evaluators, gender diversity to provide different perceptions, and choosing the appropriate age group with high perception and concentration. In general, this combination of positive factors is considered a standard and suitable approach for the sensory evaluation of food products such as sausage, and for evaluation, a 5-point hedonic scoring system (score 1 = very bad, score 2 = bad, score 3 = average, score 4 = good, score 5 = very good) was used [28].

12-2- Statistical analysis

In this research, data analysis was performed in the form of a completely randomized design, and the experiments were repeated three times. SPSS version 22 software was used for data analysis, one-

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way ANOVA was used, and Excel was used to draw the figures. To compare the mean of the data, Duncan's multi-range test was performed at a confidence level of 95%, and the values ($p < 0.05$) were considered significant.

3- Results

3-1- Measurement of total phenol

The amount of total phenolic compounds was calculated with the Folin-Ciocaltio reagent based on the mg of gallic acid per gram of extract based on equation 5. The amount of total phenol in the hydroalcoholic extract is 174.6 mg of gallic acid per gram of the extract.

(٥) $Y = 0.0001X + 0.4425$
$R^2 = 0.8422$

3-2- Assessment of antioxidant activity

In the assessment of antioxidant activity, DPPH free radical inhibition was evaluated at different concentrations of 500, 1000, 1500, and 2000 ppm of sour tea extract. In this regard, the concentrations were selected based on the researchers' experimental studies in the laboratory and conducting pilot tests. In fact, the selection of desired concentrations of sour tea extract was based on several factors. Lower concentrations (500 and 1000 ppm) were chosen to show the practical and real use of this extract in products, and higher concentrations (1500 and 2000 ppm) were chosen with the aim of evaluating the maximum antioxidant power of sour tea extract in laboratory conditions. Choosing this concentration range is possible. A comparison of the antioxidant activity of the extract with a reference antioxidant such as TBHQ was provided. The results showed that concentrations above 1500 and 2000 ppm of sour tea extract and also the concentration of 1500 ppm of TBHQ had the highest inhibition rate.

3-3- Determining the optimal capsule particle size and zeta potential

According to the obtained results, the size of particles coated with pectin and maltodextrin was between 108.517 and 646.369 micrometers. The zeta potential of the microcoated extract with pectin and maltodextrin was -85.5.

3-4- Efficiency, solubility, and capsule mass density test

The efficiency of the produced capsules was 81.7%, which indicates the high success of the microencapsulation process in maintaining the efficiency of the active ingredients in the sour tea extract. The results of the solubility test also indicate the solubility of 80% of the produced capsules. This level of solubility shows that the capsules have a good ability to dissolve and release active substances in different environments, which can be effective in improving the absorption and effectiveness of the final product. Also, the results of measuring the mass density of the capsules showed a density of 0.15 grams per cubic centimeter. This density value indicates the relative density of the capsules and the quality of the production process. Low bulk density means there is space between capsule particles, which may improve their compressibility in different applications. In general, these results indicate the high quality of the produced capsules in terms of efficiency, solubility, and bulk density.

3-5- Morphological properties of the optimal capsule

Figure ١ shows the morphology of the capsule containing sour tea extract with a scanning electron microscope, which shows prominent features. The capsules have a regular geometric shape and smooth, flawless walls, indicating a precise and controlled manufacturing process. The uniform size and dense structure of the capsules allow for a controlled and uniform release of the extract, and the crack-free surface ensures the physical stability of the

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capsules. These morphological features make the capsules very suitable for various applications, including sausage coating. Although these morphological characteristics are influenced by various factors such as wall composition, drying method, and drying speed, Since these factors can affect the surface characteristics and microstructure of microcoated materials and make changes in them, the morphology and surface structure of the examined capsules were a reflection of the interaction of these factors during the microcoating process.

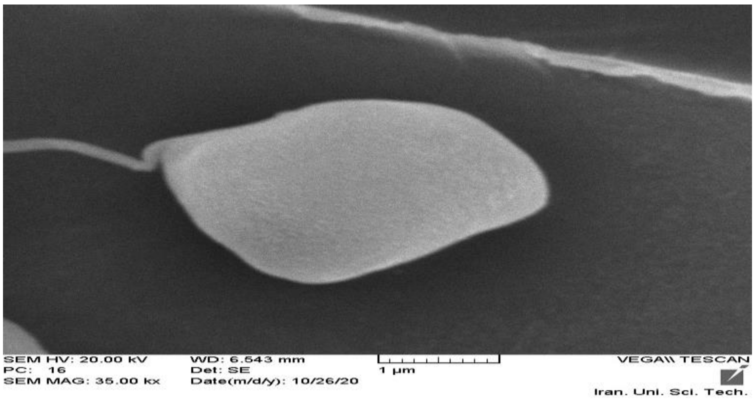

Figure ١. Capsule morphology

3-7- Thiobarbituric Acid Index (TBA)

During the storage period, the index values of thiobarbituric acid increased in the treatment containing a 1500 ppm concentration of sour tea extract and the control treatment (Figure ٢). On the zero day, the value of this index was equal to 0.31 mg of malonaldehyde per kilogram of tissue, which on the 30th day of storage was found to be 4.64 and 0.91 mg of malonaldehyde per kg in the control treatment and the treatment containing sour tea extract, respectively. Increased. During the storage period, the increase in the amount of thiobarbituric acid in the control treatment was higher than in the treatment containing sour tea extract, and this difference was also significant ($p<0.05$). On the other hand, in pilot and experimental research, it was found that the microcoated extract did not have a significant effect on the TBA index, which is one of the most important criteria for measuring the amount of oxidation of fats and the quality of food. The lack of significant change in the TBA index indicated that the microcapsule extract did not improve the ability to prevent lipid oxidation in laboratory conditions. For this reason, the data related to the TBA index is not displayed in the graphs and related results.

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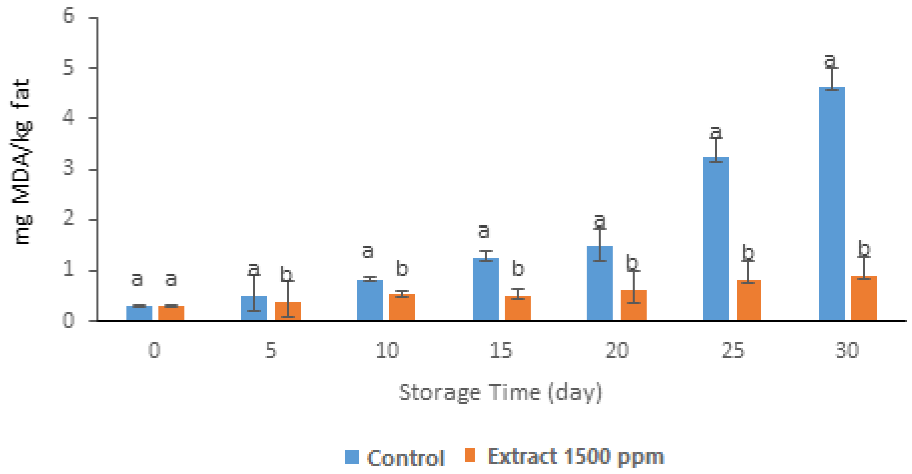

Figure ۲. The amount of thiobarbituric acid index during the storage period. Different Latin letters on the columns (a,b) indicate significant differences between the columns.

3-7- Colorimetry test

The L* factor is one of the colorimetric factors that indicates the lightness and darkness of the samples. The values of this factor are in the range of 0 to 100, so that the number zero indicates blackness and the number one hundred indicates the whiteness or brightness of the sample. In Figure ۳, changes in the colorimetric factor L* in sausage samples during storage periods of 0, 15, 30, and 45 days at refrigerator temperature are displayed. The results showed that during the storage period, the colorimetric factor L* in different samples had a decreasing trend (Figure ۳). Probably, the chemical and microbial changes during sausage storage at refrigerator temperature were the main reason for the darkening of the samples (decrease in factor L*), which can be mentioned as an indicator of product stability and quality during storage.

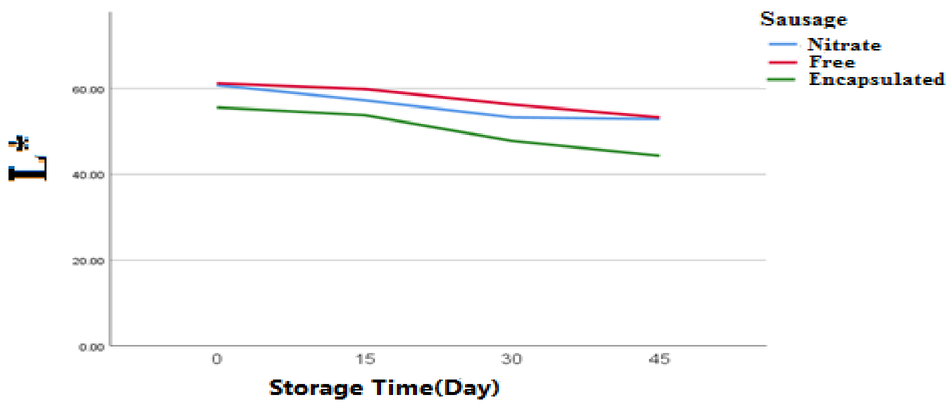

Figure 3. Changes in color index (L*) in different treatments (nitrite, free and encapsulated) during the storage process. The red, blue, and green linear graphs indicate the treatment with nitrite, free extract, and encapsulated extract, respectively.

3-8- Sensory evaluation

In the evaluation forms, five evaluators examined the characteristics related to the general acceptability of beef sausages containing nitrite, free extract, and sour tea coating and assigned points from 1 to 5. These evaluators gave their opinions based on the overall acceptance criteria of the product. The results of this research showed

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that all evaluators gave a score of 4 to the sausages containing nitrite, which indicates the high satisfaction of the evaluators with the quality of this type of sausage. Similarly, sausages containing free extract and sour tea microcoating also received a score of 4. These high scores show that the addition of sour tea extract, either free or microcoated, did not have a negative effect on the overall acceptance of the product. On the other hand, the taste of sour tea did not have a negative effect on the organoleptic properties of the product. Evaluators reported that the sour tea flavor in the final product was well balanced and did not reduce the sensory quality of the sausage. These findings indicate that sour tea extract can be used as a useful additive in the production of beef sausages without having a negative effect on consumer acceptance.

4- Discussion

Excessive consumption of nitrates in meat products, especially sausages, can have serious health effects, leading to the creation of carcinogenic chemicals, cell damage, and increased blood pressure. To reduce these side effects caused by chemical additives, using natural additives such as plant extracts and their microcoating and improving processing and packaging methods can be a solution. In this study, the effect of a free and microencapsulated extract of sour tea sepals on some physicochemical and sensory characteristics of beef sausage was investigated in order to introduce safer and natural alternative methods to improve the quality and safety of these food products.

Phenolic compounds found in fruits and vegetables have attracted much attention from researchers due to their high potential for antioxidant activity. These phenolic compounds prevent the activity of free radicals by donating a hydrogen atom [29]. The results of this research showed that the amount of phenolic compounds in sour tea extract was equal to 174.6 mg of gallic acid per gram of extract. In line with the results of this study, in research conducted by Feridouni and Shormasti, the total amount of phenolic compounds in sour tea extract was reported to be 626.57 mg of gallic acid per gram [18]. On the other hand, in another study, a low phenolic content of 69.04 mg gallic acid equivalent per liter of sour tea extract was reported [30]. These differences may be due to differences in extraction methods, plant culture conditions, and different environments that affect the final phenolic content. Therefore, choosing the right extraction methods and optimizing the conditions can play an important role in interpreting the results and the antioxidant effect of sour tea extract. On the other hand, due to the high content of phenolic compounds in sour tea extract, this extract can play an important role in improving the physicochemical and sensory characteristics of sausage. In fact, the phenolic compounds in this extract, with their antioxidant mechanism, prevent the oxidation of fat and proteins in sausage and increase the stability and durability of the product [29,30]. Also, these compounds can affect the texture and color characteristics of sausage.

The results of the present study showed that with an increase in the concentration of sour tea extract, the amount of DPPH free radical inhibition also increased. Agreeing with the findings of the present research, the results of the study of Feridouni and Shormasti (2018), which investigated the effect of nanoencapsulated sour tea extract with carboxymethyl cellulose on the quality and shelf life of chicken nuggets, showed that free radical inhibition (DPPH) increased with increasing extract concentration. finds [18]. Also, in a study conducted by Perez-Quintana et al. to use sour tea extract as a natural antioxidant in sausages, they showed that adding the extract with a concentration of 4% sour tea increases the antioxidant activity and improves the protective quality. It is made from sausage [31]. Also, the study of Marquez Rodrigues et al. (2020) showed that sour tea extract can be a healthy option to consume antioxidants and a natural alternative to increase the shelf life of meat at cold temperatures [32]. DPPH has an absorption maximum at about 515 nm and has the ability to be rapidly inactivated by antioxidants. This method, which is widely used to evaluate the ability of different compounds to inhibit free radicals, showed that sour tea has a high antioxidant power, and adding sour tea extract to beef sausage leads to an increase in antioxidant activity and improves the shelf life of these products. This, in turn, shows that sour tea can act as a natural antioxidant and a suitable alternative to artificial antioxidants. In practice, sour tea contains phenolic compounds such as phenolic acid, flavonoids, and catechins, which can easily neutralize free radicals with their electron-donating properties and thus reduce their harmful effects. Also, these compounds can prevent chain oxidation reactions by chelating metal ions. Therefore, adding sour tea extract to food can act as an effective method to prevent spoilage and reduce its quality.

Examining capsule characteristics such as particle size and zeta potential is of particular importance because these characteristics directly affect the characteristics and performance of capsules. The size of capsule particles can be important in different aspects. For example, the size of the particles can have a direct effect on the physical and chemical stability of the capsule, so choosing the right size of the particles can increase the shelf life and write and discharge control. Also, zeta potential is vital as an indicator of the dispersion stability of capsules in different environments, including biological environments. Zeta potential indicates the capsule's ability against decomposition and formation, often in environments with different pHs and the presence of ions, which can have a great impact on the strength and stability of the encapsulation system [24].

The results of particle measurement showed that the size of the particles coated with maltodextrin and pectin was between 108.517 and 646.369 micrometers. According to the results, the size of the microcoated extract is small, and the small size of the particles can increase the stability and resistance of the extract in different environments. In another study, the particle size of sour tea extract nanoencapsulated with maltodextrin-protein whey concentrate was reported as 139.03 ± 2.76 nm [18]. The measurement of zeta potential helps to evaluate the surface charge and stability of particles, which is very important in investigating the physicochemical characteristics and biological stability of particles [33]. The results of our research show that the zeta potential for the microcoated extract with maltodextrin and pectin has a strong negative surface charge equal to -85.5, which can contribute to increasing the biological stability and efficiency of the extract. The results obtained in our study can contribute to the development of improved methods for microencapsulation and extract application on biological substrates. Further analysis shows that the small size of the microcoated particles, due to the increased contact surface with the surrounding environment, can improve the absorption and bioavailability of the extract in the body. Also, the strong negative surface charge caused by the use of maltodextrin and pectin can prevent the accumulation of particles and increase their colloidal stability through electrostatic repulsion. These characteristics can lead to an improvement in the effectiveness and efficiency of the extract in biological applications.

The results of the present study showed that microcoated extracts using maltodextrin and pectin have 81.7% efficiency, 80% solubility, and 0.15 mg/cm3 density. These results show that the capsules are produced with the best performance. In fact, the results of our research show that the use of maltodextrin and pectin as microcoating

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agents for sour tea sepal extract has a good ability to increase the efficiency and solubility of capsules. Also, the appropriate density of these capsules indicates their stability. These capsules with a high efficiency of 81.7% show that, probably due to their high ability to cover and protect the active compounds in the extract, they are able to effectively maintain and preserve the active ingredient. On the other hand, the high solubility of 80% also shows that these capsules are able to effectively release the active ingredient in the right environment, which can help improve the bioavailability and thus the clinical effectiveness of this product. Their appropriate density, which is equal to 0.15 g/cm3, indicates the stability and physical compatibility of these capsules, which can positively affect their properties such as transportation and durability. These results show that the use of maltodextrin and pectin as microencapsulating agents for sour tea sepal extract can lead to the production of capsules with favorable and acceptable characteristics for various applications in the food industry.

Scanning electron microscope images of the capsules prepared using maltodextrin and pectin showed that the capsules have a distinct geometric structure and smooth walls. These features show that the microcoating process was well done and led to the production of uniform and defined capsules. The composition of the wall, drying method, and drying speed, especially in the early stages, can affect the surface characteristics and microstructure of microcoated materials and cause changes in them [21]. The smooth and homogeneous surface of the capsules indicates that the drying conditions and the interaction between the microcoating materials and the sour tea extract have been optimized. This property can contribute to the physical stability, controlled release, and efficient distribution of the coated compounds. It may also contribute to the visual appeal and ease of working with the final microcoated product.

In food, the oxidation of saturated fats leads to the production of secondary aldehyde and ketone compounds that can cause unpleasant odors in meat products. To evaluate the degree of fat oxidation in food, the TBA index is widely used. The results obtained from the present study showed that with increasing storage time, the amount of TBA increases, which indicates the accumulation of secondary oxidation products. In this regard, the results of a study showed that the addition of roselle flower extract to high concentrations (6 and 8%) can accelerate the oxidation process of lipids and have a negative effect on the physicochemical and sensory properties related to the color and texture of the product [16]. Also, the addition of antioxidant agents such as Melastomalabaricum leaf extract could not prevent the increase in the amount of reactive substances with thiobarbituric acid during the storage period [14]. In total, these studies show that the oxidation of fats during the food storage process is a complex process that can lead to the formation of aldehyde and ketone compounds and have a negative effect on the sensory properties of the product.

Another factor that can be checked for the quality of produced sausages is the colorimetric factor L*, or the brightness symbol (black to white). In fact, the higher the L*, the lighter the appearance of the sausage. In this regard, factors such as the oxidation of fats and proteins and microbial and chemical changes during sausage storage can lead to a decrease in brightness. The results obtained from the comparative descriptive study within the group showed that the colorimetric factor L* during the storage period in different samples had a decreasing trend, and in the last days of storage, the fewest color index changes were observed. Naturally, this is predictable due to the chemical changes and oxidation of fats and proteins during the storage period. Despite the limitations of

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research facilities, the possibility of a comparative study between groups was not provided in order to provide an accurate and calculable analysis in terms of the comparison of the L* symbol between groups. In agreement with the results of this study, the research conducted by Hosseinpour et al. showed that in meat storage systems without nitrite and with a low amount of nitrite during the storage period, the colorimetric factor gradually decreased [34].

The results of the present study showed that the addition of free and micro-encapsulated extracts of sour tea to beef sausage compared to sausage containing nitrite had sensory characteristics approved by the evaluators and obtained the same scores. These results are in line with the study of Siyari et al., who showed that the addition of black cumin seed essential oil coated with basil gum to colored salmon fillet did not change the smell, texture, or overall acceptance, but in relation to the color, with the addition of coatings, the sensory score was significantly increased. decreased [22]. Also, the study of Gutierrez-Cortez and Mahcha showed that there is no difference between the sensory characteristics of sausages containing bromoham extract and sausages containing nitrite [35]. These findings indicate that the use of natural substitutes, such as plant extracts, can improve the sensory characteristics of meat products to an acceptable extent. This issue is very important because the acceptance and success of a food product in the market, in addition to scientific criteria, is fundamentally related to the opinions and experiences of consumers about the taste and experience of consuming the product.

The present study was carried out in the scope of evaluating some physicochemical and sensory characteristics of beef sausage containing sour tea extract and in terms of studies in the biochemical and microbial fields, as well as measuring other indicators except thiobarbituric acid, in compliance with the operational scope determined in the research and support proposal. Finance is limited. The researchers of this project hope that it will be possible to carry out more extensive studies, especially in the fields of microbial and oxidative corruption related to this research.

5- Conclusion

In total, the results of the current research show that sour tea extract with a high content of phenolic compounds as strong antioxidants can help the stability and resistance of the extract in different conditions. Also, treatments containing sour tea extract can improve shelf life, increase physicochemical quality, and improve the sensory properties of beef sausage. On the one hand, adding sour tea extract in a free and micro-coated form to beef sausage as a natural and stable solution to maintain the quality of the meat product is very effective and useful, and in this regard, the best treatment identified in this study includes the optimal concentration. Sour tea extract has shown significant improvements in the physicochemical and sensory characteristics of beef sausage. Based on the experimental findings, the formulation that includes sour tea extract at this optimal level is introduced as the best approach. Considering the importance of the subject and the remarkable results of this research in order to solve the problem of using artificial additives in the meat products industry, the use of sour tea extract as a natural additive can significantly improve the physicochemical and sensory properties of meat products, as well as increase the attractiveness and health of these products. Therefore, this approach not only has potential benefits for the meat products industry but is also an effective factor for maintaining the health of consumers and protecting the environment.

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مقاله علمی_پژوهشی

ارزیابی برخی خصوصیات فیزیکوشیمیایی و حسی سوسیس گوشت گاو حاوی عصاره چای ترش (*Hibiscus sabdariffa* L.)

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چکیده

گرچه مطالعات زیادی نشان داده‌اند استفاده از افزودنی‌های طبیعی گیاهی و ریزپوشانی عصاره‌های گیاهی در فرآورده‌های گوشتی برای کاهش اثرات مضر افزودنی‌های شیمیایی، اهمیت زیادی دارد، اما مطالعات در این حیطه بسیار محدود است. بر این اساس، مطالعه حاضر به بررسی اثرات عصاره چای ترش (*Hibiscus sabdariffa* L.) بر بهبود خصوصیات فیزیکوشیمیایی و حسی سوسیس گوشت گاو پرداخته است. در این تحقیق، محتوای فنولی عصاره هیدروالکلی گیاه چای ترش با آزمون فولین–سیوکالتو و فعالیت آنتی‌اکسیدانی با آزمون DPPH در غلظت های مختلف ۵۰۰، ۱۰۰۰، ۱۵۰۰ و ۲۰۰۰ ppm عصاره مورد ارزیابی قرار گرفت. خصوصیات کپسول‌های حاوی این عصاره از جمله اندازه ذرات، پتانسیل زتا، کارایی، حلالیت، دانسیته توده و مورفولوژی آنها و ویژگی‌های فیزیکوشیمیایی فرآورده‌های گوشتی حاوی این کپسول‌ها مانند میزان تیوباربیتوریک اسید، رنگ و ویژگی‌های حسی ارزیابی شدند. تجزیه و تحلیل داده‌ها با استفاده از آزمون آنالیز واریانس یکطرفه انجام گرفت. نتایج نشان داد میزان ترکیبات فنولی کل در عصاره چای ترش برابر ۱۷٤.٦ میلی‌گرم گالیک اسید بر گرم عصاره بوده و بالاترین فعالیت آنتی اکسیدانی در غلظت‌های بالای ۱۵۰۰ ppm عصاره مشاهده گردید. اندازه ذرات عصاره بین ۱۰۸.۵۱۷ تا ٦٤٦.۳٦۹ میکرومتر بود و پارامترهای فیزیکوشیمیایی مانند پتانسیل زتا، کارایی، حلالیت، دانسیته توده، و مورفولوژی کپسول در محدوده مناسب قرار داشتند. در طول نگهداری، میزان ترکیب تیوباربیتوریک اسید در نمونه شاهد افزایش یافت و این اختلاف با نمونه حاوی ۱۵۰۰ ppm عصاره چای ترش معنادار بود. همچنین با افزایش زمان نگهداری، میزان فاکتور رنگی L کاهش یافت، ولی ارزیابی حسی نشان داد تیمارها امتیاز قابل قبولی کسب کردند. در مجموع نتایج این مطالعه نشان داد که عصاره چای ترش، چه به شکل آزاد و چه ریزپوشانی شده، می‌تواند به عنوان یک افزودنی طبیعی برای افزایش کیفیت و بهبود خواص حسی سوسیس گوشت گاو جایگزین نگهدارنده‌های شیمیایی مد نظر قرار گیرد.

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