Milk Fat Globule Membrane Proteins in Milk Extraction Conditions Optimization and Enzymolysis Products Antioxidant Activity Research

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ABSTRACT: As one of the important composition of milk protein, milk fat globule membrane (MFGM) proteins have many specific physiological functions. By physical method to extract MFGM proteins in milk, the extraction conditions were optimized, and the optimal physical conditions for the extraction of MFGM proteins in milk were determined, amount of sucrose added 4g, and washed 3 times. Using intercept molecular weight 10 ku and 5 ku ultrafiltration membrane carries on the preliminary purification of MFGM antioxidant peptides, respectively measured antioxidant activity, and found that 5ku <molecular weight (MW) ≤10ku had higher antioxidant activity, and measured DPPH • clearance, reducing ability. DPPH • clearance, was 70.41%, is the V C 0.8 times; reducing capacity is the V C 0.92 times.

KEYWORDS: Bovine milk; MFGM proteins; Enzymatic hydrolysis; Antioxidant peptides.

1 INTRODUCTION

Milk fat is one of the main ingredients in milk, and its content is generally accounted for 3%-5% milk, milk fat globules (MFG) is formed in the secretion breast cells, and in the form of tiny droplets exists in milk(Heid and Keenan, 2005, Keenan, 2001, Mather and Keenan, 1998). Diameter of MFG is about 0.2μm-15μm, the outside is surrounded by a layer of about 5-10 nm membrane, this membrane called milk fat globule membrane (MFGM). MFGM has the function of the emulsifying agent, and can prevent the milk fat globule aggregation and enzyme degradation(Dewettinck et al., 2008). MGFM contains proteins, enzymes, phospholipids, cholesterol, and other small molecular compositions, the MFGM proteins account for 1% -2% in the total milk protein, milk fat accounts for 2-6%(Kuchta et al., 2012). MFGM proteins are mainly consisted by xanthine oxidase (XO, 155kDa), butyrophilin (BTN, 67kDa), periodic acid dilute Cardiff 6/7 (PAS 6/7, 49-50kDa) and other proteins(Mather, 2000). Milk source antioxidant peptides can remove the body of excess reactive oxygen free radicals and protect cells and normal functions activity of mitochondria, and prevent the occurrence of lipid peroxidation in vivo (ping et al., 2009, Serbecic and Beutelspacher, 2005), and have anti-aging and cancer prevention effects.

At present, the research on the MFGM area is still in its infancy, and milk MFGM protein as an important component of milk protein, its antioxidant activity of protease hydrolyzate products had not yet been reported. Therefore, this article use physical method to extract MFGM proteins, determines the amount of sugar addition and washing times which have important influence on proteins extraction; and through the protease hydrolyzate for MFGM protease hydrolyzate products antioxidant activity were studied.

2 MATERIALS AND METHODS

2.1 Isolation of MFGM material (Le et al., 2009, Ye et al., 2002)

A 50 ml fresh milk volume was centrifuged at 8000 r/min for 10 min at 4°C to remove cells, obtaining milk fat. Milk fat was collected and then refrigerated 12 h at 4°C overnight, before it was churned into buttermilk and butter. Butter was melted with 1 volume of deionized water at 42°C and centrifuged
at 8000 r/min for 15 min at 4℃ into butter serum and butter oil. The buttermilk was adjusted to pH 4.8 using hydrochloric acid (0.01mol/L) to allow the MFGM to precipitate out. The precipitated MFGM supernatants were collected. Both the suspended MFGM pellet and supernatant was adjusted to pH 6.8 using sodium hydroxid (0.01mol/L). The purified MFGM was stored at the condition of -20℃ until analyzed.

2.2 Different sucrose content and washing times on the impact of MFGM protein

Selected 6 fresh milks each one 50 ml volume to respectively add sucrose 0g, 1g, 2g, 3g, 4g, 5g. followed 1.1 method to extract MFGM proteins. In addition to select another 5 fresh milks each one 50 ml volume to respectively wash 0 times, 1 time, 2 times, 3 times, 4 times, 5 times. And MFGM proteins were extracted followed 1.1 method.

2.3 Comparison of Different molecular weight peptides MFGM proteins enzymolysis peptides antioxidant activity

Intercept molecular weight of 10ku and 5ku ultrafiltration membrane were respectively used for the MFGM antioxidant peptides classification , it could be divided into MW (molecular weight) ≤ 5ku, 5ku < MW ≤ 10ku and MW > 10ku three peptide fragments, and the ability to clear the DPPH • for evaluation, examine the antioxidant activity of different molecular weight MFGM peptides.

2.4 Antioxidant activity tests

2.4.1 The determination of DPPH radical scavenging capacity (Sun and Ho, 2005)
A 2mL sample with 2mL DPPH ethanol solution was mixed (1 × 10-4mol / L), the reaction at room temperature for 30min, and the absorbance was measured at 517nm values. DPPH radical scavenging according to formula (1):

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\text{DPPH scavenging rate}\% = \left(1 - \frac{A_j}{A_i}\right) \times 100 \quad (1)
\]

Type: A₀, control group absorbance; Aᵢ, the sample group of absorbance; Aⱼ, absorbance blank group.

2.4.2 Determination of reducing ability
A 1mL sample was added 2.5mL concentration of 0.2mol/L, pH = 6.6 phosphate buffer and 2.5mL mass fraction 1% potassium ferricyanide, after mixed, placed in a water bath at 50 ℃ to react for 20nim, and then added 2.5mL mass fraction of 10% trichloroacetic acid, after mixed to centrifuge, 2.5mL the supernatant was added 2.5mL distilled water and 0.5mL the mass fraction 0.1% ferric chloride, the absorbance was measured at 700nm.

3 RESULTS AND DISCUSSION

3.1 Sucrose addition on the impact of MFGM protein extraction
Sucrose was added before centrifugation to milk which can increase the density of the whey to better separate the cream and the proteins. Figure 1 shows sucrose addition on the impact of MFGM protein extraction.

As shown in figure 1, the contents of casein and milk globulin in the whey contained in the MFGM protein were higher without the addition of sucrose. With the increase of adding the amount of sucrose, the content of casein and lactoglobulin decreased, it indicated that sucrose could reduce the adsorption of whey proteins in the milk fat, in particular casein and lactoglobulin, it played a key role for purification of MFGM protein. When sucrose was added for 4g in milk, the content of casein and lactoglobulin reached a minimum, so the best content of sucrose is 4 g.

3.2 The washing time on the impact of MFGM protein extraction
The separated milk fat was washed with deionized water; it could obtain higher purity MFGM protein. Figure 2 shows the washing time on the impact of MFGM protein extraction.
As shown in figure 2, Without washing the extracted MFGM protein milk fat contained in the casein and whey lactoglobulin content is higher, the number of washing increases, the content of casein and lactoglobulin decreased, indicating that washing times of MFGM separation of proteins plays an important role. When washed for 3 times, the content of casein and lactoglobulin reduced more, and therefore, the optimal number of milk fat is washed 3 times.

3.3 Antioxidant activity of different molecular weights MFGM protein enzymolysis products

Intercept molecular weight of 10ku and 5ku ultrafiltration membrane were respectively used for the MFGM antioxidant peptides classification, it could be divided into MW ≤5ku, 5ku < MW≤10ku and MW>10ku three peptide fragments, these peptide fragments were prepared concentration of 200 mg/mL solution for DPPH • radical scavenging ability measuring. Different molecular weight antioxidant peptides were used for DPPH • radical scavenging capacity as shown in figure 4.

Figure 3. The best extraction method of MFGM proteins.

As shown in figure 4, the DPPH • radical scavenging capacity of MW ≤5ku, 5ku < MW≤10ku and MW>10ku three peptide fragments respectively were 18.25%, 46.23%, 9.38%, and 5ku < MW≤10ku exhibited strong antioxidant activity. Therefore, 5ku < MW≤10ku peptides were selected on antioxidant activity of the experiment.

3.4 Antioxidant activity measurement of MFGM protein enzymolysis products mes

5ku < MW≤10ku peptides were selected to measure DPPH • clearance rate, reducing ability and were compared with the VC.

3.4.1 MFGM antioxidant peptides influence on DPPH radical scavenging rate

At present, DPPH • is considered as an indicator evaluation whether material has antioxidant capacity. MFGM antioxidant peptides and VC on DPPH • clearance was shown in figure 5.

Figure 4. MFGM antioxidant peptides and VC influence on DPPH radical scavenging rate.

It was shown as figure 5, when the concentration of VC is 50% - 300%, DPPH • scavenging rate was around 80%, its effect was not obvious. It showed that the concentration of the VC on DPPH • removal effect was not big. However, MFGM antioxidant activity gradually increased with its antioxidant peptides concentration increases, when the concentration was 300µg/mL, for DPPH • clearance rate reached 70.41%, it was 0.8 times for the VC, so MFGM antioxidant peptides showed strong antioxidant activity.

3.4.2 MFGM antioxidant peptides influence on reducing capacity

By analyzing the greater the absorbance at a wavelength of 700nm department indicated the greater reduction capability. MFGM antioxidant peptides and VC on reducing capacity were as shown in Figure 6.

Figure 5. Different concentrations of MFGM antioxidant peptides influence on reducing capacity.

It was shown as figure 6, the reducing capacity of VC and MFGM antioxidant peptides exhibited good positive correlation. With antioxidant peptides concentration increasing, VC and MFGM antioxidant peptides on reducing power gradually increased. When the concentration of VC and MFGM antioxidant peptide reached 300µg/mL, the absorbance respectively was 0.4988, 0.4612. MFGM...
antioxidant peptides and VC reducing power were closer, MFGM antioxidant peptide reducing power was 0.92 times for VC, and it indicated that MFGM antioxidant peptides showed strong reducing ability.

4 CONCLUSION

By physical method to extract MFGM proteins in milk, the extraction conditions were optimized, and the optimal physical conditions for the MFGM proteins were determined, amount of sucrose added 4g, and washed 3 times. Using intercept molecular weight 10 ku and 5 ku ultrafiltration membrane carries on the preliminary purification of MFGM antioxidant peptides, respectively measured antioxidant activity, and found that 5 ku < molecular weight (MW) ≤ 10 ku had higher antioxidant activity, and measured DPPH • clearance, reducing ability. DPPH • clearance, was 70.41%, is the VC 0.8 times; reducing capacity is the VC 0.92 times. Experimental results showed that prepared MFGM protein antioxidant peptides in this experiment had strong antioxidant activity.

5 ACKNOWLEDGMENTS

This work was supported by Development Program of 'the twelfth five-year-plan' in national science and technology for the rural development in China (Grant No. 2013BAD18B03-02).

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