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Development and Assessment of a Spice Oleoresin Blend for Substituting a Commercial Raw Spice Mix in Processed Meat Production

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Abstract

Spices play a significant role in enhancing the flavour of processed meat products, yet their raw form presents significant challenges in terms of quality and safety, including flavour inconsistencies, microbial contamination, and non-compliance with regulatory standards. In contrast, spice oleoresins are concentrated extracts with consistent and robust flavour profiles and lower contamination risks. The aim of this study was to substitute the flavour of a raw spice mix with a spice oleoresin blend, in a commercial sausage. Eight formulations of oleoresin blends (F1–F8), incorporating pepper, coriander, cardamom, and nutmeg oleoresins with starch and maltodextrin as wall materials, were developed. The blends, along with the raw spice mix, were integrated into sausages, and sensory evaluations were conducted using a 'difference from control' test to identify the blend capable of flavour substitution. Microbiological quality and physicochemical properties of the selected oleoresin blend were analyzed. Gas chromatography determined half-lives ($t_{1/2}$) for key flavour components, showing flavour retention stability in the sample. Sensory analysis showed no significant flavour difference ($P > 0.05$) between sausages with oleoresin blend F5 and those with the raw spice mix. The half-lives of key flavour constituents were 41.25, 12.38, 12.83, and 53.31 weeks for piperine, sabinene, myristicin, and β-caryophyllene, respectively. The sample exhibited bulk density of 358.57 ± 6.59 kg/m³ and tapped density of 554.88 ± 9.10 kg/m³, with poor flowability and high cohesiveness. Its wettability was 10.10 ± 0.21 minutes, and it was found to be susceptible to high-humidity environments, with the lowest hygroscopicity (%) at 43% and the highest at 91% relative humidity. Initial microbial load met safety criteria, indicating suitability for use in processed meat products. In conclusion, the findings suggest that spice oleoresin blends have the potential to effectively substitute the flavour of raw spice mixes, highlighting the need for further optimization of physicochemical properties to broaden their adoption in the food industry.

Keywords: Flavour, Processed Meat Products, Oleoresin, Sausages, Sensory Analysis, Spices

1.INTRODUCTION

Sausages are considered one of the most popular processed meat products worldwide. Different kinds of comminuted meat (beef, pork, or chicken) are combined with fat, seasonings (herbs and spices) and preservatives, and then encased either in natural or artificial casings (Bolívar-Monsalve et al., 2019; Elias et al., 2021). Manufacturers add spices to sausages to enhance and enrich the flavour, aroma, appearance, and overall sensory experience of the product. In commercial sausage production, a wide range of spice forms are used and they are usually added in dry whole, powder, or cracked forms (Lonergan et al., 2019). However, despite their crucial role as key contributors to successful meat products, the use of raw spices (dry/fresh forms) in the industry presents several challenges that affect the quality and safety of the final products (Witkowska et al., 2011).

Spices contain a diverse array of chemical compounds, each contributing to their unique aroma and flavour profiles (Brown, 2009). The levels of these flavour compounds can vary significantly from one batch to another, due to variations in agricultural practices, environmental conditions, and harvesting time (Salgueiro et al., 2010). Inconsistencies of these flavour and aroma compounds of raw spices can result in variations in flavour and sensory attributes of processed meat products, making it challenging to achieve consistent product standards.

Moreover, raw spices significantly impact the safety of the products they are used in, as they have a high potential for contamination by microorganisms and environmental contaminants such as insects, feces materials from rodents and birds, dust, and other foreign substances throughout their cultivation, harvesting, post-harvest treatments, storage, and transport (Jakubowska-Gawlik et al., 2021). Raw spices are highly susceptible to the growth of various pathogenic and food spoilage microorganisms such as *Salmonella, Escherichia coli, Staphylococcus, Clostridium, Bacillus, and Listeria* (Banerjee and Sarkar, 2003; Schweiggert et al., 2007). Manufacturers often purchase raw spices in bulk to cater to large-scale production, and it typically involves storing them for an extended period. Microorganisms can grow and contaminate spices during this time under improper temperature control or high humidity. Further, during long-term storage, raw spices undergo flavour degradation due to microbial growth, enzymatic reactions, oxidation, and moisture absorption resulting in reduced flavour intensity and the development of undesirable off-flavours (Schweiggert et al., 2007). Adulteration is another problem that increases the likelihood of quality and safety issues in spices (Salgueiro et al., 2010). Raw spices often lack comprehensive quality assurance systems to ensure their quality, posing challenges for manufacturers in assessing the purity, authenticity, and safety of the raw spices they purchase.

Recently, spice oleoresins have emerged as a promising alternative to raw spices in the food industry. Oleoresins are concentrated extracts obtained from spices through a process that involves grinding or crushing the spices and then extracting them using a solvent extraction, supercritical CO_2 extraction or other methods (Shahidi and Hossain, 2018). Spice oleoresins exhibit the full spectrum of flavour, aroma, and pungency found in fresh or dried raw spices. This is primarily attributed to their high concentration of nonvolatile and volatile flavour constituents, which encompass essential elements such as resins and gums naturally present in spices (Brown, 2009). Compared to raw spices, oleoresins have become more advantageous in many aspects. Oleoresins provide a concentrated and standardized flavour and aroma profiles consistent across different batches. This consistency ensures that final products achieve the desired flavour, eliminating variations that can occur with raw spices. Moreover, oleoresins are devoid of microbial and extraneous matter, minimizing the risk of contaminations in the final product.

However, spice oleoresins are highly concentrated and viscous, making it difficult to distribute evenly throughout the product. As a solution, they can be dispersed in neutral carriers such as hydrolyzed starches (SHPs), salt, sugar and gum Arabic (Chranioti and Tzia, 2013; Sardar and Singhal, 2012). Dispersed oleoresins are in a powdered or granulated form, which makes them convenient to handle and incorporate into food formulations. They can be easily blended with other food ingredients, ensuring uniform distribution throughout the product.

The potential of spice oleoresins as alternatives to raw spices in the processed meat industry has been relatively understudied. The processed meat industry often relies on established formulations and practices developed over time and implementing new ingredients, such as spice oleoresin mixtures might require adjustments to existing processes. This study explores the

feasibility of replacing raw spices used in a commercial sausage with their corresponding oleoresins. The primary objective is to obtain a similar flavour profile to that of raw spices and analyzing various physicochemical and microbiological parameters to determine the suitability of spice oleoresins in terms of quality, safety and stability in processed meat products.

2. MATERIALS AND METHOD

Spice oleoresins; pepper (piperine 40%: volatile oil 20%) from STAY Naturals (Pvt.) Ltd. Sri Lanka), nutmeg (30% volatile oil), cardamom (30% volatile oil) and coriander (30% volatile oil) purchased from Greenleaf Extractions (Pvt.) Ltd. India were used. As the wall materials for the dispersion of oleoresins, modified corn starch (MS) purchased from Varalakshmi Starch Industries (Pvt.) Ltd. (moisture content 11%, pH (10% aqueous solution) 6.5, cold water solubility 1.26%, viscosity 1120 Cps) and maltodextrin (MD) purchased from Roquette Riddgi Siddhi (Pvt.) Ltd. India (pH (50% solution w/v) 4.97, apparent density 0.39 g/mL, total plate count (TPC) 30 colony forming units (CFU/g), total yeast and molds count < 10 CFU/g, *Escherichia coli* absent in 1 g, and *Salmonella* absent in 25 g) were used.

2.1. Formulation of spice oleoresin blends

Spice oleoresin blends were prepared by combining pepper, nutmeg, coriander, and cardamom oleoresins with a coating mixture of MS and MD. The available blending details for 180.0 g of raw spice mix were as follows: pepper-125.0 g, nutmeg-25.0 g, coriander-20.0 g, and cardamom-10.0 g. Based on the raw spice mix blending details, literature on raw spice to oleoresin equivalents, and numerous preliminary organoleptic trials, approximate amounts of each spice oleoresin equivalent to raw spices were determined. MS and MD were then measured to adjust the weight of the blend to180.0 g, while maintaining a 3:1 ratio of MS to MD. Each oleoresin was weighed individually using an electrical balance (Shimadzu, Model No. - D307037308) and dispersed in MS and MD mixture using a laboratory mixture until a uniform blend was obtained (formulation F1). After developing F1, it was refined by gradually adjusting the amounts of oleoresins and the MS and MD added. Consequently, formulations F2, F3, F4, F5, F6, F7, and F8 were developed. Based on a preliminary assessment performed by a panel of experts at STAY Naturals (Pvt.) Ltd., three formulations were selected for further sensory studies in which they were incorporated into sausages. A 250.0 g sample from each selected formulation was developed.

2.2 Storage of samples

Prepared samples were labelled and placed in airtight laminated pouches at ambient temperature (25 °C). Sensory analysis was performed on the same day as the sample preparation. The characteristics of the sample (selected from sensory analysis) at zero storage time were analysed within one day after preparation.

2.3. Preparation of sausages

Raw materials including chicken breasts, salt, milk powder, vegetable oil and stock powder, were purchased from Central Essence Suppliers, Kandy. Isolated Soy Protein (ISP), phosphate (TSP) and non-edible artificial cellulose casings were obtained from the Department of Animal Science, University of Peradeniya. The sausages were prepared at the processing laboratory in the same department. Chicken breasts (2.4 kg) were mixed with ice water (600.0 g), fat emulsion (ice flakes (336.0 g), ISP (56.0 g), vegetable oil (156.8 g), salt (11.2 g)), ISP (164.8 g), salt (80.0 g), milk powder (40.0 g), sugar (40.0 g), chicken stock (20.0 g), and TSP (11.2 g). All ingredients were mixed in a bowl chopper (Taifun 200, Nowicki, Poland) to obtain sausage batter. The batter was separated into five batches. The raw spice mix was added to the 1st and 2nd batches at a 2% (w/w) ratio of the batter. These batches served as the control and blind control, and were identical to each other. Three spice oleoresin blends, selected through preliminary organoleptic assessments, were added to the 3rd, 4th, and 5th batches at the same ratio as the raw spice mix (2%

w/w of the batter). All batches were stuffed into artificial cellulose casings (diameter of 27 mm) and precooked (in steam at 75 °C) until an internal temperature of 72 °C was reached. After heating, the sausages were submerged in cold iced water for 15 minutes (Šojić et al., 2017) and transferred to the food-processing laboratory at the Department of Food Science and Technology, University of Peradeniya, and stored at 4 °C until analysis.

2.4. Sensory analysis

Prepared sausages were evaluated using 'difference from control test' (DFC) by 30 untrained panelists. Assessors were provided with control sample, three test samples and blind control sample. Samples were served on small white plates coded with three-digit random numbers. Sample presentation order was balanced across all panelists so that they did not receive the samples in the same order. Panelists were asked first to evaluate the control sample and then to evaluate how different the other coded samples were from the control sample by rating the difference on a scale from 0 to 6, where 0 = no difference; 1 = very slight difference; 2 = slight/moderate difference; 3 = moderate difference; 4 = moderate/large difference; 5 = large difference; and 6 = very large difference. Sensory evaluation was conducted in individual booths at the sensory evaluation laboratory at the Department of Food Science and Technology, Faculty of Agriculture, University of Peradeniya.

2.5. Statistical analysis

Sensory data were subjected to one-way ANOVA to determine if a significant difference in flavour existed between the mean difference from control scores for 3 test samples and the blind control sample, with a confidence interval of 95% (P = 0.05). Then, Dunnett's multiple comparison test was used to compare the means of the blind control and other samples to determine which test samples differed significantly from the blind control and which did not. Based on the results, the sample with no significant difference ($p > 0.05$) from the blind control was selected as the potential spice oleoresin mixture to provide a flavour equivalent to the raw spice mix.

Selected sample was subjected to further physicochemical and microbial quality analysis.

2.6. Microbial analysis

Microbial load of the selected sample at zero storage time was analyzed using standard methods; TPC; Sri Lanka Standards (SLS) 516-part 1:2013, yeast & molds count; SLS 516-part 2 section 2:2013, coliforms; SLS 516-part 3 section 1:2013, *Escherichia coli*.; SLS 516 part 12:2013, *Salmonella* spp.; SLS 516-part 5:2013, and *Staphylococcus aureus*; SLS 516-part 6 section 1:2022.

2.7. Physicochemical property analysis

2.7.1. Stability of major flavour components

2.7.1.1. Stability of piperine

To assess the stability of piperine in the sample, the total piperine content was measured over a period of 4 weeks following the American Spice Trade Association (ASTA) Method 12.1, with slight modifications.

A 0.5000 g sample of the oleoresin blend was weighed using an electronic balance (Shimadzu, Model No. D307037308) and transferred into a 100 mL volumetric flask. Approximately 70 mL of acetone (purity 95%) was added, and the mixture was swirled occasionally for 20 minutes. The flask was then filled to the mark with acetone (purity 95%) and allowed to settle. From this solution, 5 mL was pipetted into another 100 mL volumetric flask, which was then filled to the mark with acetone (purity 95%) and shaken well. Using acetone (purity 95%) as the reference solution, the absorbance of the final solution was recorded at a wavelength of 345 nm within 15 minutes using a UV-Vis spectrophotometer (Shimadzu UV-1900).

Separately, the absorbance factor (the average of the standard absorbances, Aavg) was determined using the method specified in ASTA Method 12.1. Piperine standard (Sigma-Aldrich, catalog number P49007) was used, with acetone (purity 95%) as the solvent. This absorbance factor was considered

a constant value throughout the 4-week measurement period.
The total piperine content of the sample was determined using the following equation.

% piperine = [(A_s/A_avg) × (V/W_s × 10^6)] × 100

Where,
As= Absorbance of sample
Aavg= Average of standard absorbances, each normalized to 1 µg/mL
V= Dilution volume, milliliters
Ws= Sample weight, grams

After measuring the total piperine content in the sample over a four-week period, piperine retention (%) was calculated using the following formula.

% piperine retention= (total piperine content at 'x' storage time)/(total piperine content at zero stroage time) ×100 %

A semi-log plot of percentage retention of piperine vs. time, according to Cai et al., (1998) was done to obtain the rate constant (k) as the slope of the graph. Half-life ($t_{1/2}$) for the retention of piperine was calculated from the rate constant as 0.693/k, as follows.

Half life ($t_{(1/2)}$) = 0.693/k

2.7.1.2. Stability of key volatile compounds: sabinene, myristicin, and β-caryophyllene
To assess the stability of sabinene, myristicin, and β-caryophyllene, the amounts of each analyte in the extracted oil from the oleoresin mixture were measured over a three-week period. Clevenger light arm apparatus method was used for the extraction of the oil from the sample. For this, 50 g of the sample was measured into a 500 mL distillation flask, and a sufficient amount of water (approximately 300 mL) was added. Hydro-distillation was then carried out for 6 hours. After the extraction, oil was diluted in hexane (1:10 (v/v)). The analysis of volatile compounds was carried out using a Nexis GC-2030 (Shimadzu) chromatograph equipped with LabSolutions data handling software, coupled with a flame ionization detector (FID). A capillary column (SH-STABILWAX, 30 m length, 0.25 mm internal diameter, 0.25 µm film thickness) was used. A 0.2 µL volume of the diluted sample was injected in split mode with a split ratio of 10:1. The sampling time was 1.00 minute. The injector temperature was 200 °C. Nitrogen was used as the carrier gas with a pressure of 77.7 kPa. Total flow was 104.2 mL/min, column flow rate was 1.0 mL/min, and linear velocity was 26.1 cm/sec. The column oven temperature program was as follows: the initial temperature was 50 °C, which was held for 1 minute. The temperature was then increased at a rate of 3 °C/min to 180 °C, followed by an increase of 25 °C/min to 200 °C, which was maintained for 10 minutes. The total program time was 54.13 minutes.
After measuring the amount of sabinene, myristicin, and β-caryophyllene in the sample over three weeks, the half-life of each analyte was calculated separately, using the same method as for piperine.

2.7.2. Hygroscopicity
Three different relative humidity (RH) levels were made with saturated salts in desiccators: sodium sulphate (91% RH), sodium chloride (75% RH), and potassium carbonate (43% RH). Saturated solutions were placed at the bottom of each desiccator, with excess solid salt remaining to maintain saturation, and each desiccator was kept at 25 °C. A 5.0 g sample was placed in each desiccator for 7 days. After 7 days, hygroscopic moisture was measured relative to the initial weight of the sample at all three RH levels and expressed as grams of moisture per 100 grams of dry solids (g/100 g).

2.7.3. Wettability

Wettability of the sample was determined at zero storage time according to the method described by (Fernandes et al., 2014). One gram of the sample was sprinkled over the surface of 50 mL of distilled water at 20 °C without agitation. The time taken for the sample particles to disappear from the surface of water was recorded using a stopwatch.

2.7.4 Flow characteristics

Bulk density (ρB) and tapped density (ρT) of the sample were determined at zero storage time as per the method described by (Arshad et al., 2020). Three grams of the sample (m_o) were poured into a 25 mL graduated glass cylinder, and the initial sample volume (v_b) was read directly from the cylinder. The cylinder was then tapped 200 times for the tapped density, and the final volume (v_t) was recorded. Bulk density and tapped density of the sample were calculated using the following equations.

$\rho B = m_o/v_b$

$\rho T = m_o/v_t$

Flowability was determined by Carr's index (C) and Hausner ratio (HR) using following equations.

$C = [(\rho T - \rho B)/\rho T] \times 100$

$HR = \rho T/\rho B$

2.7.5. Colour value

Colour value of the sample at zero storage time was measured using a digital handheld colorimeter (CS-10) in triplicates.

3. RESULTS AND DISCUSSION

3.1. Selection of a carrier agent

Spice oleoresins are free from many inherent disadvantages associated with their fresh or dried spice counterparts. However, they are immiscible in aqueous mediums and have a thick, viscous nature that complicates their handling and blending into food systems (Peter and Shylaja, 2012). They are also sensitive to light, heat, and oxygen, which can shorten their storage life if not stored properly (Shaikh et al., 2016; Balasubramani et al., 2015). To address these issues, oleoresins can be dispersed onto a soluble carrier matrix and converted into a dry powder form using various techniques, such as direct blending and adsorption, spray drying, freeze drying, extrusion coating, coacervation, and fluidized bed coating (Fuchs et al., 2006; Nedovic et al., 2011).

Most commonly used carriers include salt, starch, MS, MD, gum arabic, and proteins (whey protein and casein) and lipids (Krishnan et al., 2005; Fernandes et al., 2014; Arshad et al., 2020). In the present study, MD and MS were used as the carrier agents, considering their continuous supply, availability and internal cost considerations.

MD is produced through the partial hydrolysis of starch using enzymes and/or acids (Fernandes et al., 2014). MD has a neutral flavour and aroma, high water solubility, and provides a strong oxidation stability for core materials (Carneiro et al., 2013; Setyaningsih et al., 2020). Native corn starch is subjected to physical and/or chemical modifications such as destabilization and cross-linking and converted to modified starch (MS). MS possesses enhanced properties, including improved rheological characteristics, textural qualities, optical properties, and stability (Wongphan and Harnkarnsujarit, 2020). When added to food products like processed meats, modified starches act as binders to help maintain juiciness and tenderness while also improving freeze/thaw stability and texture (Totosaus, 2019; Piotrowska et al., 2004).

Several studies have investigated the suitability of maltodextrin and modified starch as carrier agents for oleoresins (Shaikh et al., 2006; Wang et al., 2014; Krishnan et al., 2005). When used as carrier agents, binary blends of starch and maltodextrin have shown greater efficiency in terms of the stability of flavours, rather than their individual use (Kanakdande et al., 2007). In the present study, a 3:1 ratio of MS to MD was used, based on previous internal experiments assessing various MS to MD ratios for their rheological properties and cost considerations.

3.2. Formulation development and preliminary sensory evaluation

In the present study, the objective was to replace the flavour of the raw spice mixture with an oleoresin blend. However, to maintain the filling properties provided by the raw spices in the sausage, the oleoresin blend needed to be used in the same amount by weight as the raw spice blend. Thus, the flavour intensity per unit in both the developed oleoresin blends and the raw spice mix needed to remain the same.

Table 1. Amounts of oleoresins, MS and MD used in different formulations

	F3 (g)	F2 (g)	F1 (g)	F4 (g)	F5 (g)	F6 (g)	F7 (g)	F8 (g)
Cardamom	0.050	0.100	0.200	0.300	0.400	0.500	0.600	0.700
Coriander	0.850	0.900	1.000	1.100	1.200	1.300	1.400	1.500
Nutmeg	1.100	1.150	1.250	1.350	1.450	1.550	1.650	1.750
Pepper	6.100	6.150	6.250	6.350	6.450	6.550	6.750	6.850
Starch	128.925	128.700	128.475	128.250	128.025	127.800	127.575	127.350
Maltodextrin	42.975	43.000	42.825	42.650	42.475	42.300	42.025	41.850
Total weight	180	180	180	180	180	180	180	180

In formulating the blends, although the amounts of oleoresins varied slightly between samples, even small changes could lead to a disproportionate shift in the flavour balance due to their concentrated nature, resulting in significantly different flavour profiles between formulations.

As per the assessment by a panel of experts at STAY Naturals (Pvt.) Ltd., formulations F2 and F3 exhibited a reduced flavour intensity in contrast to the raw spice mix. Formulations F6, F7, and F8 had an increased flavour intensity. Both formulations F7 and F8 had a highly pungent flavour, indicating a substantial quantity of added pepper oleoresin. Formulations F1, F4, and F5 were satisfactory in terms of flavour comparison with the raw spice mix. These three formulations were added to sausages for sensory evaluation to identify the most comparable sample to the raw spice mix.

Table 2. Codes of sausage samples used in sensory evaluation

Sausage Sample	Oleoresin/raw spice mix added	Code
SC (blind control)	Raw spice	C
SF1	Sample F1	384
SF4	Sample F4	491
SF5	Sample F5	567
SC (blind control)	Raw spice	785

3.3 Sensory evaluation

Based on average DFC data and calculated *P*-value, a statistically significant difference was observed between test samples and blind control ($P < 0.05$). Since a significant difference was observed, Dunnett's multiple comparison test was performed to determine which test samples exhibited significant differences and to identify the sample that closely resembled the flavour of blind control.

According to results, samples SF1 and SF4 showed statistically significant differences compared to blind control ($P < 0.05$). Sample SF5 demonstrated no statistically significant difference in flavour compared to the blind control ($P=0.238$) suggesting formulation F5 as a suitable substitute for the raw spice mix.

Table 3. Dunnett's Simultaneous Tests for Level Mean - Control Mean

Sample	Mean	Difference of Means with blind control	P – value
F1	2.567	1.500	0.000
F4	2.800	1.733	0.000
F5	1.567	0.500	0.238
C	1.067		

Table 4. Grouping Information Using the Dunnett's Method and 95 % Confidence

Factor	Mean	Grouping
C (control)	1.067	A
F4	2.800	
F1	2.567	
F5	1.567	A

Means not labelled with the letter 'A' are significantly different from the control level mean.

3.4. Microbial analysis

Raw spices used in food industry frequently harbor unacceptable levels of microorganisms (Witkowska et al., 2011; Salgueiro et al., 2010; Banerjee and Sarkar, 2003). Many studies have documented various spice-related outbreaks and product recalls due to the consumption of contaminated spices, with *Salmonella* being frequently associated with these incidents (Witkowska et al., 2011; Beuchat et al., 2013; Van Doren et al., 2013). In raw spices, presence of high moisture content, organic matter, and nutrients makes an ideal environment for microbial growth. They acquire significantly high amounts of microbes during cultivation, harvesting, drying, post-processing and storage where they are exposed to excess heat, humidity, dust, dirt and fecal materials from birds, rodents and other animals (Salgueiro et al., 2010; Tassou et al., 2012). In contrast, spice oleoresins undergo the extraction of essential oils and active compounds, effectively removing much of the moisture and organic matter that would otherwise support microbial growth (Sowbhagya, 2019). High concentrations of antibacterial compounds in oleoresins also contribute to their increased safety by reducing the likelihood of microbial growth (Figueroa-Lopez et al., 2018; Procopio et al., 2018). Further, spice oleoresins in liquid and dry powder forms are often packaged and stored in airtight containers, protecting them from environmental contaminants and reducing the risk of contamination during transportation and storage.

European Spice Association (ESA) and International Commission on Microbiological Specifications for Foods (ICMSF) have established microbiological requirements for spices and herbs. ESA specifies that *Salmonella* spp. should be absent in 25 g, *Escherichia coli* should be < 10^2 CFU/g and yeast and molds should be < 10^5 CFU/g (ESA, 2004). According to ICMSF, total bacterial count should be < 10^6 CFU/g (ICMSF, 1974). In sample F5, TPC was 1.3 x 102 CFU/g, and yeast and molds count were 7.0 x 10^2 CFU/g. Coliform bacteria were not detected in the sample, and

Salmonella was absent in a 25 g portion. *Staphylococcus aureus* was less than 10 CFU/g. Currently, there are no microbiology standards specifically established for dispersed oleoresins; however, based on the existing standards, sample F5 meets the acceptable criteria of spices for all tested parameters with its initial microbial load.

3.5. Stability of total piperine, sabinene, myristicin and β-caryophyllene

Spice oleoresins are sensitive to light, heat, and oxygen, which can lead to a reduction in their storage life if not stored properly (Krishnan et al., 2005; Balasubramani et al., 2015). For instance, poor storage life of black pepper oleoresin is attributed to oxidative and polymeric changes affecting the fatty oil component and monoterpene hydrocarbons. During prolonged storage, chemical and organoleptic changes can also take place and these chemical transformations result in a loss of colour and flavour in the oleoresin, adversely affecting its quality and desirability as a spice extract. However, dispersing oleoresins in carrier matrices enhances their stability, protects their active compounds, and ensures consistent flavour and aroma release in food applications (Balasubramani et al., 2015; Wang et al., 2014; Procopio et al., 2023).

Piperine is an alkaloid that serves as the primary pungent constituent in pepper (*Piper nigrum* L.) (Sirinivasan, 2007). Myristicin is one of the main constituents of the essential oil of nutmeg (*Myristica fragrans*) and the primary psychoactive and hallucinogenic substance in the spice (Nowak, 2016). Sabinene is the primary terpene constituent of cardamom (*Elettaria cardamomum*) essential oil, classified as a bicyclic monoterpene (Sharma et al., 2019; Zachariah and Leela, 2018). β-caryophyllene is largely found in volatile oil of black pepper (Gertsch et al., 2008).

The findings of the study indicate that the oleoresin blend tends to lose key volatile and nonvolatile components under the reported storage conditions. Stability of total piperine, sabinene, myristicin, and β-caryophyllene was assessed by plotting the natural logarithm of the percentage retention (ln% retention) against storage time in weeks, and it shows a decrease in all the constituents. Linear nature of graphs indicates that these flavour components' degradation follows first - order kinetics. Determination of reaction order was based on correlation coefficients (R^2) for each analyte. Half-life ($t_{1/2}$), which represents the time required for a value to decrease to 50% of its initial value, was calculated using the slope 'k' of the semi-log plot according to the formula $t_{1/2} = 0.693/k$. Calculated $t_{1/2}$ values in sample F5 for piperine, sabinene, myristicin, and β-caryophyllene were 41.25, 12.38, 12.83, and 53.31 weeks respectively. Retention of analytes was in the order of β-caryophyllene > piperine > myristicin > sabinene.

Table 5. Regression analysis of total piperine, sabinene, myristicin and β-caryophyllene in sample F5

Analyte	Regression equation	$t_{(1/2)}$(weeks)
Total piperine	Y = -0.0168X + 4.6122 R^2 = 0.8789	41.25
Sabinene	Y = -0.056X + 4.614 R^2 = 0.9011	12.38
Myristicin	Y = -0.054X + 4.606 R^2 = 0.9918	12.83
β-Caryophyllene	Y = -0.013X + 4.607 R^2 = 0.9657	53.31

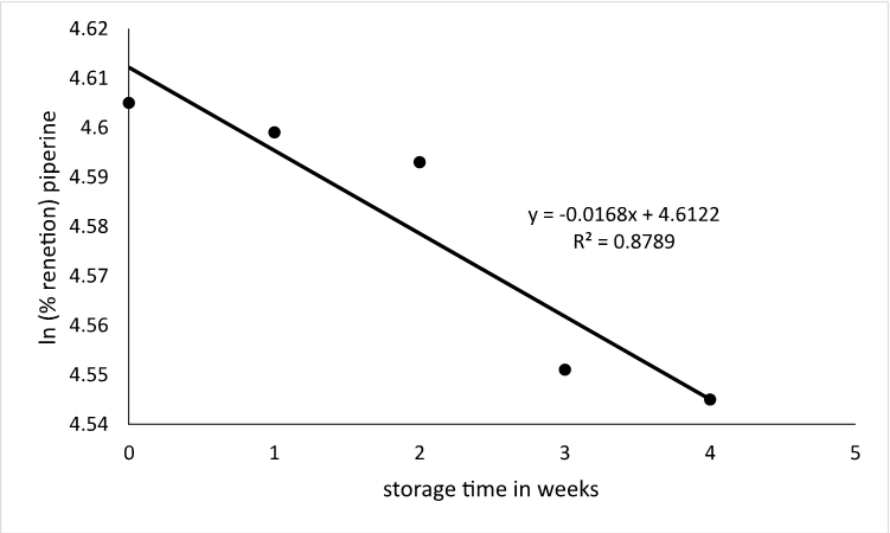

Figure 1. Stability of total piperine content in sample F5 during 4 weeks of storage

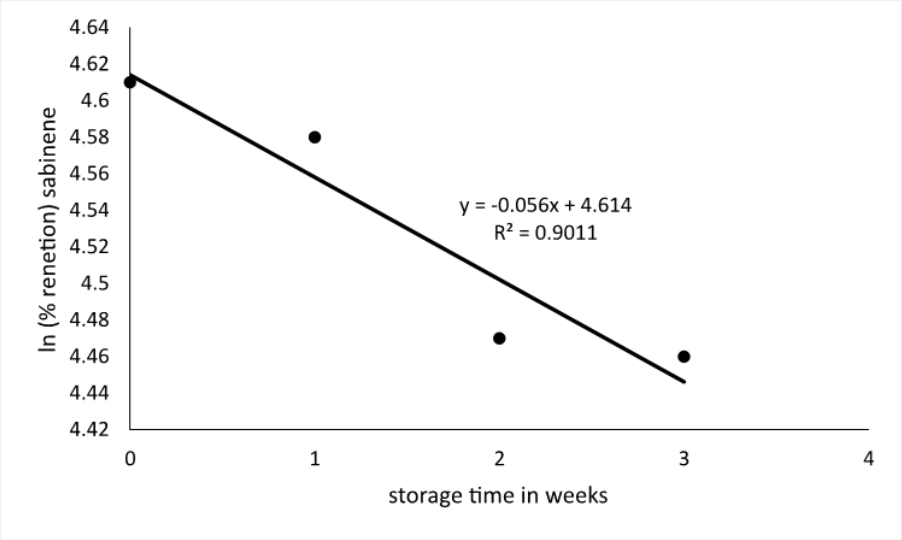

Figure 2. Stability of sabinene in sample F5 during 3 weeks of storage

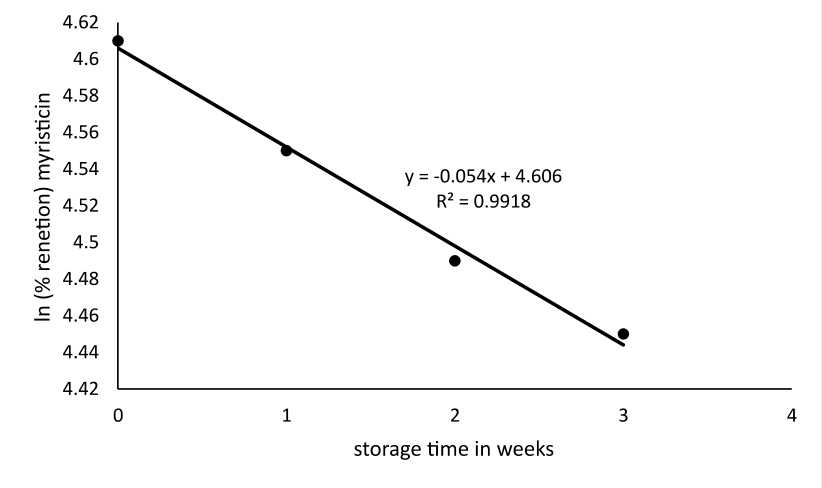

Figure 3. Stability of myristicin in sample F5 during 3 weeks of storage

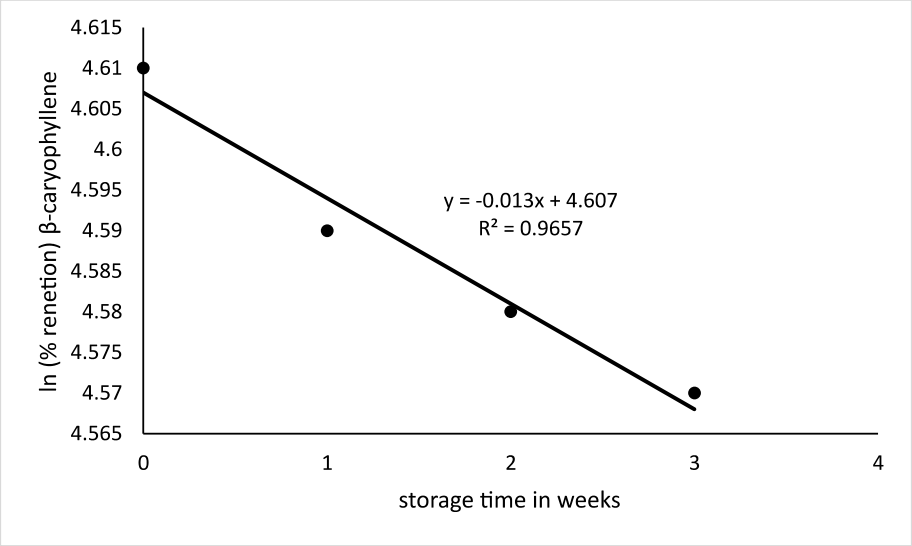

Figure 4. Stability of β- caryophyllene in sample F5 during 3 weeks of storage

3.6 Hygroscopicity

Hygroscopicity (%) of sample F5 increased significantly from week 1 to week 4 (P<0.05) at all relative humidity (RH) levels: 43%, 75%, and 91%. In addition, a significant difference in hygroscopicity (%) was observed between three RH levels each week (P<0.05). Hygroscopicity (%) of powder stored at RH 43%, 75% and 91% increased from an initial value of 3.31 %, 4.09% and 5.17% to 4.55%, 6.65% and 6.66% respectively from week 1 to week 4. Highest hygroscopicity (%) was observed in the powder which was stored at RH 91 %, and the lowest hygroscopicity (%) was observed in the powder stored at RH 43%. Observed moisture absorbance behavior aligned well with visual observations concerning the alterations in physical characteristics of sample F5 over four weeks. Sample F5, which was stored at the highest RH (91%) showed more caking and aggregates at the end of 4th week, indicating high moisture absorbance.

Results of the study demonstrate the susceptibility of oleoresin powder and its response to exposure in highly humid environments. Oleoresins are hydrophobic in nature; however, MD is highly hygroscopic and MS is moderately hygroscopic (Juarez-Enriquez et al., 2017). This can lead to moisture adsorption, resulting in unfavorable textural changes, reduced shelf life, and potential microbial growth in the powder. Therefore, it is recommended to store the powder in a low relative humidity environment.

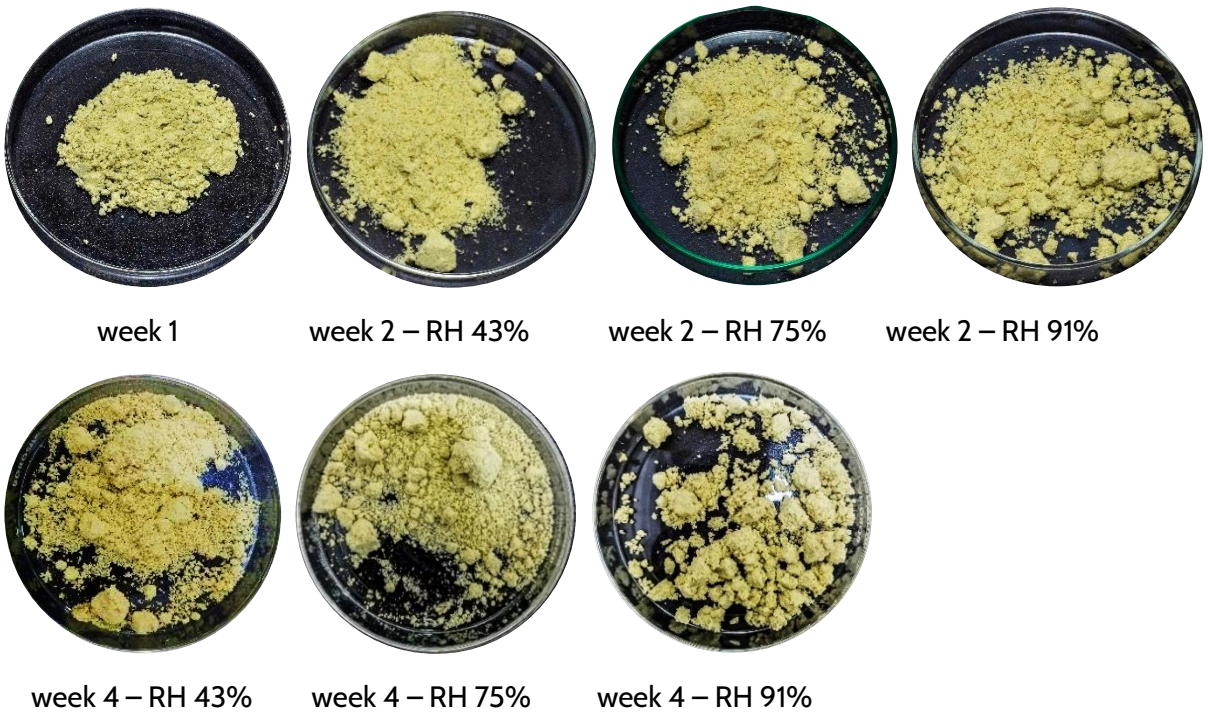

Figure 5. Changes in the physical appearance of sample F5 observed at different RH levels at 25 °C

3.7. Wettability

The wettability of sample F5 was 10.10 ± 0.21 min. Wettability of a powder is one of the fundamental aspects, interpreted as the ability of bulk powder to preferentially wet a solid surface under capillary forces . The lesser time it takes for the powder to become wet (disappear from the surface) and completely dissolve in water, the better off it will be in terms of its physical characteristics (Arshad et al., 2020). Physical properties of a powder including particle size, density, porosity and temperature have a direct impact on powder wettability (Jóźwiak et al., 2018). Powders with wetting times of less than 1 minute are referred to as easy wetting, whereas powders with wetting times of more than 5 minutes are referred to as poor wetting powders (Fitzpatrick et al., 2017). The poor wettability of sample F5 can be attributed to the hydrophobic nature and low apparent density of the dispersed oleoresins within the sample.

3.8. Flow characteristics of oleoresin mixture

Powder flow can be defined as the movement of a bulk of particles relative to each other or along the surface of the container wall. Flowability of a powder is a complex phenomenon determined by various particle properties (such as composition, density, moisture content, surface properties, size distribution, shape, surface friction, and compressibility) as well as powder properties (including size distribution, bulk density, homogeneity, and cohesiveness). Additionally, numerous internal and external factors, such as temperature, air relative humidity, hopper dimensions and design, and discharge aids, also influence powder flowability (Juliano and Barbosa-Cánovas, 2010).

Carr's index (CI) and Hausner ratio (HR) are two qualitative descriptives that can be used to characterize powder flowability through the correlation between bulk density and tapped density. According to United States Pharmacopeia standards, a powder's flowability can be rated with CI values as very good (< 15), good (15-20), fair (20-35), bad (35 - 45) and very bad (> 45). According to HR values, cohesiveness can be rated as, low (< 1.2), intermediate (1.2-1.4), and high (>1.4) (Procopio et al., 2018). Cohesion arises from interparticle attractions and internal forces within the bulk, which resist the planar sliding of one particle's

surface against another; thus, an increase in cohesion decreases flowability (Juliano and Barbosa-Cánovas, 2010).

Bulk density and tapped density of sample F5 were 358.57 ± 6.59 kg/m^3 and 554.88 ± 9.10 kg/m^3 respectively. CI index and HR values were 35.36 ± 2.25 and 1.55 ± 0.05 respectively, indicating sample F5 has poor flowability and high cohesiveness.

3.9. Colour value

Colour values obtained for sample F5 were L* = 71.69 ± 0.98; a* = - 4.48 ± 0.63; b* = 16.66 ± 0.64. L* represents the lightness ranging from a scale of 0 = black to 100 = white. Sample F5 has a relatively high L* value indicating more lightness. A positive a* value indicates a red-purple colour, whereas a negative a* value indicates green colour. A negative a* value obtained for the powder indicates it has a predominant greenish colour. A positive b* value represents yellow colour and a negative b* value represents blue colour. The positive b* value obtained for the powder indicates it has a more predominant yellow colour. These values were well in agreement with the visual aspect observed in sample F5.

4. CONCLUSION

In conclusion, this study highlights the sensory acceptance of dispersed oleoresin blends in substituting the flavour profile of conventional raw spice mixes. However, it also emphasizes the need for further research and development to optimize specific physicochemical properties of dispersed oleoresins, including flow characteristics, wettability, and storage stability. Given that oleoresins offer numerous advantages over raw spices in terms of their consistent flavour, year-round availability, ease of handling, and high safety in terms of microbiological quality, enhancements in these physicochemical properties could broader the adoption of dispersed oleoresins in commercial food products.

Ethical Approval

None.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Literature review: WMAS, MBEP
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REFERENCES

Bolívar-Monsalve, C. Ramírez-Toro, G. Bolívar, and C. Ceballos-González, "Mechanisms of action of novel ingredients used in edible films to preserve microbial quality and oxidative stability in sausages - A review," Trends Food Sci. Technol., vol. 89, no. May, pp. 100–109, 2019, doi: 10.1016/j.tifs.2019.05.011.

M. Elias, M. Laranjo, A. C. Agulheiro-Santos, M. E. Potes, and A. V. Carrascosa, "Quality of nonmeat ingredients used in the manufacturing of traditional dry-cured pork sausages," J. Food Process. Preserv., vol. 45, no. 3, pp. 1–9, 2021, doi: 10.1111/jfpp.15213.

S. M. Lonergan, D. G. Topel, and D. N. Marple, "Sausage processing and production," Sci. Anim. Growth Meat Technol., pp. 229–253, 2019, doi: 10.1016/b978-0-12-815277-5.00014-7.

A. M. Witkowska, D. K. Hickey, M. Alonso-Gomez, and M. G. Wilkinson, "The microbiological quality of commercial herb and spice preparations used in the formulation of a chicken supreme ready meal and microbial survival following a simulated industrial heating process," Food Control, vol. 22, no. 3–4, pp. 616–625, Mar. 2011, doi: 10.1016/j.foodcont.2010.10.014.

P. M. Brown, "Spices , Seasonings , and Flavors," pp. 199–210, 2009, doi: 10.1007/978-0-387-71327-4.

L. Salgueiro, A. P. Martins, and H. Correia, "Raw materials: the importance of quality and safety. A review. †," vol. 25, pp. 253–271, 2010, doi: 10.1002/ffj.

K. Jakubowska-Gawlik, W. Kolanowski, and J. Trafialek, "Evaluating suppliers of spices, casings and packaging to a meat processing plant using food safety audits data gathered during a 13-year period," Food Control, vol. 127, no. February, p. 108138, 2021, doi: 10.1016/j.foodcont.2021.108138.

M. Banerjee and P. K. Sarkar, "Microbiological quality of some retail spices in India," Food Res. Int., vol. 36, no. 5, pp. 469–474, 2003, doi: 10.1016/S0963-9969(02)00194-1.

U. Schweiggert, R. Carle, and A. Schieber, "Conventional and alternative processes for spice production - a review," Trends Food Sci. Technol., vol. 18, no. 5, pp. 260–268, May 2007, doi: 10.1016/j.tifs.2007.01.005.

F. Shahidi and A. Hossain, "Bioactives in spices, and spice oleoresins: Phytochemicals and their beneficial effects in food preservation and health promotion," J. Food Bioact., vol. 3, pp. 8–75, 2018, doi: 10.31665/jfb.2018.3149.

C. Chranioti and C. Tzia, "Binary Mixtures of Modified Starch, Maltodextrin and Chitosan as Efficient Encapsulating Agents of Fennel Oleoresin," Food Bioprocess Technol., vol. 6, no. 11, pp. 3238–3246, 2013, doi: 10.1007/s11947-012-0966-7.

S. Krishnan, A. C. Kshirsagar, and R. S. Singhal, "The use of gum arabic and modified starch in the microencapsulation of a food flavoring agent," Carbohydr. Polym., vol. 62, no. 4, pp. 309–315, Dec. 2005, doi: 10.1016/j.carbpol.2005.03.020.

B. R. Sardar and R. S. Singhal, "Characterization of co-crystallized sucrose entrapped with cardamom oleoresin," J. Food Eng., vol. 117, no. 4, pp. 521–529, 2013, doi: 10.1016/j.jfoodeng.2012.12.011.

B. Šojić et al., "Antioxidant activity of juniperus communis L. essential oil in cooked pork sausages," Czech J. Food Sci., vol. 35, no. 3, pp. 189–193, 2017, doi: 10.17221/210/2016-CJFS.

Y. Cai, M. Sun, and H. Corke, "Colorant Properties and Stability of Amaranthus Betacyanin Pigments," J. Agric. Food Chem., vol. 46, no. 11, pp. 4491–4495, 1998, doi: 10.1021/jf980457g.

R. V. D. B. Fernandes, S. V. Borges, and D. A. Botrel, "Gum arabic/starch/maltodextrin/inulin as wall materials on the microencapsulation of rosemary essential oil," Carbohydr. Polym., vol. 101, no. 1, pp. 524–532, 2014, doi: 10.1016/j.carbpol.2013.09.083.

H. Arshad, T. M. Ali, and A. Hasnain, "Comparative study on efficiency of nutmeg microencapsulation (freeze-drying method) using native and OSA sorghum starch as wall materials in combination with gum arabic," Cereal Chem., vol. 97, no. 3, pp. 589–600, 2020, doi: 10.1002/cche.10275.

K. V. Peter and M. R. Shylaja, Introduction to herbs and spices: Definitions, trade and applications, Second Edi., vol. 1. Woodhead Publishing Limited, 2012. doi: 10.1533/9780857095671.1.

J. Shaikh, R. Bhosale, and R. Singhal, "Microencapsulation of black pepper oleoresin," Food Chem., vol. 94, no. 1, pp. 105–110, Jan. 2006, doi: 10.1016/j.foodchem.2004.10.056.

P. Balasubramani, P. T. Palaniswamy, R. Visvanathan, V. Thirupathi, A. Subbarayan, and J. Prakash Maran, "Microencapsulation of garlic oleoresin using maltodextrin as wall material by spray drying technology," Int. J. Biol. Macromol., vol. 72, pp. 210–217, 2015, doi: 10.1016/j.ijbiomac.2014.08.011.

M. Fuchs et al., "Encapsulation of oil in powder using spray drying and fluidised bed agglomeration," J. Food Eng., vol. 75, no. 1, pp. 27–35, 2006, doi: 10.1016/j.jfoodeng.2005.03.047.

V. Nedovic, A. Kalusevic, V. Manojlovic, S. Levic, and B. Bugarski, "An overview of encapsulation technologies for food applications," Procedia Food Sci., vol. 1, pp. 1806–1815, 2011, doi: 10.1016/j.profoo.2011.09.265.

H. C. F. Carneiro, R. V. Tonon, C. R. F. Grosso, and M. D. Hubinger, "Encapsulation efficiency and oxidative stability of flaxseed oil microencapsulated by spray drying using different combinations of wall materials," J. Food Eng., vol. 115, no. 4, pp. 443–451, 2013, doi: 10.1016/j.jfoodeng.2012.03.033.

D. Setyaningsih, D. Kurniawan, and N. Muna, "Encapsulation of ginger oleoresin with a combination of maltodextrin and skim milk powder as wall material," in IOP Conference Series: Earth and Environmental Science, Jun. 2020, vol. 472, no. 1. doi: 10.1088/1755-1315/472/1/012016.

P. Wongphan and N. Harnkarnsujarit, "Characterization of starch, agar and maltodextrin blends for controlled dissolution of edible films," Int. J. Biol. Macromol., vol. 156, pp. 80–93, Aug. 2020, doi: 10.1016/j.ijbiomac.2020.04.056.

A. Totosaus, "The use of potato starch in meat products," Food, vol. 3, no. 1, pp. 102–108, 2009, [Online]. Available: http://www.globalsciencebooks.info/JournalsSup/09FOOD_3_SI1.html

E. Piotrowska, W. Dolata, H. M. Baranowska, and R. Rezler, "Quality Assessment of Finely Comminuted Sausages," Acta Agrophysica, vol. 4, no. 1, pp. 129–139, 2004.

Q. Wang et al., "Optimization and Evaluation of Microencapsulation of Star Anise Oleoresin," J. Food Process. Preserv., vol. 38, no. 5, pp. 2129–2136, 2014, doi: 10.1111/jfpp.12193.

S. Krishnan, R. Bhosale, and R. S. Singhal, "Microencapsulation of cardamom oleoresin: Evaluation of blends of gum arabic, maltodextrin and a modified starch as wall materials," Carbohydr. Polym., vol. 61, no. 1, pp. 95–102, Jul. 2005, doi: 10.1016/j.carbpol.2005.02.020.

D. Kanakdande, R. Bhosale, and R. S. Singhal, "Stability of cumin oleoresin microencapsulated in different combination of gum arabic, maltodextrin and modified starch," Carbohydr. Polym., vol. 67, no. 4, pp. 536–541, Feb. 2007, doi: 10.1016/j.carbpol.2006.06.023.

L. R. Beuchat et al., "Low-water activity foods: Increased concern as vehicles of foodborne pathogens," J. Food Prot., vol. 76, no. 1, pp. 150–172, Jan. 2013, doi: 10.4315/0362-028X.JFP-12-211.

J. M. Van Doren, K. P. Neil, M. Parish, L. Gieraltowski, L. H. Gould, and K. L. Gombas, "Foodborne illness outbreaks from microbial contaminants in spices, 1973-2010," Food Microbiology, vol. 36, no. 2. pp. 456–464, Dec. 2013. doi: 10.1016/j.fm.2013.04.014.

C. Zweifel and R. Stephan, "Spices and herbs as source of Salmonella-related foodborne diseases," Food Research International, vol. 45, no. 2. pp. 765–769, Mar. 2012. doi: 10.1016/j.foodres.2011.02.024.

C. C. Tassou, N. G. Chorianopoulos, P. N. Skandamis, and G. J. E. Nychas, "Herbs, spices and their active components as natural antimicrobials in foods," in Handbook of Herbs and Spices: Second Edition, vol. 2, Elsevier Inc., 2012, pp. 17–50. doi: 10.1533/9780857095688.17.

H. B. Sowbhagya, "Value-added processing of by-products from spice industry," pp. 73–80, 2019, doi: 10.1093/fqsafe/fyy029.

K. J. Figueroa-Lopez, M. M. Andrade-Mahecha, and O. L. Torres-Vargas, "Spice oleoresins containing antimicrobial agents improve the potential use of bio-composite films based on gelatin," Food Packag. Shelf Life, vol. 17, pp. 50–56, Sep. 2018, doi: 10.1016/j.fpsl.2018.05.005.

F. R. Procopio et al., "Solid lipid microparticles loaded with cinnamon oleoresin: Characterization, stability and antimicrobial activity," Food Res. Int., vol. 113, pp. 351–361, 2018, doi: 10.1016/j.foodres.2018.07.026.

F. R. Procopio, S. Klettenhammer, and G. Ferrentino, "Comparative study of cinnamon and paprika oleoresins encapsulated by spray chilling and particles from gas saturated solutions techniques: evaluation of physical characteristics and oleoresins release in food simulated media," pp. 1–21.

K. Srinivasan, "Black pepper and its pungent principle-piperine: A review of diverse physiological effects," Critical Reviews in Food Science and Nutrition, vol. 47, no. 8. pp. 735–748, Nov. 2007. doi: 10.1080/10408390601062054.

J. Nowak, M. Woźniakiewicz, M. Gładysz, A. Sowa, and P. Kościelniak, "Development of Advance Extraction Methods for the Extraction of Myristicin from Myristica fragrans," Food Anal. Methods, vol. 9, no. 5, pp. 1246–1253, 2016, doi: 10.1007/s12161-015-0300-x.

S. Sharma, J. Gupta, P. K. Prabhakar, P. Gupta, P. Solanki, and A. Rajput, "Phytochemical Repurposing of Natural Molecule: Sabinene for Identification of Novel Therapeutic Benefits Using in Silico and in Vitro Approaches," Assay Drug Dev. Technol., vol. 17, no. 8, pp. 339–351, 2019, doi: 10.1089/adt.2019.939.

T. J. Zachariah and N. K. Leela, Spices: Secondary metabolites and medicinal properties. 2018. doi: 10.1007/978-3-319-75016-3_10.

J. Gertsch et al., "Beta-caryophyllene is a dietary cannabinoid," Proc. Natl. Acad. Sci. U. S. A., vol. 105, no. 26, pp. 9099–9104, 2008, doi: 10.1073/pnas.0803601105.

E. Juarez-Enriquez, G. I. Olivas, P. B. Zamudio-Flores, E. Ortega-Rivas, S. Perez-Vega, and D. R. Sepulveda, "Effect of water content on the flowability of hygroscopic powders," J. Food Eng., vol. 205, pp. 12–17, 2017, doi: 10.1016/j.jfoodeng.2017.02.024.

B. Jóźwiak, M. Dziubiński, and M. Orczykowska, "Wettability of commercial starches and galactomannans," J. Dispers. Sci. Technol., vol. 39, no. 8, pp. 1085–1092, 2018, doi: 10.1080/01932691.2017.1382374.

J. J. Fitzpatrick, J. Salmon, J. Ji, and S. Miao, "Characterisation of the wetting behaviour of poor wetting food powders and the influence of temperature and film formation," KONA Powder Part. J., vol. 2017, no. 34, pp. 282–289, 2017, doi: 10.14356/kona.2017019.

P. Juliano and G. V. Barbosa-Cánovas, "Food powders flowability characterization: Theory, methods, and applications," Annu. Rev. Food Sci. Technol., vol. 1, no. 1, pp. 211–239, 2010, doi: 10.1146/annurev.food.102308.124155.

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