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Original research

The enrichment of emergency food rations with complexes made of curcumin/quercetin-whey protein nanofibrils to improve their antioxidant activity

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A B S T R A C T

Functional emergency food rations with health-promoting attributes can improve the performance of armed forces during military missions, especially if there would not be enough time for food consumption. Therefore, the aim of this study was to produce emergency rations enriched with functional ingredients including whey protein nanofibril (WPN) and its complexes with curcumin (C-WPN) and quercetin (Q-WPN) as bioactive antioxidant compounds. After the formulation and production of the rations, their antioxidant activity, sensory properties, and microbial attributes were investigated. Addition of curcumin and quercetin to rations significantly improved their antioxidant activity as investigated by free radical scavenging method and reducing power assay. In all these methods, rations had higher antioxidant activity in the presence of curcumin and quercetin. The microbial and sensory properties of rations also were acceptable. Therefore, the results of this study suggested that the curcumin and quercetin as biologically active ingredients can be used in the formulation of emergency food rations for increasing their antioxidant activity which is very useful for improving the performance of armed forces and soldiers during military missions and activities.

Keywords: Emergency food rations; Whey protein isolate; Natural antioxidants; Antioxidant activity

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1. Introduction

The military and combatants are affected by numerous stressors, the most important of which are nutritional deficiencies, dehydration during military operations environmental pollution, bad weather, chemicals and microbial contaminants. These tensions and stresses can negatively affect their performance and can also cause health problems (Tornero-Aguilera et al., 2017). Oxidative stress caused by these stresses can increased the oxygen free radicals in the blood and thus damage to adipose tissue, protein, DNA, mood disorders and a feeling of physical fatigue (Lollo et al., 2014). One of the most important strategies to reduce oxidative stress and also reduce their destructive effects on soldiers can be the use of emergency rations rich in antioxidant compounds. These rations can maintain the body system at a desirable mental, emotional, and physical state to have sufficient ability to manage and fight. Therefore, using a good food ration can also help improve the morale index in soldiers (Sheibani et al., 2018).

Curcumin and quercetin are the most important natural antioxidants used in dietary formulations to overcome oxidative stress in the body. Curcumin is a natural polyphenolic compound that is extracted from the rhizomes of turmeric and has numerous health effects such as antioxidant, anti-inflammatory and antimicrobial effects. It is also a non-toxic compound and is safe for the body even in high doses (Liu et al., 2017). Quercetin is also a polyphenolic substance found in vegetables and fruits such as onions, apples, grapes, citrus fruits, broccoli, tomatoes and green

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and black tea. Quercetin is one of the most abundant and important compounds in the flavonoid family because it has the highest antioxidant properties among flavonoids and is even about six times stronger than vitamin C. It also has anti-cancer, anti-viral, anti-microbial, anti-allergy and anti-hypertensive effects (Ghayour et al., 2019).

Despite having proven health-promoting properties, curcumin and quercetin have limited applications in the food formulations owing to their very low water solubility. Also, this low solubility in water causes these compounds to have very poor absorption in the human body. Therefore, these bioactive compounds need to be used together with a carrier in food formulations to improve their bioavailability (Tapal &Tiku, 2012). Food proteins are one of the most important carriers used for curcumin and quercetin to improve their solubility, chemical stability, bioavailability, and antioxidant activity (Yi et al., 2016). Among food proteins, it seems that whey protein isolate (WPI), which is a milk protein, can be used as a promising carrier for these beneficial compounds. In this regard, it was reported that the encapsulation of curcumin in WPI microparticles significantly improved its solubility (Liu et al., 2016). More recently, it was reported that the whey protein-based nanofibrillar aggregates have a higher ability to form soluble complexes with curcumin (Mohammadian et al., 2019) and quercetin (Mohammadian et al., 2020) compared to the non-fibrillated WPI. This higher binding ability was attributed to the higher surface hydrophobicity of nanofibrils in comparison with non-fibrillated counterparts which make them as more suitable carriers for the hydrophobic bioactive compounds such as curcumin and quercetin. These nanofibrils are produced from the heating of protein solutions at an acidic condition and have many improved functional and biological properties such as foaming ability, emulsifying activity, gelation properties, and antioxidant properties (Moayedzadeh et al., 2015; Mohammadian & Madadlou, 2018).

To the best of our knowledge, there is no study in the literature on the production of a functional emergency food rations containing curcumin and quercetin as natural antioxidant. Therefore, in the present study, at first curcumin and quercetin were complexed with whey protein-based nanofibrillar aggregates to improve their water dispersibility and then were used in the formulation of emergency food rations. After that, the antioxidant, sensorial, and microbial properties of the produced rations were investigated by different methods.

2. Material and Methods

2.1. Materials

WPI with more than 90% protein content was obtained from Arla Food Ingredients (Viby J, Denmark). The ingredients (wheat flour, confectionery oil, and sugar) used for the production of ration samples were purchased from a local market in Karaj, Iran. Quercetin, 2,2′-azinobis-(3-ethylbanzthiazo-line-6-sulfonate) (ABTS) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Sigma-Aldrich (Sa. Louis, MO, USA). Curcumin (with purity > 95%) was supplied by Bio Basic (Bio Basic Inc., Canada). All of the other chemical as well as the microbial culture environment were bought from Merck Co.; Germany.

2.2. Preparation of whey protein nanofibrils (WPN)

For the preparation of WPN solution, at first, the WPI dispersion with a concentration of 50 mg/mL was prepared and then was completely hydrated. After that, the pH of WPI solution was adjusted to 2.0 using HCl 8.0 M. Then, the protein solution was heated for 5 h at 85°C under a mild stirring of 100 rpm. Finally, the fibrillation process was terminated by immediate cooling of the system. The resulting solution was freeze-dried for the further applications (Mantovani et al., 2018).

2.3. Complexation of curcumin and quercetin with WPN

For the preparation of complexes made of whey protein nanofibrils and curcumin (C-WPN) or quercetin (Q-WPN), the WPN solution was charged with curcumin and quercetin in a final concentration of 1 mg/mL and stirred at 100 rpm for 5 h in a dark place at room temperature. Finally, the resulting complexes were freeze-dried and stored at 4°C for the subsequent uses.

2.4. Atomic force microscopy (AFM)

The morphology of different samples including WPN, C-WPN, and Q-WPN after preparation was investigated by an AFM apparatus (Nanowizard II instrument, JPK, Germany) equipped with a HYDRA-Cantilever. Different sample were diluted in distilled water with same pH to a final concentration of 0.05 mg/mL prior to microscopy. After that, 20 µL of diluted samples was deposited on a glass slide and air-dried. Finally, images were taken at a scan rate of 1.2 Hz. The JPK Data Processing software (version 3.4.15) was employed to process the resulting AFM images.

2.5. Preparation of emergency food rations

The food ration samples were produced according to the method of Dabbagh Moghaddam et al. (2019) with some modifications. In this study, wheat flour, confectionery oil and sugar were consistently used in the formulation of ration samples. This basic formulation was then enriched with WPN, Q-WPN, and C-WPN powders which were prepared in the previous section. For the preparation of ration samples, first the confectionery oil was placed in a container and placed inside the oven to melt completely. Simultaneously, the dry ingredients of each formulation are mixed well in a mixer for 5 min. Lecithin was then added to the melted oil and dissolved well and added to the previous mixture. Stir again for 5 min and in the last step, after adding 4-6 mL of water, the mixture was stirred for 5 min to get the final dough. The resulting mixture was poured onto aluminum foil and molded. Finally, the molds were placed in the oven at 150°C for 20 min to complete the baking process. In total, three types of rations were produced in this study. In these diets, only the protein part of the formulation was different. These rations include: rations containing whey protein nanofibrils, rations containing curcumin-nanofibril complexes, and rations containing quercetin-nanofibril complexes.

2.6. Antioxidant activity

For measuring the antioxidant activity of the produced rations, at first, the ration extracts were prepared. For this purpose, 1.0 g of the ration sample was dispersed in 10 mL of distilled water, well stirred, and then was centrifuged. The resulting supernatants were used for the antioxidant activity tests. Then, the antioxidant activity of the samples was measured by three different methods including ABTS radical scavenging activity test (Wang & Wang, 2015), DPPH free radical scavenging activity experiment (Mohammadian et al., 2020), and the reducing power assay (Taghavi Kevij et al., 2019).

2.7. Sensory evaluation

The sensory analysis of the ration samples was carried out according to Farajzadeh and Golmakani (2011) with some adjustments. Ten untrained panelists were selected to test the organoleptic properties of the ration samples. They evaluated the randomly presented samples for color, odor, taste, texture, and general acceptance with a 5-point hedonic scale ranging from 1 (most disliked) to 5 (most liked). The panelists were asked to rinse their mouth with water between testing different food ration samples.

2.8. Microbial analysis

The microbial properties of samples in term of total count, coliforms, *Salmonella*, *Escherichia coli*, and yeast and mold were evaluated using standard methods. Accordingly, 10 g of ration was homogenized with 90 mL of sterile peptone water (0.1%) using a Stomacher lab blender. Appropriate dilutions were spread on sterile petri plates containing plate count agar and incubated for 24 h at 37°C for the enumeration of total count of bacteria. Sabouraud dextrose agar medium was employed to enumerate total molds and yeasts with 7 days incubation at 25°C. MacConkey agar was used for coliforms counting, and petri plates were kept at 37°C for 24 h (Ghorbani et al., 2021). Moreover, for the enumeration of *salmonella* and *E.coli* in the samples, the method reported by Dabbagh Moghaddam et al. (2019) was employed.

2.9. Statistical analysis

All of the experiments were done in triplicates, and the resulting data were analyzed by SPSS software version 16.0 using one-way analyses of variance (ANOVA). The Duncan post hoc test was used to examine the significant differences at a significance level of 5%.

3. Results and Discussion

3.1. AFM images

The morphology of different samples including WPN, Q-WPN, and C-WPN was studied by AFM and the results are shown in Fig. 1. The whey protein nanofibrils formed in this study had a nanometric diameter (up to 10 nm) and micrometric length which was in accordance with the results of other studies in the case of whey protein nanofibrils (Mohammadian & Madadlou, 2016; Mantovani et al., 2018). Moreover, the results showed that the complexation of the nanofibrils with curcumin and quercetin had not a significant effect on their morphology. In fact the resulting nanocomplexes including C-WPN and Q-WPN also had nanometric diameter and micrometric length. This observation was in accordance with the findings of Mohammadian et al. (2020) who used the whey protein-based nanofibrillar aggregates as delivery systems for improving the solubility of quercetin. They reported that the morphology of fibrils was not significantly affected through the complexation with quercetin as a bioactive ligand. These complexes which were fabricated in this study were used in the formulation of emergency food rations which will be discussed in the following sections.

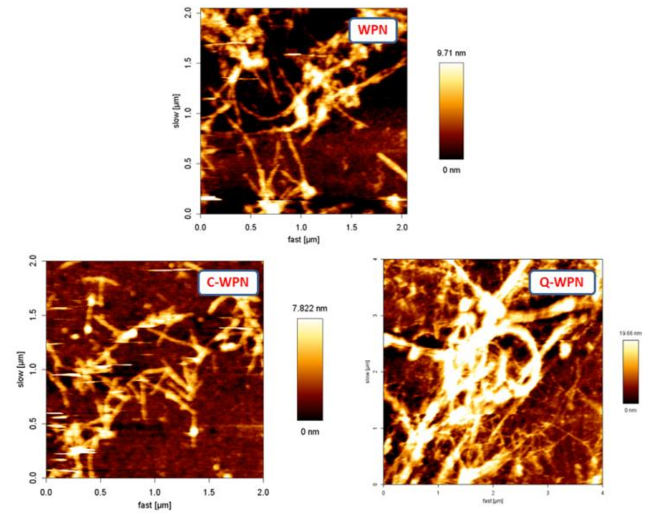

Fig. 1. AFM images of whey protein nanofibrils (WPN) and its complexes with curcumin (C-WPN) and quercetin (Q-WPN).

3.2. Antioxidant activity of rations

In the present study, emergency food rations were successfully produced in the presence of WPN, C-WPN, and Q-WPN, as shown in Fig. 2. Then the produced rations were examined for various properties including antioxidant, sensory, and microbial properties. After the preparation of ration samples, their antioxidant activity was measured by different method including DPPH/ABTS free radical scavenging activity test and reducing power assay and the results are shown in Table 1.

In all of the methods, the lowest antioxidant activity was related to the control sample without curcumin and quercetin. Addition of curcumin and quercetin (C-WPN and Q-WPN) to the rations significantly ($p < 0.05$) increased their free radical scavenging activity. The sample without curcumin and quercetin showed DPPH and ABTS scavenging activities of 34.45 and 25.50%, respectively. However, the sample enriched with curcumin showed the DPPH scavenging activity of 86.65% and ABTS scavenging activity of 90.51%. The ration enriched with quercetin also showed a DPPH scavenging activity of 75.30% and ABTS scavenging activity of 68.85%. These results indicated that the free radical scavenging activity of emergency food rations was drastically improved through the enrichment with curcumin and quercetin. A similar trend was also observed in the reducing power assay which the sample enriched with curcumin showed the highest reducing power between different ration samples. Therefore, these rations had a high reducing power and activity to inhibit free radicals, which can help prevent the occurrence of oxidative stress in the body of the consumer. The results also showed that the antioxidant activity of curcumin-enriched rations was higher than quercetin-enriched counterpart, which could be due to the higher antioxidant activity of curcumin itself compared to the quercetin

(Tapal & Tiku, 2012). In fact, curcumin and quercetin are two natural antioxidants of plant origin. The mechanism of action of these antioxidants is that they prevent the spread of oxidation chain reactions by giving a hydrogen atom to the formed free radical (Liu et al., 2017; Mohammadian et al., 2019; Moghadam et al., 2020). Therefore, curcumin and quercetin can be used as natural antioxidants in ration formulations instead of synthetic antioxidants agents which have many harmful properties for humans.

This high antioxidant activity of the rations can be important in several ways. The high antioxidant activity of rations can prevent lipid oxidation in these food rations, which leads to increased shelf life and also prevents the production of harmful oxidation products that can be dangerous to the health of the consumers (Hadi et al., 2018). In addition, the high antioxidant activity of diets can be important for the soldiers and warriors in combat and military activities. When warriors' anxiety is at its peak, they experience a series of reactions to combat stress, which in extreme warfare is a great source of significant reduction in manpower and military power, while causing severe damage. Intense physical activity also has a high impact on the occurrence of oxidative stress, muscle breakdown, inflammation and immune responses. Oxidative stress caused by these stresses in the body can have devastating effects such as increased oxygen free radicals in the blood and thus damage to adipose tissue, protein, DNA, mood disorders and a feeling of physical fatigue (Lollo et al., 2014; Hadi et al., 2020). One of the most important strategies to reduce oxidative stress and also reduce their destructive effects on soldiers can be the use of emergency rations rich in antioxidant compounds. For example, the antioxidant rations produced in the present study can be used as functional and health-promoting rations for decreasing of combat stress and increasing of consciousness of the soldiers in military actions.

Fig. 2. Emergency food rations prepared in this study from left to right: ration enriched with WPN, C-WPN, and Q-WPN.

Table 1. Antioxidant activity of food ration samples enriched with whey protein nanofibrils (WPN) and quercetin/curcumin nanofibril complexes (Q-WPN/CWPN).

Ration enriched with	DPPH scavenging activity (%)	ABTS scavenging activity (%)	Reducing power (Abs 700 nm)
WPN	34.45 ± 1.50^{c}	25.50 ± 1.13^{c}	0.10 ± 0.009^{c}
Q-WPN	75.30 ± 2.60^{b}	68.85 ± 2.51^{b}	0.25 ± 0.010^{b}
C-WPN	86.65 ± 2.02^{a}	90.51 ± 2.12^{a}	0.39 ± 0.008^{a}

Means with different letters within a column are significantly different ($p < 0.05$).

Table 2. Sensory evaluation scores of food ration samples enriched with whey protein nanofibrils (WPN) and quercetin/curcumin nanofibril complexes (Q-WPN/CWPN).

Sensory properties	Food ration enriched with		
	WPN	Q-WPN	C-WPN
Color	3.4 ± 0.54^{a}	3.8 ± 0.44^{b}	4.8 ± 0.44^{a}
Odor	4.6 ± 0.54^{a}	4.4 ± 0.54^{a}	4.6 ± 0.54^{a}
Taste	4.4 ± 0.54^{a}	4.2 ± 0.44^{a}	4.8 ± 0.44^{a}
Texture	4.6 ± 0.54^{a}	4.0 ± 0.70^{a}	4.4 ± 0.54^{a}
General acceptance	4.4 ± 0.54^{a}	4.4 ± 0.44^{a}	4.6 ± 0.44^{a}

Means with different letters within a row are significantly different ($p < 0.05$).

Table 3. Microbial properties of food ration samples enriched with whey protein nanofibrils (WPN) and quercetin/curcumin nanofibril complexes (Q-WPN/C-WPN).

Microbial test	Food ration enriched with		
	WPN	Q-WPN	C-WPN
Total count	4×10^2	2×10^2	<10
Coliforms	Negative	Negative	Negative
Salmonella	Negative	Negative	Negative
Escherichia coli	Negative	Negative	Negative
Yeast and mold	Negative	Negative	Negative

3.3. *Sensory properties of the rations*

The results of the sensory evaluation of the food rations are shown in Table 2. The results showed that all of the samples received good scores from the panelists. In general, in terms of general acceptance, the highest score was related to the sample containing curcumin, which can be due to the specific color and flavor of curcumin, which makes this product more desirable to the consumers. However, it should be noted that only in term of color, the difference between the samples was significant (p < 0.05). Therefore, the antioxidant food rations produced in this study can be considered as standard rations in terms of sensory characteristics because they contain wheat flour, which has many nutritional properties and is also inexpensive and usually is available in abundance in different conditions (Goesaert et al., 2005). The produced rations also contain whey protein, which is a protein with essential amino acids which has the highest bioavailability among different proteins. In addition, these rations contain curcumin and quercetin, which are natural antioxidants with ability to enhance the health-promoting attributes of these emergency food rations (Moghadam et al., 2020).

3.4. *Microbial properties of the rations*

The microbial properties of the produced food rations were measured after 24 h of the preparation and the results are shown in Table 3. As shown in the table, none of the samples found any of the *coliform*, *Salmonella*, mold and yeast, and *Escherichia coli* microorganisms. The total count of aerobic bacteria in the formulation also showed that the amount of these bacteria in the formulations containing curcumin and quercetin was lower because these compounds themselves have antimicrobial effects and the antimicrobial effect of curcumin is much higher than quercetin (Tapal & Tiku, 2012). The lack of growth of the microorganisms tested in this study (excluding total count) indicates unfavorable growth conditions for the microorganisms. The growth of microorganisms is influenced by various factors, the most important of which are the amount of active water, the presence of antimicrobials agents, the process of food production, and storage temperature (Dabbagh Moghaddam et al., 2019). The water activity of the substances used in the production of rations is very low, which can prevent the growth of most microorganisms that cause spoilage and disease (Farajzadeh & Golmakani, 2011). In general, the sample containing curcumin was in a better condition in terms of microbial quality, which could be due to the antimicrobial properties of curcumin itself as a bioactive ingredient.

4. Conclusion

In the present study, whey protein nanofibrils and their complexes with curcumin and quercetin as bioactive molecules were used to produce emergency food rations with an improved antioxidant activity. The results of this study showed that the addition of curcumin and quercetin to rations significantly increased their antioxidant activity. The antioxidant activity of rations was investigated by free radical scavenging and reducing power methods. In all these methods, rations had better antioxidant properties in the presence of curcumin and quercetin. The microbial and sensory properties of rations have also been acceptable. Generally, the results of our study suggested that the whey proteins, curcumin, and quercetin can be used to produce functional emergency food rations which can decrease the combat stress and increase the consciousness of the soldiers in the military activities with high levels of stress. However, it seems that very little research has been done on the production and development of functional antioxidant emergency food rations and this area still needs much more studies.

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Conflict of interest

We confirm that the authors of this manuscript have no conflict of interest with anyone.

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