TREATMENT REGIME AND BIOACTIVE RETENTION IN NONI EXTRACT ENCAPSULATED LIQUID APPLYING HOMOGENIZED SUSPENSION APPROACH

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ABSTRACT

Microencapsulated bioactive compounds from noni fruit (*Morinda citrifolia*) offer diverse health benefits. This study aimed to optimize liquid encapsulation of noni extract using a homogenized suspension approach. Parameters such as shell formulation, homogenization speed, and pasteurization were scrutinized to obtain suspension stability and achieve better bioactive retentions concerning total phenolics (TPC), total flavonoids (TFC), and total saponins (TSC). Microcapsule size and analytical characteristics of the resulting microencapsulated solution were assessed, including bioactive degradation kinetics. The findings examined that the microencapsulated solution combining maltodextrin and gum arabic exhibited the highest stability and yielded the greatest TPC, TFC, and TSC contents. Optimal homogenized suspension was determined and pasteurized for storage in a refrigerator at 10°C for 10 months, maintaining encapsulation efficiency of TPC, TFC, and TSC at 82.2%, 87.6%, and 85.9%, respectively. A robust shelf life underscored its quality and longevity, which is potential for studies on scalability and further applications

1. INTRODUCTION

With the escalating trend towards functional food development, the necessity for researching and developing plant-derived ingredients with high levels of bioactive compounds using environmentally friendly methods becomes paramount. Natural bioactive compounds, once extracted, often suffer from instability and susceptibility to degradation, limiting their industrial applications in the food sector. Furthermore, there is still a limited number of research and reports on the microencapsulation technology of bioactive compounds found in noni fruit (Widjastuti et al., 2023; Zhang, Khoo, Chen, & Quek, 2020). Noni fruit, comprising 90% water and dry matter components such as soluble solids, dietary fiber, and protein, possesses surprising levels of amino acids, particularly aspartic acid, glutamic acid, and

isoleucine, with protein content in the fruit being notably high, constituting 11.3% of its dry matter. Minerals account for 8.4% of the dry matter, primarily potassium, sulfur, calcium, and phosphorus, with traces of selenium also found in noni fruit juice (Abou Assi, Darwis, Abdulbaqi, Vuanghao, & Laghari, 2017; Chan Blanco et al., 2006).

Noni fruit is rich in vitamins, primarily ascorbic acid (24–158 mg/100g dry matter), and provitamin A (Morton, 1992). Noni fruit juice contains phenolic compounds such as damnacanthal, scopoletin, molindone, alizarin, aucubin, noordamnacanthal, rubidium, rubidium-1-methyl ether, and other anthraquinone glycosides. Damnacanthal, an anthraquinone, has been discovered with notable functions, particularly anti-cancer properties (Nualsanit et al., 2012). Thus, noni fruit has strong potential for nutritional boost, functional foods and beverages, supplements, skincare, and other long-term pharmaceutical properties.

Microencapsulation technology via homogenization presents an application-capable and effective method for encapsulating bioactive compounds suitable for use in the nutraceutical industry as functional supplements (Silva et al., 2014). A significant advantage of microencapsulation technology is the substantial enhancement of the bioactivity of these compounds and the storage duration. In microencapsulation methods, the selection of encapsulating materials significantly influences the physical and functional properties of the encapsulated products. Maltodextrin (MD), gum arabic (GA), and whey protein (WP) are the most frequently applied wall materials for encapsulation (Mousa et al., 2023; Premi & Sharma, 2017). The choice of shell materials is critical as it affects encapsulation efficiency, the stability of the microcapsules against adverse conditions, and minimal undesirable odors (Mirzaei, Pourjafar, & Homayouni, 2012). The physical and chemical properties of shell formulations and core material, i.e. noni extract in this study, must be compatible to provide the desired coating properties such as protectability and stability.

Microencapsulation of bioactive compounds of noni extract in a liquid form laid the foundation for further research into potentially directing the development of functional foods and nutraceutical products. The research findings could contribute to the enhancement of noni fruit utilization and its economic value. By determining encapsulating formula, homogenization speed and time, and pasteurization regime, this study is expected to obtain a liquid product with high levels of bioactive compounds and high stability for ensuring their retention during storage conditions and duration.

2. METHODOLOGY

2.1. Noni Fruit Extract

The research project utilized fresh noni fruit (*Morinda citrifolia* L) procured from Tien Giang province, Vietnam. Uniformly ripe noni fruits (from two-thirds to whole fruit ripened) were selectively free from bruising and damage. The noni fruits were thoroughly washed to remove impurities and stored in a freezer (-10°C). Prior to processing, the noni fruits were thawed, then sliced into 4-5 mm thick slices, and subjected to 1% ascorbic acid pretreatment before drying at 60°C for 7-8 hours until the moisture content reached below 12%. This procedure is aligned with a previous optimization publication by Kha, Nguyen, Tran, and Truong (2021). The moisture content of the samples was determined using the moisture balance method at 130°C until a constant weight was achieved. Dry noni slices

were ground, crushed, and sieved through a mesh size of 0.5 mm. The resulting noni powder was hermetically stored in a freezer (-18°C) for subsequent extraction.

The extraction process was assisted by ultrasound and enzymatic treatment (Ho, Nguyen, Nguyen, & Kha, 2022; Nguyen, Di, Phan, Kha, & Nguyen, 2024). A noni powder mass of 5 g was added with 40 mL water in a flask with parafilm cover, mixed thoroughly, and sonicated in an ultrasound bath at 37 kHz, temperature of 50°C for 10 min. Then, a pectinase and cellulase enzyme preparation (Mashzyme, Advanced Enzyme Technologies Ltd) was added at 1% (w/v). The enzymatic treatment lasted for 51 min at 60ºC before the mixture was filtered through 15-20 µm porous Whatman papers to remove solid particles and get the noni extract for later steps.

2.2. Suspension preparation for encapsulation of bioactive compounds in noni extract

The encapsulation mixture which comprised noni extract and each shell formula at a concentration of 20% (w/w) was mixed continuously at 10,000 rpm for 5 min. The shell formulation was a parameter tested with different combinations of whey protein (WP), maltodextrin (MD), and gum arabic (GA) in triplicate. Four shell formulae (ratio w/w) were employed GA:MD (1:1), GA:WP (3:1), WP:MD (1:1), and GA:WP:MD (2:1:2). The resulting encapsulation suspensions were then transferred into 100 mL glass bottles and subjected to pasteurization at 90°C for 5 min. The suspensions after pasteurization were aliquoted into test tubes for sedimentation observation (i.e. suspension stability) and subjected to analytical tests on TPC, TFC, and TSC for determination of encapsulation efficiency, particle size, and particle size distribution. Particle size and particle size distribution of the encapsulation liquid were determined using the Dynamic Light Scattering Particle Size Distribution Analyzer LA-350 (Horiba, Japan).

2.3. Evaluation of speed and time of mixing for suspension preparation

The experiment was designed following a randomized design of two factors of mixing i.e. speed and time in triplicate. The mixing speed was tested at three levels i.e. 8,000, 10,000, and 12,000 rpm, while the mixing time was tested with four periods i.e. 5, 10, 15, and 20 min. Based on the best suspension agent from the above section, the procedure was conducted similarly in all treatments. After pasteurization (90°C for 5 min), the noni encapsulated treatments were analyzed for TPC, TFC, and TSC encapsulation efficiency.

2.4. Evaluation of temperature and time of pasteurization for suspension preparation

For the pasteurization process, a randomized two-factorial design was employed with temperature and time variables in triplicate. Based on the results of the aforementioned sections, a suitable suspension solution was prepared at the most appropriate mixing speed and time. Subsequently, the suspension was treated at different temperatures and times. The pasteurization temperature consisted of three levels i.e. 70, 80, and 90°C, while the pasteurization time was tested with three periods i.e. 5, 10, and 15 min. The noni encapsulated solutions were then analyzed for TPC, TFC, and TSC encapsulation efficiency.

2.5. Stability of noni encapsulated juice

The stability of the best treatment of encapsulated noni juice was assessed by storing the suspension liquid at 10 ± 2°C and in accelerated thermal conditions at 40 and 50°C. Samples were analyzed for TPC, TFC

and TSC at various time intervals to determine the appropriate storage time by applying the Labuza equation:

$$t_2 = t_1 \times Q_{10}^{\Delta/10} \quad (1)$$

where Q_{10} was the increase in reaction rate when the temperature increased by 10°C, t_1 was the test time (h) at an accelerated temperature, t_2 was the test time (h) at a desired storage temperature, and Δ was the difference between the accelerated temperature and the desired storage temperature.

2.6. Determination of contents of bioactive compounds and encapsulation efficiency

Polyphenol analysis was conducted using the Folin-Ciocalteu method (Singleton, Orthofer, & Lamuela-Raventós, 1999), the TPC was measured at 765 nm absorbance, which was expressed in milligram of gallic acid equivalent per gram of dry weight (mg GAE/g DW). Flavonoid content was determined using the Aluminum Chloride colorimetric method (Ribarova, Atanassova, Marinova, Ribarova, & Atanassova, 2005), the TFC was measured at 510 nm absorbance, which was expressed in milligram of quercetin equivalent per gram of dry weight (mg QE/g DW). Triterpenoid saponin content was determined using the UV-Vis spectrophotometry method (Tan, Vuong, Stathopoulos, Parks, & Roach, 2014), the TSC was measured at 560 nm absorbance, which was expressed in milligram of quercetin equivalent per gram of dry weight (mg QE/g DW).

The encapsulation efficiency was calculated following recent study (Teixé-Roig, Oms-Oliu, Ballesté-Muñoz, Odriozola-Serrano, & Martín-Belloso, 2022). The encapsulation liquid (10 mL) was centrifuged in a Falcon tube at a 10,000-rpm speed for 10 min. Supernatant was collected to measure TPC, TFC, and TSC. The performance of encapsulation efficiency based on TPC (EE-TPC, %), TFC (EE-TFC, %), and TSC (EE-TSC, %) was calculated as follows:

$$\text{EE-TPC (\%)} = \frac{TPC_1 - TPC_2}{TPC_1} x100\% \quad (2)$$

$$\text{EE-TFC (\%)} = \frac{TFC_1 - TFC_2}{TFC_1} x100\% \quad (3)$$

$$\text{EE-TSC (\%)} = \frac{TSC_1 - TSC_2}{TSC_1} x100\% \quad (4)$$

Where TPC_1, TFC_1, and TSC_1 were the initial respective bioactive contents in the microencapsulation solution, and TPC_2, TFC_2, and TSC_2 were the respective bioactive contents in the supernatant.

2.7. Statistical analysis

Statistical analysis was performed using SPSS 15.0 software. Results were presented as mean ± standard deviation (SD), and differences between means were evaluated using ANOVA and LSD tests at a significance level of 5% ($p < 0.05$). Graphs were created using Excel 16.0.

3. FINDINGS AND DISCUSSION

3.1. Suspension stability and encapsulation efficiency of noni bioactive compounds

Different shell formulations with noni extract had an impact on the suspension stability and the particle size. Fig 1 shows the strongest sedimentation/precipitation within GA:WP and GA:WP:MD treatments, then a mild one within WP:MD treatment, and a good stable suspension within GA:MD treatment. The analysis results show particle sizes by median, D_{50} and mode in Table 2 with low particle size variations. By leaving the treatment tubes at room temperature for 30 min, formulations combining gum arabic and whey protein sedimented quickly by forming

precipitates around the particles, leading to larger particle sizes. Whey protein should be an effective emulsifier and stabilizer (Akhtar & Dickinson, 2007); However, due to protein coagulation or precipitation and related loss of adhesive properties, the emulsifying properties of whey protein became poorer under certain conditions (Damodaran, 2017).

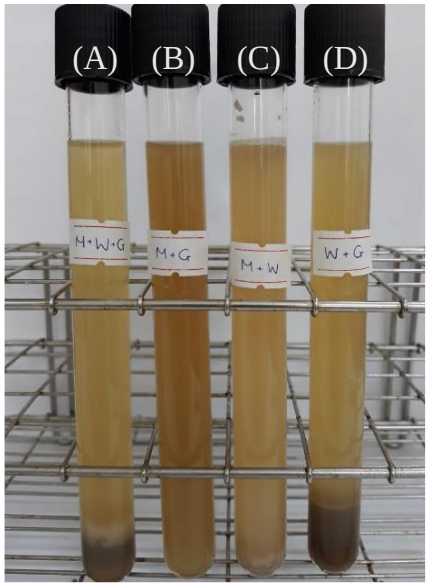

Figure 1. Sedimentation treatments of shell formulae (A) GA:WP:MD, (B) GA:MD, (C) WP:MD, and (D) GA:WP left at room temperature for 30 min

This may also be a reason for large-sized particles obtained in the encapsulated solution combining whey protein and maltodextrin as shell formulation. Additionally, combining protein and oppositely charged hydrocolloids in a solution leads to the formation of new electrostatically attractive complex(es) (McClements & Li, 2010; Xu et al., 2020) which can either dissolve in water to form a molecular solution, or precipitate to form a two-phase system. In summary, the results indicated that the stability of the suspension comprising gum arabic and maltodextrin (GA:MD) was better than that of the other formulations. These findings are consistent with previous studies on noni fruit properties and extraction techniques (Ho et al., 2022; Wang et al., 2023; Zhang, Khoo, Chen, et al., 2020).

The bioactive compounds of the liquid encapsulated samples were impacted by different shell formulations with noni extract (Table 1). The results indicated that the use of the GA:MD shell formula for encapsulating bioactive compounds in noni fruit extract yielded better results in compared with using the other three formulae as coating materials. From these results, the highest encapsulation efficiency in terms of TPC, TFC, and TSC went to the GA:MD treatment (corresponding to 79.85%, 80.5%, and 85.4%, respectively). The greatest reductions in TFC of GA:WP, WP:MD, and GA:WP:MD might be a consequence of precipitated forming pellets discarded after centrifugation. The experiment showed that WP was not easily soluble in water due to the casein molecules existing as a mixture of monomers, complexes, and aggregates in the dispersed system. These monomers cannot adequately eliminate hydrophilic surfaces when interacting with water (Singh & Sharma, 2020), thereby limiting the solubility of the protein. Gum arabic dissolved more readily in water due to its numerous hydroxyl groups, enhancing the binding/adhesion ability of water molecules on the surface of the encapsulated particles, thus shortening the coalescence time of the mixture with carbohydrates based on greater interaction with water (Kang, Lee, Kim, & Chang, 2019; Premi & Sharma, 2017; Zhang, Khoo, Swedlund, et al., 2020). Therefore, the combination of GA and MD provides better suspension stability and protection for bioactive compounds.

Table 1. Encapsulation efficiency of bioactive compounds in different shell formulae and particle size in liquid encapsulated treatments

Shell formula	Median (µm)	D_{50} (µm)	Mode (µm)	EE-TPC (%)	EE-TFC (%)	EE-TSC (%)
GA:MD	146.4	139.9	186.7	79.8 ± 2.6^{a}	80.5 ± 1.7^{a}	85.4 ± 1.9^{a}
GA:WP	165.4	157.4	185.5	72.6 ± 2.1^{bd}	46.3 ± 3.9^{b}	72.0 ± 3.1^{b}
WP:MD	182.3	179.9	143.3	70.9 ± 1.4^{bc}	66.7 ± 3.0^{c}	78.6 ± 2.5^{c}
GA:WP:MD	167.3	157.9	186.1	76.0 ± 2.1^{ad}	61.0 ± 2.1^{d}	80.3 ± 1.2^{c}

Values in the same column followed by different letters (a-d) are significantly different ($p < 0.05$)

3.2. Determination of mixing speed and time for suspension

Table 2 presents the effect of mixing speed and time on the particle size of the encapsulated particles. At the same mixing speed (except 10,000 rpm), increasing mixing time generally resulted in a decrease in particle size. Furthermore, at the same mixing time, increasing mixing speed from 8,000 rpm to 12,000 rpm also resulted in a decrease in particle size. These results aligned with the trend that the more energy supply was, the smaller the particle size was. This can be explained that the energy supplied to the system not only broke down large particles into smaller ones but also prevents their aggregation. However, for 10,000 rpm, smaller particles were obtained at 5 and 10 (the smallest among all treatments) min, then particle size kept increasing when mixing time increased to 15 and 20 min. Perhaps in this experimental condition, the 10,000 rpm speed for 10 min mixing reached a point when the highest number of smaller particles in a certain volume had an opportunity to start colliding and re-agglomerating, which led to increasing particle size as the mixing lasted longer (15 and 20 min).

For encapsulation efficiency of noni bioactive compounds in mixing speed and time treatments, statistical results showed significant differences among the suspensions ($p < 0.05$). It is possible to be seen in Table 3 that longer mixing time (15 and 20 min) mostly kept reducing the levels of bioactive compound encapsulation efficiency. The influence of mixing conditions on the phase separation of suspensions can be explained by high-speed homogenization creating turbulent flow in the solution, while simultaneously generating disruptive forces during the flow motion. Turbulent flow aided in better dispersion of particles in the continuous phase, and when the disruptive forces exceeded the surface tension of particles, they broke down into smaller ones (Lakhotia & Papoutsakis, 1992; Momin, Khan, Ghadge, & Bhise, 2017). But as the particles became smaller, the surface area increased with possibly insufficient emulsifying agents to completely cover all newly formed particle surfaces, thus increasing surface tension caused re-aggregation due to collision movements (Jafari, He, & Bhandari, 2007). Between the shorter mixing time treatments (5 and 10 min), comparable or lower EE-TPC, EE-TFC, and EE-TSC were obtained with 5 min mixing. In these conditions, the encapsulation efficiency in terms of bioactive compounds (TPC, TFC, and TSC) was found to be the highest at 80.4%, 88.4%, and 78.3%, respectively when

the noni extract GA:MD suspension was mixed at 10,000 rpm for 10 min. These should be chosen as the fixed parameters for subsequent experiments.

Table 2. Effect of mixing speed and time on particle size and bioactive contents of noni extract encapsulation using GA:MD

Speed (rpm)	Time (min)	Median (µm)	D_{50} (µm)	Mode (µm)	EE-TPC (%)	EE-TFC (%)	EE-TSC (%)
8,000	5	152.7	148.6	162.8	74.8 ± 1.9	85.0 ± 2.1	65.1 ± 3.6
	10	148.5	144.2	161.9	77.6 ± 3.6	81.4 ± 1.6	77.8 ± 2.3
	15	143.2	139.8	143.0	78.1 ± 2.5	81.9 ± 1.5	61.8 ± 5.3
	20	127.9	126.7	124.8	61.2 ± 1.9	77.0 ± 1.9	73.3 ± 1.8
10,000	5	120.8	104.8	142.0	78.5 ± 3.4	85.8 ± 1.2	71.2 ± 2.0
	10	105.4	89.5	124.6	80.4 ± 2.0	88.4 ± 1.1	78.3 ± 1.0
	15	144.9	141.5	143.2	77.0 ± 2.1	83.9 ± 1.3	67.7 ± 2.1
	20	155.6	149.4	163.3	61.9 ± 2.4	75.0 ± 2.9	73.2 ± 1.9
12,000	5	135.5	134.7	141.3	72.6 ± 3.1	78.5 ± 2.0	61.1 ± 1.2
	10	129.3	126.9	125.1	71.2 ± 2.6	79.5 ± 1.8	70.0 ± 1.6
	15	128.0	125.6	124.8	68.3 ± 2.7	71.8 ± 1.6	55.6 ± 3.0
	20	121.7	120.2	123.7	61.9 ± 3.3	69.3 ± 2.8	52.8 ± 1.0

3.3. Determination of temperature and pasteurization time for suspension

Pasteurization temperature and time influenced noni encapsulated particle size, which is presented in **Table 3**. The smallest particle size was in the treatment at 80^{o}C for 10 min, whereas the largest particle size was produced in the treatments with longer pasteurization times, i.e. 80^{o}C for 15 min, 70 and 90^{o}C at both 10 and 15 min. However, the changes in particle size were not significant. On the other hand, the effect of pasteurization process significantly influenced the encapsulation efficiency of noni bioactive compounds ($p < 0.05$). The best encapsulation efficiency in terms of TPC (82.2%), TSC (85.9%), and TFC (87.6%) was achieved at 90°C for 10 min.

In overall, there were limited negative impacts on noni bioactive compounds in this experimental system when the pasteurization went up to 90^{o}C for extended time of 10 and 15 min. Although it has been initially believed that heat treatment adversely affected the retention of phenols, several studies showed an increase in TPC depending on temperature and method (Colantuono et al., 2018; Sharma et al., 2022). However, boiling at high temperature (> 100°C) may eventually reduce the polyphenol content. Heating may disrupt the matrix containing MD and GA, leading to low stability of bioactive compounds (Minatel

et al., 2017; Pattnaik, Pandey, Martin, Mishra, & Ashokkumar, 2021). This may be due to excessively high pasteurization/sterilization temperature and time that can destroy bioactive compounds in cellular components. In this study, it can be suggested that the temperature of 90°C (below the boiling point) and the duration of 10 min should be suitable for pasteurization of the noni extract GA:MD suspension.

Table 3. Effect of pasteurization temperature and time on particle size and bioactive contents of noni extract encapsulation using GA:MD

Temp (°C)	Time (min)	Median (µm)	D_{50} (µm)	Mode (µm)	EE-TPC (%)	EE-TFC (%)	EE-TSC (%)
70	5	128.3	126.2	124.6	77.7 ± 1.9	78.1 ± 1.1	63.3 ± 3.2
	10	144.4	141.6	142.8	78.7 ± 1.6	83.0 ± 1.0	70.2 ± 2.0
	15	142.8	139.2	143.0	76.6 ± 2.0	81.0 ± 3.2	61.4 ± 1.0
80	5	140.9	138.3	142.6	78.3 ± 1.8	84.3 ± 1.2	79.2 ± 1.0
	10	115.7	112.6	109.9	80.3 ± 1.6	84.5 ± 1.3	81.7 ± 1.9
	15	135.9	132.8	141.9	77.4 ± 2.2	85.0 ± 1.8	82.2 ± 1.0
90	5	136.4	113.0	163.0	78.3 ± 1.9	84.9 ± 1.2	80.8 ± 1.5
	10	147.6	143.5	161.7	82.2 ± 1.6	87.6 ± 1.0	85.9 ± 2.4
	15	147.4	141.9	162.0	78.2 ± 1.9	86.7 ± 1.7	84.6 ± 2.5

3.4. Evaluation of suitable preservation conditions over storage time

Based on previous significant conditions to obtain a good noni extract encapsulated suspension, a small scale batch production was produced and packaged in glass bottles with screw caps, presented in Fig 2. The product exhibited high levels of bioactive compounds of TPC (32.0 mg GAE/g), TFC (97.5 mg QE/g), and TSC (245.2 mg AE/g). The results predicting the loss of bioactive compounds i.e. TPC, TFC, and TSC in the noni encapsulation solution during storage at different accelerated temperatures (40 and 50°C) and different durations are presented in Fig 3A, 3B, and 3C, respectively. The evaluation results showed that the loss of bioactive compounds in the encapsulation solution was negligible over the storage period. This indicated the effectiveness of homogenized suspension encapsulation method applied on noni extract. In this study, the storage time of the liquid product for each criterion was determined such that TPC, TFC and TSC did not decrease by less than 50%. The estimated storage durations for the product were determined at the desired temperature of 10oC based on Q10 values of 2.52, 2.40, and 2.24 for TPC, TFC, and TSC, respectively. Thus, 10 months for TPC, 10 months for TFC, and 11 months for TSC should be suggested as the storage time of these bioactive compounds in the encapsulated noni juice at 10oC in a standard condition. However, food spoilage may occur under

various storage conditions, mechanisms and/or contamination circumstances, which may also happen differently to different nutritional components of the encapsulated juice. Additionally, packaging methods and processing procedures may critically impact the shelf life of the product. More quality indices should be considered and described by multiple kinetic equations to determine stability of a product.

Figure 2. Batch production of encapsulated noni juice using GA:MD, and optimized mixing and pasteurization conditions

4. CONCLUSION

This study has demonstrated the influence of shell formulation, homogenized mixing parameters, and pasteurization conditions on suspension stability, particle size and levels of TPC, TFC, and TSC in the extract derived from noni fruit. A shell formulation consisting of maltodextrin and gum arabic in a 1:1 ratio (w/w) at a concentration of 20% was confirmed as a suitable agent for suspension preparation with noni extract. The resulting noni extract encapsulation suspension was stable during the mixing and pasteurization process evaluated in this study. The liquid encapsulated product achieved encapsulation efficiencies of 82.2%, 87.6%, and 85.9% for TPC, TFC, and TSC, respectively with relatively high stability. The liquid noni encapsulated product should possibly be stored with over 50% of the bioactive contents remaining for 10 months in a refrigerator at 10°C. This ready-to-use liquid product form needs standard quality assessments for possible consumption. Further research on large scale implementations, utilizing other encapsulation methods, and developing other product forms for diverse applications are recommended.

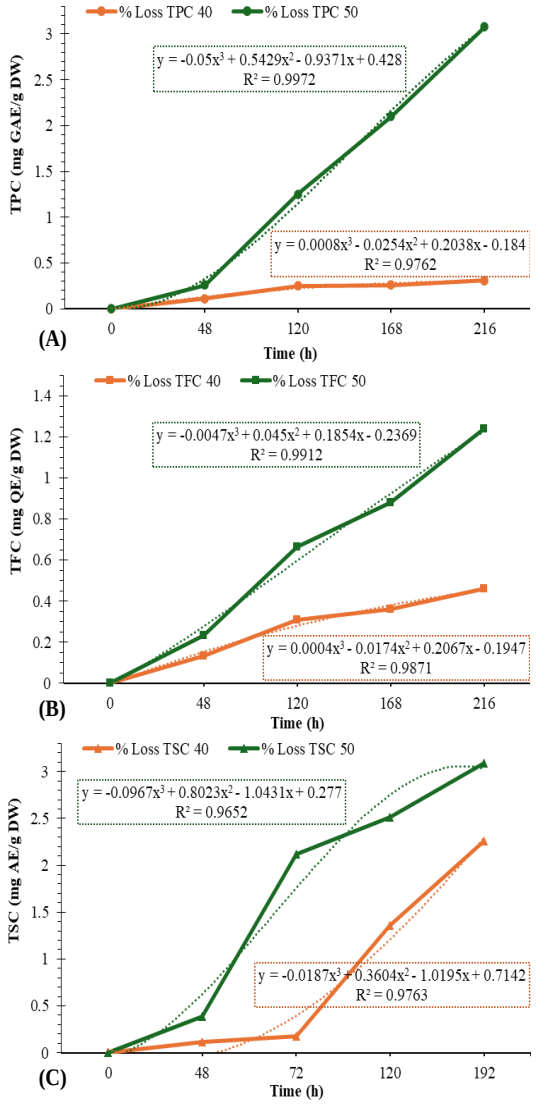

Figure 3. Loss of bioactive compound (A) TPC, (B) TSC, and (C) TSC during storage time at 40 and 50°C

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