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Encapsulation of Wine Industry By-Product by Ionotropic Gelation for Application in a Wheat Beer

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Best encapsulation conditions of wine industry by-products (WIBP) with sodium alginate by ionotropic gelling with Ca^{2+} ions were selected by a 2^4 central composite rotational design. The erosion degree of the beads immersed for 15 and 90 days in a wheat beer was reduced with lower $CaCl_2$ concentrations, higher WIBP amounts and reduced complexation times (CT). The swelling behavior of the beads was influenced by $CaCl_2$, sodium alginate, CT and WIBP concentrations. Best conditions to obtain beads with simultaneously lower swelling (606.7 %) and erosion degree (7.6 %) are: 1.5% sodium alginate, 4% WIBP, 0.26 M calcium chloride and 26 min CT. Beads containing the residue of the wine industry can be considered potential additives for a wheat beer to increase the antioxidant activity of this beverage benefiting the consumer.

1. Introduction

The global beer market is larger than the global wine market. Since beer is cheaper and with lower alcohol content, the differences in volume are much larger (Swinnen and Briski, 2017) and, among the alcoholic beverages, it is the world's most consumed one (Colen & Swinnen, 2016). Despite this beverage competition, wine and beer industries can help each other. While wine producers face the problem of disposal of the solid by-products, beer industry is in search of innovation since many artisanal beers with different tastes and additives are invading the market. Wine industry wastes, which consist mainly of solid by-products, include grape pomace and stems, and may account on average for almost 30% (w/w) of the grapes used for wine production (Colussi et al., 2012). Although biodegradable, this solid waste requires a minimum time to be mineralized, constituting a source of environmental pollutants (Christ and Burrit, 2013). The importance of reusing the solid by-product of wine industry is related to its phenolic compounds, antioxidants, anthocyanins and dyes, which are of great importance for the pharmaceutical, food and cosmetic industries (Rockenbach et al., 2011). Encapsulation of antioxidants is a proper way of avoiding its degradation by light and oxygen (Ferreira et al., 2007). The encapsulation of the wine industry by-product in alginate beads would fit into a nobler destination and beer could be one of its applications, increasing antioxidant properties to this beverage, benefiting the consumer. This approach to increase beer nutritional properties has never been reported in literature. Alginates are produced from naturally occurring alginic acids, which are copolymers comprised of linear, unbranched chains of 1,4-linked β-D-mannuronic and α-L-guluronic acid residues (Martinsen et al., 1992). An alginate bead is prepared by gelation, which occurs by cross-linking the uronic acids with divalent cations, such as calcium. Calcium alginate beads are formed by ionotropic gelation when a sodium alginate salt is added dropwise to a solution of calcium chloride (Narra, 2012). The great advantage of encapsulation is that the inert environment within the alginate polymer network allows the entrapment of a wide range of substances with minor interactions between the biopolymer and the substances (Griffith, 2000). Therefore, this work evaluated the use of WIBP encapsulated in alginate beads as an ingredient in a wheat beer. The best

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conditions of the encapsulation conditions was performed using a central composite rotatable design (CCRD) in order to reduce the degree of erosion and swelling behavior of the capsules when applied in the beer.

2. Methodology

2.1 Materials

Sodium alginate was purchased from Sigma®, with a mannuronic acid to guluronic acid residues ratio of 0.4 to 1.9 (a sodium alginate solution (2%, w/v) in water has a viscosity at 25 °C and 60 rpm (spindle no. 2) of 100-300 Pa.s). The calcium chloride was obtained from Vetec®. The artisanal wheat beer (Rio de Janeiro, Brasil, Alcohol by Volume 4,3%, international bitterness units scale 12, Initial specific gravity (SG) 1048, Final SG 1014) was purchased at a local fair for small producers (Rio de Janeiro, Rio de Janeiro, Brasil). The wine industry by-product (WIBP) consisted of Alicante Bouschet grape pomace from red wine production and was provided by Rio Sol Winery (Lagoa Grande, Pernambuco, Brazil). The pomace was dried at 60 °C for 24 h and the solid material obtained was ground and sieved with particle size less than 0.80 mm.

2.2 Encapsulation of wine industry by-product

Encapsulation conditions to obtain WIBP beads were best conditions by central composite rotatable design (CCRD) aiming to obtain better swelling behavior and erosion degree in wheat beer. The general encapsulation process by inotropic gelation was performed as described by Fontes et al. (2013). Sodium alginate was dissolved in distilled water with WIBP (concentrations determined by CCRD). The polymer solution with WIBP was then added drop-wise into gelation media consisting of 250 mL of $CaCl_2$ solution of different concentrations (% w/v) using a 25 mL hypodermic syringe (without needle), under constant stirring at room temperature. The beads, thus formed, were left in the gelation medium for a certain period (complexation time), then collected by filtration, followed by a washing procedure with distilled water. Just after preparation, with the aid of a pachymeter the size of the beads was measured (mean size of 0.32 cm).Thus, five beads were immersed in 15 mL amber bottles with 10 mL wheat beer at room temperature, and samples were taken every 15 days for 90 days to measure the degree of erosion. A 2^4 central composite rotatable design (CCRD) was carried out to verify the effects and interactions of sodium alginate, WIBP and calcium chloride concentrations, as well as reticulation time in the swelling behavior and erosion degree of the beads immersed in wheat beer. Table 1 shows the limits for each parameter studied. The experiments were performed at random conditions. “STATISTICA” (version 7.0) software was used for regression and graphical analyses of the obtained data.

Table 1: Factors and levels of experimental runs for Central composite rotatable design (CCRD) for degree of swelling and erosion.

Independent variables	Levels				
	-2	-1	0	+1	+2
Sodium alginate (% w/v)	1.5	2.5	3.5	4.5	5.5
WIBP (% w/v)	0	1	2	3	4
Calcium chloride (M)	0.15	0.25	0.35	0.45	0.55
Complexation time (min)	5	15	25	35	45

WIBP: wine industry by-product

2.3 Analytical methods

2.3.1. Swelling behavior

The increase in weight due to absorbed liquid (swelling behavior) was determined for wet beads, after 90 days of immersion in 10 mL of a wheat beer. Initial weight was determined after washing the beads with Milli-Q water just after being produced and removing the excess of water with filter paper (W_i). Then, beads were weighted after drying them at 40 °C until constant weight was achieved (W_{iDB}). The swelling degree (S_w) was determined by Eq (1) (Fontes, 2013).

$$S_w = \frac{W_s - W_{iDB}}{W_{iDB}} * 100 \quad (1)$$

where W_s is the weight of the beads in the swollen state (after 90 days immersed in beer) and W_{iDB} is the initial weight of the dry beads. The analysis was performed with four replicates.

2.3.2. Degree of erosion

The degree of erosion was determined after the immersion of beads in 10 mL of a wheat beer. After a selected time interval (15 and 90 days), the beads were withdrawn, filtered and the excess of water removed with filter paper. Three different samples were weighted for each time, and fresh samples were used for each individual time. The percentage erosion (E) was estimated as Eq (2) (Efentakis et al, 2000).

$$E\ (\%) = \frac{W_i - W_f}{W_i} * 100 \qquad (2)$$

where, W_i is the initial weight of the beads (just after preparation) and W_f is the final weight of the same partially eroded sample. The analysis was performed with three replicates.

3. Results

The characteristics of hydrophilic polymers and their ability to hydrate and form a gel layer are well known and essential to sustain and control the release of internal material from matrices. The rate and extent of this release are also dependent on the swelling (S_w) and erosion (E) of the hydrated polymer mass (Efentakis et al, 2000). Central composite rotatable design was performed to evaluate the influence of sodium alginate (SA), wine industry by-product (WIBP) and calcium chloride ($CaCl_2$) concentrations, as well as complexation time in S_w of the prepared beads and in E of the beads immersed in a wheat beer for 15 and 90 days, as shown in Table 2.

Table 2: Matrix of experimental runs for central composite rotatable design (CCRD) for swelling behaviour of the beads and erosion degree after 15 or 90 immersion days in a wheat beer.

Run	Real values$_{\text{(corresponding coded values)}}$				$E_{15\ days}$ (%)	$E_{90\ days}$ (%)	S_w (%)
	SA (% w/v)	WIBP (%)	$CaCl_2$ (M)	CT (min)			
1	$2.50_{(-1)}$	$1.00_{(-1)}$	$0.25_{(-1)}$	$15.00_{(-1)}$	3.78	13.34	1331.82
2	$2.50_{(-1)}$	$1.00_{(-1)}$	$0.25_{(-1)}$	$35.00_{(+1)}$	16.23	20.40	1050.38
3	$2.50_{(-1)}$	$1.00_{(-1)}$	$0.45_{(+1)}$	$15.00_{(-1)}$	9.14	17.81	931.62
4	$2.50_{(-1)}$	$1.00_{(-1)}$	$0.45_{(+1)}$	$35.00_{(+1)}$	1.64	7.16	793.79
5	$2.50_{(-1)}$	$3.00_{(+1)}$	$0.25_{(-1)}$	$15.00_{(-1)}$	1.18	7.36	720.40
6	$2.50_{(-1)}$	$3.00_{(+1)}$	$0.25_{(-1)}$	$35.00_{(+1)}$	2.57	9.33	791.24
7	$2.50_{(-1)}$	$3.00_{(+1)}$	$0.45_{(+1)}$	$15.00_{(-1)}$	10.23	17.84	747.96
8	$2.50_{(-1)}$	$3.00_{(+1)}$	$0.45_{(+1)}$	$35.00_{(+1)}$	8.42	15.20	787.18
9	$4.50_{(+1)}$	$1.00_{(-1)}$	$0.25_{(-1)}$	$15.00_{(-1)}$	4.84	12.21	812.41
10	$4.50_{(+1)}$	$1.00_{(-1)}$	$0.25_{(-1)}$	$35.00_{(+1)}$	5.69	13.48	790.79
11	$4.50_{(+1)}$	$1.00_{(-1)}$	$0.45_{(+1)}$	$15.00_{(-1)}$	7.09	13.06	803.10
12	$4.50_{(+1)}$	$1.00_{(-1)}$	$0.45_{(+1)}$	$35.00_{(+1)}$	8.30	13.9	801.38
13	$4.50_{(+1)}$	$3.00_{(+1)}$	$0.25_{(-1)}$	$15.00_{(-1)}$	3.95	9.65	863.40
14	$4.50_{(+1)}$	$3.00_{(+1)}$	$0.25_{(-1)}$	$35.00_{(+1)}$	14.7	20.6	823.26
15	$4.50_{(+1)}$	$3.00_{(+1)}$	$0.45_{(+1)}$	$15.00_{(-1)}$	12.15	16.90	779.59
16	$4.50_{(+1)}$	$3.00_{(+1)}$	$0.45_{(+1)}$	$35.00_{(+1)}$	7.61	11.96	654.11
17	$1.50_{(-2)}$	$2.00_{(0)}$	$0.35_{(0)}$	$25.00_{(0)}$	9.26	19.98	864.63
18	$5.50_{(+2)}$	$2.00_{(0)}$	$0.35_{(0)}$	$25.00_{(0)}$	0.36	9.57	597.21
19	$3.50_{(0)}$	$0.00_{(-2)}$	$0.35_{(0)}$	$25.00_{(0)}$	0.69	10.50	1000.45
20	$3.50_{(0)}$	$4.00_{(+2)}$	$0.35_{(0)}$	$25.00_{(0)}$	7.68	15.30	598.49
21	$3.50_{(0)}$	$2.00_{(0)}$	$0.15_{(-2)}$	$25.00_{(0)}$	1.90	30.32	578.00
22	$3.50_{(0)}$	$2.00_{(0)}$	$0.55_{(+2)}$	$25.00_{(0)}$	3.46	15.30	653.49
23	$3.50_{(0)}$	$2.00_{(0)}$	$0.35_{(0)}$	$5.00_{(-2)}$	5.87	13.68	781.08
24	$3.50_{(0)}$	$2.00_{(0)}$	$0.35_{(0)}$	$45.00_{(+2)}$	11.37	17.62	696.04
25 (C)	$3.50_{(0)}$	$2.00_{(0)}$	$0.35_{(0)}$	$25.00_{(0)}$	8.80	16.51	790.06
26 (C)	$3.50_{(0)}$	$2.00_{(0)}$	$0.35_{(0)}$	$25.00_{(0)}$	7.55	12.25	797.92
27 (C)	$3.50_{(0)}$	$2.00_{(0)}$	$0.35_{(0)}$	$25.00_{(0)}$	6.99	11.94	797.93

SA: sodium alginate concentration; WIBP: wine industry by-product concentration; $CaCl_2$: calcium chloride concentration; CT: complexation time; $E_{15\ days}$: erosion degree after 15 immersion days; $E_{90\ days}$: erosion degree after 90 immersion days; S_w: swelling degree.

From the experimental data presented in Table 3, it is possible to notice that $E_{15\ days}$ ranged 16 % and $E_{90\ days}$ 13 %. Higher erosion was already expected for more immersion days because there is more time for the polymer to dissolute. Swelling degree ranged almost 754 %, a wider range than the variation at central point (8 %). The analysis of variance was performed (Tables 3 and 4) to verify the variables that influenced the results and to obtain a predictive model. The significance of the model was verified by Fisher's statistical test (F-test), considering the level of significance of 10% ($p < 0.1$) and non-significant terms were eliminated.

Table 3: Analysis of variance (ANOVA) for composite rotatable design (CCRD) to evaluate degree of erosion in 15 and 90 days.

Factor	DF		Sum of square		Mean square		F-value		p-value	
	$E_{15\ days}$ (%)	$E_{90\ days}$ (%)	$E_{15\ days}$ (%)	$E_{90\ days}$ (%)	$E_{15\ days}$ (%)	$E_{90\ days}$ (%)	$E_{15\ days}$ (%)	$E_{90\ days}$ (%)	$E_{15\ days}$ (%)	$E_{90\ days}$ (%)
WIBP(%)(L)	1		13.59		13.59		15.82		0.06	
$CaCl_2$(M)(L)	1	1	9.08	21.27	9.08	21.27	10.57	3.26	0.08	0.22
$CaCl_2$(M)(Q)	1	1	11.65	106.13	11.65	106.13	13.56	16.26	0.07	0.06
CT(min)(L)	1	1	23.62	5.74	23.62	5.74	27.49	0.88	0.03	0.45
CT (min)(Q)	1		16.79		16.80		19.55		0.05	
WIBP(%)(L) by $CaCl_2$(M)(L)	1		25.98		25.98		30.23		0.03	
$CaCl_2$(M)(L) by CT(min)(L)	1	1	90.59	93.39	90.59	93.39	105.41	14.31	0.01	0.06
Lack of Fit	17	20	248.09	380.31	14.59	19.02	16.98	2.92	0.06	0.29
Pure Error	2	2	1.72	12.05	0.86	6.53				
Total SS	26	26	448.14	619.88	13.59	21.27				

WIBP: wine industry by-product concentration; $CaCl_2$: calcium chloride concentration; CT: complexation time; L: Linear term; Q: Quadratic term

Table 4: Analysis of variance (ANOVA) for composite rotatable design (CCRD) for swelling behaviour of the beads.

Factor	DF	Sum of square	Mean square	F-value	p-value	Factor	DF	Sum of square	Mean square	F-value	p-value
Alg. (%)(L)	1	77199.5	77199.5	3743.69	0.0003	Alg (L) by WIBP (L)	1	59230.7	59230.7	2872.32	0.0003
Alg. (%)(Q)	1	367.2	367.2	17.81	0.0518						
WIBP (%)(L)	1	158772.4	158772.4	7699.47	0.0001	Alg (L) by $CaCl_2$ (L)	1	9101.5	9101.5	441.36	0.0023
WIBP (%)(Q)	1	9667.2	9667.2	468.80	0.0021						
$CaCl_2$ (M)(L)	1	22447.7	22447.7	1088.57	0.0009	Alg (L) by CT (L)	1	903.6	903.6	43.82	0.0221
$CaCl_2$ (M)(Q)	1	12956.3	12956.3	628.30	0.0015						
CT (min)(L)	1	18607.9	18607.9	902.37	0.0011	WIBP(L) by $CaCl_2$ (L)	1	11345.4	11345.4	550.18	0.0018
CT (min)(Q)	1	783.4	783.4	37.99	0.0253						
Lack of Fit	11	204310.7	18573.7	900.71	0.0010						
Pure Error	2	41.2	20.6	3743.69		WIBP(L) by CT (L)	1	9363.0	9363.0	454.05	0.0021
Total SS	26	606734.5									

Alg.: sodium alginate concentration; WIBP: wine industry by-product concentration; $CaCl_2$: calcium chloride concentration; L: Linear term; Q: Quadratic term; DF: degree of freedom

The results presented in Table 3 showed that there was a lack of fit ($p < 0.10$) for the degree of erosion for 15 days. However, the lack of fit is not important for the development of a predictive model when the pure error presents a very low value (Rodrigues & Iemma, 2014), as observed in the present study. Another important point is that SA concentrations did not influence erosion of the beads.

The mathematical models with the real variables to predict the variation of the degree of erosion in 15 and 90 immersion days in a wheat beer are represented in Eq(3) and (4).

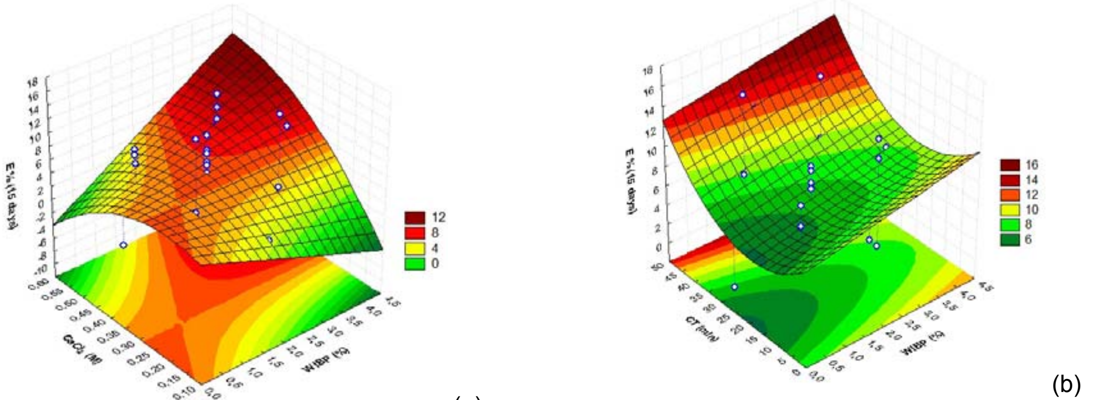
$$E_{15\ days} = -14.64 - 3.71 * \mathrm{WIBP} + 87.38 * \mathrm{CaCl_2} - 67.47 * \mathrm{CaCl_2}^2 + 0.53 * \mathrm{CT} + 0.0081 * \mathrm{CT}^2 + 12.74 * \mathrm{WIBP} * \mathrm{CaCl_2} - 2.38 * \mathrm{CaCl_2} * \mathrm{CT} \quad (3)$$

$$E_{90\ days} = -18.16 - 88.66 * CaCl_2 + 199.49 * \mathrm{CaCl_2}^2 + 0.89 * \mathrm{CT} - 2.42 * \mathrm{CaCl_2} * \mathrm{CT} \quad (4)$$

where E_{15days} and E_{90days} represents erosion degree in 15 and 90 immersion days, respectively, WIBP represents wine industry by-product concentration; $CaCl_2$ is for calcium chloride concentration and CT stands for complexation time.The data in Table 4 shows that there was lack of fit ($p < 0.10$) for the swelling behavior of the beads. However, as for the Erosion degree, the lack of fit is not important for the development of a predictive model because the pure error presents a very low value, which indicates that the model obtained is adequate to explain the process. Eq. (5) represents the mathematical model obtained using the real variables.

$$Sw(\%) = 22296.27 - 309.71\mathrm{SA} + 4.15\mathrm{SA}^2 - 533.11\mathrm{WIBP} + 21.29\mathrm{WIBP}^2 + 51.91\mathrm{CaCl_2} - 2464.40{\mathrm{CaCl_2}}^2 - 13.28\mathrm{CT} + 0.06\mathrm{CT}^2 + 60.84\mathrm{SA} * \mathrm{WIBP} + 238.50\mathrm{SA} * \mathrm{CaCl_2} + 0.75\mathrm{SA} * \mathrm{CT} + 266.29\,\mathrm{WIBP} * \mathrm{CaCl_2} + 2.42\mathrm{WIBP} * \mathrm{CT}$$

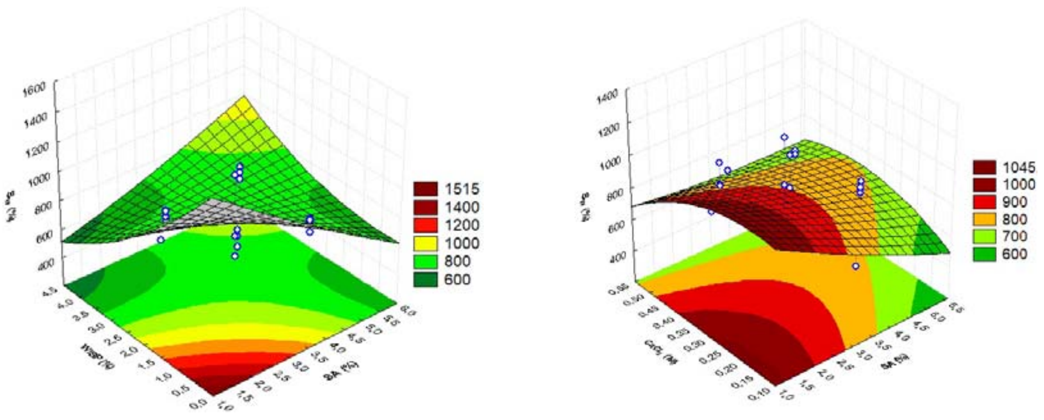
where S_w represents swelling behavior of the beads, SA is sodium alginate concentration, WIBP represents wine industry by-product concentration and $CaCl_2$ is for calcium chloride concentration and CT stands for complexation time. From the models of Eq. (3) and (4), response surfaces were obtained (Figure 1).



(a) (b)

Figure 1: Response surface for the degree of erosion (E %) after 15 immersion days in wheat beer (a), (b): as a function of wine industry by-product (WIBP) concentration, $CaCl_2$ concentration and complexation time (CT).

Figure 1 (a) depicts that when lower calcium chloride concentrations and higher WIBP concentrations were used, or the opposite, lower degrees of erosion were obtained, wich is better for the product. Due to greater complexation of alginate chains by Ca^{2+} ions, the solubility of the polymer decreases, which also results in reduced erosion. For the complexation time (Figure 1b), 20 min would be better, considering different WIBP concentrations. Therefore, to reduce erosion, lower $CaCl_2$ concentrations, higher WIBP amounts and reduced CT would be good encapsulation conditions.For the swelling behavior, Eq (5) was used to obtain the response surfaces. Figure 2(a, b) indicates that lower swelling was observed when larger concentrations of sodium alginate, medium to lower amounts of WIBP and lower $CaCl_2$ concentration were used to produce the beads. Therefore, for lower S_w, high sodium alginate and lower $CaCl_2$ concentrations should be used as well as medium WIBP and CT.



(a) (b)

Figure 2: Response surface for the swelling behavior (S_W %) as a function of wine industry by-product (WIBP) concentration, $CaCl_2$ concentration and sodium alginate concentration (SA).

A desirability function (Statistica 7.0) was used to obtain lower degree of erosion in 90 immersion days simultaneously with lower swelling behavior. A greater degree of importance was given to the lower values of the responses, because it is important that WIBP beads should not disintegrate in wheat beer before consumption. For sodium alginate, a range of 1.5 to 4% was used in the desirability function, aiming at a better formation of the spheres, since low concentrations generates imperfect beads, whereas concentrations above 4% results in difficulties in the extrusion of the polymer solution in the syringe. In order to obtain the lowest values of degree of erosion in 90 immersion days and lower swelling behavior of the beads, 1.5% of sodium alginate, 4% of WIBP, 0.26 M of $CaCl_2$ and 26 min of CT should be used to obtain the beads. In those conditions it will be possible to obtain a degree of erosion at 90 days of 7.6 % and a S_w of 606.7 %.

4. Conclusions

The production conditions of alginate beads with wine industry by-product (WIBP) is influenced by WIBP and $CaCl_2$ concentrations as well as complexation time (CT) when erosion degree of the beads immersed by 15 days is considered. When the beads are immersed for longer periods (90 days) only calcium chloride concentration and CT influence erosion. Considering 15 to 90 immersion days, the encapsulation conditions to reduce the degree of erosion in wheat beer are lower $CaCl_2$ concentrations, higher WIBP amounts and reduced CT. To avoid excessive swelling, $CaCl_2$ should be used in lower concentrations with high sodium alginate and medium amount of WIBP and medium CT. Best conditions to obtain beads with simultaneously lower swelling (606.7 %) and erosion degree (7.6 %) are: 1.5% sodium alginate, 4% WIBP, 0.26 M $CaCl_2$ and 26 min CT. Beads containing the residue of the wine industry are potential additives for wheat beer increasing the consumption of antioxidants by the final consumer.

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