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Characterization of *Capsicum* oleoresin microparticles and *in vivo* evaluation of short-term capsaicin intake

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ABSTRACT

Gum arabic, modified corn starch (EMCAP), modified malt (MALT), either blended or isolated, were assessed as encapsulating agents for *Capsicum* oleoresin. *Capsicum* oleoresin microparticles were obtained by spray drying and analysed for physicochemical properties and *in vivo*. Obtained powders were adequate for storage, given their low water activity (<0.150), hygroscopicity (<11.43 g/100 g), moisture (<4.76%) and high glass transition temperature (<98.3 °C). FT-IR analysis concluded that carbohydrates matrices were loaded after spray drying, with peaks around 2850 cm^{-1} for aromatic compounds, and bands around 1760 cm^{-1}, pointing to the presence of capsaicin inside the microparticles. All formulations exhibited high antioxidant activity, low contact angles and great solubility in water. Any adverse effect was observed in the experimental assay, neither change on the level of hepatic aminotransferases. The intake of a High-Fat Diet (HFD) supplemented with *Capsicum* oleoresin microparticles decreased weight gain when compared to the HFD control.

Introduction

Obesity is a major concern now spanning every age group. It is an underlying factor for diabetes, cardiovascular diseases and various metabolic disorders arising due to a sedentary lifestyle and lack of proper nutrition and diet awareness.

Many compounds impact weight loss through the thermogenic effect. One such compound, capsaicin, has shown promising results in the treatment of obesity related chronic diseases (Adaszek et al., 2019): the intake of *Capsicum* oleoresin was related to reduction of adipose tissue and inflammation, by Lee et al. (2017), and by Rogers et al. (2018). Authors observed changes on multiple gene expression, activation of AMP-activated protein kinase and inhibition of glycerol-3-phosphate dehydrogenase in white adipose tissue.

Capsicum oleoresin is the primary source of capsaicin, while also rich in phenolic compounds with high antioxidant capacity. Capsaicin is a pungent substance widely used in cooking as a flavouring agent, with high viscosity and low solubility in water, while also vulnerable to temperature, light and oxygen availability, which all obstruct its direct applications into food products (Berke & Shieh, 2010).

Encapsulation techniques are used to overcome those drawbacks of lipophilic food ingredients, such as spray drying, widely used due to its low-cost and ease of implementation in mass production. Powdered microparticles containing capsaicin have already been produced from red chili oleoresin (Cruz-Olivares et al., 2008) and chili seed oil (Wang et al., 2017), with the spray dried hydrophobic compounds showing enhanced stability, solubility, and enhanced shelf-life.

Many natural and modified carbohydrates are employed as carrier agents for encapsulation. In spray drying, gum arabic is an good material for its film-forming, high emulsifying and thermal stability features (Santiago et al., 2018). Starch sources are also commonly used in emulsification as well for their low cost and practicality. starch sodium octenyl succinate (OSA) is composed of hydrophobic alkenyl and hydrophilic carboxyl groups, making it a strong emulsifier agent (Wang et al., 2017). New experiments with blends of biopolymers are of great interest, due to their possible improvements on the antioxidant activity, encapsulation efficiency and film formation (Boonlao et al., 2020; Comunian et al., 2019).

The methodology designed for this study aimed at improving the *Capsicum* oleoresin protection and evaluating its functional properties

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using a combination of carbohydrates as wall materials. *Capsicum* oleoresin microparticles were obtained by spray drying using gum arabic (GA), octenyl succinic anhydride (OSA) corn starch (EMCAP), modified malt (MALT), either individually and blended (GA:EMCAP, GA:MALT, EMCAP:MALT). Further, microparticles were evaluated in terms of their physicochemical properties, morphology, thermal characteristics, capsaicin retention, encapsulation efficiency and antioxidant activities.

Although few studies about the protective effect of *Capsicum* oleoresin in obesity, there is neither any certainty that microencapsulation by spray drying could modify its bioavailability and thus, impacts its biological response. Therefore, a preliminary study was carried out: C57BL/6J mice, fed for 28 days on a high-fat (HF) diet supplemented with different concentrations of microparticles (10 or 20 mg/ kg of PV) were employed, in order to assess the acceptability of the diet and its toxicological response.

Material and methods

Material

Gum arabic and octenyl succinic anhydride (OSA)-modified starch (EMCAP^{TM}) were kindly donated by Nexira (São Paulo, Brazil), and Cargill (Campinas, Brazil), respectively. Modified malt (MALT) was obtained by ultrasound, (45 W/5 min) using stearic acid (2% w/w), in a previous work (Anthero, Comunian, Bezerra, & Hubinger, 2021). *Capsicum* oleoresin was purchased from Synthite Industries Ltd. (Kerala, India), the capsaicin standard was acquired from Cayman Chemical (Ann Arbor, USA, Purity > 95%) and corn oil (Liza, Cargill, Campinas, Brazil) was purchased from a local supermarket. Other standards as 2,4, 6-Tris(2-Pyridyl)-S-Triazine (TPTZ) and Trolox (x (Hidroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) were acquired from Sigma-Aldrich (St. Louis, USA). All chemicals were of analytical grade.

Microparticles powder: Production and characterization

The production of microparticles

The dispersion of the wall materials (gum arabic, OSA modified corn starch, and modified malt) and blends, containing 15% solids, was dissolved in distilled water under magnetic stirring overnight. Six formulations (5% oleoresin, 95% wall materials) were made, according to the description in the Table 1. Dispersions composed of aqueous (95%) and oil phase (5%) were produced using a Silverson Laboratory Mixer L5M-A (Buckinghamshire, UK) at 5,000 rpm or 7650x g (Allegra 25-R, Beckman Coulter Inc., São Bernardo do Campo, Brazil), for 10 min. Emulsions (500 g) were then submitted to the Mini Spray Dryer B-290 (Büchi, Flawil, Switzerland) using a two-fluid atomizer nozzle of 0.7 mm. The drying air flow rate was of 35 m^3/h, with an inlet temperature of 160 °C. Air pressure was kept at 0.06 Mpa, with an emulsion feed flow of 15 mL/min, while the outlet temperature varied from 75 °C to 78 °C. Spray-dried powders were stored in sealed flasks at −10 °C.

Table 1
Microparticles formulation.

Treatments	Wall material	Composition
GA	GA (Arabic gum)	100 g of emulsion containing:15% total solid, in which 95% is wall material and 5% is *Capsicum* oleoresin
EMCAP	EMCAP (OSA-modified corn starch)	
MALT	Modified Malt	
GA: EMCAP	GA: EMCAP (1:1)	
GA: MALT	GA: MALT (1:1)	
EMCAP: MALT	EMCAP: MALT (1:1)	

Microparticles characterization

Moisture and water activity

The moisture of the particles was determined by the moisture determination balance (Shimadzu brand, model Moc63u, Kyoto, Japan). While drying at 105 °C, powder samples (2 g) were scaled, until their weight difference was of<1%. Water activity was measured using an Aqualab model 3TE digital hygrometer (Decagon, Pullman, USA) at 25 °C, based on the chilled mirror dew point technique.

Hygroscopicity

Powders hygroscopicity was determined according to the methodology described by Cai & Corke (2000) with some modifications. Approximately 1 g of powder was placed in a desiccator containing saturated NaCl solution, at 25 °C, until constant weight. After 7 days, the hygroscopicity was calculated and expressed as grams of moisture adsorbed on 100 g of dry solids.

Solubility measurement. Approximately 0.2 g of microparticles (in dry basis) were added to 20 mL of distilled water and kept under stirring for 5 min. The solution was centrifuged at 10,000 rpm or 15,300x g (Allegra 25-R, Beckman Coulter Inc., São Bernardo do Campo, Brazil), for 10 min. An aliquot of 5 mL of the supernatant was transferred to a pre-weighed petri dish and dried at 105 °C for 24 h. Thus, solubility was calculated based on the weight difference.

Contact angle. The contact angle was determined in order to characterizing the wettability of the powder. The effect of different biopolymers on the hydrophilicity of *Capsicum's* oleoresin microparticles was measured using the Track-S tensiometer (Teclis, Longessaigne, France). Tablets of 14 mm thickness were produced with 150 mg of the sample using 80 kN of hydraulic pressure force (Shimadzu Corporation, Kyoto, Japan). A drop of water with a volume of 10 μL was applied to the surface of the microparticle and the consequent reduction of the angle was measured for 30 s.

Particles mean diameter and size distribution and their microstructure. Particle size was measured by light scattering technique in the Mastersizer 2000 instrument (Malvern Instruments Ltda, Malvern, UK), using the SIROCCO optical unit with dry air dispersion. Readings were repeated six times and mean values of the volumetric diameter and the Span index were determined. The microstructure of the particles was analysed using the Scanning Electron Microscope (SEM) by LEO Electron Microscopy (Model Leo 440i, Cambridge, UK). The samples were covered with gold, and the analysis was performed in 2.00 KX magnification with a voltage acceleration of 15.00 kV.

Glass transition temperature. Glass transition temperature (Tg) was measured in triplicate by differential scanning calorimetry (DSC) analysis (model Q100, TA Instruments, New Castle, USA). Four milligrams of powder were placed into an aluminum pan and sealed. An empty pan was used as reference. The procedure consisted of cooling down to −70 °C (10 °C/min), keeping an isothermal for 1 min and, at last, heating up to 120 °C (10 °C/min), twice. Hence, the last running temperature was balanced to 25 °C. Tg was obtained from the second curve using software Universal Analysis 3.9 (TA Instruments, USA) (Consoli et al., 2019).

FT-IR analysis. Chemical structures of wall materials, free *Capsicum* oleoresin, and microparticles were analysed by Infrared spectroscopy. The data were collected in the interval between 4,000 and 400 cm^{-1} (IRPrestige-21, Shimadzu, Kyoto, Japan) and the results were obtained by IRSolution software, version 1.60. Tablets were prepared in triplicate by manual hydraulic pressing, containing 100 mg of potassium bromide (KBr) and 1 mg of the oven-dried sample (105 °C/48 h).

Oleoresin retention. An amount of 15 mL of hexane was added to 1.5 g of powder and mixed in a vortex for 2 min. The blend was filtered through n°.1 filter paper and the collected powder was rinsed with 15 mL of hexane twice. Thus, the samples were subjected to hexane evaporation in a circulating air oven at 60 °C, until constant weight. The encapsulation efficiency of oleoresin (EE%) was calculated by the weight difference, according to Equation (1):

$$EE\ (\%) = \frac{(Total\ oil\ content - surface\ oil\ content)}{total\ oil\ content} x\ 100 \quad (1)$$

Capsaicin content. A sample of 0.25 g of the obtained microparticles was mixed to 2.5 mL of Milli-Q water in the Falcon tube. Thus, it was homogenized for 1 min using a vortex stirrer AP56 (Phoenix Luferco, Araraquara-SP, Brazil). Thereafter, 7.5 mL of methanol were added to the sample, with immediate stirring for another 1 min. These samples were kept in an ultrasound bath for 15 min and then centrifuged for 15 min at 10,000 rpm or 15,300x g (Allegra 25-R, Beckman Coulter Inc., São Bernardo do Campo, Brazil), at 20 °C. The supernatant was filtered through 0.45 μm polytetrafluoroethylene (PTFE) syringe filters to posterior High Performance Liquid Chromatography (HPLC) analysis.

The content of capsaicin was obtained using a HPLC of Agilent 1100 series (Agilent Technologies, Santa Clara, USA) equipped with a diode array detector (DAD G1315B). An aliquot (10 μL) of each sample was injected in a Poroshell 120 EC-C18 column 4.6x100 mm, 2.7 μm (Agilent, Santa Clara, USA). Capsaicin was determined by monitoring in 280 nm, at 25 °C. The mobile phase was composed of a blend of methanol: water (85: 15, v/v), while the flow rate was of 1 mL.min^{-1}, as described by De Aguiar et al. (2016). A calibration curve (R^2 0.998) was determined using diluted capsaicin, in concentrations varying from 7.14 to 142.8 μg/L. Quantification was obtained by Chromeleon software 6.8 and the capsaicin concentration was expressed as mg capsaicin/g powder. Each extraction was performed in triplicate.

Antioxidant activity of microparticles by FRAP and ORAC methods.

a) FRAP

The FRAP reagent was produced by a mixture of acetate buffer (300 mmol L^{-1}) (pH 3.6), TPTZ (10 mmol), HCl solution (40 mmol L^{-1}) and $FeCl_3$ (20 mmol L^{-1}). After, FRAP was mixed to 0.01 mL of aqueous microparticle extract (MP) (0.5 of MP: 2.5 mL of deionized water: 7.5 mL of methanol). After 30 min at 37 °C, the absorbance of the sample at 595 nm was determined in a microplate reader (NOVOstar, BMG Labtech®, Germany) (Benzie & Strain, 1996). The results were expressed in μmol of Trolox equivalent (TE)/g of microparticle using a calibration curve varying from 10 to 400 μmol Trolox/L (y = 0.000651x + 0.000223) with an R^2 = 0.999.

b) ORAC

Antioxidant activity was determined by the hydrophilic-ORAC method. The preparation of the samples and the determination of the antioxidant activity by ORAC (Oxygen Radical Absorbance Capacity Assay) were conducted by the methodology described by Ou et al. (2013). Approximately, 0.5 g of microparticles were homogenized with 2.5 mL of deionized water and then, 7.5 mL of methanol were added for complete extraction of bioactive compounds. An aliquot (0.2 mL) of this solution was homogenized with fluorescein (5.7 μmol/L), as a target for free radical attack, and with 2,2′-azobis-amidino-propane dihydrochloride (24 mM) as a peroxyl radical generator, at 37° C. The Trolox (5 μM) was used as a standard control and readings were taken on a microplate reader (NOVOstar, BMG Labtech®, Germany) with the following fluorescent filters: 485 nm for the excitation wavelength and 520 nm for emission wave. ORAC values were obtained in μmol of Trolox equivalent/g using standard curves varying from 5 to 75 μmol Trolox equivalent/g, (y = 0.35x + 1.15) and an R^2 0.99.

Effect of microparticles on weight gain and acute toxicity in the diet of mice

For this assay, formulations containing modified malt and gum arabic as a carrier agent were chosen as a dietetic supplement for the pilot test. The formulation blend was selected at random. Purpose of this assay was to assess if the animals would accept a diet supplemented with the obtained microparticles and, thus, to evaluate the acute toxicity of the encapsulated compound regardless of the wall material. Only two formulations were chosen to experiment to reduce the number of animals required to experimental design. Thus, microparticles loaded with capsaicin were made into two different solutions, while additional corn oil was mixed to the least concentrated of them, in order to keep the same solid content in the emulsions and to check for any effect of this type of oil on the acute toxicity and weight gain in mice.

Formulation preparation

The wall materials (modified malt and gum arabic) were previously dissolved in distilled water under magnetic agitation for 12 h. Two emulsions were prepared: one using only *Capsicum* oleoresin as oil phase (OC) and another one containing OC plus corn oil (1:1, w/w), named Formulation 1 (F1) and Formulation 2 (F2), respectively. Dispersions were atomized according to the section on microparticles production and, thus, the powdered particles were ready for use.

Experimental design

The study was approved by the Institutional Animal Care and Use Committee (protocol #5154–1/2019, CEUA, UNICAMP, Brazil), thus following the institutional ethical and the Brazilian National Council for the Control of Animal Experimentation (CONCEA) guidelines. Old mice C57BL/6J (28 days) were kept on a 12-h light/dark cycle, at a temperature of 25 ± 2 °C, with free access to food and water. The mice were divided into five groups (n = 6), as follows: Control group or Diet 1: animals received high-fat diet only without any microparticles; Diet 2: a high-fat diet containing 10% microparticles; Diet 3: a high-fat diet containing 20% microparticles; Diet 4: a high-fat diet containing 10% microparticles with corn oil; and Diet 5: a high-fat diet containing 20% microparticles with corn oil. The diets were formulated (Table 2) following the American Institute of Nutrition, to preserving the animal's modified nutritional status (AIN-93 M) to high-fat profiles (Reeves, 1997).

During the experimental period (28 days), food intake was monitored three times a week and body weight gain was measured once a week. At the end, after 6 h of fasting, the animals were euthanized; heart, kidney, spleen, mesenteric adipose tissue, and liver were removed and weighed; also, samples from the liver were placed into formalin solution for further histological analysis. Blood fasting glucose was measured using the G.Tech Lite apparatus (Infopia Co., Ltd, Gyeonggi-do, South Korea). The blood samples were collected in appropriate

Table 2
Composition diet for feeding mice groups during 28 days of experimental assay.

Groups	Composition of the diet	*Capsaicin in the diet (%)
Diet 1	High fat	–
Diet 2	High fat diet containing 10% of *Capsicum* oleoresin microparticles composed by MALT: GA	0.0022%
Diet 3	High fat diet containing 20% of *Capsicum* oleoresin microparticles composed by MALT :GA	0.0044%
Diet 4	High fat diet containing 10% of *Capsicum* oleoresin microparticles composed by MALT: GA with corn oil	0.0014%
Diet 5	High fat diet containing 20% of *Capsicum* oleoresin microparticles composed by MALT: GA with corn oil	0.0028%

*Percentage of capsaicin present into diet in relation to 1 kg of high fat diet.

tubes, from which serum was obtained by centrifugation (10,000 rpm or 15,300x g for 15 min) using the Allegra 25-R (Beckman Coulter Inc., São Bernardo do Campo, Brazil), separated and thus immediately frozen at −80 °C.

Acute toxicity. The enzymes aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were identified in the serum of the animals by a Labtest commercial kit (ELISA, USA), following the manufacturer instructions.

Hepatic lipid oxidation. The liver sample was used to assessing Thiobarbituric acid reactive substances (TBARS), according to Ohkawa, Ohishi and Yagi (1979). A standard curve (1.25 – 50 nmol malondialdehyde (MDA)) was obtained using 1,1,3,3 - tetraethoxypropane. The measurement was determined at 532 nm and the results expressed as nmol MDA mg^{-1} tissue.

Histological assay. Liver samples were kept for 24 h at room temperature (24 °C) in 10% buffered formalin, preserved in 70% ethanol and embedded in paraffin. Slices were cut into sections of 5 μm thickness and stained with hematoxylin and eosin for visualization of the hepatocyte morphology, as previously described (Flister et al. 2018). NAFLD activity score (NAS) (Kleiner et al. 2005) was used for the semiquantitative analysis of the three defined criteria of NASH: steatosis (0–3), ballooning (0–3) and lobular inflammation (0–2), using a Leica DMI 4000B microscope (Heerbrugg, Switzerland).

Statistical analysis

All physicochemical analyses were performed at least in triplicated, and the results were subjected to analysis of variance (ANOVA) using the Minitab 18®. In addition, the significant differences (p-value < 0.05) between the treatments were evaluated using Tukey's test.

Results and discussion

Physicochemical properties of microparticles: Moisture content, water activity, hygroscopicity

Table 3 shows moisture and water activity (a_w) values for all particles obtained by spray drying. Capsaicin microparticles presented low values for moisture content and a_w, ranging from 2.81 ± 0.62 to 5.36 ± 0.21 and from 0.105 ± 0.006 to 0.150 ± 0.004, respectively. Low values for water activity can affect the microorganisms' growth and contribute to the low rate of degradation of the encapsulated compound. Similar results for a_w and moisture were also observed for spray-dried microparticles (Consoli et al., 2019; Comunian et al., 2019).

Hygroscopicity values showed minimal differences among formulations (Table 3). Microparticles containing MALT as a carrier agent presented the lowest values for hygroscopicity (6.95 g water/100 g powder), while commercial materials, either blended or isolated (GA, EMCAP, GA: EMCAP, GA: MALT), presented the highest values.

Gum and modified starches present high hygroscopicity due to the presence of carboxylic groups, which increase water adsorption. Food powders obtained in this research showed a low tendency to become liquid or hardened, making them suitable for storage and handling in food processing.

Glass transition temperature

Glass transition temperature (T_g) is an essential parameter for powdered products, especially in food containing carbohydrates. Particulate systems are glassy structure (solid) or a rubbery (liquid-like) state. The change from very thick glass to a rubbery state occurs at a given T_g, which is specific for each material. When particles are stored above the T_g, there occurs high mobility of molecules and thus the powder will change into a "gummy state". As a result, there is agglomeration, crystallization, loss of volatiles, structural transformations and increased rates for chemical and enzymatic changes on the food product (Bhandari & Howes, 1999).

In this research, *Capsicum* oleoresin microparticles presented different glass transition profiles, as shown in Fig. 1. Formulations containing only one wall material presented glass temperature values at 122.4 °C (MALT), 100.7 °C (GA), and 98.3 °C (EMCAP).

Powders formed by MALT show the highest T_g upon comparison to every other formulation. The difference in the composition of MALT concerning other pure wall materials explains this behaviour, for MALT contains fibers, sugars, granules of starch, and protein. Regarding EMCAP and GA microparticles, high values for water activity and low values for T_g were observed. The obtained results agree with the values

Table 3
Physicochemical analysis, size diameter, oleoresin encapsulation efficiency, capsaicin retention and antioxidant activity for microparticles

Measurements	Treatments					
	GA	EMCAP	MALT	GA: EMCAP	GA: MALT	EMCAP: MALT
Moisture (g water/100 g powder)	$3.83^{bcd} \pm 0.73$	$4.76^{ab} \pm 0.49$	$4.48^{abc} \pm 1.59$	$5.36^{a} \pm 0.21$	$2.81^{d} \pm 0.62$	$3.19^{cd} \pm 0.63$
a_w	$0.139^{a} \pm 0.032$	$0.145^{a} \pm 0.024$	$0.113^{a} \pm 0.016$	$0.150^{a} \pm 0.004$	$0.105^{a} \pm 0.006$	$0.128^{a} \pm 0.034$
Solubility (%)	$93.92^{a} \pm 1.85$	$94.50^{a} \pm 4.42$	$74.55^{c} \pm 2.30$	$83.16^{b} \pm 2.58$	$82.44^{bc} \pm 7.72$	$78.51^{bc} \pm 3.54$
Hygroscopicity (g of adsorbed moisture/100 g of sample)	$10.00^{a} \pm 1.00$	$10.81^{a} \pm 0.92$	$6.95^{b} \pm 1.57$	$11.43^{a} \pm 0.94$	$11.12^{a} \pm 0.64$	$7.49^{b} \pm 0.30$
Contact angle Θ (°)	$46.6^{b} \pm 0.9$	$65.6^{a} \pm 1.6$	$64.6^{a} \pm 1.9$	$69.7^{a} \pm 5.9$	$49.3^{b} \pm 1.4$	$68.5^{a} \pm 1.3$
$D_{[4,3]}$ (μm)	$22.5^{d} \pm 1.2$	$21.7^{d} \pm 0.7$	$186.7^{a} \pm 0.3$	$14.7^{e} \pm 0.5$	$37.9^{c} \pm 2.5$	$44.0^{b} \pm 1.0$
SPAN (dimensionless)	$0.6^{d} \pm 0.2$	$1.7^{c} \pm 0.1$	$6.3^{a} \pm 0.3$	$2.3^{c} \pm 0.3$	$4.3^{b} \pm 0.2$	$6.9^{a} \pm 0.3$
*OEE (%)	$84.4^{b} \pm 2.7$	$91.6^{a} \pm 3.1$	$68.5^{c} \pm 2.3$	$89.1^{ab} \pm 0.9$	$89.7^{ab} \pm 2.2$	$90.7^{a} \pm 1.1$
*CR (mg capsaicin/ g powder microparticle)	$3.49^{a} \pm 0.2$	$3.33^{a} \pm 0.2$	$2.97^{b} \pm 0.3$	$2.35^{b} \pm 0.7$	$3.48^{a} \pm 0.1$	$3.46^{a} \pm 0.2$
ORAC (μmol TE/g microparticle)	$3.83^{bcd} \pm 0.73$	$4.76^{ab} \pm 0.49$	$4.48^{abc} \pm 1.59$	$5.36^{a} \pm 0.21$	$2.81^{d} \pm 0.62$	$3.19^{cd} \pm 0.63$
FRAP (μmol TE/g microparticle)	$0.139^{a} \pm 0.032$	$0.145^{a} \pm 0.024$	$0.113^{a} \pm 0.016$	$0.150^{a} \pm 0.004$	$0.105^{a} \pm 0.006$	$0.128^{a} \pm 0.034$

*Means that do not share a letter are significantly different. a_w: Water activity; OEE: Oleoresin Encapsulation Efficiency; CR: Capsaicin retention. GA: Arabic gum, EMCAP: modified corn starch-OSA(EMCAP®); MALT: Stearic acid-modified malt; GA: EMCAP, GA: MALT and EMCAP: MALT.

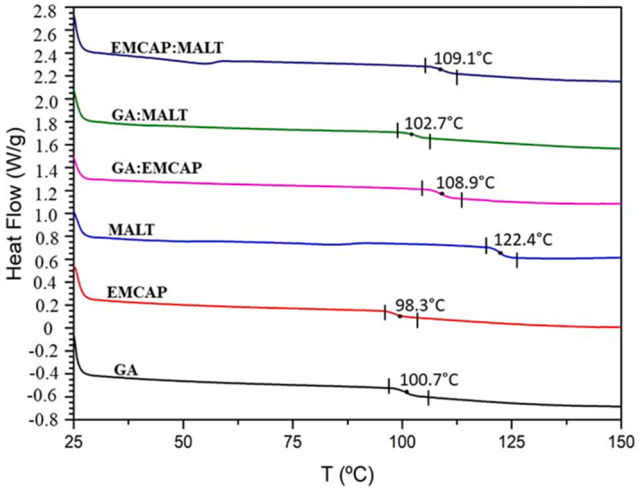


Fig. 1. Microparticles glass transition temperature *GA: Arabic gum, EMCAP: modified corn starch-OSA(EMCAP®); MALT: Stearic acid modified malt; GA:EMCAP, GA:MALT and EMCAP:MALT.

obtained by Chen et al. (2019), which observed a glass transition temperature at 97.19 °C for beta-carotene microparticles encapsulated by OSA starch (corn starch esterified with octenyl succinic acid).

Blends of these wall materials (EMCAP: MALT, GA: MALT, and GA: EMCAP) resulted in a slight increase in T_g in comparison to formulations containing only isolated forms due to an interaction of biopolymers. Several other authors employed a distinct combination of wall materials on spray-dried particle formation and observed a similar effect on the glass transition temperature (Porras-Saavedra et al., 2018). According to Bhandari & Howes (1999), the addition of high molecular weight substances to solid mixture foods promotes an increase in T_g.

Contact angle

The contact angle is an indirect measure related to wettability, i.e., the water adsorption capacity of the particle surface. High values for contact angle imply low wettability, while low contact angle (<90°) imply a wetting behaviour (Wenzel, 1949). This is an important physical property for powdered foods because it is linked to the capacity of their re-constitution in water (Arshad et al., 2018).

Powders obtained by different carbohydrates resulted in contact angle values ranging from 46.6° ± 0.9 to 69.7° ± 5.9, as seen in Table 3. Treatments containing gum arabic only and gum arabic blended with modified malt showed statistically ($p < 0.05$) lower contact angles compared to every other formulation. This result may be associated with the chemical structure of the gum arabic, for it is a hydrocolloid with highly branched polysaccharide chains, mainly acid arabinogalactans, which contribute to its anionic nature and greater affinity to water (Bemiller, 2019). Consoli et al. (2019) reported similar results (~46°) for resveratrol microparticles composed of Maillard-glycated conjugates (produced by sodium caseinate, maltodextrin and glucose syrup). Otherwise, for microparticles formulated by corn starch esterified with OSA (EMCAP) and malt esterified with stearic acid (MALT), or for their blends (EMCAP:MALT), the contact angles (°) were higher than those for formulations containing gum arabic. The results confirmed the impact on the hydrophobicity of hydrophobic groups of modified starches.

Solubility

The solubility is associated with the ability of the powder to disperse in water. An oil encapsulated by emulsion, followed by spray drying, displays high solubility in water, resulting in quick release of the encapsulated compound into the medium, which simplifies its application in water-based food products (Fernandes et al., 2016).

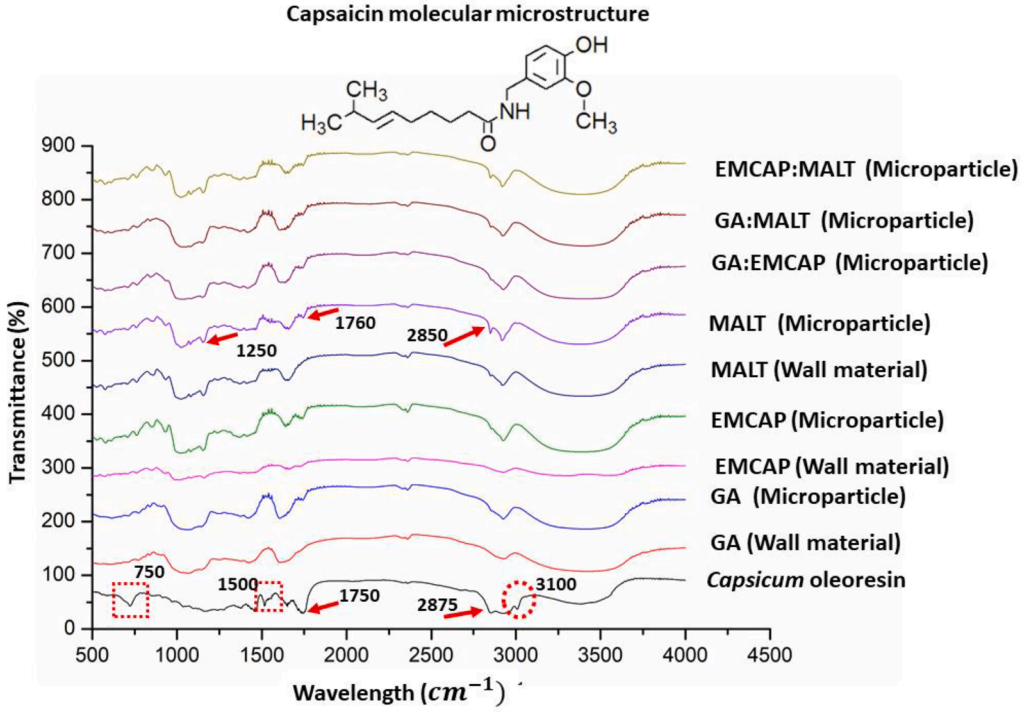
Post-encapsulated *Capsicum* oleoresin presented solubility indexes ranging from 74.5 ± 2.30 to 94.5 ± 4.42%, as shown in Table 3. The lowest value was found for a sample containing malt as a coating material, which could be related to the presence of insoluble solids. In contrast, commercial wall materials showed high solubility values due to their chemical structure.

When the commercial materials (GA and EMCAP) were combined to MALT, there was a slight increase in the solubility index, due to a decrease in the concentration of insoluble solids. Overall, carbohydrates present high solubility in water. Similar results were found for ginger essential oil encapsulated by gum arabic, maltodextrin, and inulin, with values ranging from 81.4 to 84.6% for all blends (Fernandes et al., 2016 and for nutmeg oleoresin microencapsulated by gum arabic and modified sorghum starches-OSA, which showed a solubility of over 75% (Arshad et al., 2018).

Additionally, *Capsicum* oleoresin-loaded microparticles presented a good solubility in water due to low oil content. According to Mohammed et al. (2017), microparticles containing 5% oil phase presented an average solubility of 80%, also showing decreases in solubility for increases in the ratio of oil of microparticles.

Chemical structure by Fourier-transform infrared spectroscopy (FT-IR)

FT-IR is a qualitative and versatile technique that is able to identify organic groups in short time, with ease of sample preparation and accurate results. Fig. 2 shows a molecular structure of capsaicin, FT-IR



*GA: Arabic gum, EMCAP: modified corn starch-OSA(EMCAP®); MALT: Stearic acid modified malt; GA:EMCAP, GA:MALT and EMCAP:MALT

Fig. 2. Microparticles chemical structure *GA: Arabic gum, EMCAP: modified corn starch-OSA(EMCAP®); MALT: Stearic acid modified malt; GA:EMCAP, GA:MALT and EMCAP:MALT.

spectra for *Capsicum* oleoresin, OC microparticles and wall materials.

The spectrum of pure *Capsicum* oleoresin showed bands around 3100, 2875, 1750, 1500, 750 cm^{-1}, thus pointing to the presence of an aromatic ring, polar groups (hydroxyl, amide, and carbonyl groups), and a hydrophobic group (Freitas et al., 2018). Aromatic groups, represented in the 3000–3150 cm^{-1} spectral region, are the most important bands for this oleoresin. Meanwhile, the stretching vibrations of C—H groups in 2875 cm^{-1} region represent saturated aliphatic compounds (Nagavekar & Singhal, 2019).

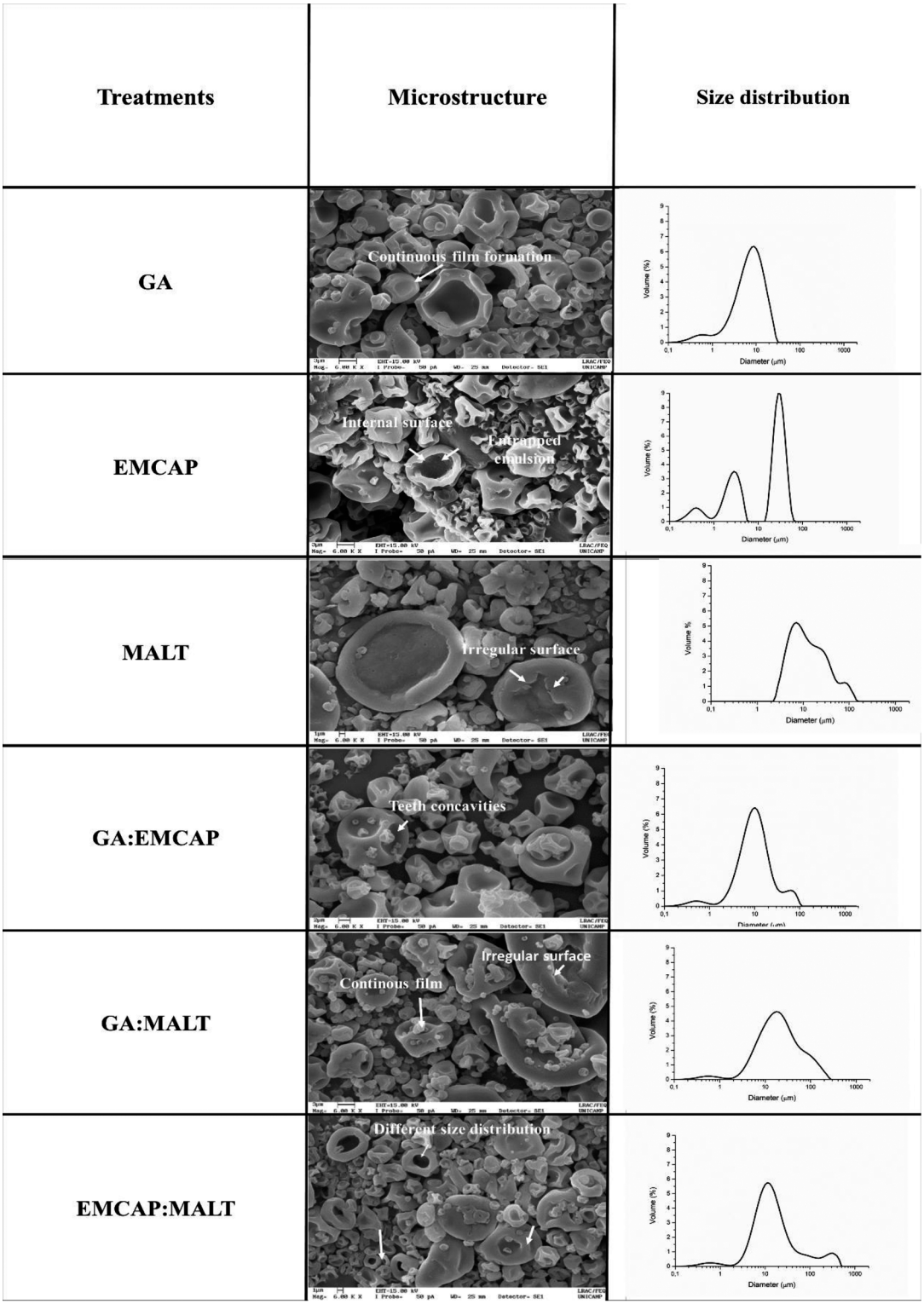
According to Techawinyutham et al. (2019), the signal peaks in 2854 to 2925 cm^{-1} relate to capsaicin (CAP), the main compound responsible for *Capsicum* oleoresin pungency. The peak at 1750 cm^{-1} corresponds to the stretching vibration of C═O (carbonyl group) and the one at 1510 cm^{-1} suggests flexion of N—H group. In addition, a band in the 750 cm^{-1} characterizes regional vibration of C—H and C—C, recognized as the aromatic bonds of the capsaicin phenyl ring. The main band correlated to samples of pure wall materials (GA, EMCAP, and MALT) is placed around 995 cm^{-1}, comprehending a typical polysaccharide absorbing region (Binsi et al., 2017). At last, the loading profile of microparticles presented a band deformation of the carbonyl group and the hydroxyl group, implying that *Capsicum* oleoresin was likely bound to chemical groups of the wall materials. The same behaviour was reported by Freitas et al. (2018) for capsaicin-loaded albumin nanoparticles.

Microstructure of microparticles, size distribution and volumetric mean diameter

Scanning electron microscopy was used for assessment of the differences among *Capsicum* oleoresin microparticles made with various carbohydrates as wall materials. The microstructure of microparticles is seen in Fig. 3. Microparticles formulated by modified corn starch (EMCAP) or/and gum arabic show spherical geometry, teeth concavities in the surface and no pores. EMCAP particles also show small pits where the emulsion is likely located. In general, commercial wall materials (GA; EMCAP; GA: EMCAP) present good film forming properties, resulting in particles with smoother surfaces, as similarly described by Alcântara et al., 2019.

When modified malt is used as a carrier agent, due to its insoluble fraction, a blood cell structure and greater roughness in particle coating are seen. This effect decreased for MALT combined to commercial materials (GA: MALT; EMCAP: MALT) and it is associated to a low content of insoluble solids, which thus forms particles with smoother structures.

Different sizes were observed for all microparticles formulations, according to graphic data obtained from Fig. 3, in accordance with the micrograph images mentioned above. The formulation containing gum arabic only presented two peaks that ranged from 0.1 to 1 μm and a third and greater peak varying from 1 to 50 μm. Microparticles containing



*GA: Arabic gum, EMCAP: modified corn starch-OSA(EMCAP®); MALT: Stearic acid modified malt; GA:EMCAP, GA:MALT and EMCAP:MALT

Fig. 3. Microparticles morphology and size distribution *GA: Arabic gum, EMCAP: modified corn starch-OSA(EMCAP®); MALT: Stearic acid modified malt; GA: EMCAP, GA:MALT and EMCAP:MALT.

EMCAP in their formulation showed three peaks, the first ranging from 0.1 to 1 μm, the second from 1 to 10 μm and the third from 10 to 100 μm. The MALT exhibited a very large size range, with diameters ranging from 5 to 120 μm when compared to other formulations. The high range of particles containing MALT are related mostly to the composition of malt. Modified malt is a complex material composed of insoluble solids, which all contribute to the formation of agglomerates.

The combination of GA: EMCAP materials resulted in particles with varying sizes and polymodal behaviour with a small peak between 0.1 and 1.5 μm, a second peak varying from 1.5 to 100 μm, typical of polysaccharides mixtures (Goëlo et al., 2020). On the other hand, the incorporation of MALT into formulation containing EMCAP or GA was observed a differential volume distribution with one short peak consisting of a small average size of particles, and another peak with a wide range of a large volume reaching values over 200 μm, which implies agglomeration of the powders.

Table 3 shows the mean volumetric diameter (D_{43}) and the SPAN values for all formulations. The treatment containing gum arabic only in its composition presented the lowest D_{43} (14.7 ± 0.5 μm), whereas MALT particles presented an average diameter of 186.7 ± 6.3 μm. The distribution graph of microparticles containing MALT evidenced the presence of small particles and microparticles with a considerable size, which contributed to obtaining a high D_{43} average value (186.7 ± 0.3). In addition, coating of microparticles by modified malt presented more agglomerates due to its rich composition in low molecular weight sugars.

Influence of different biopolymers on Capsicum oleoresin encapsulation efficiency and capsaicin retention

Microencapsulation efficiency implies the degree of entrapment of the emulsion by the wall material and quantify the amount of bioactive compound is encapsulated in the microparticle (Binsi et al., 2017, Goëlo et al., 2020). The encapsulation efficiency (OEE) of *Capsicum* oleoresin varied from 68.5% to 91.6%, pointing the influence of carbohydrates on the oleoresin retention (Table 3). Data showed higher OEE values for GA and EMCAP in relation to other treatments. These results are related to the strong emulsifying property of these materials and to their ability to form continuous films on the microparticle. As a result, the development of a continuous film entailed a stable system with better entrapment of the emulsion, reaching high values to oleoresin retention. In respect of microparticles composed by MALT, a low oleoresin efficiency encapsulation of 68.5% was observed. Possible reason for this trend can be associated with big droplet size of emulsions and surface imperfections of microparticles (Jafari, Assapdoor, He, & Bhandari, 2008; Linke, Linke, Hinrichs, & Kohlus, 2019).

Commercial materials in combination with modified malt (GA: MALT; EMCAP: MALT) promoted a significant increase ($p < 0.05$) in the *Capsicum* oleoresin encapsulation efficiency. This improvement was associated to smoother particle covering, synergy between the hydrophobic groups of wall materials and better rearrangement of the carbohydrates, which all generated a strong emulsion entrapment.

A similar trend for OEE (from 84.2 ± 1.5% to 96.2 ± 0.2%) was reported for *Nigella sativa* L. oleoresin microparticles produced by *spray drying* using gum arabic and maltodextrin (1: 1) as wall materials. The authors associated the high encapsulation efficiency and the strong capacity to form a continuous film to gum arabic and the low viscosity of the solution to maltodextrin (Edris et al., 2016).

Among the compounds present in *Capsicum* oleoresin, the capsaicin (*trans*-8-methyl-*N*-vanillyl-6-nonenamide) has proved several health benefits, being a compound of great interest by scientific research. That way, the purpose of the *Capsicum* oleoresin encapsulation was also to produce microparticles carried with capsaicin with potential application as a functional ingredient. Thus, microparticles with high capsaicin content were obtained even using distinct carbohydrates matrixes, as is shown in Table 3. The obtained values were within the range from 2.35 to 3.49 mg capsaicin/g microparticles, i.e., the encapsulation capsaicin retention could be associated to *Capsicum* oleoresin entrapment into particle, and the efficiency of spray drying method to encapsulate both *Capsicum* oleoresin and capsaicin. Other examples of microparticles rich in hydrophobic ingredients produced by spray drying were found in the scientific literature, such as chia oil (Alcântara et al. 2019), astaxanthin (Boonlao et al. 2020), and resveratrol (Consoli et al. 2019).

Antioxidant activity of microparticles

ORAC and FRAP methods showed that all microparticles rich in capsaicin and various other bioactive compounds had high antioxidant capacity (Table 3). However, significant differences among formulations were observed. GA presented the highest value, by ORAC, for antioxidant activity (AA), of 89.4 ± 9.7 (μmol TE/ g microparticle); meanwhile, the mixtures GA: EMCAP; GA: MALT; EMCAP: MALT showed an antagonistic effect, thus resulting in a decreased antioxidant activity. The same trend was reported previously in the literature. Higher values for AA by ORAC were obtained for propolis microparticles carried by gum arabic than those for the formulation containing maltodextrin as an encapsulating material (Andrade et al., 2018).

Otherwise, FRAP analysis resulted in values ranging from 21.3 to 88.9 μmol TE/g microparticles, which also implied a synergic behaviour between mixtures of wall materials. Blends composed by GA: MALT and EMCAP: MALT exhibited higher values for AA (88.9 ± 3.8, and 72.3 ± 2.6 μmol TE/ g of microparticles, respectively).

These outcomes are due to the content of oleoresin in the microparticle, the antioxidant capacity of oleoresin compounds and each of the distinct wall materials used. Andrade et al. (2018) concluded that the reaction mechanism used in ORAC analysis has high reactivity to aromatic substances and compounds that do not act as electron donors. In contrast, FRAP measures the ability of the sample to donate electrons; therefore, its strength may be correlated to the phenolic substances that form the product.

AA values for *capsicum* microparticles are in accordance with the results in the literature. So, California red peppers presented averages of 14.78 μmol TE/g of pepper by the ORAC test and 79.62 μmol of eq. $FeSO_4$/ g of pepper by FRAP analysis.

Overall, spices such as peppers and their by-products are known as good antioxidant agents regarding oils and fats, and hence their powerful off-taste reduction from oxidative degradation. Spices are of adequate employment by the food industry since they are natural ingredients that can, thus, be applied to clean label products.

Effect of capsaicin microparticles consumption on in vivo experimental model

Various studies using different experimental models showed that capsaicin and *Capsicum* oleoresin are not toxic. However, the bioaccessibility and bioavailability of capsaicin are modified after the microencapsulation of *Capsicum* oleoresin (El Abbassi et al., 2016). Thus, the present study aimed at evaluating the effects of diets supplemented with *Capsicum* oleoresin either encapsulated with corn oil or not, in terms of food intake, body weight gain and acute toxicity parameters.

Each experimental group received different diets, i.e., with different percentages of capsaicin, representing 0.0022% of diet 2, 0.0044% of diet 3, 0.0014% of diet 4 and 0.0028% of diet 5, as detailed in Table 2.

The consumption was assessed weekly, for 28 days, as shown in Fig. 4A. No significant difference in intake was observed for diets, except for group 4, which showed a reduction in dietary consumption in the fourth week. Thus, the addition of capsaicin-rich microparticles did not affect the animals' dietary intake, which could become an issue due to the pungency of the compound.

Fig. 4B shows the total weight gain of the animals during the four experimental weeks. Overall, the addition of the capsaicin microparticles in the animals' diet promoted smaller weight gains, dose-

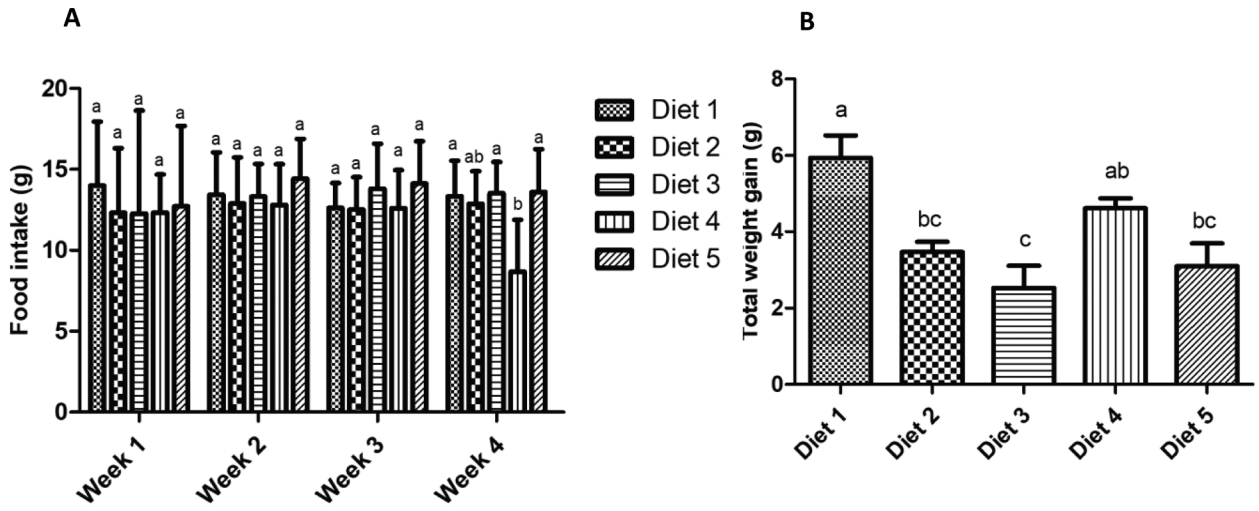


Fig. 4. A) Food Intake per week. B) Total weight gain by the mice after 28 days.

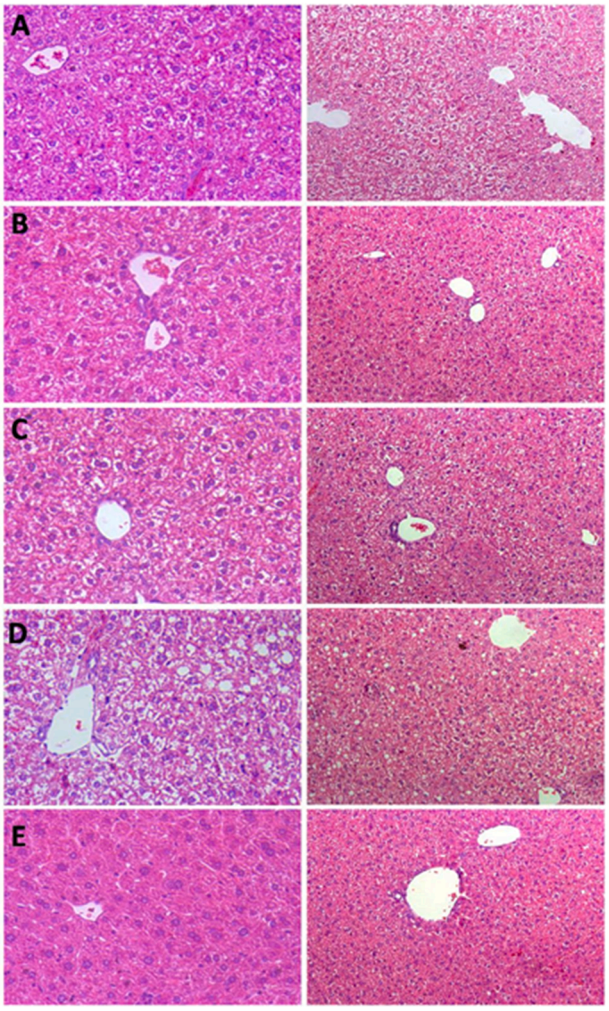
dependent, in animals upon comparison to the control group (diet 1). Only group 4, which contains the lowest capsaicin content, does not show a statistical difference concerning weight gain when compared to the control group.

The consumption of a high-fat diet (high-fat and high-calorie) is an experimental model widely used to induce obesity in experimental models. The difference in dietary and caloric intake was not observed among groups. *Capsicum* oleoresin promoted less weight gain and provoked changes in metabolic or thermogenic activity, as previously reported in the literature (Ohyama et al., 2016). That way, the high-fat diets with a capsaicin content ranging from 0.0022 to 0.0044% (diets 2, 3, and 5) may contribute to a lower weight gain in animals. A similar result was observed in a study by Tan et al. (2014), who attended a reduction in body weight gain in Sprague Dawley rats with 30 mg capsaicin/kg during a five-week experiment. The authors observed that the intake of microencapsulated capsaicin had a significant effect on body weight reduction when compared to the consumption of non-encapsulated capsaicin. In another study authors also reported that food intake from a high-fat diet supplemented with *Capsicum's* oleoresin nanoemulsions was able to reduce the final body weight of Sprague Dawley rats, decrease the total adipose tissue and reduce the level of plasma triglycerides (Kim et al., 2014)

No impact of different diets on the tissue mass of the animals (liver, mesenteric adipose tissue, spleen, heart) or fasting blood glucose, with no difference observed among the groups (Supplementary Material – Table S1). AST and ALT analysis show if different diets caused any toxicity in animals, since increases in plasma levels of cytosolic aminotransferase enzymes indicate cellular liver damage. In addition, increases in the AST/ ALT ratio are often associated to liver damage (non-alcoholic steatohepatitis) (Sorbi et al., 1999). The AST and ALT levels were high only in the control group and for diet 4. The AST/ ALT ratio was high only in diet 4 (Supplementary Material – Table S1), which corroborates with the lower protection of these diets on the gain body weight.

Lipid oxidation results did not show statistically significant differences among groups regarding the level of hepatic TBARS, which values varied from 57.7 ± 6.2 to 59.2 ± 5.8 nmol MDA/ mg of tissue (Supplementary Material – Table S1). However, statistical differences were observed in the histological evaluation of the liver samples of the animals. The liver sections stained with HE showed that 28 days of ingestion of HFD induced dramatic accumulation of fat in the liver (hepatic steatosis), as well as the formation of cell ballooning, which is indicative of reversible liver damage, in spite of the absence of inflammatory infiltrates in the samples Fig. 5E. Diet 5 (Fig. 5E) containing *Capsicum* oleoresin and corn oil, promoted a better liver protection concerning fat accumulation and cellular ballooning than other diets (diet 1, 2,3, and 4). Future studies are necessary to investigating if this protective effect is associated to an improvement of the bioavailability of capsaicin by the addition of medium-chain oil. Clegg et al. (2013) observed that the ingestion of a meal containing chili or pepper, sources of capsaicin, associated to medium-chain triglycerides increased energy expenditure and thermogenesis in healthy individuals when compared to the consumption of a meal containing chili or pepper associated to sunflower oil.

Therefore, this research reached the objective of producing microparticles with functional benefits, rich in capsaicin. Total weight gain of the animals and the histology of the hepatic tissue showed a positive dose-dependent protective effect of *Capsicum's* oleoresin against the damage caused by obesity, induced by a high-fat and high-calorie diet. However, additional studies focusing on metabolic pathways and cell signalling can clarify the related mechanisms.



	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
Steatosis	$2.5^{a} \pm 0.83$	$1.83^{a,b} \pm 1.16$	$1.67^{a,b} \pm 1.03$	$1.83^{a,b} \pm 1.16$	$0.33^{b} \pm 0.51$
Balloning	$1.33^{a} \pm 0.51$	$1.17^{a,b} \pm 0.40$	$0.50^{b,c} \pm 0.54$	$1.33^{a} \pm 0.51$	$0.17^{c} \pm 0.40$
Inflamation	$0.17^{a} \pm 0.40$	$0.33^{a} \pm 0.51$	$0.00^{a} \pm 0.00$	$0.17^{a} \pm 0.40$	$0.17^{a} \pm 0.40$

Fig. 5. Hepatic histological analysis Sections of liver samples stained with H&E for visualization of hepatocytes morphologies and score for steatosis, ballooning and inflammation assessed in mice fed a (A) high-fat diet without microparticles of OC (Diet 1,n = 6), (B) a high-fat diet containing 10% of microparticles of OC (Diet 2, n = 6), (C) a high-fat diet containing 20% of microparticles of OC (Diet 3, n = 6), (D) High-fat diet containing 10% of microparticles of OC added of corn oil (Diet 4, n = 6) and (E) a high-fat diet containing 20% of microparticles of OC added of corn oil (Diet 5, n = 6) for 28 days from weaning. Magnification: 200x and 400x. Values represent mean ± S.D. (*Means that do not share a letter are significantly different).

Conclusions

In this study, all carbohydrates affected the powder properties related to wettability, particle size, morphology, antioxidant activity and capsaicin retention. Regardless of the formulation, these materials proved to be great carriers' agents for capsaicin, thus turning these powders into potentially appealing ingredients. All formulations presented high contact angle, great solubility in water, low water activity and hygroscopicity. FT-IR analysis showed that capsaicin was successfully bound to the structure of the carbohydrates. Along with these results, animal assays using a high-fat diet (HFD) supplemented with microparticles rich in capsaicin evidenced that the consumption of the latter promoted a decrease in body weight gain compared to the HFD control. This outcome points to a promising employment of *Capsicum* oleoresin microparticles to controlling obesity and nonalcoholic steatohepatitis. Future studies on the *Capsicum* oleoresin microencapsulation, using different techniques, under various conditions and with distinct carrier materials are necessary, though, to improving the encapsulation efficiency and antioxidant activity. Assessing capsaicin microparticles regarding their gastrointestinal release and transport mechanism may also be helpful to assign satiety.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence

the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2021.100179.

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