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Microencapsulation by Freeze-Drying of Potassium Norbixinate and Curcumin with Maltodextrin: Stability, Solubility, and Food Application

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ABSTRACT: Stability of potassium norbixinate and curcumin by microencapsulation with maltodextrin DE20 and freeze-drying was evaluated as a function of exposition to light, air, different pH, water solubility, and in food applications. The best results were obtained with microencapsulated potassium norbixinate 1:20, which, when vacuum-packed and in the presence of natural light, showed color retention of 78%, while microencapsulated curcumin 1:20 showed color retention of 71%. Differential scanning calorimetry and thermogravimetry provided an indication of interaction between colorants and maltodextrin. Photoacoustic spectroscopy (PAS) showed that free and microencapsulated colorants exhibited high rates of absorption throughout the measured spectral region. This work evidenced that the freeze-drying process is favorable for microencapsulation of curcumin by maltodextrin, providing improved solubility to the microencapsulated colorant. Both microencapsulated colorants showed relevant results for use in a wide range of pH and food applications. The PAS technique was useful for the evaluation of the stability of free and microencapsulated colorants.

KEYWORDS: *potassium norbixinate, curcumin, maltodextrin, microencapsulation*

INTRODUCTION

Color is one of the most important attributes in the food industry, affecting the products' acceptability by consumers. Reports of health hazards due to the toxicity of synthetic colorants have caused changes in the food industry toward increased usage of natural colorants.[1,2]

Annatto (*Bixa orellana* L.) represents about 90% of the natural colorants used in Brazil and 70% worldwide. In 2008, it was estimated that global production was 17,500 tons, with Brazil accounting for approximately 12,000 tons. However, only 25% of the total industrial annatto seeds are used in the preparation of water-soluble extracts. The dairy industry is the biggest consumer as they are used to color cheeses and yogurt, as well as other products such as sausages, salami, salad dressings, breakfast cereals, pasta, sweets, ice creams, chocolates, soft drinks, and liqueurs.[3−5]

The water-soluble annatto extract can be isolated from annatto seeds (exocarp) by abrasion or by agitation in aqueous alkaline solution at temperatures below 70 °C. With alkaline hydrolysis of *cis*-bixin, sodium norbixinate ($C_{24}H_{26}Na_2O_4$) or potassium norbixinate ($C_{24}H_{26}K_2O_4$) is obtained. Bixin is the major pigment in annatto and represents about 80% of the carotenoids in the seed coat. The color of the hydrosoluble annatto extract varies from red to brown, and its stability is affected by light exposition, thermal processes, oxygen, and pH.[6−10] Norbixinate (sodium or potassium salt of norbixin) widens the spectrum of use of annatto seeds because it is soluble in water.[11,12]

Curcumin ($C_{21}H_{20}O_6$), a yellow−orange natural polyphenol, is obtained by solvent extraction of the rhizomes of *Curcuma longa*. To obtain concentrated curcumin powder, oil-resin crystallization is carried out from a tubercle with a purity of approximately 95%. It is widely used as food coloring agent. It may provide protection against several chronic diseases, including cancer and HIV infection, as well as neurological, cardiovascular, and skin diseases.[13] This colorant is insoluble in water and ether, it is degraded in alkaline solution, and unstable in the presence of light, factors that usually limit its application as a food colorant.[14] India is the world's largest producer of *Curcuma longa* tubercles, and its production is estimated to be between 250 to 300 tons/year. Most of these tubercles are consumed for flavor, and only a small fraction (1−1.5 tons) is converted to extracts.[5]

The food industry has encouraged the development of new forms of conservation of these pigments, most importantly, the use of antioxidants and microencapsulation. Microencapsulation techniques may help to resolve functional problems, as they offer products with improved solubility and oxidative stability. It is a technique in which droplets of liquid, particles, or gas bubbles are trapped in a continuous polymer film,[15,16]

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which can also be accomplished by means of freeze-drying of an emulsion/solution with a microencapsulation agent. This method creates products of excellent quality because it minimizes the changes associated with high temperatures.[17,18] Analytical studies of microencapsulated curcumin have been conducted with both differential scanning calorimetry (DSC) and thermogravimetry (TG), as well as the solubility studies and the measurements of photochemical stability and the stability in alkaline mediums.[5,19−21]

Maltodextrins with different molecular weights are products of hydrolyzed starch, and these matrices are commonly used as agents for microencapsulation. They are classified by their dextrose equivalent (DE) and due to physicochemical properties and low cost are widely used in the food industry. Both hydrophilic and hydrophobic components can be microencapsulated with maltodextrins.[22−24]

There is a lack of studies in the literature investigating potassium norbixinate and its microencapsulation. In addition, there are no reports applying the photoacoustic spectroscopy (PAS) technique evaluating the stability of maltodextrin DE20 microencapsulated colorants. Therefore, with the purpose of providing the food industry with more stable natural colorants, this study aimed to use the freeze-drying technique to obtain the microencapsulation of potassium norbixinate and curcumin in maltodextrin DE20. The complexes' stability against exposure to light, air, different pH, water solubility, and food applications was evaluated with DSC, TG, scanning electron microscopy (SEM), and the PAS technique.

MATERIALS AND METHODS

Samples and Reagents. Potassium norbixinate (purity 85%) and curcumin (purity 95%) was provided by Christian Hansen (Valinhos, SP, Brazil), while maltodextrin DE20 was obtained from Corn Products (São Paulo, SP, Brasil). All solvents were of analytical grade.

Spectrophotometric Determination and Quantification of Colorants. The method of the Joint Expert Committee on Food Additives, FAO/WHO,[8] was applied to the examination of the spectrophotometric absorption peaks of potassium norbixinate. The curcumin peaks were verified according to the procedure described by Marcolino et al.[5]

The absorption spectra for potassium norbixinate were obtained from 10 μg/mL samples dissolved in 0.5% KOH. From this solution, aliquots of 10 mL were removed to obtain absorbance data in a UV−vis spectrophotometer (Cary 50, Varian, USA) at a wavelength of 482 nm, using 0.5% KOH as the blank. Aliquots were obtained at concentrations between 4.3−10 μg/mL.

For curcumin samples, 4 μg/mL was dissolved in a solution of ethanol and water with the ratio of 4:1. Aliquots of 10 mL were removed to obtain absorbance data in a spectrophotometer using ethanol and water as the blank in a ratios of of 4:1. Aliquots were removed from the solution such that the concentrations obtained varied from 0.8 to 4 μg/mL. The curcumin concentrations were obtained in the range of maximum wavelength. This procedure was adapted from that described by Marcolino et al.[5] The spectra were obtained in triplicate. All solutions were kept protected from light.

Colorant Microencapsulation. The procedures used in the colorant microencapsulation have been adapted from the method described by Desobry et al.[25] and Wang et al.[20] For potassium norbixinate microencapsulation, maltodextrin DE20 was used in 1:10 and 1:20 proportions to the colorant. The 1:10 proportions were weighed with 1.2 g of potassium norbixinate dissolved in 10 mL of distilled water, which was added to a solution containing 10.0 g of maltodextrin DE20, previously dissolved in 40 mL of distilled water heated up to 60 °C. For the 1:20 proportion samples, 20 g of maltodextrin DE20 was substituted following the previous procedure.

To obtain a 1:20 curcumin solution, 1.1 g of colorant was dissolved in ethanol, which was added to a solution containing 20.0 g of maltodextrin DE20, previously dissolved in 40 mL of distilled water at 60 °C. The used weight for both colorants was adjusted according to the purity indicated by the manufacturer.

The solutions for the preparation of microcapsules were shaken separately, and a mechanical shaker (Fisatom/Model FIS715A, Sao Paulo, Brazil) was used for the homogenization for 30 min at 2500 rpm. For the colorant curcumin, after a period of stirring, the solution containing the microcapsules was concentrated in a rotary evaporator (Tecnal/TE211, Piracicaba, Brazil). Each one of the solutions of both colorants was previously frozen with liquid nitrogen and submitted to a freeze-drying process (Christ-Alpha 1-4LD Plus/Martin Christ, UK). The operating condition of the freeze-dryer was −50 °C for a period of up to 24 h for all samples. After the drying period, a fine dry powder was obtained. The powders containing the microcapsules with the potassium norbixinate in the ratios of 1:10 and 1:20 and curcumin in the ratio of 1:20 were stored under refrigeration and protected from light for subsequent analyses.

Stability to Light and Atmospheric Air of Potassium Norbixinate and Curcumin. This test was conducted to simulate the behavior of the colorants when exposed to light and air under commercial conditions. The pure and microencapsulated colorants were analyzed with regard to storage in daylight and darkness, based on procedures described by Matioli and Rodrigues-Amaya.[26] The pure and microencapsulated colorants were packed in polyethylene bags, with an area of 100 mm^2, in which 1.5 g of each sample was added and subsequently exposed to natural light and darkness for 30 days. For colorants vacuum-packaged, a vacuum sealer (JetVac/Model Jet 20, Brasil) was used. After exposure during 5, 15, 25, and 30 days, samples were collected, and the retained percentage of the colorants was analyzed. For the retention percentage of curcumin, samples were prepared in a solution of ethanol and water in 4:1 proportions. For samples of potassium norbixinate, they were prepared in a 0.5% KOH solution. The readings were performed in a UV−vis spectrophotometer (Cary 50-Varian, USA) at the maximum absorption wavelength, and the percentage of colorant concentrations monitored during the experimental period were analyzed by linear regression.

Colorant Stability in Different pH Ranges. The pure and microencapsulated colorants were analyzed varying the pH from 1 to 9, according to the method employed by Wang et al.[20] Solutions were prepared with 0.5% of each colorant with or without maltodextrin dissolved in water, with the exception of curcumin, which was dissolved in ethanol and water at a 4:1 ratio (adapted from the procedure described by Paramera et al.[21]). The solution aliquots were measured for each pH. Each of the solutions at different pH values was analyzed in a spectrophotometer at the wavelength of maximum absorption.

Curcumin Solubility in Water. Solutions were prepared with 0.3% curcumin or curcumin with maltodextrin DE20 (1:20). Subsequently, we measured the time of colorant solubilization in distilled water, adopting 5 min slight agitation, following the method used by Wang et al.[20]

Scanning Electron Microscopy of Pure Colorants and Microencapsulated Formulations. The pure colorants and microcapsules obtained after the freeze-drying process were subjected to scanning electron microscopy (Shimadzu/SS model-550/Shimadzu Corporation, Japan). To obtain the photomicrographs, an acceleration voltage of 10 kV was used. The samples were placed on the surface of a double-faced conductive tape, and before the measurements, they were gold metalized. The used image amplifications were 50, 200, and 450 times.[27,28]

Microencapsulation Evaluation by Differential Scanning Calorimetry (DSC) and Thermogravimetry (TG). Samples of potassium norbixinate, potassium norbixinate microencapsulated with maltodextrin DE20 (1:10 and 1:20) as well as curcumin, curcumin microencapsulated with maltodextrin DE20 (1:20), and maltodextrin DE20 were placed into platinum capsules and analyzed by simultaneous DSC and TG techniques. The equipment (STA 409 PG Luxx/NETZSCH, Selb, Germany) was operated from room

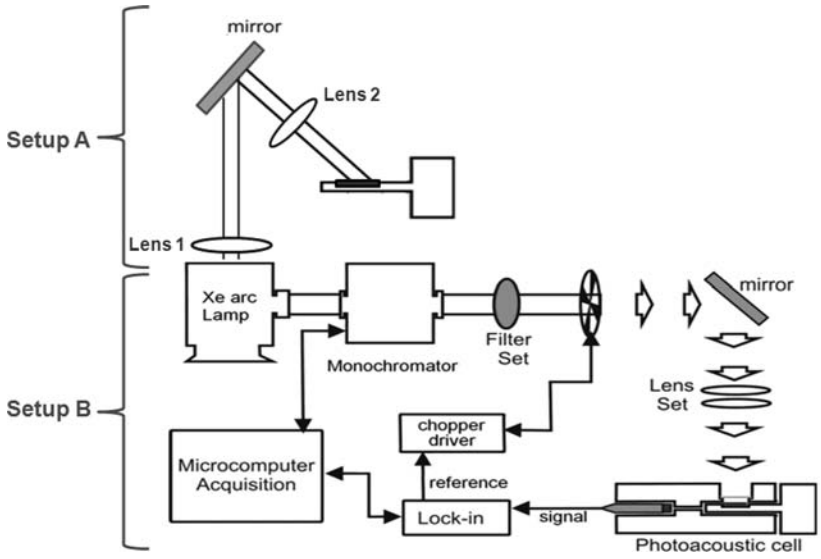

Figure 1. Experimental setup of the photoacoustic spectroscopy analysis.

temperature up to 500 °C, with a heating rate of 10 °C/min in nitrogen atmosphere (20 mL/min).

Evaluation of the Optical Absorption Spectra of Colorants and Formulations Using Photoacoustic Spectroscopy (PAS). The PAS measurements were performed using a custom-built experimental setup, shown in Figure 1. This technique assessed the protection exerted by the maltodextrin film over the natural colorants potassium norbixinate and curcumin. The photoacoustic spectra were obtained at zero time and after 2 h of white light illumination.

The monochromatic light for optical absorption spectra measurements was obtained from a 1000 W xenon arc lamp (Oriel Corporation 68820) and a monochromator (model 77250; Oriel Instruments). The light beam was modulated with a mechanical chopper (Stanford Research Systems SR540). The photoacoustic cell was custom-designed to have a minimum volume. It was made of an aluminum block, machined to hold samples with maximum dimensions of about 5 mm in diameter and 1 mm thick, which allows light to enter through a highly transparent quartz window. The microphone chamber was 15 mm from the cell and connected to the sample-holder chamber by means of a 1 mm-diameter duct. The capacitive microphone was a very sensitive 12 mm-diameter Brüel & Kjaer model 2639, which provides a high gain of 50 mV/Pa and a flat performance frequency response from 1 Hz to 10 kHz. The lock-in amplifier was from EG&G Instruments (model 5110). All photoacoustic spectra were obtained at a modulation frequency of 25 Hz and recorded between 200 and 800 nm. Data acquisition was done with a personal computer, and the PAS spectra were normalized with respect to the carbon black signal.[29]

Initially, the powder samples were placed on the specimen holder and inserted into the photoacoustic cell, obtaining the first absorption spectrum. After the measurement, without removing the sample, the specimen holder was positioned in such a way that the sample was irradiated, during 2 h, with light from a solar simulator with a xenon lamp. The dose of light which reached the sample was about 0.4 W/cm^2. After the period of exposure to light, the specimen holder was again placed in the photoacoustic cell, and a new optical absorption spectrum was obtained.

Food Applications. The established standards in relation to the acceptable daily intake (ADI) for norbixinate obtained from annatto extracts are between 0 and 0.6 mg/kg of the body weight (expressed as norbixin), and for curcumin, they are between 0 and 3 mg/kg of the body mass.[8]

The food applications of pure and microencapsulated potassium norbixinate were performed in pasta. The pasta was prepared by mixing and kneading of a mass consisting of 100 parts of wheat flour (*T. aestivum*) and 32 parts of water. After mixing, the mass was extruded in the form of spaghetti, to which no colorant had been added. To the second mixture, 5 mg/L of potassium norbixinate was added, while in the third, 5 mg/L of potassium norbixinate microencapsulated 1:20 was added. The pasta samples were packed and kept under refrigeration at 6 °C.

The application in food of pure and microencapsulated curcumin was carried out in vanilla ice cream. The ice cream was obtained using 2 L of pasteurized milk, cream, emulsifiers, neutral links, flavoring, and sugar. Three samples were prepared in triplicate: in the first no colorant was added, in the second 5 mg/L of curcumin was added, and in the third 5 mg/L of microencapsulated curcumin was added. The ice cream samples were kept frozen at −20 °C.

In the determination of the color of the samples, three measurements were carried out with five replications. Measurements were taken after 0, 7, and 15 days from the end of the product preparations. The equipment used was a Minolta CR-400 colorimeter (Konica Minolta, Inc., Osaka, Japan) with the following specifications: reading area of 8 mm, CIE D65 illuminant (natural daylight) at an angle of 10° standard CIE observer. The colorimeter supplied parameters L^* (lightness), a^* (red−green component), and b^* (yellow−blue component). Colors were directly measured on the surface of the samples, which were homogeneously distributed in a thickness of 15 mm.

Sensorial Analysis. The sensory evaluation of pasta and ice cream was performed using an acceptance test with the participation of 80 untrained evaluators for each food, that is, students, teachers, and employees at the State University of Maringá (Maringá-Paraná/Brazil). Each of the evaluators received samples and water to consume in the interval between the analyses. They also received a statement for recording their assessment, which contained a nine-point hedonic scale (1 = "extremely dislike"; 9 = "extremely like") and purchase intent (would certainly buy/definitely would not buy) for both products produced.[30]

In the sensorial analysis of pasta, each evaluator received the test samples and also a sample of commercial spaghetti (PAVIOLI-Canoas-RS/Brazil) as a standard for comparison. The samples were precooked and stored at 65 °C until sensory analysis. To achieve the sensory tests, the present study was approved by the Standing Committee on Ethics in Research Involving Human Beings of Maringá State University (Protocol CAAE no. 0339.0.093.000-11).

Statistical Analysis. All experiments were carried out using a randomized design. The results were subjected to analysis of variance (ANOVA) with the type of sample as the source of variance and to Tukey's test of means (5% significance level), using the software Statistica 10.0/2010 (Statsoft, Inc., Tulsa, OK, USA).[31]

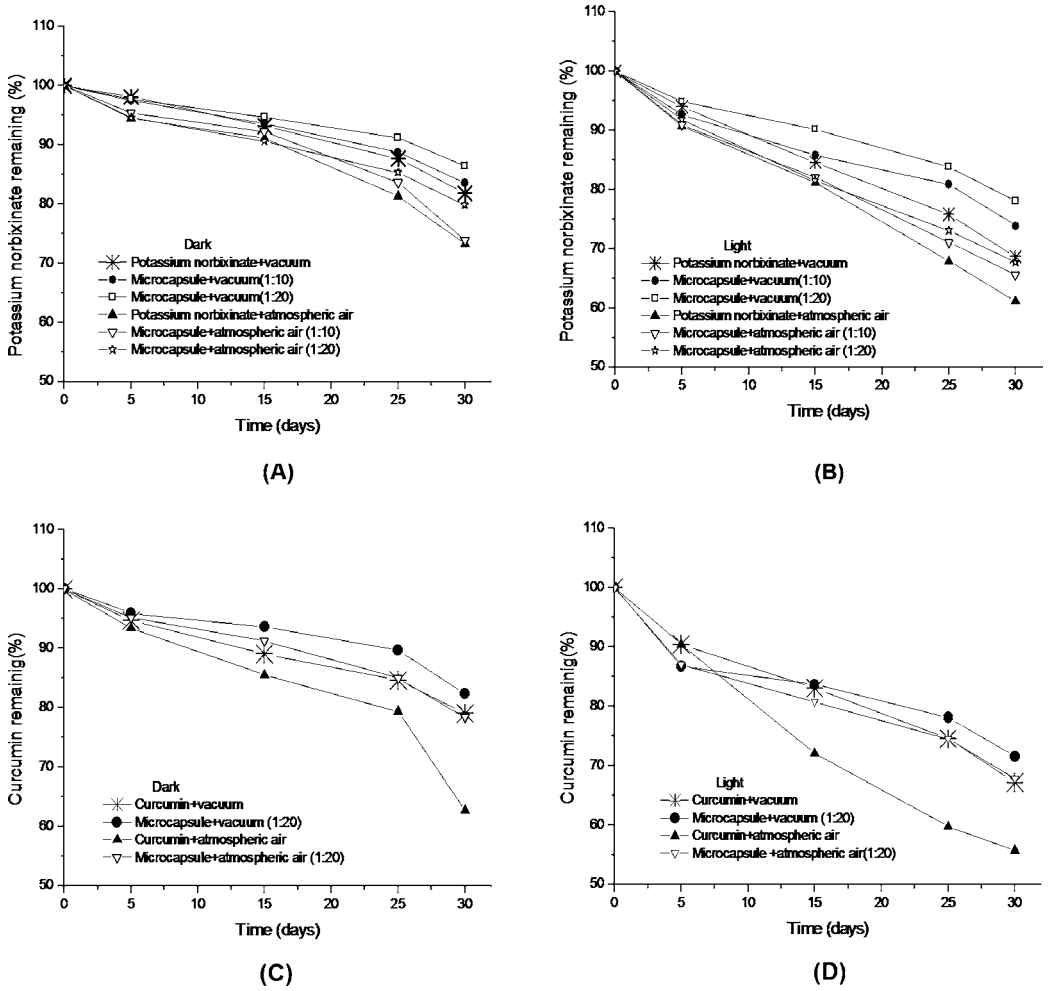

Figure 2. Stability of free potassium norbixinate and microencapsulated with maltodextrin DE20 at 1:10 and 1:20 proportions and stored in the dark in the presence of oxygen and vacuum conditions (A) and stored in light (natural light) in the presence of oxygen and vacuum conditions (B). Stability of free curcumin and microencapsulated with maltodextrin DE20 (1:20) in the dark in the presence of oxygen and vacuum conditions (C), and stored in light (natural light) in the presence of oxygen and vacuum conditions (D). The error associated with each measurement was 5%.

RESULTS AND DISCUSSION

Spectrophotometric Determination of Free Colorants. The absorption spectra of potassium norbixinate were consistent with the literature,[8] with two peaks particularly observed, the first at 453 nm and the second at 482 nm. For curcumin obtained in ethanol solution, a maximum absorption peak at 425 nm was observed. According to Dyerssen et al.,[32] at pH 3−7, curcumin in a 1:1 proportion of ethanol and water exhibits a maximum in absorption at 430 nm, suggesting that the its absorption peak is dependent on the solvent and pH range.[33,20]

Potassium Norbixinate's and Curcumin's Stability to Light and Atmospheric Air. The stability of the free and microencapsulated colorants, stored in darkness and exposed to natural light, in the presence of atmospheric air and also in vacuum packaged form, is shown in Figure 2.

When comparing free and microencapsulated potassium norbixinate in ratios of 1:10 and 1:20, in light and dark conditions and in the presence or absence of air, it was observed that the microencapsulated norbixinate 1:20 showed the best results. The percentage of remaining microencapsulated norbixinate 1:20 in the presence of light and atmospheric air was 68%, while in the dark it was 80%. This sample when examined under light and vacuum conditions resulted in a percentage of remaining colorant of 78%, whereas in the dark and vacuum conditions, the remaining colorant was 90%. Therefore, the microencapsulated norbixinate 1:20 in the dark and under vacuum conditions had 22% greater retention of color for the sample stored under both light and air (Figure 2A and B). The highest losses of colorant retention were observed in the free potassium norbixinate, stored in the presence of light and in the presence of air, as well as the same colorant microencapsulated with maltodextrin 1:10 with decay rates of approximately 40% and 35%, respectively (Figure 2B).

Carvalho et al.[34] reported that in extracts prepared from annatto seeds stored for approximately one year at 30 °C in the presence of light and in packages with different rates of oxygen, the color was reduced by 10% during storage in the first 2−3 weeks, stabilizing later. Studies carried out by Scheidt and Liaaen-Jensen[35] showed that the exclusion of atmospheric air using an inert gas or vacuum is highly recommended for annatto to minimize the risk of destruction or the occurrence of undesired reactions in testing. The results presented for this colorant suggest that the presence of atmospheric air influences the degradation of the colorant, being more pronounced in the presence of light.

The best remaining percentage of curcumin was obtained with the microencapsulated sample in the dark and packaged

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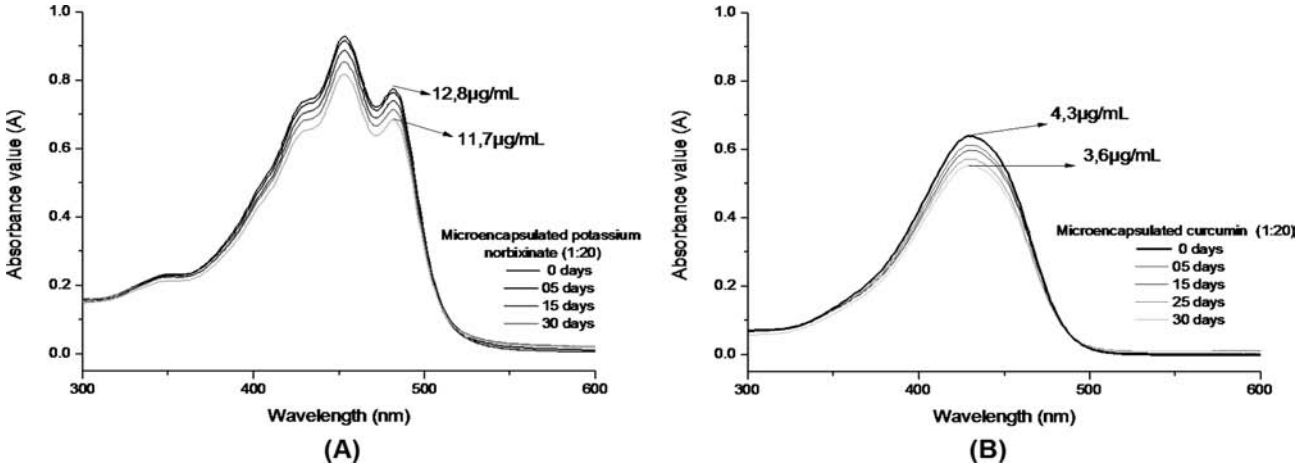

Figure 3. Absorption spectra of potassium norbixinate microencapsulated with maltodextrin DE20 prepared in 0.5% KOH solution at a ratio of 1:20 (A) and curcumin microencapsulated with maltodextrin DE20 prepared in a solution of ethanol and water at a 4:1 ratio and at a 1:20 ratio (B) within 0−30 days.

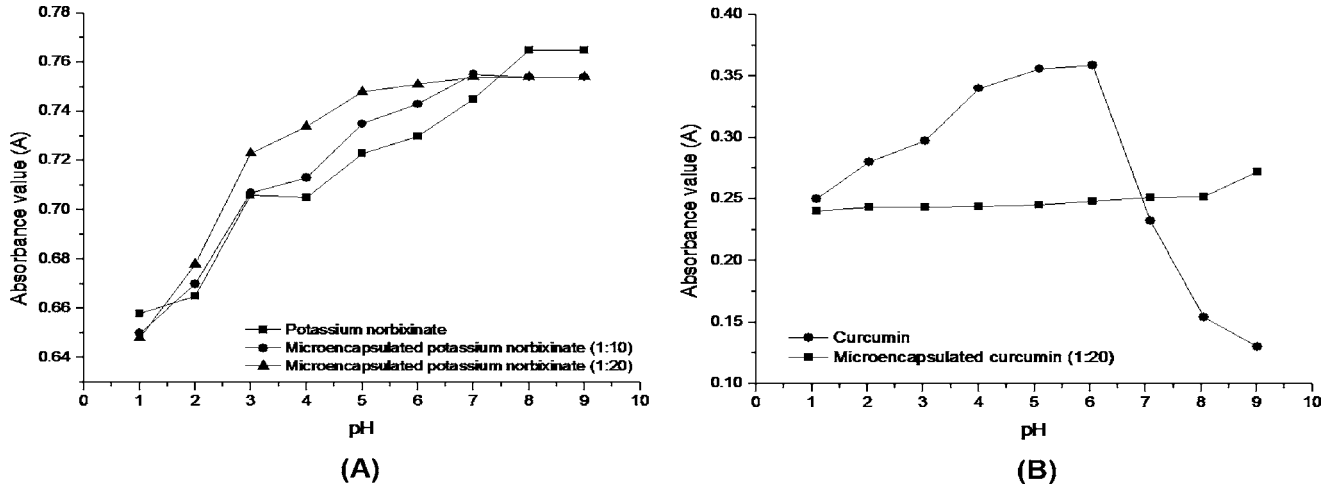

Figure 4. Stability of free and microencapsulated colorants at different pH values: free potassium norbixinate and microencapsulated with maltodextrin DE20 at the 1:10 and 1:20 ratios (A) and free curcumin and microencapsulated with maltodextrin DE20 at a 1:20 ratio (B).

under vacuum, with a rate of color retention of 85% (Figure 2C). For the microencapsulated curcumin 1:20, in the presence of light and air, the retention rate of color was 70% (Figure 2D). The protective effect of maltodextrin was observed for all samples since starch hydrolysates can protect the microencapsulated materials against oxidation due to their ability to form films, to induce plasticity, and to promote reducing power. The plasticity prevents the breakdown of the matrix, which can make the ingredient susceptible to oxidation.[36]

Microencapsulated curcumin, stored in both light or darkness and under vacuum, when compared to pure curcumin in the presence of air, showed a greater color retention rate of approximately 20% (Figure 2C and D). Marcolino et al.[5] when studying the stability of curcumin complexed with β-cyclodextrin found that light is not the major interfering factor in the process of curcumin color loss since the dye when stored in the dark showed color loss similar to the colorant stored in daylight. The same behavior was observed in this study with the use of maltodextrin.

The best results obtained above, specifically curcumin and potassium norbixinate, both microencapsulated with maltodextrin 1:20, were monitored by reading the absorption spectra, as can be seen in Figure 3A and B, respectively.

The determination of the microencapsulated norbixinate started with a colorant concentration of 12.80 μg/mL, and 30 days afterward, the concentration was 11.7 μg/mL, which is quite a small decrease rate for this period. For the microencapsulated curcumin, the result of the concentrations evaluated after 30 days showed an even smaller decay of only 0.7 μg/mL.

Solubility of Free and Microencapsulated Curcumin. Considering that potassium norbixinate is naturally water-soluble, the solubility test was carried out only for curcumin. Wang et al.,[20] using water as the solvent, observed that solubility was not achieved using free curcumin, whereas in curcumin microencapsulated with maltodextrin at the 1:20 ratio, the solubility was obtained after 1 min and 35 s without producing precipitation or agglomerates. This result shows that the freeze-drying process combined with maltodextrin provides an improvement in solubility of this colorant.

Stability of Free and Microencapsulated Colorants at Different pH Values. Solutions of free and microencapsulated potassium norbixinate under conditions of pH 1−5 showed a similar significant increase in color proportional to the pH elevation. However, it was observed that the microencapsulated

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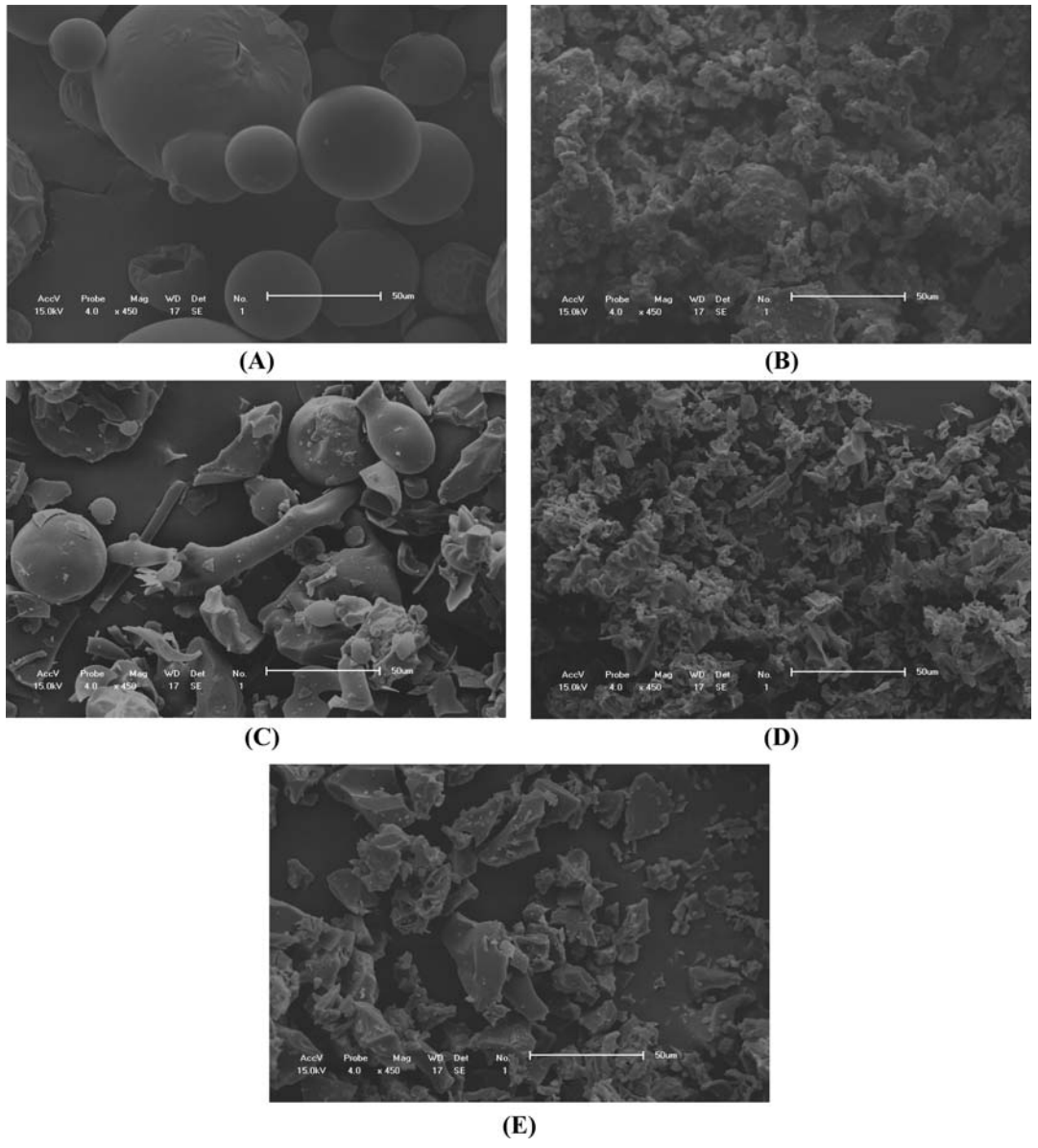

Figure 5. Scanning electron microscopy: free potassium norbixinate (A); free curcumin (B); commercial maltodextrin DE20 (C); potassium norbixinate with maltodextrin DE20 at a 1:20 ratio (D); and curcumin with maltodextrin DE20 at a 1:20 ratio (E). A, B, and C were obtained by a spray-dryer and D and E obtained by a freeze-dryer.

colorant, at ratios of 1:10 and 1:20, was stable from pH 7 and 5, respectively (Figure 4A).

Microencapsulated curcumin was stable in the range between pH 1−8, with a slight increase in color at pH 9. With free curcumin, it was observed that the pH significantly affects the colorant, showing an increase in absorbance to pH 6 and subsequent accentuated decline to pH 9 (Figure 4B). Studies by Wang et al.[20] in the range of pH 1−6 demonstrated a decrease of 15% in absorbance values for free curcumin. In curcumin microencapsulated with gelatin and with modified starch, using a spray-drying process, the decays were 3.3% and 2.6%, respectively. These authors reported that curcumin can be easily deposited in acidic conditions, which restricts its application in food. When present in alkaline conditions, the curcumin color changes to brick-red, also affecting its food application; however, these effects were not observed in the present study in the pH range of 2−8. The possible formation of curcumin microcapsules with maltodextrins, by freeze-drying, suggests a protection from acidic and basic pH ranges without precipitation and color change.

Scanning Electron Microscopy of Free and Microencapsulated Colorants. The topography and morphology of solids are important physical properties. They influence both the visual and sensory aspects and influence the processing, storage, and applications of products. Additionally, they can show the specific regions where chemical and physical interactions occur.[37]

Figure 5 shows the 450 times zoomed image of free and microencapsulated colorants. The globular form of the free potassium norbixinate is due to the drying process of the commercial colorant by a spray-dryer (Figure 5A). However, free curcumin showed powder agglomeration (Figure 5B). The commercial maltodextrin is also obtained by a spray-dryer (Figure 5C). The morphology of the potassium norbixinate microcapsules (Figure 5D) and microcapsulated curcumin (Figure 5E) showed likeness to wood chips or flakes after

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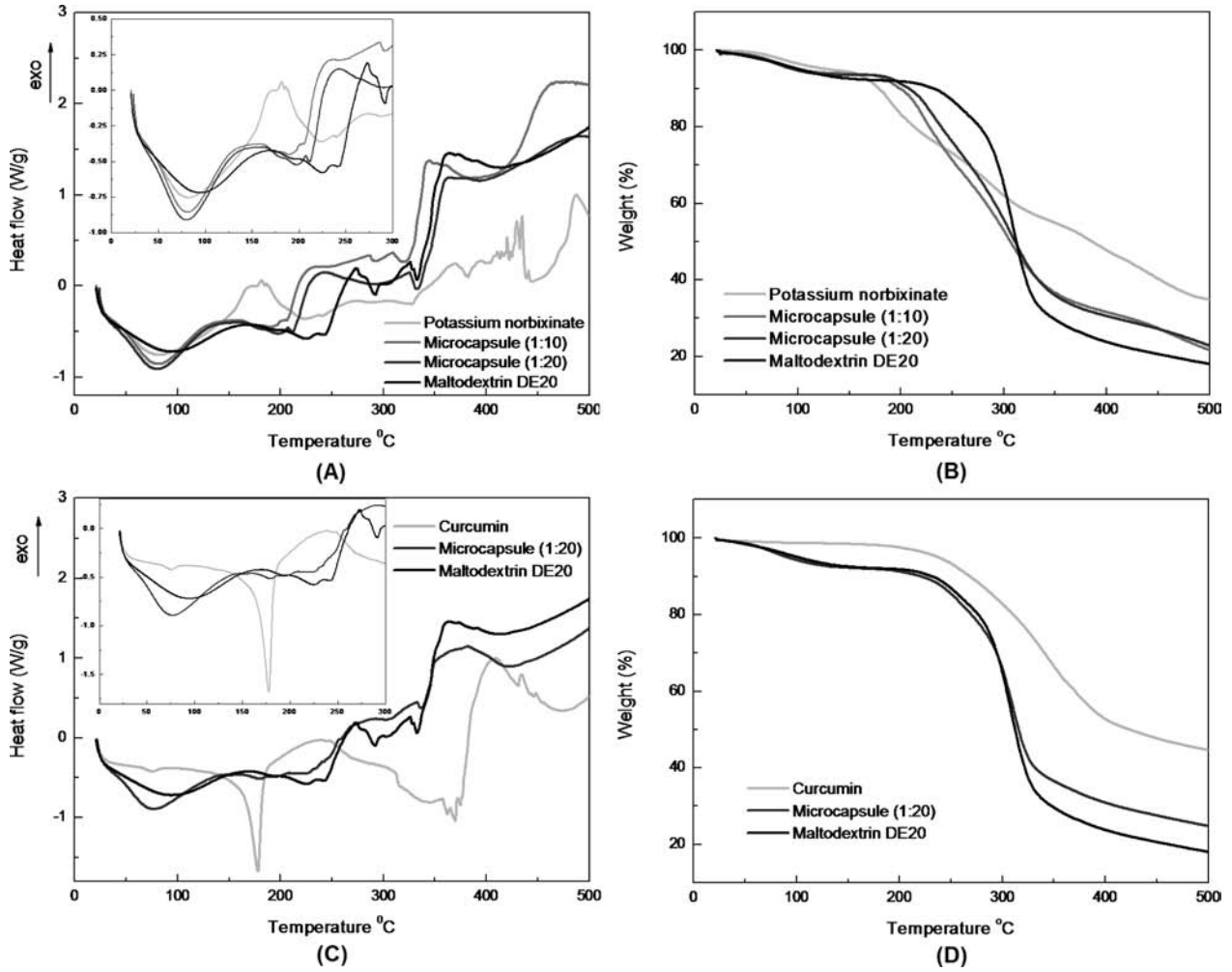

Figure 6. Differential scanning calorimetric thermogram (A) and thermogravimetry thermogram (B) of free potassium norbixinate and microencapsulated with maltodextrin DE20 at ratios of 1:10 and 1:20. Differential scanning calorimetric thermogram (C) and thermogravimetry thermogram (D) of free curcumin and microencapsulated with maltodextrin DE20 at a ratio of 1:20.

freeze-drying, confirming the forms previously observed by Farias et al.[38] for this type of drying process.

For the microencapsulated curcumin, a suitable morphology and a relatively smooth surface was observed, while for the microencapsulated potassium norbixinate the surface was observed to be rougher, with irregular agglomerates. This may be due to better interaction between curcumin, which is oil soluble, and maltodextrin. Farias et al.[38] used maltodextrin and gelatin to encapsulate α-tocopherol by freeze-drying and reported that, in addition to gelatin, maltodextrin is also appropriate to efficiently microencapsulate soluble particles.

Microencapsulation Evaluation by DSC and TG. Figure 6 shows DSC and TG analysis performed for characterization and evaluation of the possible formation of microencapsulation of natural colorants with maltodextrin. In Figure 6A, the DSC analysis for potassium norbixinate at 80 °C revealed an endothermic peak, which possibly represents a loss of water from the free colorant. The exothermic peak observed between 146 and 220 °C for free colorant is not present for the microencapsulated colorant in 1:10 and 1:20 proportions, which means a possible interaction between colorant and microencapsulate agent, practically identical for the two proportions. The exothermic peak for free potassium norbixinate, which may possibly be associated with decomposition due to the possible degradation of the chromophores in the main chain of the molecule, occurred at temperatures from 120 °C, enhancing the degradation at 145 °C. The thermogram of maltodextrin shows a second endothermic peak at 230 °C, which suggests the melting point and further decomposition of the molecule. In the microencapsulated colorant, this peak is shifted to a temperature around 215 °C, suggesting the interaction between the colorant and maltodextrin.

The TG curve analysis confirmed the data in the DSC analysis. The onset temperature of mass loss is shifted from 160 °C for the free colorant to 190 °C for the microencapsulated colorant (Figure 6B), effectively showing the protective action of maltodextrin.

For curcumin, the endothermic peak was observed at 177 °C, which appears in the DSC curve, indicating the melting temperature (Figure 6C), as reported in the literature.[5,19] Following the curve, an exothermic peak appeared, probably due to curcumin thermal decomposition at 245 °C. In the curve representing the microcapsule, the endothermic peak is not observed, probably indicating a protection exerted by maltodextrin. The endothermic peaks of the microencapsulated curcumin and maltodextrin, at 75 and 98 °C, respectively, represent the loss of water. The initial temperature of loss of

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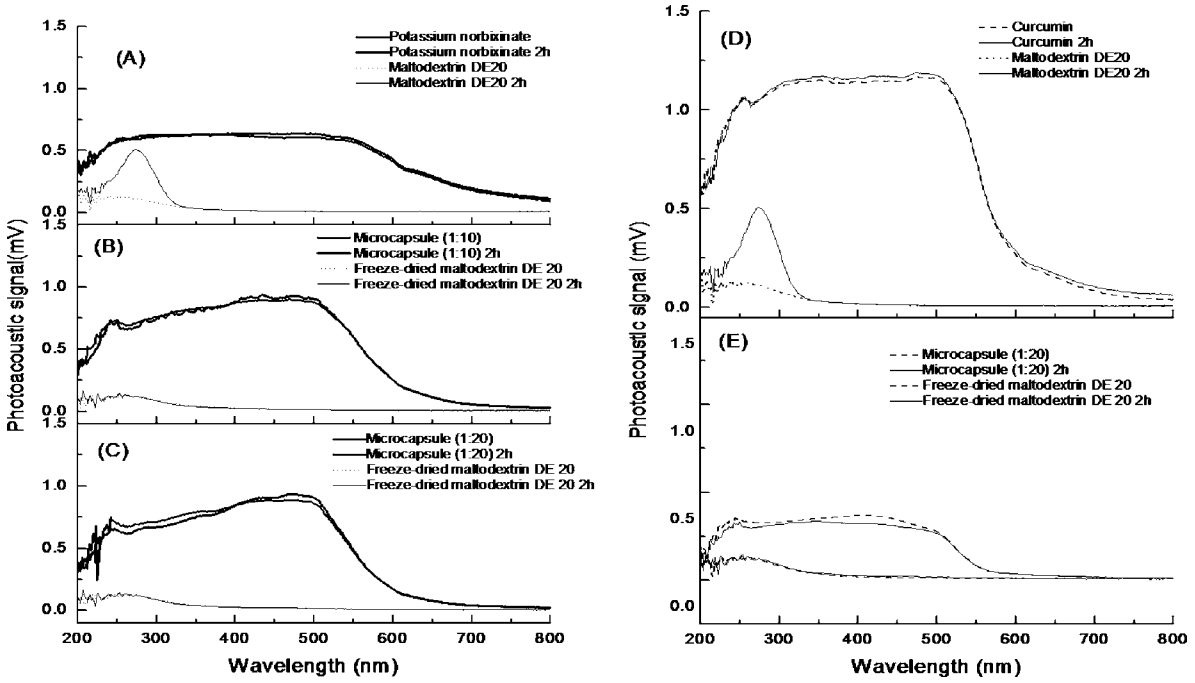

Figure 7. Photoacoustic spectra of UV−vis absorption at the zero time sample and after 2 h exposed to white light: free potassium norbixinate; maltodextrin DE20 (A); microencapsulated potassium norbixinate (1:10); maltodextrin DE20 lyophilized (B); microencapsulated potassium norbixinate (1:20); maltodextrin DE20 lyophilized (C); free curcumin and maltodextrin DE20 (D); and microencapsulated curcumin (1:20) and maltodextrin DE20 lyophilized (E).

mass of free curcumin was around 207 °C, while for the microencapsulated curcumin, it was at 220 °C (Figure 6D). The results observed in the DSC and TG curves for the thermal analysis with free and microencapsulated curcumin (Figure 6C and D) show an interaction of the colorant with maltodextrin, suggesting higher stability to the microcapsules.

Optical Absorption Spectra Evaluation of Free and Microencapsulated Colorants Using PAS. PAS in the spectral regions of UV, vis, and IR has been used for determining the qualitative and quantitative compositions of the free and microencapsulated compounds.[3] By this technique, annatto in commercial spice was analyzed[39] and more recently the determination of the triplet state energy of bixin (annatto).[40] In this study, the first step was to obtain the optical absorption spectra of potassium norbixinate, curcumin, maltodextrin, and microcapsules formed between the maltodextrin and colorants, as shown in Figure 7. In a second step, the possible protection exerted by maltodextrin on color and behavior when exposed to light from a solar simulator was evaluated. In Figure 7, the two colorants, both free and microencapsulated, exhibited higher rates of absorption in the entire evaluated spectra. Only the maltodextrin had its absorption restricted up to the ultraviolet spectral region (350 nm).

An increase in the photoacoustic signal was observed, especially between 400 and 550 nm for the sample of potassium norbixinate after exposure to light (Figure 7A), indicating photoinstability of the colorant. This same behavior was observed for the microencapsulated potassium norbixinate (Figure 7B and C) at both 1:10 and 1:20 ratios. Free and microencapsulated curcumin were also unstable in the presence of light (Figure 7D and E) since the photoacoustic signal to the free and microcapsule colorant increased in the region between 300 and 500 nm.

Studies by Najar et al.[41] reported that light is the main factor in the degradation of annatto extracts. In the case of curcumin, it is decomposed when exposed to UV−vis by breaking the link β-diketone, leading to the formation of smaller phenolic compounds, in other words, vanillin, ferulic acid, feruloylmetano acid, and vanillic aldehyde, while the major photodegradation product is a cyclization product, formed by the loss of the hydrogen atoms.[42] Rusig and Martins[43] found in their work that light was the most significant factor in the degradation of curcumin when compared with other factors such as pH stability. Therefore, light factors remain a subject of research aimed to minimize the undesirable effects caused on the colorant.

A relevant observation was found with maltodextrin used in this study. An absorption peak in the UV spectral region of 274 nm was detected after exposure for 2 h in white light (Figure 7A and D). However, this behavior did not occur when the maltodextrin was prepared by the same process in microcapsules, suggesting that freeze-drying influences the behavior of maltodextrin after exposure to white light in the UV region. Further studies should be conducted to evaluate the stability of compounds microencapsulated by maltodextrins through the freeze-drying preparation process.

Applications in Food of Free and Microencapsulated Colorants: Colorimetry. On the basis of the values shown in Table 1, it was observed that the colorimetric data obtained for the prepared ice cream without colorant, with free and microencapsulated curcumin, showed significant differences only for the parameter b^*. Specifically, an increase in yellow color was observed when the ice cream was prepared with microencapsulated curcumin. This result was expected, considering that the solubility test of microencapsulated curcumin presented very applicable results in relation to free curcumin. In the course of time, the addition of micro-

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Table 1. Color Parameters Obtained during Storage for Ice Cream Formulations[a]

samples	storage (days) 5	10	15
	Without Colorants		
L^*	86.2 a ± 0.2	86.9 ab ± 0.192	86.4 a ± 0.1
a^*	−2.2 a ± 0.1	−2.1 ab ± 0.0	−2.2 b ± 0.1
b^*	6.9 a ± 1.2	7.2 a ± 0.1	7.7 a ± 0.2
	Curcumin		
L^*	85.3 a ± 0.2	85.1 b ± 0.2	84.4 a ± 0.1
a^*	−5.3 ab ± 0.4	−6.2 b ± 0.3	−6.5 a ± 0.3
b^*	27.5 a ± 2.1	30.5 a ± 1.5	31.7 a ± 1.6
	Microencapsulated Curcumin		
L^*	83.5 a ± 0.6	84.0 a ± 0.7	83.3 a ± 0.5
a^*	−2.5 a ± 0.8	−2.7 a ± 0.5	−2.9 a ± 0.2
b^*	27.5 a ± 4.3	29.9 a ± 2.3	33.8 a ± 2.1

[a]The same letters (a,b) in the same line indicate that there were no significant differences between the samples (p <0.05). L^* = Lightness (0 = black, 100 = white), a^* = red−green component, and b^* = yellow−blue component.

encapsulated curcumin promoted an increase in the color of ice cream of about 23% after 2 weeks of storage. Marcolino et al.[5] also achieved positive results with curcumin complexed with β-cyclodextrin applied in the preparation of yogurt.

Regarding the pasta, it was noted in Table 2 that the sample with microencapsulated potassium norbixinate showed a

Table 2. Color Parameters of Pasta Formulations Obtained during Storage[a]

samples	storage (days) 5	10	15
	Microencapsulated Norbixinate (1:10)		
L^*	70.7 a ± 0.1	67.4 a ± 0.3	65.3 a ± 0.1
a^*	9.5 a ± 0.1	11.7 a ± 0.2	13.4 a ± 0.2
b^*	35.0 a ± 0.0	38.0 a ± 0.5	39.2 a ± 0.0
	Norbixinate		
L^*	74.6 a ± 0.3	73.7 b ± 0.0	73.4 a ± 0.0
a^*	8.5 b ± 0.1	8.7 ab ± 0.0	8.8 a ± 0.1
b^*	34.3 a ± 0.1	37.9 a ± 0.1	39.3 a ± 0.1
	Without Colorant		
L^*	85.9 a ± 0.0	81.1 a ± 0.1	80.4 a ± 0.0
a^*	1.7 a ± 0.0	2.0 a ± 0.1	2.8 a ± 0.1
b^*	34.1 a ± 0.1	35.0 a ± 0.0	35.4 a ± 0.0
	Commercial		
L^*	77.1 ab ± 0.2	76.3 b ± 0.3	76.8 a ± 0.0
a^*	1.3 a ± 0.0	1.7 a ± 0.0	1.4 a ± 0.0
b^*	34.1 a ± 0.1	35.0 a ± 0.0	35.4 a ± 0.0

[a]The same letters (a,b) in the same line indicate that there were no significant differences between the samples (p <0.05). L^* = lightness (0 = black, 100 = white), a^* = red−green component, and b^* = yellow−blue component.

stronger coloration for the parameter a^* (red) compared with the other samples. The pasta commercial samples and pasta without colorant showed similar values for all parameters. After 15 days of storage, the pasta prepared with the microcapsules presented a significant increase of around 42%. This same sample showed an increase of 52% in color compared with the pasta prepared with the free colorant. Ferreira et al.[7] observed that the significant differences with respect to color parameters evaluated for potassium norbixinate without protection of the microcapsules were dependent on time and temperature.

Sensory Analysis of Food with Free and Microencapsulated Colorants. The pasta with the addition of microencapsulated potassium norbixinate was well accepted, receiving the best grades for the attributes of color, texture, and flavor (Table 3). It is noteworthy that the tasters accepted the

Table 3. Acceptance Testing for the Color, Texture, and Flavor Attributes of the Pasta Formulations[a]

pasta sample	attributes color	texture	flavor
with norbixinate	6.39 a ± 1.59	6.84 a ± 1.32	6.75 a ± 1.55
without colorant	5.49 a ± 1.87	5.73 a ± 1.89	6.00 b ± 1.83
microencapsulated norbixinate	7.49 a ± 1.33	6.95 a ± 1.46	6.85 ab ± 1.68
commercial	6.96 a ± 1.55	6.65 a ± 1.67	5.89 ab ± 2.46

[a]The same letters (a,b) in the same line indicate that there were no significant differences between the samples (p <0.05).

texture of all pasta samples similarly, including the samples containing the colorant and also the maltodextrin. Regarding the flavor attribute, samples containing colorant were the most accepted. Another important fact observed was that the pasta with the added microencapsulated colorant remained the same color after the cooking process, which was not the case with pasta with the added free potassium norbixinate.

Regarding the sensory analysis of vanilla ice cream, the data shown in Table 4 resulted in a value of 7.66 for the color on a 9-point scale, although for all evaluated attributes (color, texture, flavor, and aroma) there was no significant difference between samples (p < 0.05). In the work published by Marcolino et al.,[5] a sensory analysis of fresh cheese was performed from a sample containing the colorant curcumin complexed with β-cyclodextrin, added at 20 mg/L in the formulation of this sample. The results showed increased approval for the flavor attribute (60%) followed by color (43%).

Both, the pasta prepared with microencapsulated potassium norbixinate and the ice cream prepared with microencapsulated curcumin resulted in greater intention to purchase and proved to be significantly different from the other samples.

The results obtained in this research confirm that the colorant microencapsulated potassium norbixinate with maltodextrin DE20 showed better results in the ratio 1:20. This sample when stored in the dark and under vacuum conditions had 22% greater retention of color in relation to the stored sample in the presence of light and atmospheric air. Microencapsulated curcumin exposed to a wide range of pH was more stable compared with free curcumin. Another very important result was the solubilization of microencapsulated curcumin with maltodextrin. Additionally, the thermal stability observed in DSC and TG curves confirmed the best results for the microencapsulated curcumin. The application of PAS showed that the free and microencapsulated colorants presented high rates of absorption in the entire spectral range evaluated. This technique proved to be relevant to the degradation study of free and microencapsulated colorants exposed to light since it is simple, nondestructive (preserves the sample), and is innovative in the photosensitivity study of natural colors. From the sensory point of view, products made

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Table 4. Acceptance Testing for the Color, Texture, Flavor, and Aroma Attributes of the Ice Cream Formulations[a]

	attributes			
ice cream sample	color	texture	flavor	aroma
curcumin	7.31 a ± 1.42	7.54 a ± 1.34	7.75 a ± 1.15	7.21 a ± 1.36
without colorant	6.81 a ± 1.68	7.61 a ± 1.33	7.56 a ± 1.34	6.99 a ± 1.35
microencapsulated curcumin	7.66 a ± 1.30	7.45 a ± 1.38	7.58 a ± 1.53	6.86 a ± 1.53

[a]The same letters (a,b) in the same line indicate that there were no significant differences between the samples (p <0.05).

with colorants microencapsulated with maltodextrin had good acceptability and purchase intent. Therefore, freeze-drying is a favorable process for the microencapsulation of natural colors with maltodextrin, in addition to being a protective low-cost agent, and a good choice for the stabilization of natural colorants used in food industry.

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ABBREVIATIONS USED

DE, dextrose equivalent; DSC, differential scanning calorimetry; TGA, thermogravimetry analysis; PAS, photoacoustic spectroscopy; ADI, acceptable daily intake; FAO/WHO, Food and Agriculture Organization of the United Nations/World Health Organization

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