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ORIGINAL PAPER

New Insights in the Use of Trehalose and Modified Starches for the Encapsulation of Orange Essential Oil

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Abstract During the encapsulation of aromas by spray-drying, some volatile components may be lost; consequently, the sensory profile could be modified in the final product. Therefore, the selection of the carrier matrix for the encapsulation is crucial to obtain high aroma retention and long shelf-life stability, reduce aroma oxidation and increase physical stability of the powder. With the aim of studying the use of trehalose for the encapsulation of orange essential oil, emulsions were prepared containing mixtures of trehalose–maltodextrin (TMD) and sucrose–maltodextrin (SMD). Two modified starches were used as emulsifiers. The emulsions were spray-dried and stored at 25 and 37 °C. The aroma retention was evaluated using head space–solid phase microextraction/gas chromatography–mass spectrometry (HS-SPME/GC-MS) and by sensory evaluation. In addition, some physical properties of orange essential oil powders, such as water sorption and glass transition temperature (T_g), were determined. The sensory profiles obtained for TMD and SMD were different: TMD formulations retained mainly limonene, while SMD retained mostly α-pinene and myrcene. The modified starches (Capsul and Hi Cap) used as emulsifiers also affected the retention of certain volatiles. Therefore, the selection of components in the carrier matrix is relevant to the retention of aromas in orange oil powders. Regarding the physical properties, TMD formulations presented better characteristics in comparison to SMD, particularly due to the higher T_g values. The high aroma retention levels and the good physical characteristics are promising results in relation to the inclusion of the developed formulations in dehydrated citric juices.

Keywords Orange essential oil · Trehalose · Encapsulation · Spray-drying · Emulsifiers · Glass transition temperature

Introduction

The flavor compounds used in the food industry are mainly in the form of liquid at room temperature. Most of these flavors exhibit considerable sensitivity to air, light, moisture, irradiation and elevated temperature. The microencapsulation of volatile ingredients is beneficial to limit aroma degradation or loss during processing and storage (Jafari et al. 2008). Spray-drying is a common technique for food encapsulation (Murugesan & Orsat, 2011) and is frequently used for aromas micorencapsulation (Soottitantawat et al., 2005). For hydrophobic flavors, an emulsification step has to be performed before spray-drying. In order to generate emulsions kinetically stable for a reasonable period of time, emulsifying components have to be included in the formulations (Mc Clements, 1999). Some modified starches, such as those obtained from waxy maize by derivatization with octenyl succinic anhydride (OSA), have been used to stabilize emulsions and to encapsulate sensitive ingredients (Bhosale & Singhal, 2006; Cova et al. 2010). In addition to the good emulsifying properties, they showed high volatile retention capacity, and were

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recommended for the encapsulation of citric essential oils among other flavors (Aguilar-Chavez, 2007).

The flavor retention of encapsulated spray-dried powders is governed by various aspects, of which the carrier matrix is an important factor. Maltodextrins are commonly used for this application, as they have high glass transition temperature (T_g) and provide good product stability (Bhandari & Hartel, 2005; Jaya & Das, 2009). Disaccharides like sucrose are sometimes included in the formulations to improve aroma retention characteristics (Menting et al. 1970). Spray-dried orange oil powder showed that the presence of sucrose in the carrier formulation affected storage stability in a negative way due to the low T_g (Busso Casati et al. 2007). Trehalose is a non-reducing disaccharide that has a higher T_g (Cardona et al. 1997) than sucrose (Roos, 2002) which could contribute to the physical stability of spray-dried powders. The addition of trehalose to dehydrated strawberry and apricot purees resulted in lower loss of aroma when compared with sucrose (Komes et al. 2003; Komes et al. 2005).

Orange essential oil has been encapsulated by spray-drying using different carrier matrices and different drying conditions. Flores-Martínez et al. (2004) used N-Lok and arabic gum 15–45 % (w/w) matrix and 5 % (w/w) orange oil, and observed better retention yield with N-Lok. Edris and Benrgnstahl (2001) have encapsulated orange oil by first preparing a triple emulsion o=w=o=w and then spray-drying using sodium caseinate and lactose as shell material. This work showed that the drawback of preparing a second emulsion was the dilution of the flavor oil, and the much lower payload in the spray-dried microcapsule. Kim and Morr (1996) encapsulated orange oil using soy protein isolate (SPI), gum arabic (GA), sodium caseinate (SC) and whey protein isolate (WPI) as carrier matrices. The authors concluded that SPI rendered the most stable formulation against oxidation and GA the least stable. Also, WPI and SPI were the most effective as orange oil microencapsulants. Risch and Reineccius (1988) studied the effect of emulsion sizes of orange peel oil on flavor retention and shelf life using gum arabic or Amiogum 23 as carriers. Their results suggested that a smaller emulsion size yielded larger orange oil retention and smaller amount of surface oil, but did not produce a longer shelf life. Galmarini et al. (2008) analyzed spray-dried orange oil encapsulated in different amorphous matrices comprised of maltodextrin and different combinations with sucrose, trehalose, lactose, modified starch and gum arabic. Their study included sensory analysis and electronic nose. The sensory analysis results showed that matrix composition determined the aromatic profile of spray dried encapsulated orange flavors.

The aim of the present work is to compare the performance of different carrier matrices (mixtures of maltodextrin with trehalose or sucrose) and different emulsifying agents (the modified starches Capsul and Hi Cap) for the encapsulation of orange essential oil. The aromatic profiles and the retention of aroma compounds after spray-drying and storage were evaluated. Some physical properties of the powders were also studied.

Materials and Methods

Materials

Food grade materials were used unless otherwise indicated.

Sucrose (analytical grade) and trehalose were from Merck (Darmstadt, Germany) and Cargill Inc. (Wayzata, MN, USA), respectively. Capsul was from Gelfix (Buenos Aires, Argentina). Maltodextrin DE 12, Hi Cap and orange essential oil (obtained from orange peel) were from Saporiti (Buenos Aires, Argentina). Linalool (analytical grade) was from Extrasynthese (Genay Cedex, France).

Hi Cap 100 and Capsul are two modified starches derived from waxy maize, and obtained by derivatization with octenyl succinic anhydride (OSA). The average molecular weights of the used modified starches are 335,000 for Hi Cap and 84,000 for Capsul.

Preparation of Formulations

Preparation of Emulsions

Pre-emulsions were formed by adding 5 % orange oil to solutions formed by 60 % water, 3 % modified starch (Capsul or Hi Cap) and a mixture (1:1) of 16 % maltodextrin plus 16 % sucrose (sucrose–maltodextrin, SMD) or 16 % maltodextrin plus 16 % trehalose (trehalose–maltodextrin, TMD). Pre-emulsions were stirred for 2 min at 25 °C and 750 rpm. Emulsions formation was completed using a VCX 750 ultrasonic processor (Vibra Cell Sonics, Newton, CT, USA) at a frequency of 20 kHz and amplitude of 30 % during 20 min. Four formulations were obtained: $SMD_{Hi\ Cap}$, SMD_{Capsul}, $TMD_{Hi\ Cap}$ and TMD_{Capsul}.

Spray-Drying

The emulsions were spray-dried using a laboratory-scale, Mini Spray Dryer Büchi B290 (Flawil, Switzerland). The operational conditions of the drying process were inlet air temperature 175±3 °C, outlet air temperature 83±3 °C, flow rate 8 ml min^{-1}, air pressure 3.2 bar and nozzle diameter 1.5 mm (Sosa et al. 2011). The obtained powders were collected into sealed polyvinylidene chloride (PVDC) bags and then stored at −20 °C. The PVDC bags were chosen due to their excellent barrier properties against water vapor and thus protect the hygroscopic powders.

The initial water activity of the different powders was between 0.1 and 0.2. The powders were loaded into vials and transferred into desiccators, which were kept for 15 days

at 25 °C, over saturated salt solutions that provided constant relative humidities (RH) of 11 % (LiCl), 22 % (CH_3COOK) and 33 % ($MgCl_2$) (Greenspan, 1977).

Storage

The 25-ml vials were loaded with 25 g of powders equilibrated at 11 % RH and were hermetically sealed and stored at 25 and 37 °C. At certain times (0, 3, 6 and 9 months), the vials were removed for the different determinations. The RH (11 %) used was selected in order to assure that all the formulations would be in the glassy state at the storage conditions.

Characterization of Powders

Water Content

Titration was carried out with a Karl Fischer titrator DL 31 from Mettler-Toledo (Zurich, Switzerland), according to Sosa et al. (2011). Three replicate measurements were done.

Thermal Transitions

Glass transitions were determined by differential scanning calorimetry (DSC) using a DSC 822e Mettler Toledo calorimeter (Schwerzenbach, Switzerland). The instrument was calibrated with water (0.0 °C), indium (156.6 °C), lead (327.5 °C) and zinc (419.6 °C). All measurements were performed at a heating rate of 10 °C min^{-1}. Approximately 10 mg of each sample was placed in 40 μl aluminum pans which, in turn, were hermetically sealed. An empty pan served as reference. Glass transitions were recorded as the onset temperature of the discontinuities in the curves of heat flow versus temperature. An immediate rescan was run for each sample to eliminate relaxations and improve interpretation of the thermograms (Lievonen et al. 1998). Thermograms were evaluated using STARe thermal analysis program (Mettler-Toledo v. 4.01.). An average value of at least two replicates was reported.

Scanning Electron Microscopy

A Zeiss microscope Supra 40 (Oberkochen, Germany) was used. The samples were placed in an aluminum support and coated with gold nanoparticles by using a sputter coater (Cressington Scientific Instruments 108; Watford, UK). The vacuum levels for the sputter coater and the microscope were 0.07 and 8×10^{-6} mbar, respectively). The images were taken with the detector within the lens, using an acceleration voltage of 3.00 kV. Duplicate samples were measured, and four magnifications were analyzed for the same image (1, 2, 5 and 10 KX). The samples were kept in evacuated desiccators at room temperature until the gold coating.

Volatiles Analysis

Instrumental Methodology for the Study of Aromas: HS-SPME/GC-MS

The volatile compounds were isolated from the headspace of the vials using head space–solid phase microextraction (HS-SPME). This technique has been reported to be cheap, solventless, fast and with a high reproducibility, limits of detection and sensitivity (Balasubramanian & Panigrahi, 2011). A Supelco fiber holder with a 100 μm polydimethylsiloxane (PDMS) coated with fused-silica fiber was employed.

The parameters used in this work were based on Jordan et al. (2001). The powder (567±10 mg) was placed into a 18 ml vial and reconstituted with distilled water (165±10 ml). As internal standard, 20 μl of linalool (Genay Cedex, France) was added. A saturated 36 % (v/v) NaCl solution was added. Samples were stirred for 5 min for complete reconstitution. The vials were sonicated at 40 °C. The fiber was exposed to the headspace for 30 min, then it was removed and the volatiles were thermally desorbed during 0.6 min in the injection port of the gas chromatograph (GC) with a flame ionization detector (FID) and a mass spectrometry detector (MS), GC-FID-MS instrument.

The GC-MS analysis of the volatiles was carried out on a Perkin Elmer (Massachusetts, USA) Clarus 500 apparatus, equipped with one injector (split/splitless) connected by a flow splitter to two capillary columns: (a) polyethylene glycol MW ca. 20,000 and (b) 5 % phenyl–95 % methyl silicone, both 60 m×0.25 mm with 0.25 μm of fixed phase (J&W Scientific, Folson, USA). The polar column was connected to a FID, whereas the non-polar column was connected to a FID and a quadrupolar mass detector (70 eV) by a vent system (MsVent™) Perkin Elmer.

The identification of volatiles was performed using two methods: comparing the mass spectra from the typical libraries (Adams, 2007; Wiley/NIST, 2008) and measuring the retention indices of a homologous series of alkanes (C6–C20) obtained by injection in the two columns in the same operating conditions.

Limonene content was used as marker to determine the total orange oil content in each formulation. For this purpose, calibration curves for limonene were constructed using the same components of the continuous phase of the four formulations to avoid matrix interference.

Sensory Evaluation

Panel Training Twenty five assessors (20 females and 5 males, 19–23 years old) from the Facultad de Ciencias Agrarias, Pontificia Universidad Católica Argentina were trained in discrimination testing and aroma descriptive methods using standards (see Table 1 for definition, attributes and standard recipe) and aroma matching.

Table 1 Definition, Attribute and Standard Recipe used by the Trained Panel to describe the Aroma Profile of Encapsulated Orange Essential Oil

Attribute	Standard récipe	Definition
Fresh orange	Filter paper soaked in fresh orange oil (Saporiti, Argentina), placed in a sealed glass flask	Aroma evocative of natural, fresh recently pressed orange juice
Juice powder	50 g of juice powder orange (Tang, Argentina) reconstituted in 100 ml of distilled water	Related to the aroma of orange juice obtained from reconstituted juice poder
Citrus	Filter paper soaked in Gamma terpinene essence oil gamma terpinene (IFF, Argentina) placed in a sealed glass flask	Aroma associated to strong piercing, citric smell
Vitamin C	Orange flavored vitamin C (Redoxón, Argentina)	Related to the aroma of medicine and vitamin complex
Lemon	Filter paper soaked in citral (Saporiti) placed in a sealed glass flask	Associated with a strong and penetrating aroma of citrus
Plastic	Fresh orange juice placed in a plastic recipient, heated for 2 min in a microwave oven and stored at room temperature	Aroma associated to plastic containers
Pine	Filter paper soaked in mircene (Firmenich, Argentina), placed in a sealed glass flask	Pine aroma associated
Fruity	No standard was used	Fruit aroma associated
Floral	Filter paper soaked in dodecanol, placed in a sealed glass flask	Aroma associated with flowers/green fruit
Glue	Filter paper soaked in ethyl acetate, placed in a sealed glass flask	Aroma associated with adhesive
Orange peel	Filter paper soaked in pure orange essence oil orange peel (Ledesma, Argentina), placed in a sealed glass flask	Aromatics associated to orange peel
Woody	Filter paper soaked in orange pure essence oil valencia orange (Ledesma), placed in a sealed glass flask	Suggestive of the odour of tree bark
Pungency	Orange flavoured, effervescent antacid salts (Uvasal, Argentina)	Tingling sensation on the surface of the nose mucosa
Green orange	No standard was used	Characteristic odor of unripe orange
Ripe orange	No standard was used	Aroma associated with ripe orange
Marmalade	Orange juice, peel and sugar heated at 85 °C for 60 min	Aromatics associated to marmalade
Cooked	Filter paper soaked in cooked flavor (Firmenich), placed in a sealed glass flask	Aroma associated with cooked sweet orange/marmalade
Tangerine	5 g of juice powder tangerine (Clight, Argentina) reconstituted in 100 ml of distilled water	Aromatics related to tangerine juice
Candy	Sin estándar	Aroma associated with orange candy
Solvent	Filter paper soaked in acetone, placed in a sealed glass flask	Aroma associated to solvent and paint
Grapefruit	5 g of juice powder grapefruit (Clight) reconstituted in 100 ml of distilled water	Aromatics related to grapefruit juice

Sorting Task The similarity among samples was evaluated using a sorting task, which is a simple method of categorizing products that share similar characteristics into the same group. The assessors analyzed separately the matrices containing trehalose and sucrose (Table 2). The test was done in duplicate in two sessions using the sorting task with description methodology (Chollet et al. 2011). Sample (powder reconstituted with distilled water to its original volume) of 1 ml was placed in glass bottles with lids and presented in a random order. The assessors were asked to smell all the bottles (14 samples per matrix) and to group the samples that were perceived as similar, making as many groups as they wanted. At least three attributes had to be selected from the standards (Table 1) to describe each group. Testing took place in individual booths kept at 22 °C, in daylight (6,500 K).

Aroma Profile The check-all-that-apply (CATA) method was used (Valentin et al. 2012). The assessors received two training sessions in aroma recognition and then evaluated the samples in triplicate. They received a list of attributes (Table 1) and selected those that best described the samples. A descriptive, semi quantitative method was used because of the limitations of intensity scoring in low-intensity complex aromas (Campo et al. 2010).

The powder reconstituted to original volume was placed in glass bottles with lids and presented in random order. The

Table 2 Encoding Used for Identification of the Formulations and Treatments (times and Temperatures of Storage)

Samples	Treatments						
	Zero time	3 months		6 months		9 months	
		25 °C	37 °C	25 °C	37 °C	25 °C	37 °C
TMD_{Capsul}	1[a]	5	9[a]	13[a]	17[a]	21	25
$TMD_{Hi\ Cap}$	2[a]	6	10[a]	14[a]	18[a]	22	26
SMD_{Capsul}	3[a]	7	11[a]	15[a]	19[a]	23	27
$SMD_{Hi\ Cap}$	4[a]	8[a]	12	16[a]	20[a]	24	28

[a] Formulations selected for aroma profile

assessors sniffed the headspace and selected the attributes from the list. At the same time, the evaluators could smell the standards (Table 1) in order to help in the profiling process.

Data Analysis

Analysis of variance was carried out by using IBM SPSS statistics v. 20.0 software (IBM, 2011, Armonk, New York, USA). Sorting task data were analyzed by applying a multidimensional scaling method. Aroma profile data were analyzed by citation frequency, chi-square distribution and factorial correspondence analysis (FCA) followed by hierarchical cluster analysis using Infostat v. 2008 (Di Rienzo et al., 2008). Instrumental and sensory data were compared by means of a partial least square (PLS) regression.

Results and Discussion

Physical Characteristics

Water sorption, glass transition temperature (T_g), and microstructure were determined for the different spray-dried formulations stored at RH between 11 % and 33 %. This RH range was selected because dehydrated powders (like the present formulations) are usually kept in low moisture conditions to avoid deleterious physical changes. Figure 1 shows the water sorption isotherms obtained after 15 days of humidification at 25 °C; and T_g values obtained for the formulations containing trehalose (a) and sucrose (b). The different formulations presented similar water sorption behavior independent of the modified starch used as emulsifier (Hi cap or Capsul). Powders containing trehalose showed slightly higher water sorption at each RH than those containing sucrose. The water sorption values were lower than those observed for similar formulations containing encapsulated citral flavor (Sosa et al., 2011).

As expected T_g values for SMD powders were lower than those observed for TMD. No differences on the T_g values were observed between the powders containing the different modified starches, which could be due to the low starch concentration in the formulations. In addition, it was found that the glassy state at room temperature (25 °C) could be maintained up to 33 % RH and 22 % RH for TMD and SMD formulations respectively. T_g values were higher than those observed for encapsulated citral powders (Sosa et al., 2011). However, this could be related to the lower water content of the encapsulated orange oil powders.

The external morphology of the powders is shown in Fig. 2. The formulations containing Hi Cap presented spherical particles with a smooth surface, with no surface oil. These results agree with previous results for encapsulated bergamot oil (Penbunditkul et al. 2011) and D-limonene (Soottitantawat et al., 2005) using Hi Cap as carrier matrix. Formulations containing Capsul showed elongated particles with smooth surface.

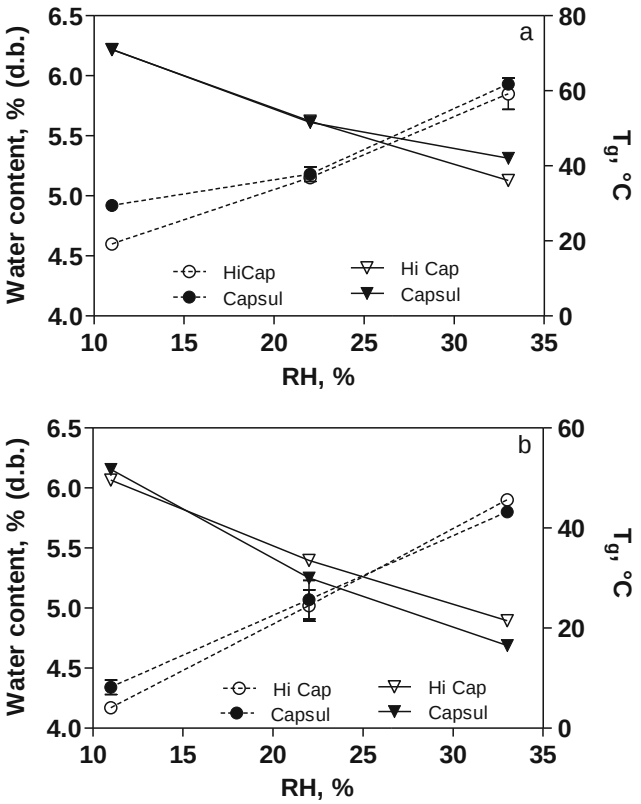

Fig. 1 Water Content (*dashed lines*) and Glass Transition Temperature (*solid lines*) as a Function of RH for the Formulations Containing Trehalose (**a**) and Sucrose (**b**)

Volatile Analysis

Global Flavor Analysis by GC

After spray dying and humidification, all the formulations lost approximately 20 % of the orange flavor. Although no statistical differences were observed among the different powders, $TMD_{Hi\ Cap}$ presented the highest global retention (78.8 %) and SMD_{Capsul} showed the lowest global retention (72.3 %).

Figure 3 shows the percentage of orange oil retained by the four formulations during storage at 25 °C (Fig. 3a) and 37 °C (Fig. 3b). A gradual decrease in flavor percentage was observed over time, the aroma loss being higher at 37 °C than at 25 °C. TMD formulations retained considerably higher percentages of aroma than sucrose ones. A particular behavior was observed regarding the two modified starches employed as emulsifiers. For sucrose formulations, the retention behavior was independent of the chosen starch, whereas in the presence of trehalose, higher losses were detected with Capsul compared to Hi Cap containing samples. Thus, the choice of emulsifier has to be

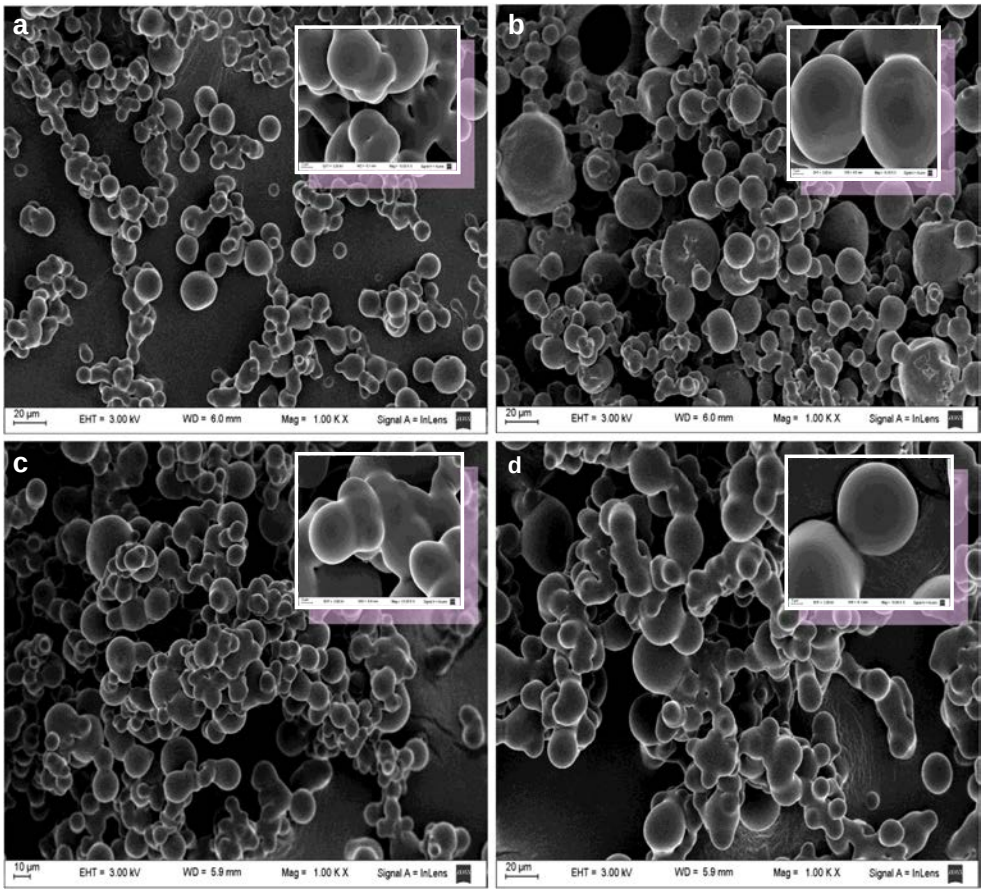

Fig. 2 SEM Micrographs of the Surface of the Powder Particles Containing Encapsulated Orange Essential Oil Using a 1,000× Magnification for the Formulation TMDCapsul (**a**), TMDHi Cap (**b**), SMDCapsul (**c**) SMDHi Cap (**d**). The Magnification of the Inset Figures is 5,000×

taken into account according to the other components selected for the carrier matrix.

Chromatographic Profiles

Figure 4 shows the main volatile components detected in the head space analysis performed after spray-drying and humidification at 11 % RH. The volatile components that were found in higher proportions were limonene and myrcene, whereas lower proportions of α-pinene, sabinene, heptanal and n-octanol were detected. Linalool was also detected, but it was used as the internal standard. All these components have been cited in the literature as characteristic of orange (Radford et al. 1974; Ahmed et al. 1978; Jordan et al., 2001). The terpene group was composed of limonene, myrcene, sabinene and α-pinene. These components were considered by Shaw (1991) to be important contributors to the aroma of orange juice. Furthermore, the main components present in orange peel are terpenes (mostly monoterpenes), of which limonene is the most abundant, ranging between 79 % and 83 % of the total volatiles (Ikeda et al. 1962). Heptanal was the only aldehyde present among the volatiles. Heptanal contributes significantly to the aroma of orange essential oil and is regarded as one of the identity standards of this oil (Arctander, 1969; Boelens & van Gemert, 1987; Shaw, 1991). According to these authors, a decrease in heptanal concentration means a significant reduction in the quality of aroma.

In general, similar retention values were registered for SMD and TMD. In the case of limonene, higher retention was observed in the presence of Hi Cap in comparison with Capsul containing formulations.

Figure 5 shows the concentration of the different volatile components of orange oil encapsulated in TMD (Fig. 5a, c, e) and SMD (Fig. 5b, d, f) during storage time at 25 °C. In general, there was a decrease in the concentration of all volatiles during storage, and this effect was more marked at 37 °C (not shown). In addition, specific effects were observed due to the type of matrix used.

To simplify the analysis, the volatiles were separated into three groups: those having high (Fig. 5a–b), medium (Fig. 5c–d) and low (Fig. 5e–f) retention. The high retention group contained limonene. Formulations containing trehalose exhibited higher limonene concentrations than sucrose ones. $TMD_{Hi\ Cap}$ was the most efficient formulation in relation to limonene retention. The medium retention group contained myrcene, in accordance with Selli et al. (2002) who found myrcene to be the second most abundant component in the orange peel. There was a remarkable myrcene concentration decrease during storage for all the studied formulations, and it was almost completely lost after 9 months of storage at 37 °C

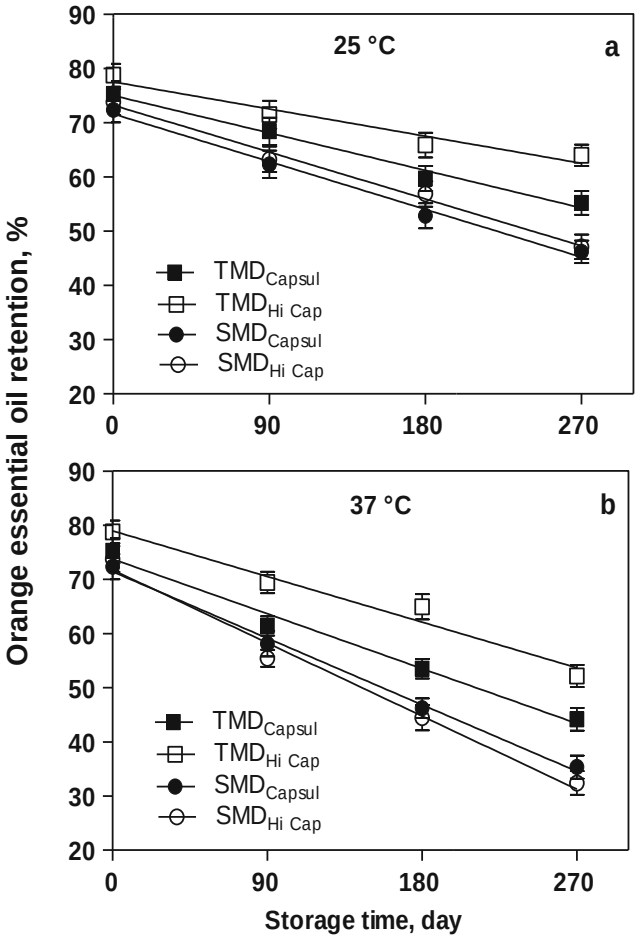

Fig. 3 Percentage of Orange Essential Oil Retained in the Formulations: TMDCapsul (■), TMDHi Cap (□), SMDCapsul (●), SMDHi Cap (○), Stored at 25 °C (**a**) and 37 °C (**b**)

(not shown). A particular behavior was observed in the different formulations. The initial myrcene concentration was lower in TMD_{Capsul} compared to $TMD_{Hi\ Cap}$. In contrast, the initial concentration was higher in $SMD_{Hi\ Cap}$ than in SMD_{Capsul}. However, the losses during storage were higher for SMD_{Capsul} than for $SMD_{Hi\ Cap}$.

The low retention group (Fig. 5e–f) was composed of n-octanol, heptanal, α-pinene and sabinene. n–octanol was retained only in the TMD_{Capsul} formulation for 3 months at 25 °C. In the rest of the analyzed cases, n-octanol was not detectable during storage. The high volatility of the alcohol might have led to its rapid loss during storage. Similar results were observed by Coggins et al. (1969). Heptanal was found only in the $TMD_{Hi\ Cap}$ formulation, and its concentration decreased significantly over time, becoming undetectable after 3 months storage. There was a remarkable α-pinene and sabinene decrease during storage. α-pinene and sabinene contribute to the aroma of orange oil giving it pine and spicy notes, respectively (Mazid et al. 2011). Higher sabinene retention was observed for SMD formulations during the studied storage time, and it was independent of the starch used. In the case of TMD_{Capsul}, sabinene was only retained up to 3 months, while for $TMD_{Hi\ Cap}$, it was retained at least for 9 months. A similar behavior was observed for α-pinene.

The combination of sugar/starch used as carrier matrix renders different retention results depending on the volatile component. Therefore, the matrix composition should be taken into account in order to optimize the retention of the desired volatile components for a certain product.

Sensory Evaluation

According to the sorting task results, four major groups of samples were formed. Within each group certain samples were very similar. Therefore, to perform the sensory aromatic profile only those samples perceived as different were considered (Table 2).

Figure 6 shows the factorial correspondence analysis (FCA) of the sensory aromatic profile. The first two factors of the FCA explained 71.7 % (48.1 % and 23.6 %, respectively) of

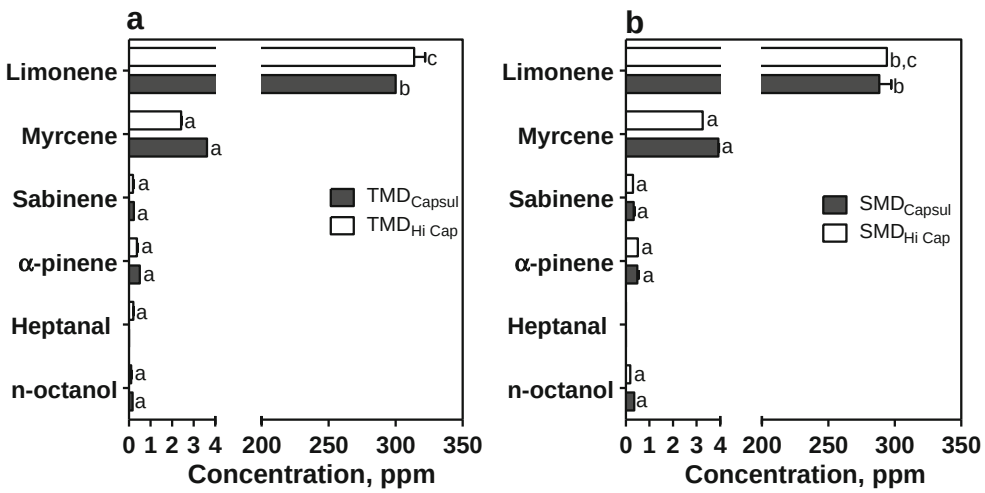

Fig. 4 Concentrations of the Main Volatile Components of Orange Oil Encapsulated in the Formulations TMD (**a**) and SMD (**b**) Detected in the Head Space Analysis Performed after Humidification at 11 % RH

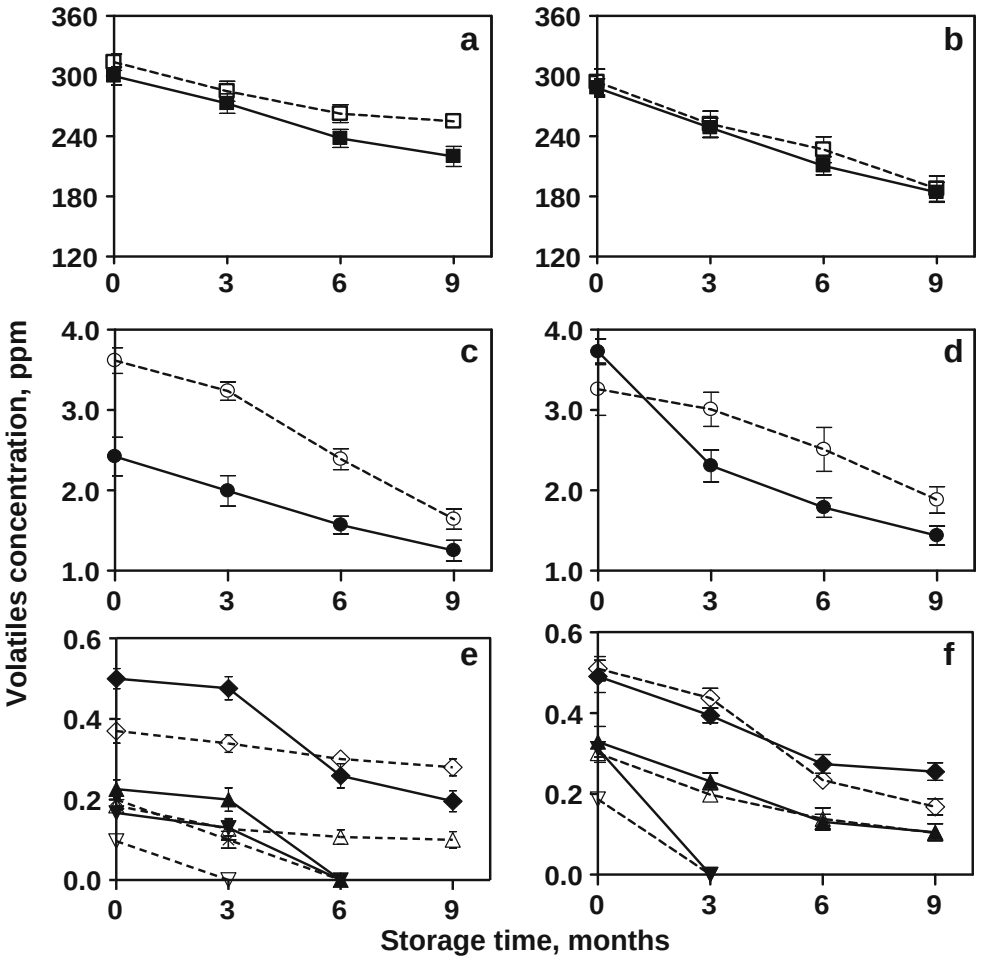

Fig. 5 Concentration of the Volatile Components of Orange Oil: Limonene (■, □), Myrcene (●, ○), α-pinene (◊, ♦), Sabinene (△, ▲), n-octanol (▼, ▽) and Heptanal (*), Encapsulated in the Formulations TMD (**a**, **c**, **e**) and SMD (**b**, **d**, **f**) along Storage Time at 25 °C. The *Solid Lines* and *Filled Symbols* Correspond to Formulations Containing Capsul, and the *Dashed Lines* and *Empty Symbols* Correspond to Hi Cap Data

the total variation among samples. The formulations containing different sugars presented diverse aromatic profiles. SMD formulations were grouped in the two quadrants on the right side of the plot, while TMD formulations were located on the left quadrants. SMD formulations retained attributes like green orange and vitamin C, determining pungency sensation. These attributes decreased during storage causing changes in the flavor profile leading to aromas of ripe orange. TMD formulations retained aromas related with juice powder, peel and lemon and their intensity also decreased during storage.

Cluster analysis determined the grouping of the samples into four groups, two for SMD and two for TMD formulations (represented by ellipses in Fig. 6). One group contained SMD samples 3, 4 and 8, showing that $SMD_{Hi\ Cap}$ and SMD_{Capsul} could retain the initial characteristics in the first 3 months of storage at 25 °C. Sample 7 (SMD_{Capsul} at 25 °C) was not included in the profile, because it was very similar to sample 3 in the sorting task test. The second group included SMD samples which showed a decrease in the intensity of the attributes perceived at time zero (samples 11, 15, 16, 19 and 20). In the case of TMD powders, one group included samples corresponding to time zero (samples 1 and 2), 3 months of storage at 37 °C (samples 9 and 10) and 6 months at 25 °C (sample 13). The samples stored at 25° C for 3 months were not included in the profile because they were very similar to the samples at zero time (sorting task test). The stored samples within this group were perceived in the same way as for the initial condition, the main attributes being lemon and orange peel. The second TMD group consisted of samples which were stored for 6 months (samples 14, 17 and 18) and were mainly associated with the descriptor juice powder. These results show that TMD-based formulations can be stored for a longer time than SMD without the perception of the aroma profile being affected. Some differences were found between starches at 25 °C/6 months storage, for which TDM_{Capsul} (sample 13) was perceived as similar to the samples at zero time but $TDM_{Hi\ Cap}$ was perceived differently from the initial conditions.

Comparison of Chromatographic and Sensory Data

Figure 7 shows the relation between volatile components determined by HS-SPME-GC-MS and the sensory attributes, analyzed by partial least square (PLS) regression. The first two factors of the PLS explained 71.7 % (45.0 % and 26.7 %,

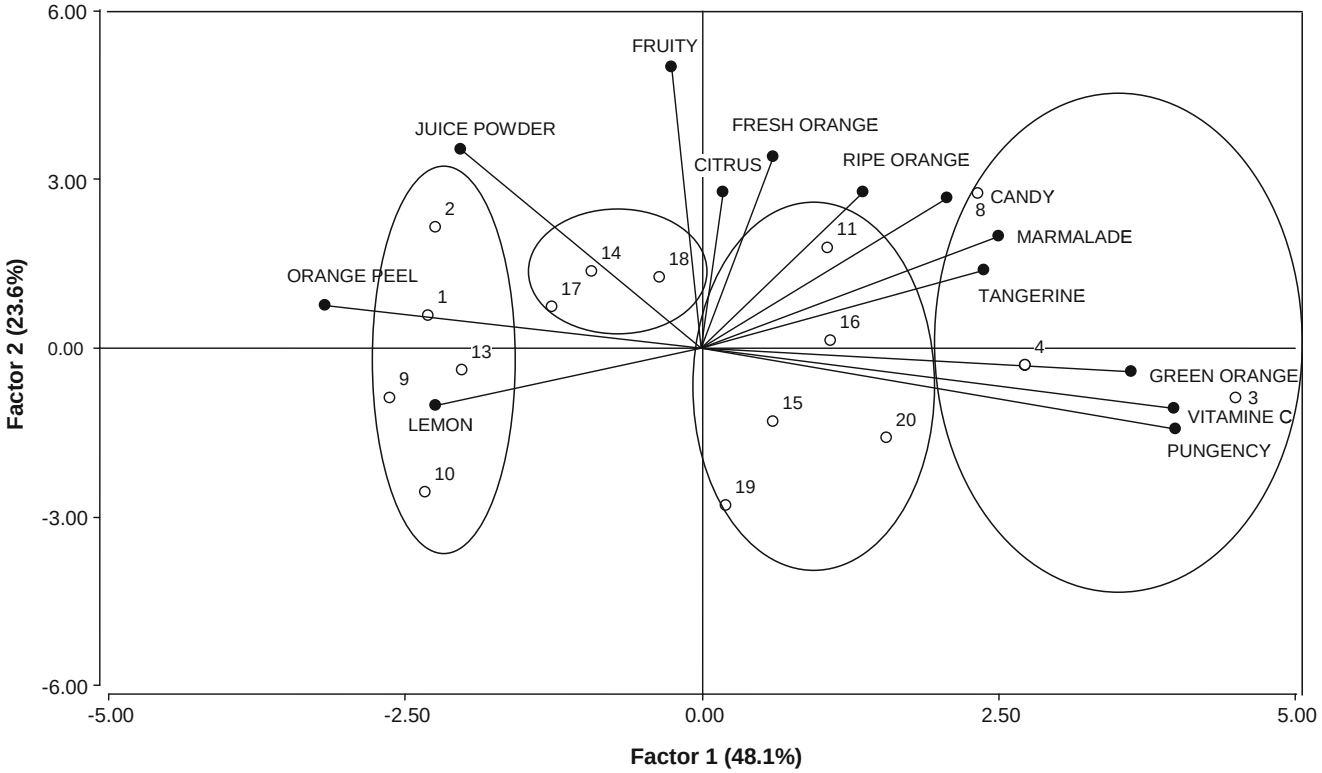

Fig. 6 FCA of Samples (O) and Aromatic Profile Attributes (●)

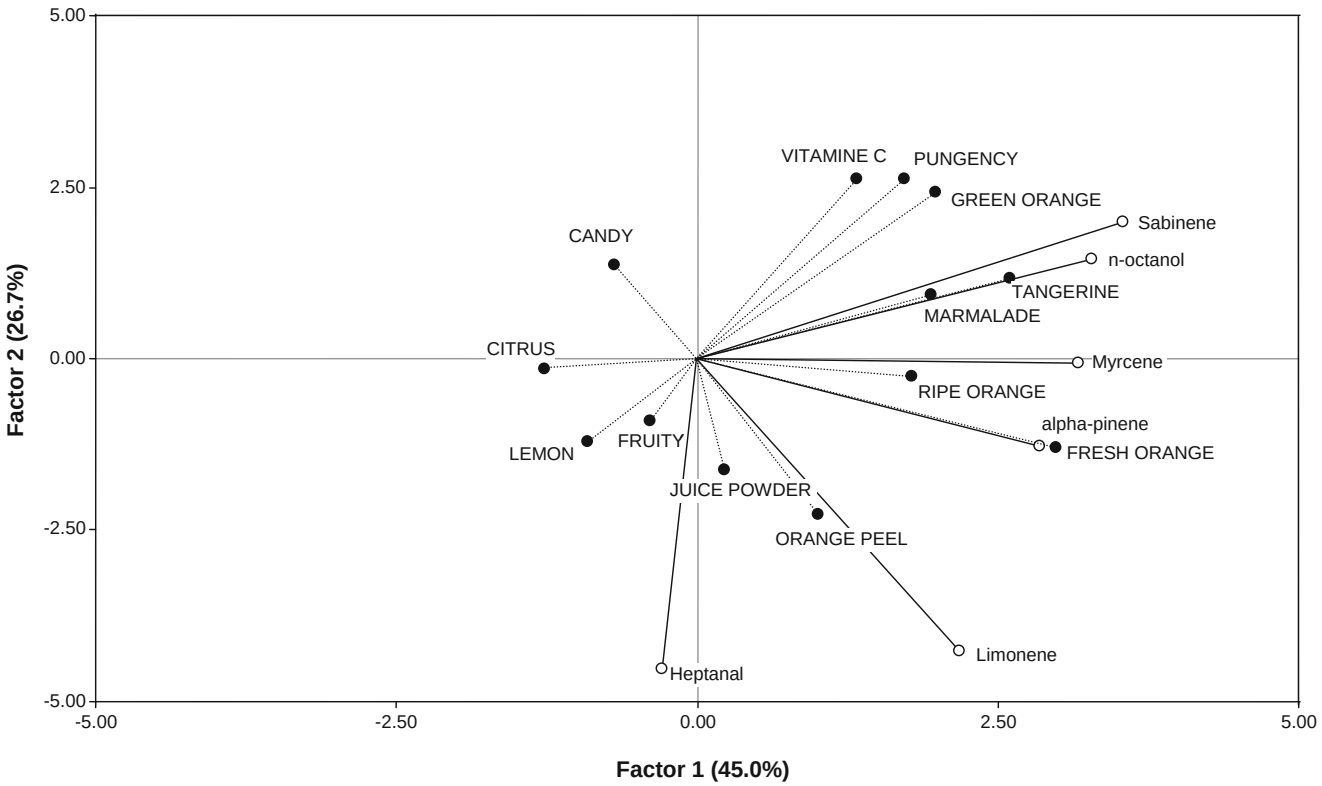

Fig. 7 Partial Least Square (PLS) Regression between Aromatic Profile Attributes (●) and Chemical Compounds Tested by HS-SPME-GCMS (O)

respectively) of the variance between the sensory attributes (X) and the components identified by chromatography (Y). A high correlation was found between the sensory attribute fresh orange and the volatile component α-pinene. A similar correlation was observed between sensory attributes ripe orange, orange marmalade and tangerine, with the volatile compound myrcene. Also, the sensory attributes orange peel and juice powder were correlated with the volatile compound limonene. TMD formulations retained an aroma mainly of lemon and orange peel, characteristic of limonene. The characteristics of the original aroma were maintained during storage even when the intensity of these attributes had decreased.

Heptanal contributes significantly to the aroma of orange essential oil and it has a penetrating fruit odor (Shaw, 1991). The $TMD_{Hi\ Cap}$ formulations were represented by heptanal.

Conclusions

In this work, four formulations to encapsulate orange essential oil were successfully obtained, showing high aroma retention levels. The sensory profiles obtained were very different for TMD and SMD. These results coincided with the differences observed in the chromatographic profiles, which showed differential retention of aromatic components for the different formulations. This fact indicates the relevance of the selected sugar for the development of encapsulated aromas, and the possibility of designing different powdered aromas from the same original aromatic source.

In some cases, the type of starch used as an emulsifier affected the retention of volatiles, indicating that although they were added in a low concentration, their influence has to be considered in the design of formulations for aroma encapsulation.

The formulations containing trehalose showed higher T_g values than those containing sucrose, indicating that in the presence of trehalose, the encapsulated aromas could be stored in broader temperature/RH conditions without altering the physical characteristics of the powders.

These results are promising in relation to the incorporation of the developed formulations to dehydrated citric juices.

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