



Optimization of microencapsulation conditions of transglutaminase by freeze drying

Hilal Isleroglu¹ · Izzet Turker¹ · Banu Koc² · Mehmet Tokatli¹

Revised: 3 July 2019 / Accepted: 16 July 2019 / Published online: 16 October 2019
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Abstract Transglutaminase (TG), which is an important enzyme for food processing, can enhance the firmness, viscosity and water binding capacity of food products by catalyzing the cross-linking reaction of proteins. Since preservation of the enzyme activity is essential, the production of microencapsulated powder form of TG can be a great challenge to maintain its initial activity. In this study, TG was microencapsulated using a freeze drying technique and the effects of homogenization conditions and coating material ratios on the enzyme activity were investigated using D-optimal combined design. Mannitol, gum arabic and casein were chosen as coating materials and different homogenization times (1–5 min) and homogenization rates (11,200–20,000 rpm) were applied. The optimum conditions which ensure the maximum enzyme activity have been determined as 11,200 rpm of homogenization rate, 1.27 min of homogenization time, and in addition a mixture of mannitol, gum arabic and casein with ratios 38.2, 40.2, and 21.6%, respectively. Most of the activity loss occurred in the homogenization stage and the coating

materials preserved enzyme activity during freeze drying. At the optimum point, the remaining activity of the microencapsulated TG was 93% while that of the crude (without coating materials) TG was 64% at the same drying conditions. Moreover, the effects of the microencapsulation conditions on the physical properties of powder such as moisture content, color, particle, bulk and tapped densities, porosity and flowability were determined.

Keywords Transglutaminase · Freeze drying · Microencapsulation · Enzyme activity · D-optimal design

Introduction

Transglutaminase (EC 2.3.2.13, protein-glutamine γ -glutamyltransferase) is an enzyme that can create significant changes in food proteins via acyl transfer reactions, deamidation and crosslinking reactions (Kuraishi et al. 2001). These changes in protein structure are one of the main subjects of enhancing the functional properties of food macromolecules and producing new compounds. Transglutaminase (TG) can increase the hardness of final products, lead to enhanced texture and stability by reducing syneresis, and can increase emulsifying properties, gelation ability and water-binding capacity. Increases in the hardness values of the samples are due to the basic function of transglutaminase, which consists of cross-linking milk proteins covalently. Consequently, the formation of new covalent bonds results in gels with different structures constructed by the stabilization of 3D networks (Gaspar and Góes-Favoni 2015). Moreover, the addition of some essential amino acids in the form of lysine-methionine and lysine-arginine peptides to the modification process can make the food products more valuable in terms of nutrition

✉ Hilal Isleroglu
hilal.isleroglu@gop.edu.tr

Izzet Turker
izzet.turker2615@gop.edu.tr

Banu Koc
banukoc@gantep.edu.tr

Mehmet Tokatli
mehmet.tokatli@gop.edu.tr

¹ Food Engineering Department, Faculty of Natural Sciences and Engineering, Tokat Gaziosmanpasa University, 60150 Tokat, Turkey

² Fine Arts, Gastronomy and Culinary Arts, Gaziantep University, 27310 Gaziantep, Turkey

(Motoki and Seguro 1998; Kieliszek and Misiewicz 2014). TG is widely used for the reconstruction of meat products through cross-linking. TG usage can reduce losses during cooking and improve the textural properties of meat products (Kuraishi et al. 2001; Gaspar and Góes-Favoni 2015). Lower grade meat products such as deboned meat and collagens can be converted into more valuable products by TG. The pale, soft and exudative meats' gelling ability and textural properties can be enhanced by the crosslinking of myofibrillar proteins, which is affected by TG (Katayama et al. 2006). TG is used in the dairy industry for obtaining a better texture in cheese and preventing whey separation in yoghurt production (Motoki and Seguro 1998; Kuraishi et al. 2001; Yokoyama et al. 2004). Also, the deamidation of gliadin in pasta produced with wheat flour by TG can reduce the prevalence rate of celiac disease (Gharibzahedi et al. 2019). TG is naturally and abundantly found in animal tissues, as well as in blood and plant tissues and it can be produced by microbial strains such as *Streptomyces mobaraensis*, *S. cinnamomeum*, *S. ladakanum*, *S. lividans*, *S. platensis*, *S. sioyaensis*, *S. hygrosopicus*, *Bacillus subtilis*, *Pseudomonas putida* and *Zygomonas mobilis* (Zhu et al. 1998; Taguchi et al. 2002; Lin et al. 2006; Cui et al. 2007; Bourneow et al. 2012; Kieliszek and Misiewicz 2014). Microbial TG can be produced with high productivity and purity without any requirement for difficult separation techniques compared to other transglutaminases which are extracted from plant and animal sources (Macedo et al. 2011).

When enzymes are isolated from their natural environments, it is hard to maintain their stability and they generally lose their activity during storage in water solutions. Encapsulation is a successful technique to overcome these instability issues compared to the free enzymes in solution, and encapsulated enzymes are easily separated and reusable over several cycles with little loss of activity (Vikartovska et al. 2007). Although spray drying is frequently used in the encapsulation of flavors, lipids and pigments, this process is limited to use for materials with low thermal resistance biomaterials such as microorganisms, essential oils, protein-based components and enzymes (Gharsallaoui 2007). The spray drying process can damage temperature-sensitive components such as enzymes or probiotic bacteria when compared to freeze drying (Knorr 1998). Freeze drying is one of the best preservation techniques for heat-labile materials such as enzymes (Adamiec et al. 2006). Although freeze drying is commonly used for preparing stable enzyme powders, it can cause different types of stresses such as low temperature stress, dehydration stress and ice crystal formation which can deactivate and destabilize the enzymes (Ramos et al. 1997). When considered from this point of view, microencapsulation of the enzyme by freeze drying can be

a challenge to avoid these kinds of problems (Kawai and Suzuki 2007). There have been several studies in the literature relating to microencapsulation of enzymes using freeze drying (Izutsu et al. 1994; Desai and Park 2005; Anjani et al. 2007; Liu et al. 2011; Zhang and Zhong 2018). Different coating materials have been used to prevent the activity of enzymes during microencapsulation by freeze drying (Arakawa et al. 2001). The most commonly used coating materials are gum arabic, maltodextrin, emulsifying starches, whey protein, etc. (Ray et al. 2016). Mannitol is a widely used bulking agent for freeze drying as the crystallization results in a good cake structure; however, its protection ability for proteins is less than other amorphous sugars and sugar alcohols (Debulis and Klibanov 1993; Izutsu et al. 1994; do Vale Morais et al. 2016). Gum arabic, with its low viscosity, high solubility and surface-active qualities, is very useful for the encapsulation of enzymes (Amid et al. 2014; Andrea et al. 2016). Furthermore, some milk proteins such as sodium caseinate are reported as suitable coating materials for bioactive materials (Heidebach et al. 2010; Vinceković et al. 2017). Although these materials have many of the desirable properties of an encapsulating agent, no single wall material supplies all the properties required of an ideal encapsulating agent (Desai and Park 2005). Therefore, approaches for improving encapsulating properties have focused on blends of these materials for microencapsulation. The objective of this study was to examine the effects of homogenization time, homogenization rate and coating material compositions on TG activity during microencapsulation by freeze drying. Moreover, the physical properties of the enzyme powder such as moisture content, color, particle, bulk and tapped densities, porosity and flowability were determined under different microencapsulation conditions.

Materials and methods

Materials

A commercial microbial transglutaminase (Benosen, Tegen 20X, Turkey) powder was used to prepare a crude enzyme solution. To remove the impurities, the enzyme dispersion (20%, w/w) was subjected to a dialysis using dialysis tubing cellulose membrane (Sigma-Aldrich, 14,000 MW cut-off, USA) at 4 °C until a constant dry matter content (10%) was reached. After the dialysing process, the obtained crude enzyme solution with 10% dry matter was used for all microencapsulation processes. Commercial powder forms of mannitol, gum arabic, and casein were chosen as coating materials, and stock solutions of these materials were prepared with dry matter content of 15, 15

and 10%, respectively. These stock solutions were prepared one night before the microencapsulation processes and kept at 4 °C for rehydration.

Activity assay

The enzymatic activity of TG was determined using the colorimetric hydroxamate method with the specific substrate of *N*-carbobenzoxy-L-glutamylglycine (Z-Gln-Gly). For this purpose, 200 µl of 200 mM Tris-HCl, 25 µl of 12.5 mM glutathione, 25 µl of 125 mM hydroxylamine and 75 µl of 37.5 mM of Z-Gln-Gly solutions were added to 100 µl of enzyme solution and incubated for 1 h at 37 °C. At the end of the incubation, 425 µl of solution (15% TCA-5% FeCl₃) was added to the reaction medium to stop the reaction, and centrifuged at 11,000 rpm for 5 min. Then, the absorbance of the supernatant phase was determined at 525 nm. The calibration of the absorbance-hydroxamate curve was carried out using L-glutamic acid γ-monohydroxamate as a standard. One unit of TG activity in a one ml of sample (U/ml) was described as the amount of enzyme which caused the formation of 1.0 µmole of hydroxamate per minute by catalyzing the reaction between Z-Gln-Gly and hydroxylamine at pH 6.0 and 37 °C (Bourneow et al. 2012). The initial activity of the crude enzyme solution was found to be 7.45 ± 0.04 U/ml.

Microencapsulation processes

A laboratory scale homogenizer (IKA-Works, Ultra Turrax T-18 Basic, Germany) was used for homogenization of the enzyme solution and coating materials with different compositions. Three different homogenization times (1, 3 and 5 min) and three different homogenization rates (11,200, 15,600 and 20,000 rpm) were applied as the homogenization process conditions. The crude enzyme solution dry matter to coating material ratio was kept constant at 1:1 and the final dry matter of the mixtures (coating material + crude enzyme solution) were adjusted to 10% for all conditions. The coating material compositions and homogenization process conditions are given in Table 1. The homogenized mixtures (30 ml) at different conditions were transferred to metal plates and frozen for 3 h at – 80 °C. After freezing, the freeze drying process was carried out for 18 h in a freeze dryer (CHRIST, Alpha 1–4 LSC, Germany). The main drying was conducted for 16 h at 1 mbar and secondary drying was applied subsequently for 2 h with a shelf temperature at 30 °C under a vacuum of 0.01 mbar.

The effect of the total process (homogenization + freeze drying) on the TG activity was defined as ‘remaining activity (%)’ and calculated by using Eq. 1. The activity of the final product was determined by rehydrating

the final product to the same dry matter content as the initial mixture. Moreover, to determine the individual effects of the homogenization and freeze drying steps on enzyme activity, the ‘remaining activity after homogenization’ and ‘remaining activity after drying’ were defined. The ‘remaining activity after homogenization’ was calculated by dividing the activity after homogenization to the activity of the crude enzyme solution and the ‘remaining activity after drying’ was calculated by dividing the activity of the final product by the activity after homogenization.

Remaining Activity (%)

$$= \frac{\text{Activity of final product (U/ml)}}{\text{Activity of crude enzyme solution (U/ml)}} \times 100 \quad (1)$$

Moisture content

The moisture content of the microencapsulated enzyme powder was determined by an infrared moisture analyzer (Shimadzu, MOC63u, Japan) at 90 °C and correlated previously by the air drying method at the same temperature.

Particle, bulk and tapped densities

Particle density (ρ_p) of the powder sample was analyzed according to the liquid pycnometer method using toluene. The bulk density (ρ_b) of the samples were determined by measuring the weight of the powder and the corresponding volume. Approximately 2 g of powder sample was placed in a 10 ml graduated cylinder. The bulk density was calculated by dividing the mass of the powder by the volume occupied in the cylinder. For the tapped density (ρ_t), the cylinder was tapped vigorously by hand until no further change in volume occurred (Jinapong et al. 2008).

Porosity

The porosity (ε) of the powder samples were calculated using the relationship between the tapped (ρ_t) and particle (ρ_p) densities of the powders as calculated using Eq. 2 (Jinapong et al. 2008).

$$\varepsilon = \frac{(\rho_p - \rho_t)}{\rho_t} \times 100 \quad (2)$$

Flowability

The flowability of powder was evaluated using the Carr’s Index (CI, percent compressibility) (Eq. 3) and the Hausner Ratio (HR) (Eq. 4) (Carr 1965; Hausner 1967).

Table 1 Experimental D-optimal combined design for coating material composition and homogenization conditions

Exp. no	MA (%)	GA (%)	CS (%)	HT (min)	HR (rpm)	Remaining activity after homogenization (%)	Remaining activity after drying (%)	Remaining activity (%)
1	100	0	0	5	11,200	91.60 (± 0.23)	87.56 (± 0.20)	80.20 (± 0.18)
2	100	0	0	5	15,600	88.51 (± 0.50)	90.20 (± 0.51)	79.84 (± 0.45)
3	33	33	33	5	15,600	89.33 (± 0.18)	94.71 (± 0.10)	84.61 (± 0.09)
4	33	33	33	1	15,600	96.28 (± 0.50)	86.61 (± 0.24)	83.38 (± 0.23)
5	0	100	0	5	15,600	90.51 (± 0.09)	89.77 (± 0.30)	81.25 (± 0.27)
6	0	50	50	3	20,000	82.61 (± 0.23)	93.90 (± 0.33)	77.57 (± 0.27)
7	50	50	0	5	11,200	92.14 (± 0.41)	89.31 (± 0.88)	82.29 (± 0.45)
8	50	50	0	1	15,600	95.80 (± 0.27)	89.34 (± 0.39)	85.59 (± 0.14)
9	100	0	0	5	20,000	81.61 (± 0.23)	92.47 (± 0.02)	75.47 (± 0.23)
10	33	33	33	1	11,200	97.33 (± 0.32)	92.34 (± 0.07)	89.88 (± 0.23)
11	0	50	50	1	20,000	85.36 (± 0.05)	94.07 (± 0.21)	80.30 (± 0.23)
12	0	50	50	5	11,200	83.19 (± 0.32)	97.50 (± 0.75)	81.11 (± 0.32)
13	33	33	33	5	20,000	75.92 (± 0.41)	96.85 (± 0.50)	76.41 (± 0.09)
14	0	0	100	1	15,600	86.58 (± 0.32)	97.39 (± 0.30)	84.32 (± 0.05)
15	0	0	100	1	11,200	90.78 (± 0.09)	92.09 (± 0.34)	83.60 (± 0.23)
16	50	50	0	5	20,000	75.38 (± 0.32)	95.38 (± 0.59)	73.75 (± 0.54)
17	50	0	50	1	15,600	95.93 (± 0.68)	90.31 (± 1.06)	86.63 (± 0.41)
18	0	100	0	5	11,200	88.48 (± 0.50)	92.80 (± 0.88)	82.11 (± 0.32)
19	100	0	0	1	20,000	86.85 (± 0.18)	90.95 (± 0.17)	78.99 (± 0.32)
20	0	100	0	1	15,600	90.69 (± 0.23)	91.33 (± 0.63)	82.83 (± 0.36)
21	0	0	100	5	11,200	87.57 (± 0.09)	95.56 (± 0.46)	83.69 (± 0.32)
22	50	0	50	5	11,200	88.21 (± 0.50)	93.55 (± 1.04)	82.51 (± 0.45)
23	0	0	100	5	20,000	78.90 (± 0.23)	94.90 (± 0.24)	74.88 (± 0.41)
24	0	100	0	1	20,000	87.35 (± 0.18)	94.31 (± 0.35)	82.38 (± 0.14)
25	0	0	100	3	11,200	94.62 (± 0.23)	95.08 (± 0.70)	89.97 (± 0.45)
26	100	0	0	3	11,200	93.67 (± 0.09)	91.46 (± 0.33)	85.68 (± 0.23)
27	17	67	17	3	11,200	94.35 (± 0.05)	97.32 (± 0.38)	91.82 (± 0.32)
28	33	33	33	5	11,200	88.39 (± 0.50)	95.71 (± 0.13)	84.59 (± 0.36)
29	100	0	0	3	15,600	91.48 (± 0.18)	92.01 (± 0.02)	84.17 (± 0.18)
30	50	0	50	1	11,200	96.39 (± 0.41)	90.88 (± 0.94)	87.60 (± 0.54)
31	50	0	50	3	20,000	84.17 (± 0.27)	94.27 (± 0.84)	79.34 (± 0.45)
32	50	50	0	5	11,200	88.45 (± 0.05)	93.98 (± 0.40)	83.13 (± 0.32)
33	0	0	100	5	15,600	85.93 (± 0.27)	96.80 (± 0.20)	83.18 (± 0.09)
34	0	100	0	1	11,200	98.29 (± 0.14)	88.57 (± 0.26)	87.06 (± 0.14)
35	0	0	100	1	20,000	88.31 (± 0.18)	95.67 (± 0.45)	84.48 (± 0.22)
36	50	0	50	3	11,200	94.20 (± 0.27)	92.60 (± 0.84)	87.23 (± 0.54)
37	100	0	0	1	15,600	89.74 (± 0.18)	91.43 (± 0.03)	82.05 (± 0.13)
38	100	0	0	3	20,000	82.28 (± 0.13)	92.24 (± 0.37)	75.89 (± 0.18)
39	0	100	0	5	15,600	86.24 (± 0.31)	93.43 (± 0.23)	80.57 (± 0.49)
40	50	50	0	5	20,000	79.89 (± 0.22)	94.20 (± 0.07)	75.26 (± 0.27)
41	100	0	0	1	11,200	97.48 (± 0.22)	89.62 (± 0.62)	87.36 (± 0.40)
42	50	0	50	5	20,000	81.51 (± 0.22)	93.82 (± 0.65)	76.48 (± 0.31)
43	0	0	100	3	15,600	91.27 (± 0.04)	96.65 (± 0.25)	88.22 (± 0.27)
44	0	100	0	3	20,000	83.13 (± 0.13)	94.75 (± 0.28)	78.77 (± 0.36)
45	50	50	0	1	20,000	88.26 (± 0.22)	94.35 (± 0.80)	83.27 (± 0.49)
46	33	33	33	3	15,600	93.12 (± 0.40)	97.59 (± 0.57)	90.87 (± 0.13)
47	0	100	0	1	15,600	91.09 (± 0.09)	93.98 (± 0.40)	85.61 (± 0.45)
48	0	50	50	5	15,600	85.16 (± 0.40)	94.62 (± 0.77)	80.57 (± 0.27)

Table 1 continued

Exp. no	MA (%)	GA (%)	CS (%)	HT (min)	HR (rpm)	Remaining activity after homogenization (%)	Remaining activity after drying (%)	Remaining activity (%)
49	0	50	50	1	11,200	97.84 (± 0.27)	91.77 (± 0.39)	89.79 (± 0.13)
50	0	50	50	5	20,000	82.64 (± 0.27)	95.32 (± 0.26)	78.77 (± 0.04)
51	50	50	0	5	15,600	83.58 (± 0.31)	92.52 (± 0.13)	77.33 (± 0.18)
52	50	0	50	5	15,600	84.26 (± 0.13)	94.72 (± 0.58)	79.80 (± 0.36)
53	33	33	33	1	20,000	89.92 (± 0.22)	97.65 (± 0.11)	87.81 (± 0.31)
54	50	0	50	1	20,000	89.74 (± 0.54)	95.14 (± 0.92)	85.38 (± 0.31)
55	50	50	0	1	20,000	90.01 (± 0.18)	92.66 (± 0.49)	83.40 (± 0.27)
56	0	50	50	1	15,600	91.68 (± 0.45)	92.94 (± 0.11)	85.20 (± 0.31)
57	50	50	0	1	11,200	97.57 (± 0.27)	91.79 (± 0.12)	89.56 (± 0.13)
58	0	100	0	5	20,000	82.10 (± 0.09)	91.78 (± 0.54)	75.35 (± 0.36)
59	50	50	0	3	15,600	94.20 (± 0.40)	93.36 (± 0.21)	87.95 (± 0.18)

MA mannitol, GA gum arabic, CS casein, HT homogenization time, HR Homogenization rate

$$CI = \frac{(\rho_t - \rho_b)}{\rho_t} \times 100 \tag{3}$$

$$HR = \frac{\rho_t}{\rho_b} \tag{4}$$

Color

The color of the TG powder samples (CIE *L*, *a*, and *b* values) was measured with a colorimeter (3NH Colorimeter NR-200, China).

Experimental design and statistical analysis

To investigate the effects of the independent variables namely the coating materials ratio [mannitol (*C*₁), gum arabic (*C*₂) and casein (*C*₃)] and homogenization conditions [homogenization time (*X*₁) and homogenization rate (*X*₂)] on the enzyme activity during the microencapsulation process a ‘D-optimal combined design’ was performed which included 59 experiments (Table 1). The remaining activity data of the runs was selected as a dependent variable. The optimum process conditions providing the maximum remaining activity were estimated using the desirability function method. All experimental data was fitted to a second-order polynomial model. Significant terms in the models were found through the analysis of variance (ANOVA) by using Design Expert-version 7.0 software at a confidence level of 95% and the model adequacies were checked by R², adj-R², adequate precision and coefficient of variation (C.V.%). At the estimated optimum microencapsulation conditions that provided the maximum remaining activity, three experiments were conducted and one sample *T* test was performed to verify the optimization.

Results and discussions

The experimental design and the remaining activity values after the homogenization and freeze drying processes are given in Table 1. Experimental results showed that the remaining activity on completion of the entire process, which is the key parameter of microencapsulation efficiency, ranged between ~ 74 and 92%. To determine the individual effect of the homogenization conditions, the TG activity after homogenization was compared to the crude enzyme activity. It was observed that when both the homogenization time and homogenization rate were increased, the remaining activity after homogenization decreased dramatically (Table 1). The low remaining activity after the homogenization values were obtained when the homogenization time was 5 min and the homogenization rate was 20,000 rpm (75.38–82.64%). On the other hand, when homogenization was applied for 1 min at 11,200 rpm, the initial crude enzyme activities were substantially preserved (90.78–98.29%). The increasing shear effects due to the high homogenization rate and time might cause damage to protein by changing their secondary and/or tertiary structures through unfolding or by disruption of the quaternary structure of a multi-subunit protein and this damage may have caused a loss of enzyme activity (Ghadge et al. 2005; Thomas and Geer 2011). Because of the loss of enzyme activity through the homogenization process, it is necessary to pay attention to the use of the enzyme in dairy products where the homogenization process is frequently applied.

To observe the individual effects of the freeze drying stage on TG activity, the activity after the homogenization process was compared to the activity of the final powder product. The remaining activity after freeze drying values ranged between 86.61 and 97.65%, which indicated that the

proportion of the different coating materials affected the final enzyme activity. When the coating material ratios were equal, the highest remaining activity after drying was determined at 3 min of homogenization time and 15,600 rpm of homogenization rate. However, the lowest remaining activity was obtained at homogenization conditions of 11,200 rpm for 1 min when the same coating material compositions were used (Table 1). This phenomenon can be explained by inadequate homogenization which was not enough to bring together the coating materials and enzyme molecules. Hence, the remaining activity after drying that indicates microencapsulation efficiency was affected by not only the coating material composition, but also the homogenization conditions. In the experiment where three different coating materials were used in equal proportions, the materials protected the enzyme more effectively because of the different physicochemical effects of the coating materials and the remaining activity values were found to be higher. Coating materials such as casein, mannitol and gum arabic have different effects on the protection of biological materials against oxidation, as well as mechanical, cold and drying stresses. This can minimize the loss of enzyme activity (Amid et al. 2014; Shiga et al. 2014; Glab and Boratyński 2017).

Optimization of the microencapsulation process was carried out using experimental remaining activity values which describe the effects of the entire process on TG activity. The experimental data arranged according to D-optimal combined design was evaluated to determine the optimum composition of the coating materials and the homogenization conditions that ensure maximum enzyme activity. To perform this operation, Design Expert-version 7.0 software (Stat-Ease Inc., MN, USA) was utilized for numerical optimization. The statistical significance of all effects and regression equation coefficients calculated for the remaining activity are given in Table 2. The calculated special cubic \times quadratic models of the remaining activity were statistically significant at $p < 0.01$ level whereas the lack of fit of the models was not significant (Table 2). Regression analysis and ANOVA were conducted for fitting the model and to examine the statistical significance of the model terms. The effects that were not significant ($p > 0.05$) were stepped down from the models without damaging the model hierarchy. The equation representing the descriptive model for the remaining activity is shown using Eq. 5. Moreover, R^2 , R^2 -adj and coefficient of variation (C.V.) were calculated to check the adequacy of the model. The coefficient of determination (R^2) value is quite high (> 0.983), indicating that a high proportion of variability was explained by the data and the RSM models were adequate. Table 2 shows that R^2 and adj- R^2 did not differ clearly, which indicates that the insignificant terms

have not been included in the model. The relative dispersion of the experimental points from the predictions of the model which is defined as the C.V.% was calculated as 1.70%.

$$\begin{aligned} \text{Remaining activity (\%)} = & \\ & + 81.9 * C_1 + 82.2 * C_2 + 87.7 * C_3 + 27.4 * C_1 C_2 \\ & - 6.0 * C_1 C_3 - 2.1 * C_1 X_1 + 3.3 * C_1 X_2 - 2.5 * C_2 X_1 \\ & + 2.7 * C_2 X_2 - 1.8 * C_3 X_1 + 1.6 * C_3 X_2 \\ & + 199.1 * C_1 C_2 C_3 + 1.8 * C_2 C_3 X_1 - 0.2 * C_2 X_1^2 \\ & - 5.4 * C_3 X_1^2 - 11.2 * C_2 C_3 X_1 X_2 - 24.0 * C_1 C_2 X_1^2 \\ & + 4.3 * C_1 C_2 C_3 X_1 X_2 - 158.3 * C_1 C_2 C_3 X_1^2 \end{aligned} \quad (5)$$

Analyzing the results, coating material composition (mannitol to gum arabic ratio) and homogenization time interactions were the most effective independent variables affecting the remaining activity. Perturbation plots (Fig. 1a) showed that the remaining activity was increased with the increase in mannitol, gum arabic and casein; however, a saddle system was identified because of the interaction of the composition of the coating materials and process condition (Fig. 1b). This was explained by the remaining activity which had the lowest value at the maximum and minimum levels of homogenization time (at the central point of homogenization rate). Sun-Waterhouse et al. (2013) also stated that the type of coating materials had a significant impact on the encapsulation efficiency of the microcapsules. The effect of the composition of the coating material on the remaining activity is further revealed in Fig. 2 which made it clear that the increase in the gum arabic to mannitol and casein ratio led to an increase in the remaining activity of the enzyme.

The counter plots and 3-D graphs of the predicted model for TG activity are illustrated in Fig. 2. According to Fig. 2, TG activity was significantly influenced by the composition of the coating materials and the interactions between MA–GA, MA–HT, MA–HR, GA–HT, GA–HR, CS–HT and CS–HR ($p < 0.01$). The remaining activity decreased with an increase in the homogenization rate and time when the highest ratios of mannitol to gum arabic were applied or vice versa (Fig. 2). These findings were consistent with the results of the ANOVA and perturbation plots (Table 2 and Fig. 1).

Numerical optimization was performed for the mixture and the process parameters of the microencapsulation process to obtain the highest remaining activity. To perform this operation, Design Expert-Version 7.0 software (Stat-Ease Inc., MN, USA) was utilized. According to the desirability function, optimum point conditions were chosen as 11,200 rpm for the homogenization rate, 1.27 min of homogenization time, and the mixture of mannitol, gum

Table 2 ANOVA results for remaining activity and physical properties of microencapsulated TG

Variation source	Remaining activity (%)	Moisture (%)	<i>L</i>	<i>a</i>	<i>b</i>	ρ_p (kg/m ³)	ρ_b (kg/m ³)	ρ_t (kg/m ³)	CI (%)	HR	ϵ (%)
	<i>p value</i>										
Model	< 0.0001	< 0.0001	0.0129	0.0014	0.0011	0.0529	0.0006	0.0025	0.8417	0.4420	< 0.0001
Linear mixture	0.0069	< 0.0001	0.0009	0.0019	0.0020	0.1376	< 0.0001	< 0.0001	0.9778	0.9540	< 0.0001
<i>X</i> ₁	0.0115	–	0.6903	0.2753	0.4480	0.0726	–	0.0413	–	–	–
<i>X</i> ₂	0.0244	–	0.8347	0.4169	0.2012	0.1527	–	0.5549	–	–	–
<i>C</i> ₁ <i>C</i> ₂	0.0023	0.6933	–	0.0020	0.0021	0.9936	< 0.0001	< 0.0001	–	0.4563	< 0.0001
<i>C</i> ₁ <i>C</i> ₃	0.0190	0.1210	–	0.1140	0.1301	0.0104	0.0016	0.0215	–	0.1622	0.1442
<i>C</i> ₁ <i>X</i> ₁	< 0.0001	–	0.6128	–	–	0.9924	0.0752	0.9897	0.8807	0.9877	–
<i>C</i> ₁ <i>X</i> ₂	< 0.0001	–	0.9677	–	–	0.4291	0.7613	0.3264	0.4099	0.3368	–
<i>C</i> ₂ <i>C</i> ₃	0.3258	0.0009	–	0.1521	0.1137	0.2211	0.7399	0.8861	–	0.8210	0.8002
<i>C</i> ₂ <i>X</i> ₁	< 0.0001	–	0.4338	–	–	0.0250	0.9526	0.0591	0.8047	0.6052	–
<i>C</i> ₂ <i>X</i> ₂	0.0004	–	0.5562	–	–	0.5577	0.8537	0.3623	0.1705	0.4187	–
<i>C</i> ₃ <i>X</i> ₁	0.0009	–	–	–	–	–	0.0868	–	0.9437	0.7725	–
<i>C</i> ₃ <i>X</i> ₂	0.0118	–	–	–	–	–	0.1025	–	0.4520	0.4602	–
<i>C</i> ₁ <i>C</i> ₂ <i>C</i> ₃	0.0061	0.0005	–	–	–	0.0991	0.5146	0.6353	–	0.9021	–
<i>C</i> ₁ <i>C</i> ₂ <i>X</i> ₁	0.0541	–	–	–	–	0.3482	0.8567	0.0947	–	0.0197	–
<i>C</i> ₁ <i>C</i> ₂ <i>X</i> ₂	0.1827	–	–	–	–	0.0454	0.9294	0.8323	–	0.4168	–
<i>C</i> ₁ <i>C</i> ₃ <i>X</i> ₁	0.1146	–	–	–	–	0.6720	0.0481	0.0269	–	0.2981	–
<i>C</i> ₁ <i>C</i> ₃ <i>X</i> ₂	0.5015	–	–	–	–	0.8771	0.7108	0.9489	–	0.2456	–
<i>C</i> ₁ <i>X</i> ₁ <i>X</i> ₂	0.4258	–	0.0227	–	–	0.0466	0.1540	0.3902	–	0.5596	–
<i>C</i> ₂ <i>C</i> ₃ <i>X</i> ₁	0.9463	–	–	–	–	0.9162	0.7041	0.9596	–	0.4626	–
<i>C</i> ₂ <i>C</i> ₃ <i>X</i> ₂	0.7278	–	–	–	–	0.3003	0.4410	0.1022	–	0.0524	–
<i>C</i> ₂ <i>X</i> ₁ <i>X</i> ₂	0.4095	–	0.7340	–	–	0.1756	0.2865	0.2570	–	0.7582	–
<i>C</i> ₃ <i>X</i> ₁ <i>X</i> ₂	0.0217	–	0.8272	–	–	0.5534	0.9227	0.9807	–	0.5446	–
<i>C</i> ₁ <i>X</i> ₁ ²	0.0834	–	0.1087	–	–	–	0.0119	0.0146	–	0.5117	–
<i>C</i> ₂ <i>X</i> ₁ ²	0.0456	–	0.0285	–	–	–	0.5400	0.7507	–	0.6032	–
<i>C</i> ₃ <i>X</i> ₁ ²	0.0034	–	0.1032	–	–	–	0.0833	0.0689	–	0.1639	–
<i>C</i> ₁ <i>C</i> ₂ <i>C</i> ₃ <i>X</i> ₁	0.1750	–	–	–	–	0.7798	0.1113	0.1753	–	0.9527	–
<i>C</i> ₁ <i>C</i> ₂ <i>C</i> ₃ <i>X</i> ₂	0.8267	–	–	–	–	0.0155	0.1057	0.3174	–	0.6709	–
<i>C</i> ₁ <i>C</i> ₂ <i>X</i> ₁ <i>X</i> ₂	0.3445	–	–	–	–	0.2031	0.0035	0.0852	–	0.2317	–
<i>C</i> ₁ <i>C</i> ₃ <i>X</i> ₁ <i>X</i> ₂	0.5542	–	–	–	–	0.7529	0.3749	0.3325	–	0.9576	–
<i>C</i> ₂ <i>C</i> ₃ <i>X</i> ₁ <i>X</i> ₂	0.0290	–	–	–	–	0.0326	0.2997	0.0729	–	0.0972	–
<i>C</i> ₁ <i>C</i> ₂ <i>X</i> ₁ ²	0.0011	–	–	–	–	–	0.2059	0.2059	–	0.6895	–
<i>C</i> ₁ <i>C</i> ₃ <i>X</i> ₁ ²	0.1888	–	–	–	–	–	0.7556	0.7575	–	0.2036	–
<i>C</i> ₂ <i>C</i> ₃ <i>X</i> ₁ ²	0.2574	–	–	–	–	–	0.3640	0.1683	–	0.1751	–
<i>C</i> ₁ <i>C</i> ₂ <i>C</i> ₃ <i>X</i> ₁ <i>X</i> ₂	0.0078	–	–	–	–	0.6251	0.0105	0.0132	–	0.6251	–
<i>C</i> ₁ <i>C</i> ₂ <i>C</i> ₃ <i>X</i> ₁ ²	0.0172	–	–	–	–	–	0.0598	0.0906	–	0.6078	–
Lack of fit	0.1635	0.8755	0.6267	0.7874	0.7155	0.6089	0.7694	0.6051	0.5317	0.7809	0.2817
R ²	0.9831	0.7343	0.5487	0.3807	0.3876	0.8334	0.9752	0.9655	0.1180	0.8451	0.6458
Adj-R ²	0.9020	0.7037	0.3111	0.2816	0.2896	0.4315	0.8559	0.8001	– 0.0884	0.1016	0.6124
C.V.%	1.70	7.33	4.34	40.83	23.18	3.41	13.27	15.41	15.99	9.32	4.76
Adeq. precision	13.04	11.06	8.77	6.65	6.84	6.98	12.78	10.65	3.10	4.99	12.63

*X*₁ homogenization time (min), *X*₂ homogenization rate (rpm), *C*₁ mannitol (%), *C*₂ gum arabic (%), *C*₃ casein (%)

arabic and casein with ratios 38.2, 40.2 and 21.6%, respectively. The predicted remaining activity was determined to be 91.82%. Validation of the optimum point

response prediction was carried out by the freeze drying of TG at the selected optimum conditions with three repetitions. According to the experimental results at optimum

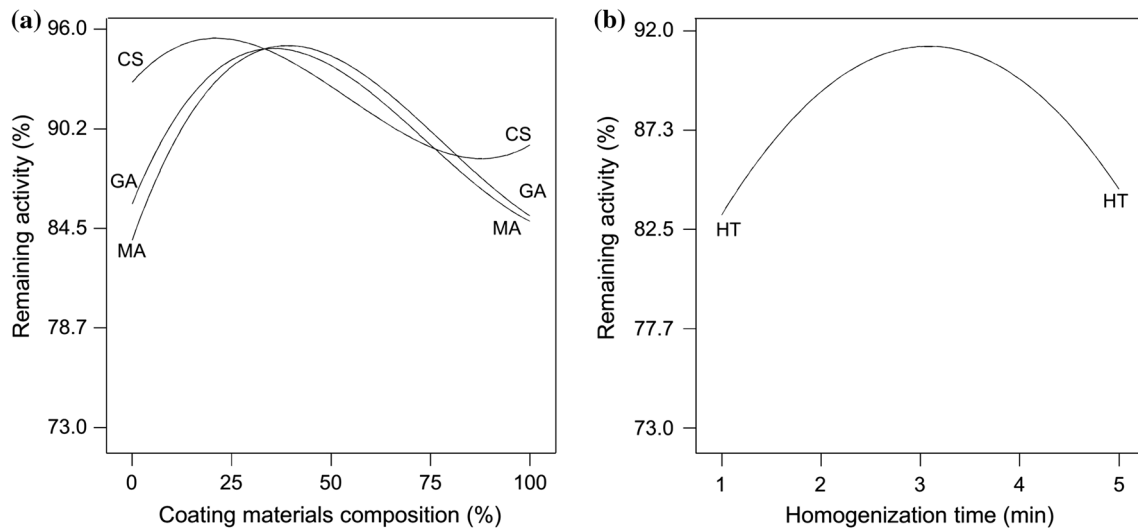


Fig. 1 **a** Effect of coating materials composition on remaining activity (%) at 11,200 rpm of homogenization rate and 3 min of homogenization time, **b** effect of homogenization time on remaining activity (%) at 11,200 rpm of homogenization rate and triple mixture of MA–GA–CS (33.33% for each coating material)

point conditions, the experimental ($93.04 \pm 0.04\%$) and predicted remaining activity values did not differ statistically ($p > 0.05$). The crude TG solution without coating materials was also freeze dried under the same drying conditions. The remaining activity value was found to be $63.86 \pm 0.04\%$, which was significantly lower when compared to that of the microencapsulated TG at the optimum point ($p < 0.05$).

The physical properties of the microencapsulated TG powder were determined for every single run of the experimental design. The physical characteristics of different TG powders in terms of moisture content, color values, particle density, bulk and tapped densities, porosity and flowability values are given in Table 3 and the ANOVA results have been previously represented in Table 2. The moisture content of the samples varied in the range of 4.74–7.42% (wet basis) which was within the limits for moisture specification of dried powders in the food industry (Koç et al. 2014). The results showed that when gum arabic and casein mixture were used as coating materials, the moisture values increased while lower moisture values were obtained when mannitol was the only coating material. The larger ice crystal formation during freezing with mannitol may lead to better sublimation during freeze drying (Debulis and Klibanov 1993). On the other hand, when casein was used as coating material, higher moisture content values were observed which may be related to the high water binding capability of casein micelles (Kneifel and Seiler 1993). The ANOVA results for moisture content showed that the model was statistically significant ($p < 0.01$) whereas the lack of fit was not significant ($p > 0.05$). The effect of linear mixture, GA–CS interaction and MA–GA–CS interaction was statistically

significant at the level of a 99% confidence interval ($p < 0.01$). The color of the different samples was determined in terms of CIE L , a and b values. The L , a and b values were in the range of 78.79–99.33, 0.53–8.12 and 5.80–22.30, respectively. The current study showed that the L values of the powder increased with an increase in the mannitol ratio whereas the b values of the samples increased with an increase in the gum arabic ratio. All color values were statistically significant in respect of the model ($p < 0.05$). The differentiation of L , a and b values could be due to the composition of the coating materials, which can be supported by the ANOVA results. It was observed that the linear mixture had a significant effect for L , a and b values ($p < 0.05$). The L values were also affected by the interactions of GA–HT–HR and GA–HT². The interaction of MA–GA had also significant effect on the a and b values (Table 2). The particle density results ranged between 1206 and 1476 kg/m³ and the coating material or process variables had no significant affect on the particle density ($p > 0.05$). However, MA–CS, GA–HT, MA–GA–HT, MA–GA–CS–HR and GA–CS–HT–HR interactions had a significant effect on the particle density values of microencapsulated TG powders. The bulk and tapped densities of the samples varied from 50.15–288.5 kg/m³ and 71.71–250.7 kg/m³, respectively. In dried food products, bulk density can be related to moisture content and this mostly correlated with the drying method (Koç et al. 2008). Accordingly, the low bulk density values of a sample may increase the risk of oxidation during storage because of the fact that there is more air within the powder particles (Fitzpatrick 2005). The differences in bulk and tapped density can arise from the proportion of MA or GA to CS. When mannitol was used as the coating material, the

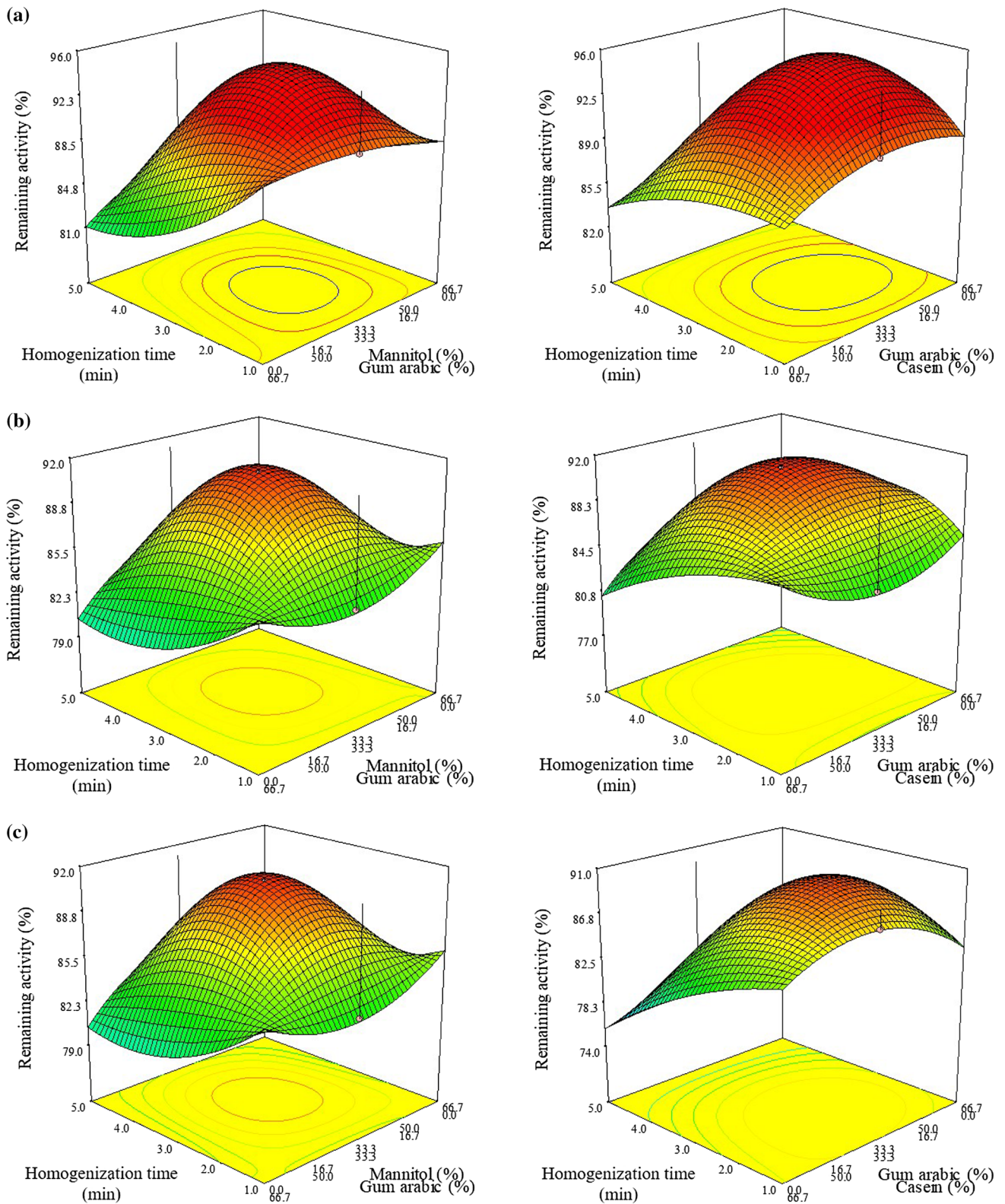


Fig. 2 Calculated effect of homogenization time and mixture composition on TG activity at homogenization rate of **a** 11,200 rpm, **b** 15,600 rpm and **c** 20,000 rpm

bulk density was increased, while GA–CS decreased the bulk density of the samples. The ANOVA table showed

that the model was significant ($p < 0.05$), while lack of fit was not significant ($p > 0.05$) for the bulk and tapped

Table 3 Physical properties of TG powders produced at different conditions

Exp. no	Moisture (%)	L	a	b	ρ_p (kg/m ³)	ρ_b (kg/m ³)	ρ_t (kg/m ³)	CI (%)	HR	ε (%)
1	5.50 (± 0.04)	92.81 (± 0.51)	1.92 (± 0.08)	11.45 (± 0.09)	1453 (± 0.44)	116.2 (± 1.07)	179.8 (± 7.20)	35.32 (± 2.00)	1.547 (± 0.048)	87.63 (± 0.49)
2	5.44 (± 0.05)	99.32 (± 1.14)	2.41 (± 0.27)	13.04 (± 0.76)	1254 (± 14.28)	121.3 (± 1.12)	189.8 (± 2.83)	36.11 (± 0.36)	1.565 (± 0.009)	84.86 (± 0.05)
3	6.29 (± 0.08)	93.29 (± 0.22)	6.41 (± 0.40)	18.10 (± 0.30)	1385 (± 23.24)	124.9 (± 9.26)	194.9 (± 15.94)	35.90 (± 0.49)	1.561 (± 0.012)	85.93 (± 0.91)
4	6.45 (± 0.06)	91.58 (± 0.44)	5.53 (± 0.29)	17.41 (± 0.68)	1461 (± 44.49)	168.0 (± 6.46)	250.2 (± 2.86)	32.86 (± 1.82)	1.489 (± 0.040)	82.88 (± 0.33)
5	6.01 (± 0.00)	84.84 (± 0.73)	6.60 (± 0.24)	18.83 (± 0.25)	1398 (± 126.39)	111.6 (± 2.75)	188.6 (± 0.90)	40.83 (± 1.18)	1.690 (± 0.034)	86.45 (± 1.16)
6	7.03 (± 0.03)	93.32 (± 1.44)	4.04 (± 0.37)	15.75 (± 0.65)	1251 (± 9.82)	50.1 (± 3.79)	71.7 (± 3.59)	30.11 (± 1.79)	1.430 (± 0.037)	94.27 (± 0.33)
7	6.82 (± 0.10)	91.90 (± 0.37)	6.84 (± 0.27)	20.28 (± 0.43)	1287 (± 36.29)	181.8 (± 2.26)	292.4 (± 1.55)	37.82 (± 1.10)	1.608 (± 0.029)	77.26 (± 0.76)
8	4.99 (± 0.23)	90.16 (± 0.52)	7.30 (± 0.17)	21.91 (± 0.07)	1465 (± 7.39)	200.1 (± 9.01)	302.0 (± 3.33)	33.74 (± 3.71)	1.510 (± 0.085)	79.39 (± 0.12)
9	6.97 (± 0.15)	98.61 (± 1.11)	4.44 (± 0.46)	17.32 (± 0.36)	1300 (± 73.83)	162.7 (± 4.23)	260.3 (± 9.84)	37.48 (± 0.74)	1.600 (± 0.019)	79.97 (± 0.38)
10	5.98 (± 0.04)	90.50 (± 0.53)	7.58 (± 0.55)	22.04 (± 0.19)	1333 (± 4.92)	175.5 (± 1.71)	264.8 (± 7.98)	33.69 (± 2.64)	1.509 (± 0.060)	80.13 (± 0.67)
11	6.81 (± 0.35)	88.61 (± 1.56)	4.37 (± 0.06)	14.65 (± 0.36)	1207 (± 16.63)	75.6 (± 0.66)	126.5 (± 4.15)	40.22 (± 2.48)	1.674 (± 0.070)	89.52 (± 0.20)
12	6.46 (± 0.38)	85.69 (± 0.57)	3.51 (± 0.08)	13.46 (± 0.33)	1312 (± 13.40)	89.0 (± 0.81)	160.0 (± 1.78)	44.37 (± 0.11)	1.797 (± 0.004)	87.80 (± 0.01)
13	5.07 (± 0.27)	86.89 (± 1.31)	3.69 (± 0.12)	13.71 (± 0.22)	1476 (± 14.90)	147.5 (± 2.76)	246.6 (± 5.29)	40.16 (± 2.40)	1.672 (± 0.067)	83.30 (± 0.19)
14	7.42 (± 0.19)	86.12 (± 0.50)	3.49 (± 0.07)	13.28 (± 0.32)	1352 (± 38.19)	107.3 (± 4.69)	179.5 (± 4.55)	40.25 (± 1.10)	1.673 (± 0.031)	86.71 (± 0.71)
15	7.16 (± 0.20)	89.94 (± 2.63)	3.37 (± 0.08)	10.78 (± 0.57)	1298 (± 0.01)	129.8 (± 5.28)	197.6 (± 10.07)	34.30 (± 0.68)	1.523 (± 0.016)	84.77 (± 0.78)
16	5.22 (± 0.22)	87.06 (± 1.21)	2.46 (± 0.24)	10.58 (± 0.33)	1387 (± 18.49)	190.0 (± 3.95)	315.8 (± 10.52)	39.80 (± 3.26)	1.663 (± 0.090)	77.24 (± 0.45)
17	6.44 (± 0.16)	89.38 (± 0.38)	4.22 (± 0.03)	13.82 (± 0.11)	1439 (± 0.35)	139.4 (± 1.31)	204.2 (± 5.80)	31.69 (± 1.30)	1.464 (± 0.028)	85.81 (± 0.40)
18	5.21 (± 0.40)	81.25 (± 0.65)	1.51 (± 0.21)	10.75 (± 0.82)	1459 (± 3.37)	102.8 (± 2.93)	173.9 (± 3.29)	40.87 (± 2.81)	1.692 (± 0.080)	88.08 (± 0.20)
19	5.47 (± 0.21)	90.36 (± 0.56)	4.42 (± 0.07)	14.36 (± 0.30)	1415 (± 16.90)	176.3 (± 4.80)	344.5 (± 1.95)	48.81 (± 1.68)	1.954 (± 0.064)	75.64 (± 0.43)
20	5.19 (± 0.16)	85.94 (± 0.49)	2.55 (± 0.24)	13.52 (± 0.65)	1392 (± 18.74)	117.8 (± 2.45)	229.0 (± 4.50)	48.53 (± 2.08)	1.944 (± 0.079)	83.54 (± 0.55)
21	6.90 (± 0.23)	93.06 (± 1.43)	4.11 (± 0.50)	13.88 (± 1.06)	1274 (± 13.51)	106.0 (± 0.11)	169.9 (± 1.61)	37.63 (± 0.53)	1.603 (± 0.014)	86.66 (± 0.01)
22	6.06 (± 0.44)	87.74 (± 0.83)	2.44 (± 0.09)	11.44 (± 0.18)	1392 (± 18.09)	144.2 (± 8.94)	235.4 (± 8.15)	38.67 (± 5.92)	1.633 (± 0.158)	83.08 (± 0.81)
23	7.21 (± 0.05)	92.62 (± 1.16)	2.09 (± 0.08)	11.30 (± 0.27)	1284 (± 21.32)	59.6 (± 0.16)	95.5 (± 0.48)	37.59 (± 0.48)	1.602 (± 0.012)	92.56 (± 0.16)
24	4.97 (± 0.21)	87.17 (± 1.39)	5.87 (± 0.10)	16.60 (± 1.67)	1322 (± 32.32)	105.4 (± 3.92)	169.5 (± 7.45)	37.80 (± 0.42)	1.608 (± 0.011)	87.17 (± 0.88)
25	6.67 (± 0.20)	86.59 (± 0.12)	3.25 (± 0.11)	11.9 (± 0.36)	1306 (± 17.32)	84.8 (± 1.40)	116.3 (± 1.45)	27.08 (± 2.11)	1.372 (± 0.040)	91.10 (± 0.01)
26	5.17 (± 0.28)	90.78 (± 0.95)	2.96 (± 0.09)	11.35 (± 0.12)	1373 (± 2.39)	201.9 (± 10.50)	353.1 (± 1.64)	42.82 (± 2.71)	1.749 (± 0.083)	74.28 (± 0.16)
27	5.95 (± 0.06)	87.93 (± 0.82)	7.13 (± 0.43)	17.75 (± 0.24)	1387 (± 19.77)	189.8 (± 3.88)	300.3 (± 8.22)	36.75 (± 3.02)	1.582 (± 0.076)	78.35 (± 0.90)
28	5.58 (± 0.29)	89.68 (± 0.38)	6.24 (± 0.53)	17.20 (± 0.83)	1332 (± 28.20)	199.0 (± 1.78)	345.6 (± 2.28)	42.40 (± 0.14)	1.736 (± 0.004)	74.05 (± 0.38)
29	5.09 (± 0.10)	92.84 (± 1.65)	3.08 (± 0.14)	11.65 (± 0.54)	1385 (± 14.04)	202.5 (± 9.61)	311.4 (± 3.50)	35.00 (± 2.36)	1.538 (± 0.056)	77.51 (± 0.48)
30	6.67 (± 0.04)	91.76 (± 1.15)	3.62 (± 0.08)	12.49 (± 0.35)	1393 (± 12.96)	181.2 (± 0.05)	261.9 (± 4.16)	30.79 (± 1.12)	1.445 (± 0.023)	81.21 (± 0.12)
31	6.74 (± 0.05)	79.13 (± 1.75)	1.20 (± 0.24)	7.76 (± 0.67)	1391 (± 18.83)	157.7 (± 4.38)	241.4 (± 4.29)	34.67 (± 0.65)	1.531 (± 0.015)	82.65 (± 0.07)
32	5.25 (± 0.14)	80.33 (± 1.46)	2.61 (± 0.40)	10.91 (± 0.77)	1342 (± 48.89)	222.8 (± 5.60)	369.9 (± 9.96)	39.72 (± 3.14)	1.660 (± 0.086)	72.41 (± 1.75)
33	7.03 (± 0.02)	90.20 (± 1.86)	0.53 (± 0.07)	8.42 (± 0.57)	1338 (± 28.60)	50.5 (± 1.63)	90.7 (± 3.03)	44.25 (± 3.65)	1.796 (± 0.118)	93.22 (± 0.37)
34	5.26 (± 0.04)	78.79 (± 3.31)	3.03 (± 1.20)	12.32 (± 0.99)	1358 (± 42.51)	139.0 (± 3.04)	232.1 (± 9.03)	40.07 (± 1.02)	1.669 (± 0.028)	82.91 (± 0.13)
35	7.08 (± 0.03)	89.24 (± 1.86)	1.62 (± 0.21)	11.12 (± 0.74)	1466 (± 10.66)	100.9 (± 0.93)	200.5 (± 0.01)	49.68 (± 0.46)	1.987 (± 0.018)	86.32 (± 0.10)
36	6.78 (± 0.12)	85.75 (± 0.20)	1.51 (± 0.29)	9.89 (± 0.34)	1382 (± 17.97)	151.7 (± 0.77)	266.5 (± 2.77)	43.07 (± 0.30)	1.757 (± 0.009)	80.72 (± 0.45)

Table 3 continued

Exp. no	Moisture (%)	<i>L</i>	<i>a</i>	<i>b</i>	ρ_p (kg/m ³)	ρ_b (kg/m ³)	ρ_t (kg/m ³)	CI (%)	HR	ϵ (%)
37	4.85 (± 0.06)	83.49 (± 0.45)	0.86 (± 0.12)	8.80 (± 1.29)	1423 (± 23.21)	168.3 (± 0.61)	310.3 (± 3.18)	45.74 (± 0.75)	1.843 (± 0.026)	78.20 (± 0.58)
38	4.75 (± 0.14)	91.70 (± 0.24)	1.80 (± 0.34)	10.86 (± 0.82)	1359 (± 36.29)	174.5 (± 2.08)	303.5 (± 1.11)	42.52 (± 0.47)	1.740 (± 0.014)	77.65 (± 0.68)
39	5.30 (± 0.04)	84.27 (± 1.61)	3.80 (± 0.30)	15.06 (± 0.25)	1420 (± 1.33)	155.8 (± 4.55)	274.3 (± 2.23)	43.22 (± 1.20)	1.761 (± 0.037)	80.68 (± 0.18)
40	5.46 (± 0.05)	86.71 (± 0.88)	2.66 (± 0.44)	12.47 (± 0.34)	1326 (± 3.33)	181.2 (± 9.51)	374.1 (± 4.29)	51.54 (± 3.10)	2.064 (± 0.132)	71.79 (± 0.39)
41	5.46 (± 0.09)	92.82 (± 0.58)	2.50 (± 0.58)	12.08 (± 0.25)	1329 (± 12.92)	180.9 (± 1.64)	271.3 (± 2.46)	33.33 (± 0.00)	1.500 (± 0.000)	79.59 (± 0.01)
42	6.42 (± 0.06)	96.07 (± 1.04)	3.10 (± 0.38)	13.88 (± 0.44)	1303 (± 13.82)	170.3 (± 2.87)	249.3 (± 4.40)	31.67 (± 2.36)	1.464 (± 0.051)	80.88 (± 0.13)
43	7.24 (± 0.05)	90.60 (± 0.12)	2.79 (± 0.06)	13.09 (± 0.19)	1353 (± 8.76)	72.7 (± 3.44)	113.4 (± 1.26)	35.86 (± 2.33)	1.559 (± 0.057)	91.62 (± 0.15)
44	5.64 (± 0.06)	91.07 (± 1.09)	6.18 (± 0.70)	18.48 (± 1.03)	1381 (± 6.78)	133.1 (± 1.95)	198.7 (± 19.54)	32.65 (± 7.61)	1.493 (± 0.169)	85.62 (± 1.34)
45	5.69 (± 0.18)	88.27 (± 1.27)	4.26 (± 0.36)	15.76 (± 0.57)	1323 (± 27.36)	240.0 (± 1.08)	380.5 (± 11.79)	36.88 (± 2.24)	1.585 (± 0.056)	71.25 (± 0.30)
46	5.09 (± 0.09)	87.60 (± 0.58)	4.50 (± 0.58)	16.07 (± 0.18)	1382 (± 2.51)	195.1 (± 0.62)	303.9 (± 14.96)	35.71 (± 3.37)	1.558 (± 0.082)	78.01 (± 1.04)
47	5.64 (± 0.02)	84.36 (± 0.80)	5.27 (± 0.24)	17.05 (± 0.71)	1375 (± 15.62)	139.3 (± 15.58)	225.9 (± 27.05)	37.45 (± 14.39)	1.621 (± 0.378)	83.58 (± 1.78)
48	7.03 (± 0.18)	90.34 (± 1.49)	5.93 (± 0.92)	19.67 (± 0.61)	1343 (± 8.38)	101.9 (± 14.50)	143.4 (± 14.27)	29.09 (± 3.06)	1.407 (± 0.061)	89.32 (± 1.13)
49	6.60 (± 0.39)	80.34 (± 1.52)	2.23 (± 0.45)	11.53 (± 0.80)	1381 (± 21.12)	173.2 (± 3.68)	320.0 (± 2.23)	45.86 (± 1.53)	1.847 (± 0.052)	76.83 (± 0.52)
50	7.43 (± 0.34)	85.89 (± 0.98)	2.81 (± 0.30)	13.58 (± 0.41)	1330 (± 26.82)	136.0 (± 3.11)	272.4 (± 10.30)	50.03 (± 3.03)	2.004 (± 0.122)	79.52 (± 0.36)
51	5.26 (± 0.03)	93.15 (± 0.91)	7.39 (± 0.04)	21.82 (± 0.12)	1446 (± 13.44)	288.5 (± 7.19)	443.6 (± 7.20)	34.97 (± 0.56)	1.538 (± 0.013)	69.32 (± 0.78)
52	6.60 (± 0.19)	95.04 (± 6.52)	5.65 (± 0.34)	19.13 (± 0.28)	1416 (± 21.33)	209.3 (± 1.67)	326.3 (± 2.01)	35.87 (± 0.91)	1.559 (± 0.022)	76.95 (± 0.49)
53	5.18 (± 0.06)	94.96 (± 0.42)	7.38 (± 0.02)	21.98 (± 0.16)	1462 (± 37.90)	215.9 (± 3.84)	334.6 (± 9.31)	35.42 (± 2.95)	1.549 (± 0.071)	77.10 (± 1.23)
54	6.69 (± 0.01)	99.33 (± 0.10)	5.13 (± 0.39)	18.82 (± 0.33)	1427 (± 12.54)	158.4 (± 5.65)	262.1 (± 6.68)	39.57 (± 0.61)	1.654 (± 0.017)	81.64 (± 0.31)
55	5.43 (± 0.06)	93.61 (± 1.15)	7.17 (± 0.03)	21.52 (± 0.18)	1451 (± 13.46)	278.2 (± 7.20)	387.1 (± 9.58)	28.15 (± 0.08)	1.392 (± 0.002)	73.32 (± 0.91)
56	7.20 (± 0.09)	94.32 (± 0.60)	6.62 (± 0.43)	20.33 (± 0.33)	1424 (± 25.50)	119.3 (± 4.70)	189.4 (± 3.01)	37.05 (± 1.48)	1.588 (± 0.037)	86.70 (± 0.03)
57	5.52 (± 0.13)	93.70 (± 0.95)	6.37 (± 0.49)	20.58 (± 0.51)	1363 (± 9.34)	228.0 (± 3.91)	344.7 (± 5.02)	33.84 (± 0.17)	1.511 (± 0.004)	74.72 (± 0.20)
58	5.38 (± 0.08)	88.85 (± 0.80)	8.12 (± 0.81)	22.30 (± 0.94)	1402 (± 18.25)	143.3 (± 5.00)	188.3 (± 10.54)	23.86 (± 1.61)	1.314 (± 0.028)	86.57 (± 0.58)
59	5.06 (± 0.06)	94.34 (± 1.13)	7.05 (± 0.15)	21.27 (± 0.19)	1283 (± 20.65)	279.9 (± 0.89)	442.9 (± 5.33)	36.81 (± 0.98)	1.583 (± 0.025)	65.46 (± 0.98)

ρ_p particle density, ρ_b bulk density, ρ_t tapped density, *CI* Carr Index, *HR* Hausner Ratio, ϵ porosity

densities (Table 2). The interactions of MA–CS, MA–CS–HT, MA–HT² and MA–GA–CS–HT–HR had a significant effect on the bulk and tapped densities ($p < 0.05$). Moreover, the interaction of MA–GA–HT–HR statistically affected the bulk density of the samples. Powder flowability can be explained by the Carr Index (CI) and Hausner Ratio (HR). CI (%) values can be in the range of < 15 , 15–20, 20–35, 35–45 and > 45 , and flowability is classified as very good, good, fair, bad and very bad, respectively. Powder cohesiveness based on HR can be in the range of < 1.2 , 1.2–1.4 and > 1.4 , where cohesiveness is classified as low, intermediate and high, respectively (Jinapong et al. 2008). In this study, CI values of microencapsulated TG powders ranged between 23.86 and 51.54%, which can be classified between fair-very bad. In particular, for the freeze-dried samples, the powder particles have a higher contact surface and more cohesive forces can arise, which makes the powders resistant to flowing (Fitzpatrick 2005). The current study revealed that the composition of the coating material or the process variables did not have any significant effect on the CI ($p > 0.05$). Likewise, the HR values ranged between 1.31 and 2.06 and different powders were classified between intermediate-high cohesiveness. According to the HR findings, process variables and use of different coating materials did not have any significant effect ($p > 0.05$). Porosity, which is an important parameter for powdered products, has an impact on rewetting control especially for microencapsulated powders. Microencapsulated TG powders had porosity values between 65.46 and 94.27% and porosity of the samples increased when the casein ratio was higher. On the other hand, by usage of more mannitol, the porosity decreased. The ANOVA table represents the results for porosity and the model was significant ($p < 0.05$) whereas the lack of fit was not significant ($p > 0.05$). Also, the linear mixture and MA–GA interaction had a significant effect on the porosity of the samples.

Conclusion

In this study, the microencapsulation conditions of TG by freeze drying were optimized and the physical characteristics of different powder products were determined. The microencapsulation parameters namely the homogenization time and homogenization rate were investigated as independent variables. It was observed that a higher homogenization time and homogenization rate reduced enzyme activity. However, very low homogenization times and rates were not efficient enough to generate microencapsulates, which increased the activity loss. For this reason, an optimization study was carried out and the optimal conditions for producing powder TG with minimal activity loss

were determined. The microencapsulated forms of TG were more resistant to the stresses which were caused by freeze drying when compared with the crude enzyme. Moreover, physical characteristics varied depending on the use of different coating materials. The current study indicated that microencapsulation of TG reduced the activity loss and determination of the optimum homogenization conditions helped to obtain better microencapsulated powders.

Acknowledgements The authors acknowledge the Scientific and Technological Research Council of Turkey (TUBITAK, Project No: 115O216) for financial support.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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