Co-joined starch modification and β-carotene dispersion *in situ* by planetary ball milling

González, Luciana Carla[1,2]; Loubes, Maria Ana[1,2]; Santagapita, Patricio Román[1,2,3,*]; Tolaba, Marcela Patricia[1,2,*]

[1] University of Buenos Aires, Faculty of Exact and Natural Sciences, Industry Department, Buenos Aires, Argentina.

[2] CONICET - University of Buenos Aires, Institute of Food Technology and Chemical Processes (ITAPROQ), Buenos Aires, Argentina.

[3] University of Buenos Aires, Faculty of Exact and Natural Sciences, Organic Chemistry Department, Buenos Aires, Argentina

*Corresponding authors. Address: Facultad de Ciencias Exactas y Naturales - Universidad de Buenos Aires - Intendente Güiraldes 2160 - Ciudad Universitaria - C1428EGA - Ciudad Autónoma de Buenos Aires - Argentina; Phone number: 54 11 5825-9033; prs@di.fcen.uba.ar; mtolaba@di.fcen.uba.ar.

Abbreviations:

N: native

BM: ball milled

TM: traditional dispersion method

G: gelatin

PBM: planetary ball mill

IM: innovative dispersion method

Keywords: β-carotene, encapsulation, modified starch, planetary ball mill, rice starch

Abstract

The objective was to evaluate an innovative dispersion method (IM) as the planetary ball milling (PBM) to obtain starch modification altogether with *in situ* β-carotene dispersion. Native (N) and ball milled (BM) rice starches were first evaluated as encapsulating matrices of β-carotene, by means of traditional dispersion method (TM) using a rotor-stator device. Ball milled starch with gelatin

This is the author manuscript accepted for publication and has undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1002/star.202000007.

(BM+G) preserved β-carotene structure and produced the highest encapsulated β-carotene content (0.154 mg/g), being four times the N starch content (0.035 mg/g). By IM, β-carotene structure was partially (69 %) preserved by using the BM+G matrix. Encapsulated β-carotene contents >86% (equivalent to 0.50 mg/ g starch) and encapsulated/surface ratio > 6.7 were achieved, obtaining at least 3.0 and 16.3 fold increments compared to TM, respectively. Results from IM revealed the potential of PBM to reach a high degree of β-carotene dispersion in a physically modified starch improving the encapsulation performance with respect to TM.

1. Introduction

Encapsulation is a strategy to limit the degradation of bioactive compounds and to deliver poorly soluble components into aqueous systems [1, 2]. Maltodextrin and modified starches with protein addition are widely used as wall materials, due to their emulsifying power, low cost, high availability and good volatile retention [3-5].

Encapsulation often involves the dispersion of the bioactive compound in the encapsulating matrix (emulsification step) followed by freeze or spray drying of the emulsion [3, 6]. One of the most important factors in microencapsulation is the physical stability of the emulsion, which depends on droplet size, droplet surface charge and viscosity [7]. For emulsification, high-shear homogenizers are usually applied [8, 9]. To obtain micro- and nano-sized emulsions, an extra micro-fluidization or sonication treatment is required. These methods are usually applied due to their high performance, low time-consumption, and good scalability in the industry [10]. However, there are currently no publications on homogenization by high-impact milling. The ability of high impact milling to produce starch modifications affecting the morphology, crystalline structure and functional properties of starch has been probed [11-13]. Therefore, the use of PBM is here proposed as a novel method for starch modification and β-carotene dispersion *in situ*.

The objectives were: a) to evaluate N and BM starches to identify the best wall material using the TM method. Six matrices (N, N+G, BM, BM+G, BM+g, BM+g+G) were formulated to study the effects of gelatin addition (G) and hydrothermal gelatinization (g) of BM starch; b) to explore the potential of the PBM to produce β-carotene encapsulated powder adopting a traditional method as control.

2. Materials and methods

2.1. Materials

Rice starch of food grade (Remy B7, Beneo GmbH, Germany) with amylose content of 18.4 g/100 g and gelatin Parafarm (150 bloom) were supplied by Saporiti S.A. (Buenos Aires, Argentina). Starch composition (dry basis) was provided by the manufacturer: 88.7% carbohydrates, 13.7% moisture, 1.0% protein, 0.0% lipid, 0.2% ash. β-carotene powder was purchased from Xi´ an Best (Xi´ an Best Bio-Tech Co. Ltd, Shaanxi, China). Analytical grade acetone, hexane and bi- distilled water were used.

2.2. Starch modification

Ball milled starch was obtained in a planetary ball mill Retsch PM 100 (Retsch GmbH, Haan, Germany) with jug (500 ml) and balls (diameter: 5 mm) of zirconium oxide, at fixed rotation speed (400 rpm), with balls:starch and water:starch ratios of 5:1 and 2.2:1 (w:w), respectively. Grinding protocol involves 10 min of milling with pauses of 15 min to complete 40 min of milling time [11, 14]. In order to obtain (BM+g) starch, BM starch aqueous slurry (5 % w/w) was gelatinized by hydrothermal treatment at 95°C for 20 min [8].

2.3. Matrices preparation

β-carotene powder was added to the starch slurry in a ratio of 1:750 (w:w, dry basis) according to Spada, Marczak, Tessaro and Noreña [15]. The emulsification by TM involved 10 min homogenization of starch slurry (starch-to-water ratio of 1:19 or 5 % w/w) at 15.500 rpm in a rotor-stator Ultra-Turrax T18B (IKA®-Werke GmbH & Co. KG, Staufen, Germany). Such starch concentration favors a good dispersion in the rotor-stator device. For the IM method the starch slurry, gelatin and β-carotene were loaded into the PBM jar and the mixture was processed for 5, 10 or 15

minutes, at 400 rpm, with balls:starch of 5:1 and starch:water of 1:2.2 (31.2 % w/w) to assures the ball-particle collision in the PBM [8, 11, 14]. Homogenized dispersions obtained by TM or IM method were then frozen, freeze-dried (48 h,−40°C, 50 μbar) in a Rificor L-A-B3 freeze-dryer (RIFICOR de Rímolo N. y Orueta R. S.H., Buenos Aires, Argentina) and stored in sealed vials at room temperature. All TM and IM tests were made by triplicate. Six matrices were prepared with and without 1 % w/w gelatin (G): N, N+G, BM, BM+G, BM+g and BM+g+G.

2.4. β-carotene content and carotene stability index

The method of Rodriguez-Amaya [16] with slight modifications was adopted. Surface carotene was extracted with hexane (aliquots of 2 mL) from the freeze-dried matrix (80 mg) by shaking 2 min at 250 rpm in an orbital shaker; the extract was measured at 450 nm with spectrophotometer model V-630 UV-Vis (JASCO Inc., MD, USA). The remaining matrix was dispersed in water (2 mL) and sonicated (10 s, amplitude 100%, cycle 0.5) in an ultrasonic homogenizer UP100H (Hielscher Ultrasonics GmbH, Teltow, Germany) to release encapsulated carotene. Encapsulated carotene was extracted with 3 mL of hexane:acetone:ethanol (50:25:25) and shook at 150 rpm for 2 min [17]. The hexane phase was measured at 450 nm in the spectrophotometer. For surface and encapsulated β-carotene, an average value of triplicates was reported as mg β-carotene/g starch using a standard absorbance curve of β-carotene in hexane. Encapsulated β-carotene (%) is the amount of encapsulated respect to the total amount of β-carotene (encapsulated and surface).

Pure β-carotene in hexane (adopted as reference) presents characteristic intensities ratio (III/II) based on absorption UV-Vis peaks at 450 nm (II) and 477 nm (III), respectively [18]. If isomerization or structural modification takes place the III/II index changes respect to the reference value. The percentage change (III/II %) was reported as a measure of stability index.

2.5. Statistical analysis

Analysis of variance (ANOVA) was performed with Statgraphics Centurion version XVI (Statgraphics Technologies, Inc., Virginia, USA), comparing the means by the least significant difference test of Fisher (LSD), with a confidence level of 95%.

3. Results and discussion

3.1. Matrix selection by traditional dispersion method

The low performance of N matrix and the positive effect of BM starch on β-carotene retention can be appreciated from the values of encapsulated and surface contents of β-carotene for different matrices (Figure 1.a). Starch granule structure was modified by ball milling to yield BM starch (100% gelatinized), which showed a significant reduction in particle size (80%) and crystallinity (85%) in comparison with N starch [14]. Due to its high level of water solubility (200-fold higher than that of N starch), BM starch produced homogeneous and viscous liquid suspension favoring the encapsulation [14]. β-carotene retention increased 48.6% by using BM starch instead of N starch. However, this value is lower than the increment (60%) reported for BM amaranth starch [8].

The effect of hydrothermal treatment on the encapsulating capacity of BM starch was negligible due to the high gelatinization degree of BM starch [14].

In agreement with literature reports [3, 5, 8], a significant effect of gelatin addition on β-carotene encapsulation was observed due to the key role of gelatin in the emulsification of β-carotene. Starch matrices N+G and BM+G showed 3.7 and 3.0 times the encapsulated contents in N and BM matrices, respectively. The best performance was 29% of encapsulated β-carotene and an encapsulated/surface ratio of 0.4, which was obtained by using BM+G starch.

As regard β-carotene stability index (Figure 1.b), the use of gelatin and BM starch contributed for preservation of β-carotene structure.

The best result was obtained by using BM+G starch, which presented an excellent stability index but a still low encapsulated/surface ratio denoting the limitation of the TM method. Therefore, the potential of PBM was explored in the next section.

3.2. Innovative dispersion method: planetary ball milling

As regard IM method, no significant effect of process time on β-carotene content was observed (Table 1). The maximum encapsulated β-carotene content was 89% (0.51mg β-carotene /g starch). Remarkable encapsulated/surface (E/S) ratios were found by IM (6.7 - 8.1), and the highest values were observed for the samples processed 5 or 10 min. The proposed method succeeded to disperse and encapsulate β-carotene in the starch matrix regardless the processing time. However, due to the thermo-mechanical damage produced by PBM [8, 11, 14], the isomerization indexes (S and E III/II in Table 1) showed at least 30 % of degradation in comparison with the reference, indicating that β-carotene could not be fully protected by this method (at present processing conditions).

3.3. Innovative method vs. traditional method

Table 1 shows encapsulation results for BM+G matrix. A huge increment in the encapsulated β-carotene content was obtained upon dispersion trough PBM, from 29 ± 1% (0.17 mg β-carotene/ g starch) up to as high as 89 ± 1 % (0.51 mg β-carotene/ g starch). Moreover, astonishing increments in the encapsulated/surface ratios were obtained, up to 8.1 ± 0.7, with respect to the 0.41 ± 0.04 obtained with TM.

However, it must be noticed that there was a significant reduction in surface and encapsulated III/II% values in comparison with TM results, indicating that IM favored the isomerization of β-carotene molecules. The differences between both methods lied not only in the great homogenization capacity of the PBM but also in the different starch concentration used (TM: 5.0% w/w; IM: 31.2% w/w). The

increase of wall material concentration positively affected the matrix drying as well as the retention of the bioactive compounds [19].

4. Conclusions

Through a high impact milling process, it was possible to modify rice starch and increase its encapsulation performance with respect to the native starch. For the first time, the potential of the planetary ball mill to modify the wall material and to disperse a bioactive compound *in situ* was studied optimizing treatment time and process conditions. Although the proposed method failed to fully protect β-carotene, these preliminary results are promising and can be improved by changing the process variables and extended to other compounds.

5. Acknowledgments

The authors acknowledge the financial support from Buenos Aires University and National Council of Scientific Research of Argentina. This work has been funded by PME-2006-01685, UBACYT (Project UBACYT 20020170100367BA) and PICT-2017-1744.

The authors declare that there is no conflict of interest regarding the publication of this article.

6. References

[1] Liang, R., Shoemaker, C. F., Yang, X., Zhong, F., Huang, Q., Stability and bioaccessibility of beta-carotene in nanoemulsions stabilized by modified starches. *Journal of Agricultural and Food Chemistry* 2013, *61*, 1249-1257.

[2] Murugesan, R., Orsat, V., Spray drying for the production of nutraceutical ingredients—a review. *Food and Bioprocess Technology* 2012, *5*, 3-14.

[3] Wang, X., Yuan, Y., Yue, T., The application of starch-based ingredients in flavor encapsulation. *Starch - Stärke* 2015, *67*, 225-236.

[4] Palma-Rodríguez, H. M., Alvarez-Ramírez, J., Vargas-Torres, A., Using Modified Starch/Maltodextrin Microparticles for Enhancing the Shelf Life of Ascorbic Acid by the Spray-Drying Method. *Starch - Stärke* 2018, *70*, 1700323.

[5] Zhu, F., Encapsulation and delivery of food ingredients using starch based systems. *Food Chemistry* 2017, *229*, 542-552.

[6] Desobry, S. A., Netto, F. M., Labuza, T. P., Comparison of spray- drying, drum- drying and freeze- drying for β- carotene encapsulation and preservation. *Journal of Food Science* 1997, *62*, 1158-1162.

[7] Drosou, C. G., Krokida, M. K., Biliaderis, C. G., Encapsulation of bioactive compounds through electrospinning/electrospraying and spray drying: A comparative assessment of food-related applications. *Drying Technology* 2016, *35*, 139-162.

[8] Roa, D. F., Buera, M. P., Tolaba, M. P., Santagapita, P. R., Encapsulation and stabilization of β-carotene in amaranth matrices obtained by dry and wet assisted ball milling. *Food and Bioprocess Technology* 2017, *10*, 512-521.

[9] Przybysz, M. A., Onacik-Gür, S., Majtczak, K., Dłużewska, E., The stability of spray-dried microencapsulated β-carotene in the mixture of gum Arabic, OSA type modified starch and maltodextrin. *Italian Journal of Food Science* 2016, 716-732.

[10] Rezvankhah, A., Emam-Djomeh, Z., Askari, G., Encapsulation and delivery of bioactive compounds using spray and freeze-drying techniques: A review. *Drying Technology* 2020, *38*, 235-258.

[11] González, L. C., Loubes, M. A., Tolaba, M. P., Incidence of milling energy on dry-milling attributes of rice starch modified by planetary ball milling. *Food Hydrocolloids* 2018, *82*, 155-163.

[12] He, S., Qin, Y., Walid, E., Li, L.*, et al.*, Effect of ball-milling on the physicochemical properties of maize starch. *Biotechnology Reports* 2014, *3*, 54-59.

[13] Kim, S. K., Kim, H. Y., Pasting properties of colored rice starch by ball-mill treatment. *Journal of Crop Science and Biotechnology* 2014, *17*, 161-166.

[14] Loubes, M. A., Dry and wet milling of rice in planetary mill, PhD Thesis, Industry Department, School of Exact and Natural Sciences, University of Buenos Aires, Buenos Aires, Argentina 2015.

[15] Spada, J. C., Marczak, L. D. F., Tessaro, I. C., Noreña, C. P. Z., Microencapsulation of β-carotene using native pinhão starch, modified pinhão starch and gelatin by freeze-drying. *International Journal of Food Science & Technology* 2012, *47*, 186-194.

[16] Rodriguez-Amaya, D. B., *A guide to carotenoid analysis in foods*, ILSI press Washington 2001.

[17] Aguirre Calvo, T. R., Busch, V. M., Santagapita, P. R., Stability and release of an encapsulated solvent-free lycopene extract in alginate-based beads. *LWT* 2017, *77*, 406-412.

[18] Rodriguez-Amaya, D. B., Kimura, M., *HarvestPlus handbook for carotenoid analysis*, International Food Policy Research Institute (IFPRI) Washington, DC 2004.

[19] Corrêa-Filho, L. C., Lourenço, M. M., Moldão-Martins, M., Alves, V. D., Microencapsulation of β-carotene by Spray Drying: Effect of Wall Material Concentration and Drying Inlet Temperature. *International Journal of Food Science* 2019, *2019*, 12.

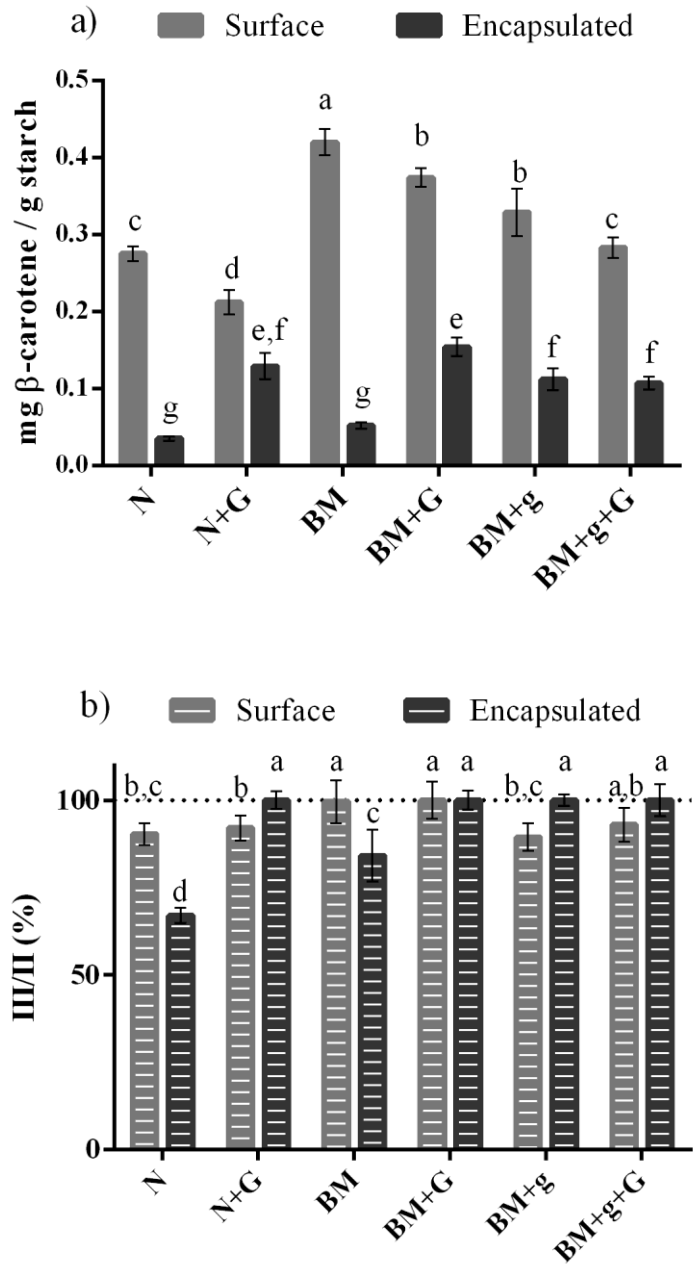
Table 1. Surface (S) and encapsulated (E) β-carotene content, E/S ratio and surface and encapsulated β-carotene stability index (III/II%), in traditional (TM) and innovative method (IM) for β-carotene in ball milled starch matrix added with gelatin (BM+G).

	TM	IM		
		5 min	10 min	15 min
S β-carotene (%)	71 ± 5^{A}	$11 \pm 1^{b,B}$	$11 \pm 1^{b,B}$	$14 \pm 1^{a,B}$
E β-carotene (%)	29 ± 1^{B}	$89 \pm 1^{a,A}$	$88 \pm 2^{a,b,A}$	$86 \pm 1^{b,A}$
E/S ratio	0.41 ± 0.04^{C}	$8.1 \pm 0.7^{a,A}$	$8.0 \pm 1.1^{a,A}$	$6.7 \pm 0.4^{b,B}$
S III/II (%)	100 ± 5^{A}	$84 \pm 9^{b,B}$	$91 \pm 4^{a,b,A,B}$	$99 \pm 5^{a,A}$
E III/II (%)	100 ± 3^{A}	$61 \pm 1^{b,C}$	$68 \pm 4^{a,B}$	$69 \pm 1^{a,B}$

Standard deviation values are included. Different letters indicate differences between mean values ($p < 0.05$): a-c among IM results and A-C between TM and IM results.

Figure captions

Figure 1. Encapsulation performance by rotor-stator dispersion method (control) of **a)** surface and encapsulated β-carotene content in different rice starch matrices and **b)** stability index (III/II%) of β-carotene in different rice starch matrices. (N: native starch, BM: ball milled starch, G: gelatin, g: gelatinized). Standard deviation values are included. Different letters on the bars indicate significant differences between mean values ($p < 0.05$).



An innovative method to obtain starch modification and β-carotene dispersion *in situ* was tested by planetary ball milling. β-carotene structure was partially (69 %) preserved, a 16-fold increase in the encapsulated/ surface ratio and a 3-fold increase in the encapsulated β-carotene content were obtained compared to traditional rotor-stator homogenizer.