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Starch-based spherical aggregates: screening of small granule sized starches for entrapment of a model flavouring compound, vanillin

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Abstract

Spherical aggregates ranging in size from 7.5 to 45 μ obtained from four small sized starch granules, isolated in the laboratory from amaranth (*Amaranthus paniculatus* L.), quinoa (*Chenopodium quinoa* L.), rice (*Oryza sativa* L.) and colocasia (*Colocasia esculenta* L.) in the presence of polysaccharide bonding agents such as gum Arabic, carboxymethyl cellulose and carrageenan at 0.1–1.0% were obtained by spray drying a 20% starch dispersion at 120 °C for entrapment of a model flavouring compound, vanillin at 5 and 10% based on starch (bos). Uniform sized spherical aggregates were observed at 1% concentration of all the bonding agents. Gum Arabic at 1.0% with amaranth starch gave the best entrapment of vanillin followed by carboxymethyl cellulose and carrageenan. The recovery/retentions of vanillin at 5 and 10% bos were found to be similar, though slightly high in the case of the latter. The extent of entrapment of vanillin for various starches decreased in the order of amaranth > colocasia > chenopodium > rice. These trends parallel the amount of amylose in the starches under study, and indicate a negative correlation of amylose on the extent of entrapment of vanillin held within the spherical aggregates.

Keywords: Spherical aggregates; Entrapment; Vanillin; Starches; Bonding agents

1. Introduction

With the emergence of controlled release technology, additives that are sensitive to temperature or pH can be used very conveniently in food systems, in the form of microcapsules. The most preferred method to achieve controlled release in the food is microencapsulation. It involves enclosure of liquid flavourings in a carrier matrix to provide dry, free-flowing materials. It facilitates the addition of flavours to dry products such as instant beverages and bakery mixes. It can also provide protection against oxidation, heat and light to otherwise sensitive constituents (Versic, 1988). The microcapsules may range from microns to several millimetres in size (0.2–5000 μm) and have a multitude of shapes, depending on the materials and methods used to prepare them (Balassa & Fanger, 1971). The simplest of the microcapsule may consist of a core surrounded by a wall or barrier of uniform or non-uniform thickness. The core may be composed of just one or several different types of ingredients and the wall may be single or multilayered.

Recently, a novel property of small sized starch granules capable of holding and releasing sensitive materials such as flavours has been reported. Termed as 'spherical aggregates', these granules combine into potentially useful interesting spheres, when spray dried as a 30% starch suspension with a 0.1–1.0% of bonding agent such as a protein or a polysaccharide under conditions that prevent gelatinisation of starch. These aggregates resemble popcorn balls, and contain open spaces in the form of interconnecting cavities that can be filled up with food ingredients such as flavours. These materials are retained even after washing with ethanol and subsequent drying. These are projected to be very useful in prolonged release of flavours such as in chewing gum. The ease of production by normal food processing methods gives it an added advantage (Zhao & Whistler, 1994).

The present work reports on the amount of a model flavouring compound, vanillin held in the spherical aggregates prepared from four small sized starches viz. amaranth (*Amaranthus paniculatus* L.), chenopodium (*Chenopodium quinoa* L.), rice (*Oryza sativa* L.) and

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colocasia (*Colocasia esculenta* L.) using carrageenan, gum Arabic and sodium carboxymethyl cellulose as the bonding agents at 0.1–1.0%.

2. Materials and methods

2.1. Materials

Amaranth seeds, rice grains and fresh colocasia tubers were procured from the local market of Mumbai city. Chenopodium seeds were obtained from National Botanical Research Institute (NBRI), Lucknow. Gum Arabic was procured from M/s. Drytech processes (I) Pvt. Ltd., Mumbai. Sodium salt of carboxymethyl cellulose (low viscosity, 30–70 cPs) was obtained from M/s. Central Drug House, New Delhi. Carrageenan was obtained from M/s. Himedia Laboratories Pvt Ltd, Mumbai. Standard amylose was procured from M/s. Sigma Chemical Co., St Louis, USA. Standard amylopectin was procured from M/s. Calbiochem-Novabiochem Corporation, CA, USA. Vanillin, used as a model-flavouring agent, was procured from M/s. Helmvanil, China. All other chemicals used were of AR grade.

2.2. Methods

2.2.1. Isolation of starches from raw materials under study

Starches from amaranth and chenopodium seeds were isolated by the alkali steeping method described by Yanez and Walker (1986). Rice starch was isolated as per the procedure described by Juliano (1984). Starch from colocasia tubers was isolated by method described by Moorthy (1991) with slight modifications.

2.2.2. Estimation of amylose/amylopectin

Amylose and amylopectin content was estimated colourimetrically as described by McCready and Hassid (1943).

2.2.3. Preparation of spherical aggregates

Twenty grams of starch, isolated from amaranth, colocasia, chenopodium and rice were dispersed in 80 ml water containing 0.1, 0.5 and 1.0 g of binding agents such as gum Arabic, sodium carboxymethyl cellulose, and carrageenan individually. The slurry was stirred for 5 min using a shear homogeniser to ensure uniform mixing. As vanillin is insoluble in water beyond 1%, it was first dissolved in minimum amount of propylene glycol and then the solution was mixed with the starch–polysaccharide mixture. This mixture was homogenised in a shear homogeniser for 5 min to ensure uniform distribution of vanillin in the network of starch–polysaccharide mixture. Vanillin was added at 5 and 10% based on starch (bos). The slurry containing all the components was spray dried in a Buchi-190 Model Mini Spray dryer (Buchi, Switzerland), (inside chamber dimension: 100 cm high, 60 cm diameter) equipped with 0.5 mm diameter nozzle. The pressure of compressed air for the flow of spray was adjusted to 5 bars. The inlet and outlet temperatures were maintained constant at 120 ± 2 and 76 ± 2 °C, respectively, to avoid gelatinisation of starch. A peristaltic pump was used to feed the spray dryer at 300 g/h. The spherical aggregates so prepared were collected from the collecting chamber. These powders were filled in airtight, self-sealable polyethylene pouches, and stored in a dessicator containing calcium chloride to prevent moisture absorption until further studies.

2.2.4. Analysis of spray-dried spherical aggregates

2.2.4.1. Preparation of standard curve for vanillin. From a standard solution of vanillin (10 mg/100 ml of distilled water), a range of 0.001–0.01 mg/ml was prepared by appropriate dilutions. 5 ml of each of this diluted solution was pipetted into 50 ml standard volumetric flask. To each flask, 5 ml Folin-Dennis reagent (100 g sodium tungstate, 20 g phosphomolybdic acid, 50 ml phosphoric acid in 1 l distilled water) was added and mixed thoroughly for 5 min. The volume was made up by 1N Na_2CO_3 solution, mixed thoroughly, transferred to 250 ml conical flasks and placed on a shaker for 30 min. The solutions were filtered through dry Whatman No. 1 filter paper. The blue colour of the filtrate was measured at 640 nm. The standard curve plotted in the range of 0.001–0.01 mg/ml (North, 1949) gave a regression equation as $Y = 2.2431X$ ($R^2 = 0.96$), where Y is the optical density at 640 nm, and X is the concentration of vanillin (mg)

2.2.4.2. Analysis for surface vanillin in spray-dried spherical aggregates. To evaluate the ability of these native starches as a flavour carrier, the spray-dried spherical aggregates were subjected to analysis for surface vanillin (SV) and entrapped vanillin (EV) by the method given by North (1949) with slight modifications. 0.25 g of spherical aggregates were washed with 25 ml of absolute alcohol and the filtrate made up to 50 ml in a volumetric flask with distilled water. This was used to estimate SV using 1 ml aliquot as above.

2.2.4.3. Analysis for entrapped vanillin in spray-dried spherical aggregates. The residue left from the above washings was dissolved in distilled water, the volume made up to 100 ml in a standard volumetric flask, and used to estimate EV using 1 ml aliquots as described earlier.

2.2.4.4. Analysis for total vanillin in spray-dried spherical aggregates. The total time required for washing with alcohol, filtration, and making solutions, both in alcohol and water for estimation of SV and EV, respectively, were approximately 1 h. Losses of vanillin occurred during this period. Hence, total vanillin (TV) was estimated after dissolving 0.1 g of spray-dried spherical aggregates in 100 ml distilled water in a standard volumetric flask.

Table 1
Physico-chemical properties of the starches used in the study

Parameters	*Amaranthus paniculatus*	*Colocasia esculenta*	*Chenopodium quinoa*	*Oryza sativa*
Size (μm)	0.75–1.25	1.0–2.2	0.6–1.5	3–10
Shape	Spherical	Oval	Spherical	Polygonal
Amylose	2.5	17.5	22.5	39.0
Amylopectin	97.5	82.5	77.5	61.0

Values are mean of two determinations.

Vanillin was estimated in 1 ml of each sample as described earlier.

2.2.4.5. Scanning electron microscopy (SEM). Particle size and structure of spray-dried spherical aggregates were evaluated with Scanning Electron Microscope-probe, Model No. SU30, (Cameca, France). The spherical aggregates were attached to SEM stubs of 1″ diameter using two-sided adhesive tape. The specimen were coated with gold–palladium (plasma deposition method) and examined on the SU30 model at 15 kV.

3. Results and discussion

The physicochemical properties of the starches used in the study are shown in Table 1. It can be seen that the starches from amaranth, chenopodium and colocasia tubers had a small granule size ranging from 0.6 to 2.2 μm. Rice starch had a slightly bigger granule size ranging from 3 to 10 μm. These are in accordance with the reported literature (Ahmed, Singhal, Kulkarni, & Pal, 1996; Moorthy, 1991; Zhao & Whistler, 1994). While amaranth and chenopodium starches were spherical in shape, colocasia starch was oval and rice starch was polygonal. These starches had varying amylose contents, ranging from 2.5% for amaranth starch to 39.0% for rice starch. Amaranth starch is known to be a low-amylose starch (Lorenz, 1990), while rice starches are known to vary in amylose contents from 14 to 32% (Belitz & Grosch, 1999). The rice sample used in this study was a local variety, whose amylose content is as yet unreported. The values of amylose for colocasia were 16.5% (Moorthy, 1991). The sample of chenopodium seeds used in the present work was a random sample obtained from plant breeding studies. This may be the reason for a higher amylose content of 22% as compared to reported values of 11% (Lorenz, 1990).

Vanillin at 5 and 10% bos was mixed with 20% starch slurry in the presence of different bonding agents to

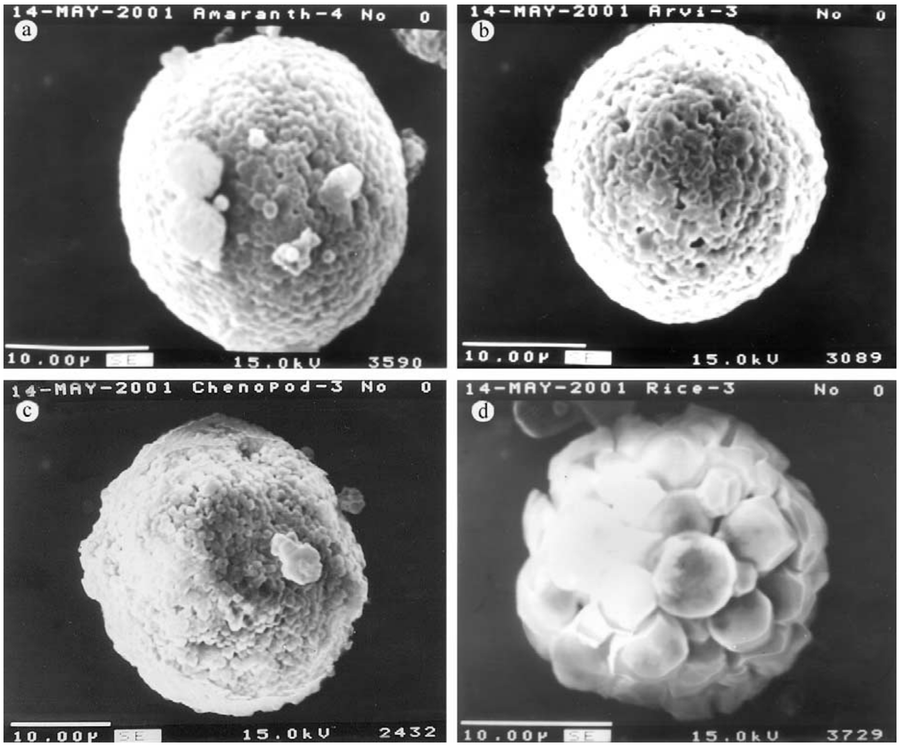

Fig. 1. SEM of spherical aggregates from (a) amaranth starch, (b) colocasia starch, (c) chenopodium starch, and (d) rice starch with 1% gum Arabic as the bonding agent.

Table 2
Effect of bonding agents on vanillin entrapment by spherical aggregates prepared from amaranth starch

Bonding agent	Concentration of the bonding agent (%)	Entrapped vanillin (%) at		Surface vanillin (%) at		Total vanillin (%) at	
		5%	10%	5%	10%	5%	10%
Gum Arabic	0.1	39.0	39.4	2.6	2.5	48.0	52.1
	0.5	78.0	79.2	2.8	2.6	85.0	86.8
	1.0	83.0	82.8	3.2	3.1	94.0	95.6
CMC	0.5	39.2	39.8	6.5	5.9	78.0	79.6
	1.0	42.4	42.8	5.2	5.4	85.0	86.8
Carrageenan	0.5	36.5	35.9	6.8	6.6	68.0	66.8
	1.0	38.4	40.1	4.9	5.2	76.5	77.4

Vanillin at 5 and 10%, bos; results are mean of two individual determinations.

Table 3
Effect of bonding agents on vanillin entrapment by spherical aggregates prepared from Colocasia starch

Bonding agent	Concentration of the bonding agent (%)	Entrapped vanillin (%) at		Surface vanillin (%) at		Total vanillin (%) at	
		5%	10%	5%	10%	5%	10%
Gum Arabic	0.1	30.4	32.5	3.0	3.3	43.5	43.8
	0.5	68.5	68.2	3.0	3.1	77.5	76.9
	1.0	72.2	74.0	2.7	2.8	80.5	81.3
CMC	0.5	58.0	59.3	5.3	5.5	70.0	71.5
	1.0	65.4	65.2	5.0	5.3	76.5	76.9
Carrageenan	0.5	48.2	47.6	6.8	7.0	61.0	62.8
	1.0	60.4	62.1	5.2	6.6	70.0	72.5

Vanillin at 5 and 10%, bos; results are mean of two individual determinations.

prepare spherical aggregates. These are shown in Fig. 1a–d, which shows the scanning electron micrographs (SEM) of a single spherical aggregate of amaranth, colocasia, chenopodium and rice starch, respectively, prepared with 1% gum Arabic as bonding agent. The SEM observations were similar to that reported by Zhao and Whistler (1994).

Tables 2–5 show the amount of vanillin entrapped within the spherical aggregates, SV and TV with amaranth, colocasia, chenopodium and rice starches, when prepared with gum Arabic (0.1, 0.5 and 1.0%), CMC (0.5 and 1.0%) and carrageenan (0.5 and 1.0%), respectively. It can be seen that for all the starches, entrapment of the vanillin within the aggregates for different bonding agents was of the under gum Arabic >

Table 4
Effect of bonding agents on vanillin entrapment by spherical aggregates prepared from chenopodium starch

Bonding agent	Concentration of the bonding agent (%)	Entrapped vanillin (%) at		Surface vanillin (%) at		Total vanillin (%) at	
		5%	10%	5%	10%	5%	10%
Gum Arabic	0.1	30.4	31.2	3.2	3.0	40.0	42.1
	0.5	60.5	62.0	3.0	3.0	72.5	73.6
	1.0	68.4	69.3	2.8	3.1	76.5	78.1
CMC	0.5	51.5	52.1	6.8	6.9	62.5	63.4
	1.0	63.4	63.9	4.7	4.5	70.0	71.5
Carrageenan	0.5	41.2	40.5	7.2	8.1	50.0	52.4
	1.0	50.2	52.1	5.0	6.2	61.5	64.2

Vanillin at 5 and 10%, bos; results are mean of two individual determinations.

Table 5
Effect of bonding agents on vanillin entrapment by spherical aggregates prepared from rice starch

Bonding agent	Concentration of the bonding agent (%)	Entrapped vanillin (%) at		Surface vanillin (%) at		Total vanillin (%) at	
		5%	10%	5%	10%	5%	10%
Gum Arabic	0.1	22.7	24.9	5.2	6.0	30.0	30.2
	0.5	57.3	59.8	5.0	5.4	65.0	67.2
	1.0	62.4	65.2	4.8	5.0	69.0	72.4
CMC	0.5	50.2	51.3	8.2	9.2	60.0	61.8
	1.0	53.1	56.1	5.7	5.4	56.5	57.4
Carrageenan	0.5	31.4	34.2	9.3	10.4	42.5	43.2
	1.0	46.8	49.5	8.4	9.3	57.0	59.8

Vanillin at 5 and 10%, bos; results are mean of two individual determinations.

CMC > carrageenan. Correspondingly, the amount of SV was in the order gum Arabic < CMC < carrageenan. The pattern for TV was similar to that of EV in all cases. TV was higher than the sum of EV and SV, indicating losses of the order of 10–35% during the analysis depending on the nature of the starch as well as the binding agents. The amount of TV, EV and SV with amaranth starch alone, in the absence of bonding agent was estimated, and found to be 52.4, 15.5 and 26.8%, respectively. Since these values were appreciably lower than for the spherical aggregates prepared with all the bonding agents, further exercises with the other starches included in the study was not undertaken.

It is interesting to note that the results were almost identical with respect to EV, TV and SV, when vanillin was incorporated at 5 and 10% bos. Another fact worth noting is a sharp increase in the amount of EV, when gum Arabic was increased as the bonding agent from 0.1 to 0.5%. Further increase to 1.0% did increase the EV, but the extent of increase depended more on the starch type. For instance, with amaranth starch, the EV was 78.0 and 83.0% when gum Arabic was increased from 0.5 to 1.0%. Corresponding values for chenopodium starch was 60.5 and 68.4% for gum Arabic at 0.5 and 1.0%, respectively. An increase in concentration from 0.5 to 1.0% CMC and carrageenan as bonding agents also increased the EV. The incremental increase was also dependent on the starch type (Tables 2–5).

In order to understand the role of the starch type on the EV, SV and TV, attempts were made to correlate the amylose content of the starches with the experimental parameters for all the bonding agents at all concentrations used in the study. Fig. 2 shows one such result where the amylose content of the starches in the study has been correlated to the TV, EV and the SV in the spherical aggregates prepared using 1.0% gum Arabic as

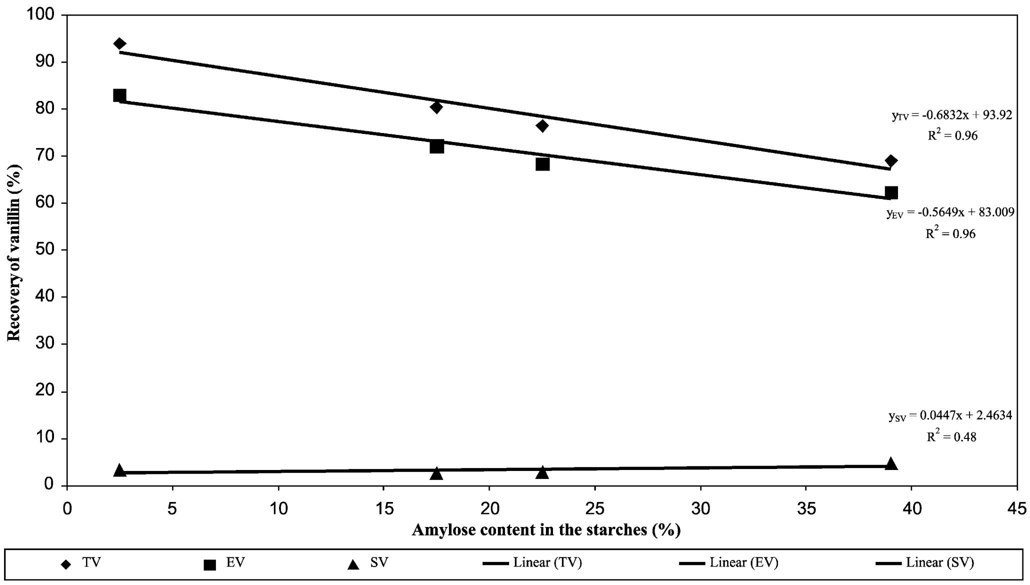

Fig. 2. Correlation of EV, TV and SV in the spherical aggregates with the amylose content of the starches using 1.0% gum Arabic as the bonding agent.

Table 6
Regression analysis of TV, EV and SV in the spherical aggregates (5% vanillin, bos) with the amylose content of the starches under study

Binding agents (%)	SV	EV	TV
Gum Arabic at 0.1%	$Y = 0.0715X + 2.0435$ ($R^2 = 0.86$)	$Y = -0.4375X + 39.539$ ($R^2 = 0.98$)	$Y = -0.4996X + 50.554$ ($R^2 = 0.96$)
Gum Arabic at 0.5%	$Y = 0.0601X + 2.2253$ ($R^2 = 0.76$)	$Y = -0.5822X + 77.937$ ($R^2 = 0.90$)	$Y = -0.5558X + 86.325$ ($R^{2=}0.99$)
Gum Arabic at 1.0%	$Y = 0.0447X + 2.4634$ ($R^2 = 0.48$)	$Y = -0.5649X + 83.009$ ($R^2 = 0.96$)	$Y = -0.6832X + 93.92$ ($R^2 = 0.96$)
CMC at 0.5%	$Y = 0.0526X + 5.6275$ ($R^2 = 0.44$)	$Y = 0.2606X + 44.416$ ($R^2 = 0.25$)	$Y = -0.5082X + 77.98$ ($R^2 = 0.89$)
CMC at 1.0%	$Y = 0.013X + 4.8853$ ($R^2 = 0.22$)	$Y = 0.2618X + 50.742$ ($R^2 = 0.14$)	$Y = -0.7925X + 88.147$ ($R^2 = 0.98$)
Carrageenan at 0.5%	$Y = 0.0698X + 6.1027$ ($R^2 = 0.77$)	$Y = -0.1747X + 42.884$ ($R^2 = 0.14$)	$Y = -0.726X + 70.166$ ($R^2 = 0.93$)
Carrageenan at 1.0%	$Y = 0.095X + 3.9389$ ($R^2 = 0.72$)	$Y = 0.1741X + 45.402$ ($R^2 = 0.08$)	$Y = -0.5542X + 77.541$ ($R^2 = 0.92$)

Values within parenthesis indicate the correlation coefficient.

Table 7
Regression analysis of TV, EV and SV in the spherical aggregates (10% vanillin, bos) with the amylose content of the starches under study

Binding agents (%)	SV	EV	TV
Gum Arabic at 0.1%	$Y = 0.0942X + 1.7816$ ($R^2 = 0.82$)	$Y = -0.3941X + 40.029$ ($R^2 = 0.99$)	$Y = -0.5967X + 54.208$ ($R^2 = 0.99$)
Gum Arabic at 0.5%	$Y = 0.0759X + 1.9782$ ($R^2 = 0.81$)	$Y = -0.5392X + 78.287$ ($R^2 = 0.87$)	$Y = -0.5369X + 87.064$ ($R^2 = 0.98$)
Gum Arabic at 1.0%	$Y = 0.0534X + 2.4125$ ($R^2 = 0.63$)	$Y = -0.4876X + 82.76$ ($R^2 = 0.94$)	$Y = -0.6304X + 94.695$ ($R^2 = 0.92$)
CMC at 0.5%	$Y = 0.0953X + 4.9329$ ($R^2 = 0.75$)	$Y = 0.2713X + 45.097$ ($R^2 = 0.26$)	$Y = -0.5045X + 79.355$ ($R^2 = 0.86$)
CMC at 1.0%	$Y = -0.0024X + 5.1987$ ($R^2 = 0.01$)	$Y = 0.3359X + 50.156$ ($R^2 = 0.24$)	$Y = -0.8122X + 89.698$ ($R^2 = 0.99$)
Carrageenan at 0.5%	$Y = 0.1072X + 5.8407$ ($R^2 = 0.89$)	$Y = -0.0818X + 41.216$ ($R^2 = 0.04$)	$Y = -0.6753X + 70.059$ ($R^2 = 0.91$)
Carrageenan at 1.0%	$Y = 0.1096X + 4.5912$ ($R^2 = 0.89$)	$Y = 0.2022X + 46.83$ ($R^2 = 0.11$)	$Y = -0.5032X + 78.728$ ($R^2 = 0.91$)

Values within parenthesis indicate the correlation coefficient.

the bonding agent, when vanillin was used at 5.0% bos. It is very clear that TV and EV show a negative correlation with the amylose content, indicating amylose to be one of the factors influencing the entrapment of the model flavouring compound used in this study. No previous reports on this aspect are available. The only available report on spherical aggregates emphasizes the size of the starch granules. The starches used in our study were all made up of small-sized starch granules, more or less of the same size. To confirm the effect of amylose,a regression analysis was performed for all the bonding agents at all the concentrations used in the study. Tables 6 and 7 show the results obtained when vanillin was used at 5 and 10% bos. It is seen that with gum Arabic as the bonding agent at all the concentrations used in the study and vanillin loading at both 5 and 10% bos, TV and EV correlated with the amylose content of the starches ($R^2 > 0.9$), while SV did not show any such correlation. With carrageenan and CMC as the bonding agents at 0.5 and 1.0%, the amylose content of the starches could be negatively correlated ($R^2 \geq 0.9$) only with the TV. With SV and EV, the correlation was quite poor. The differences in the correlations of TV and EV with different bonding agents is difficult to explain on the basis of available literature, and needs to be further investigated.

The retention of the flavours depends on the physico-chemical properties such as the molecular weight of the carriers used during spray drying. In case of maltodextrins, lower DE and higher molecular weight positively influence the entrapment of volatiles (Goubet, LeQuere, & Voilley, 1998). The greater retention observed with high molecular weight has been explained by a reduction of diffusivity of the solute during spray drying (Reineccius & Coulter, 1969), and an increase in the rate of formation of a dry crust at the surface of the carrier (Bangs & Reineccius, 1990). Amylose, a linear chain polymer of α-D-glucopyranosyl residues linked 1 → 4 has a molecular weight that can vary between 1.5×10^5 and 7.5×10^5 Da (Belitz & Grosch, 1999). Amylopectin is a branched polymer with residues linked at the 6-position of the glucose residues of the principal chain. The molecular weight of amylopectin is very high, 10^7–7×10^8 Da. Starches containing higher amounts of amylose are likely to have lower molecular weights. This may be responsible for the higher entrapment of vanillin in starch based spherical aggregates that contain lower amylose content.

4. Conclusion

Gum Arabic at 1.0% with amaranth starch gave the best entrapment of vanillin followed by carboxymethyl cellulose and carrageenan. Vanillin was chosen as a model compound in the present study. Whether this can be extended to flavour compositions containing a complex mixture of chemically distinct compounds in a wide range of concentrations remains to be confirmed. This warrants further study on entrapment of various flavours in the spherical aggregates. Further, the reasons for gum Arabic giving a better entrapment than CMC and carrageenan needs to be explored, which would then enable the use of a better bonding agent. The correlation of the molecular weight of the starch, if any, with the entrapment of flavouring compounds also need to be evaluated. These are all fertile areas of future research.

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