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Effect of molecular weight on hypolipidemic and hypoglycemic activities of fermented *Auriculaia auricula* supernatant

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Abstract

Auriculaia auricula is considered as species of Mu Er in Chinese medicine and today are used interchangeably. *A. auricula* polysaccharides have been shown anti-cholesterol and anti-hyperglycemia. The aim of this research was to investigate the hypolipidemic and anti-hyperglycemic effects of extracts from different molecular weight fragments of *A. auricula* fermentation supernatant (AAFS). *A. auricula* was through mixed fermentation by *Saccharomyces* cerevisiae and *Bifidobacterium*. The results shown that with the molecular weight of AAFS increases, the ability to lower blood lipids and blood glucose gradually increases. In the lowering blood lipids, the AAFS of above 300 kDa has the strongest sodium bovine cholate binding capacity and sodium taurocholate binding capacity. The AAFS of 100-300 kDa has the strongest sodium glycocholateas binding capacity. In the lowering blood glucose, the AAFS of 100-300 kDa has the strongest inhibition of α-amylase and α-glucosidase activity. The AAFS of above 300 kDa has the highest index of the glucose dialysis retardation at 30min, and 60min. The AAFS of 100-300 kDa has the highest index of the glucose dialysis retardation at 90min. The study shows that the ability of hypolipidemic and anti-hyperglycemic of AAFS can change with molecular weight, and the contents of substance has changed.

Keywords: *Auriculaia auricula*; fermentation; hyperglycemia; hyperlipidemia.

Pratical Application: Development of *A. auricula* functional food.

1 Introduction

Hyperglycemia and hyperlipidemia as a result of an imbalance between peripheral insulin sensitivity and peripheral insulin sensitivity in internal and organization are a group of prevalent disease symptom which is easy to lead to diabetes (Reilly & Rader, 2003). Found in study that there is a obviously relationship between diabetes and lipid prole abnormalities (Roşu et al., 2000). The standard of HDL cholesterol and increase of LDL cholesterol have relationship with high blood glucose levels and this is a reasion of increasing risk of cardiovascular disease. The hyperlipidemia is a pivotal factor of atherosclerosis, thrombus and many lipid abnormal symptom such as obesity, coronary heart disease and brain infarction and peripheral vascular disease. According to reports that high levels of cholesterol and blood lipid reduces antioxidative enzyme activities (Revenko et al., 1991). Therefore, it is critical for modern person to contral their blood sugar and lipid levels in order to decreasing the risk of diabetes and cardiovascular diseases (Sudasinghe & Peiris, 2018). The effective method for reducing high blood sugar involves the supply of insulin or other drugs with decreasing blood sugar function in combine with recommendations for diet regulation and exercise training (Caprio & Fonseca, 2014).

A. auricula (Family: *Auriculaia*; common name: Jew's ear, wood ear, jelly ear) is a species of edible fungus found all over the world. As a result of their physiologically beneficial bioactive compounds, *A. auricula* has been taken to be a precious natural resources because of its valuable edible value and medicinal value. Such as antioxidant (Lu et al., 2018a), anti-aging (Zhang et al., 2016), hypolipidemic (Reza et al., 2015), hypoglycemic (Lu et al., 2018b), resist fatigue, anticancer (Huang & Nie, 2015), anti-inflammatory (Dereje et al., 2011), hepatoprotective (Tong et al., 2015) and immunoregulation (Zeng et al., 1994) effects.

Saccharomyces cerevisiae and *Bifidobacterium* were proved to be symbiotic. According to our previous research on the function of *A. auricula* about hyperglycemia and hyperlipidemia, *the A. auricula* was mixed fermentation by *Saccharomyces cerevisiae* and *Bifidobacterium* got better bile salts binding capacity, cholesterol adsorption capacity and sodium cholate binding capacity in vitro. Several authors stated the one source of probioactive is a compound that appears as a result of the modification of the foodmatrix itself by the probiotic culture. Probiotics could produce bioactives that would improve the nutritive and functional value (Champagne et al., 2018). Probiotic strains of *Lactobacillus* and *Saccharomyces* have an extensive safety record for their consumption by the healthy population, and the *Saccharomyces* have been considered as one of the microorganisms which present probiotic potential (Cassanego et al., 2018). With the fermentation of *Saccharomyces cerevisiae* advancing, some substances in the medium will also change in content, such as reducing suger (Sun et al., 2019). The present study was designed to inspect the difference about different molecular weight fragment of the liquid of *A. auricula* after fermentation on reducing hyperglycemia and hyperlipidemia. Moreover, the difference about substance content in different molecular weight fragment of the liquid of *A. auricula* after fermentation compared with *A. auricula* was also analysed.

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2 Materials and methods

2.1 Materials

The materials of *A. auricula* were provided from Jiagedaqi Forestry Bureau, Daxinganling District, Heilongjiang Province. The material was authenticated at Institute of Microbiology, Heilongjiang Academy of Sciences. The α-amylase and α-glucosidase was provided by Shanghai Yuanye Biological Technology Co., Ltd. The enzyme activity of α-amylase and α-glucosidase was analysed by pre-test. The standard cholate samples, including sodium cholate, sodium taurocholate and sodium glycocholateas was provided Beijing Biotopped Science & Technology Co., Ltd.

2.2 Preparation of different molecular weight A. auricula fermentation extracts

1 grams of *A. auricula* power which sifted through a 200 mesh screen was mixed with 100mL distilled water. The mixture was fermented by *Bifidobacterium* and *Saccharomyces* cerevisiae at 30 °C for 60h to obtain the *A. auricula* fermentation broth. The different molecular weight *A. auricula* fermentation extracts (0-10 kDa, 10-30 kDa, 30-50 kDa, 50-100 kDa,100-300 kDa, > 300 kDa) was getted by different molecular weight ultrafiltration centrifuge tubes, 4000rpm, 10min.

2.3 Analyze testing the inhibition of α-amylase activity

The dry matter of AAFS was dissolved in phosphate buffer (pH 6.8) and confected into 0.4 mg/mL concentrations. The reaction system including the AAFS solutions (0.04mL, 0.4 mg/mL) and α-amylase (0.2 mL, 6 U/mL) was activated by the addition of the starch solution (0.1 mL, 1%, w/v) and activated for 10 minutes under the condition of 37 °C water bath. Until the reaction was stoped by adding the agentia of DNS. After the reaction system was reacted in boiling water for 5 minutes and diluted with 10mL distilled water, the absorbance was measured at 540 nm (Luyen et al., 2018), and the α-amylase inhibition rate was calculated by following formula:

$$\alpha-\text{amylase inhibition}(\%)=\frac{A_0-(A_1-A_2)}{A_0}\times 100\%$$

A_0 was the absorbance of the phosphate buffer; A_1 was the absorbance of the reaction system including enzymatic reaction system and sample; and A_2 was the absorbance of the α-amylase enzymatic reaction system.

2.4 Analyze testing the inhibition of α-glucosidase activity

The dry matter of AAFS was dissolved in phosphate buffer (pH 6.8) and confected into 0.4 mg/mL concentrations. The α-glucosidase (0.05 mL, 7 U/mL) was blended with the AAFS solutions (0.04mL, 0.4 mg/mL). The mixture was activited 10 minutes under the condition of 37 °C water bath. Then reacted by adding PNPG (0.05 mL, 10 mg/mL) and the reaction system was reacted at 15 minutes under the condition of 37 °C water bath (Ilias et al., 2018). And the reaction was ended by adding the reagent of sodium carbonate (0.01 mL, 1 mol/L). The dialysate's absorbance was measured at 405 nm, and the α-glucosidase inhibition rate was calculated by following formula:

$$\alpha-\text{glucosidase inhibition}(\%)=\frac{A_0-(A_1-A_2)}{A_0}\times 100\%$$

A_0 was the absorbance of the phosphate buffer; A_1 was the absorbance of the reaction system including enzymatic reaction system and sample; and A_2 was the absorbance of the α-glucosidase enzymatic reaction system.

2.5 Analyze testing the glucose dialysis retardation index

The dry matter of AAFS was dissolved in phosphate buffer (pH 6.8) and confected into 0.4 mg/mL concentrations. The glucose solutions (10mL, 0.1 mol/L) was premixed with the AAFS solutions (1mL, 0.4 mg/mL) and shaked at 37 °C, 120rpm for 1 h. Then the mixture was put into dialysate bag (10cm, 7000D) which was put into 150mL deionized water and shaked at 37 °C, 120rpm for 1 h. The absorbance was measured at 490 nm by phenol sulfuric acid assay at 30min, 60min and 90min (Fuentes-Alventosa et al., 2009). The index of glucose dialysis retardation was calculated by following formula:

$$\text{Glucose dialysis retardation index}(\%)=\left(1-\frac{A_1}{A_2}\right)\times 100\%$$

A_1 was the glucose dialysis of the sample group, A_2 was the glucose dialysis of the experimental group.

2.6 Bile salts binding capacity (BBC)

The sodium bovine cholate, sodium taurocholate and sodium glycocholate wa used as detection agents to detect the in vitro bile salt binding capacity of AAFS in a simulated human bile system (Gomez et al., 2018). The calculation of BBC as following:

$$\text{BBC}(\%)=\frac{(C_1-C_2)}{C_1}\times 100\%$$

C_1 was the concentration of the original bile salt in the reaction solution; C_2 was the concentration of the bile salt remaining in the reaction solution.

2.7 Comparison of total sugar content

The dry matter of AAFS and *A. auricula* supernatant (AAS) was dissolved in distilled water and confected into 0.4 mg/mL concentrations. The solution of AAFS and AAS (1mL, 0.4mg/mL) was placed in 10 mL test tubes, 1 mL of 5% phenol and 6mL H_2SO_4 was added and shaken and mixed quickly. Then the mixture was placed at room temperature for 30min and the absorbance was measured at 490 nm. The total sugar content was calculated by standard curve.

2.8 Comparison of reducing sugar content

The dry matter of AAFS and AAS was dissolved in distilled water and confected into 0.4 mg/mL concentrations. The solution of AAFS and AAS (2mL, 0.4mg/mL) was mixed with DNS reagents and reacted in boiling water for 5 min (Isarawut et al., 2018). The reaction system was cooled in ice water and then diluted to a volume of 25 mL. The absorbance was measured

at 560 nm and the reducing sugar content was calculated by standard curve.

2.9 Comparison of polysaccharide content

The dry matter of AAFS and AAS was dissolved in distilled water and confected into 0.4 mg/mL concentrations. The solution of AAFS and AAS (1mL, 0.4mg/mL) was added into 10 mL absolute ethyl alcohol and placed into 50 mL centrifugal tubes. And then the mixture was centrifuged at 4000 r/min for 10 min. Then the supernatant was abandoned and the precipitates were washed by absolute ethyl alcohol for 3 times. It was dissolve to 50 mL by distilled water and then take 2mL liquid diluted to a volume of 10mL. The polysaccharide content was determined by phenol-sulfuric acid, like 2.4.1.

2.10 Comparison of polyphenol content

The dry matter of AAFS and AAS was dissolved in distilled water and confected into 0.4 mg/mL concentrations. The solution of AAFS and AAS (2mL, 0.4mg/mL) was premixed with 2mL Foline-phenol and Na_2CO_3 solution (5mL, 20% w/v) and then diluted to a volume of 50mL. The reaction was reacted at 50 °C for 45min and the absorbance was measured at 765 nm (Sun et al., 2014).

2.11 Comparison of flavonoids content

The dry matter of AAFS and AAS was dissolved in distilled water and confected into 0.067 mg/mL concentrations. The solution of AAFS and AAS (1mL, 0.067mg/mL) was premixed with Na_2NO_2 (1mL, 5% w/v) for 6min and added Al $(NO_3)_3$ (1mL, 10% w/v) for 6min and then mixed in NaOH (10mL, 10% w/v) for 15min. The absorbance was measured at 510 nm and the flavonoids content was calculated by standard curve.

2.12 Statistical analysis

The data analysis was performed by one-way ANOVA of SPSS 20.0 software. And P-values <0.05 was considered to be statistically significant.

3 Results and discussion

3.1 Inhibitory effects of different molecular weight AAFS on α-amylase

As shown in Figure 1, the different molecular weight AAFS was confirmed that have a certain degree of inhibitory effects of α-amylase effect. It was shown that AAFS of have the strongest inhibition of α-amylase activity that can reach 66.74%, and there is no significant difference between 100-300 kDa and above 300 kDa (p>0.05). A-amylase is one of the most important substances secreted in the pancreas and salivary glands that plays a momentous role in gastro-intestinal digestion. The polysaccharides are the main component of *A. auricula*. Several researches have shown that the biological function and characteristics of polysaccharides are concerned with their structure and composition (Ma et al., 2013), especially the molecular weight plays a major part (Liu et al., 2018). Therefore, the inhibition of α-amylase by different molecular weight AAFS may be associated with differences among polysaccharides of different molecular weight. Some research reported that the polysaccharides' molecular chain should be within a reasonably scope to maintain its biological activity and function (Calazans et al., 2000). The AAFS of 100-300 kDa and above 300 kDa can inhibit more effectively the activity of α-amylase.

3.2 Inhibitory effects of different molecular weight AAFS on α-glucosidase

It was shown in Figure 2 that AAFS of 100-300 kDa have the strongest inhibition on the activity of α-glucosidase that can reach 79.64% and there is no significant difference between 100-300 kDa, 50-100 kDa and above 300 kDa (p>0.05). A-glucosidase is one of

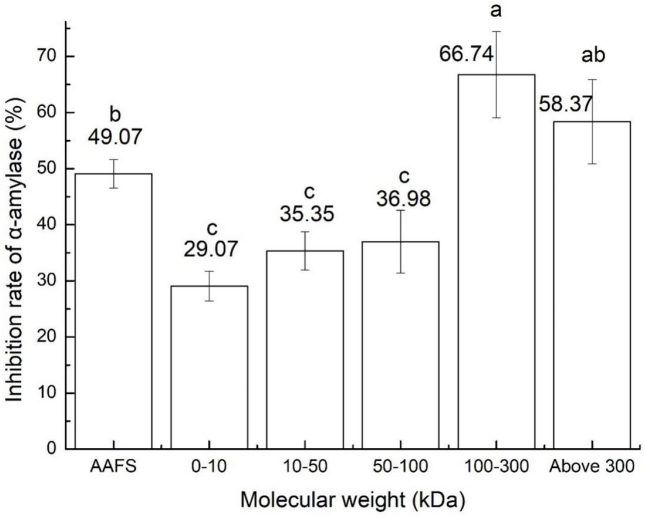

Figure 1. The inhibitory effects of different molecular weight AAFS on α-amylase activity. The results were statistically compared by Duncan's multiple-range test. Means with different letters in the figure indicate significant differences (P <0.05).

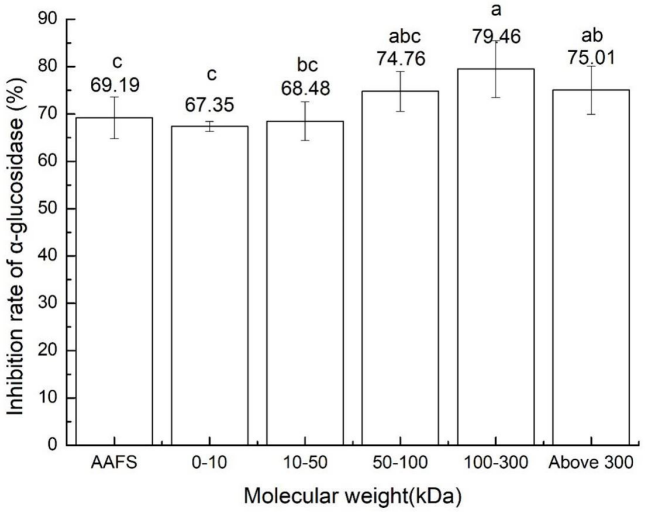

Figure 2. The inhibitory effects of different molecular weight AAFS on α-glucosidase activity. The results were statistically compared by Duncan's multiple-range test. Means with different letters in the figure indicate significant differences (P <0.05).

the representative enzyme in postprandial carbohydrate digestive enzymes, which helps to digest postprandial carbohydrates in the body (Lavelli et al., 2016). Minbae et al. (2016) show that the extraction from edible fungus can as the α-glucosidase inhibitor has an antiglycemic effect. And the inhibitor was examed an antiglycemic effect in diabetic rats. The greater molecular weight AAFS can decrease the absorption of carbohydrates by inhibiting activity of α-glucosidase to lower the postprandial blood glucose levels effectively (Ghani, 2015).

3.3 The index of glucose dialysis retardation of different molecular weight AAFS

The effects of AAFS on the index of glucose dialysis retardation are displayed in Figure 3. As shown, the AAFS and above 300 kDa has the highest index of glucose dialysis retardation at 30min, 60min and the index of glucose dialysis retardation can attain respectively 30.34%, 32.30%. In the index of glucose dialysis retardation of different molecular weight AAFS between these two time periods, there is no significant difference between above 300 kDa and 100-300 kDa ($p>0.05$). The AAFS of 100-300 kDa has the highest index of glucose dialysis retardation at 90min and the index of glucose dialysis retardation can attain 36.94%. There is no significant difference between above 300 kDa and 100-300 kDa ($p>0.05$). A previous research showed that the delayed index of glucose diffusion and absorption is influenced by viscosity of the digesta in intestinal tract (Edwards et al., 1987) and that viscous polysaccharides may induce the intestinal absorb of nutrients by augmenting the apparent thickness of the unstirred water layer (Fan et al., 2015). This discrepancy of different molecular weight AAFS in the aspect of glucose dialysis retardation index was due to differences in physical characteristics, such as water holding capacity, gelling property, and the viscosity of the solution. Based on these results, larger molecular weight polysaccharide fractions, particularly 100-300 kDa and above 300 kDa, are likely to dissolve and create network-link in solvent, thus delaying the diffusion rate of glucose and the absorption of glucose in the gastrointestinal tract is prevented (Oh & Yoon, 2018).

3.4 Bile salts binding capacity (BBC)

The research results of BBC are displayed in Figure 4. The AAFS of above 300 kDa has the strongest sodium bovine cholate binding capacity and sodium taurocholate binding capacity that can reach 68.65%, and 88.58%. There is no significant difference between above 300 kDa and 100-300 kDa ($p>0.05$). The AAFS of 100-300 kDa has the strongest sodium glycocholateas binding capacity that can reach 96.47% and there is no significant difference between above 300 kDa and 100-300 kDa ($p>0.05$). The AAFS with different molecular weight can bind bile salts in the human intestine. Thus inhibiting their reuptake by the intestinal ileum cells and reducing the production of fat. As the molecular weight increases, the index of BBC continues to rise. This may be due to the high molecular weight of AAFS in the composition and structure of polysaccharides with stronger bile salts binding capacity (Vinti Goel Ph, 1998). Susanne Nauman's research show that the in vitro model examed desplays the effect of adsorption and viscosity on bile acid reabsorption reduction. It was affirmed by the relevance of release dynamics with adsorption and viscosity properties. Accordingly, in according to the in vivo experiment findings, the viscosity effect has a decisive effect on the release rate of high fiber barley products (Naumann et al., 2018).

3.5 Total sugar content

The results were statistically compared by Duncan's multiple-range test. Means with different letters in the table indicate significant differences ($P<0.05$).

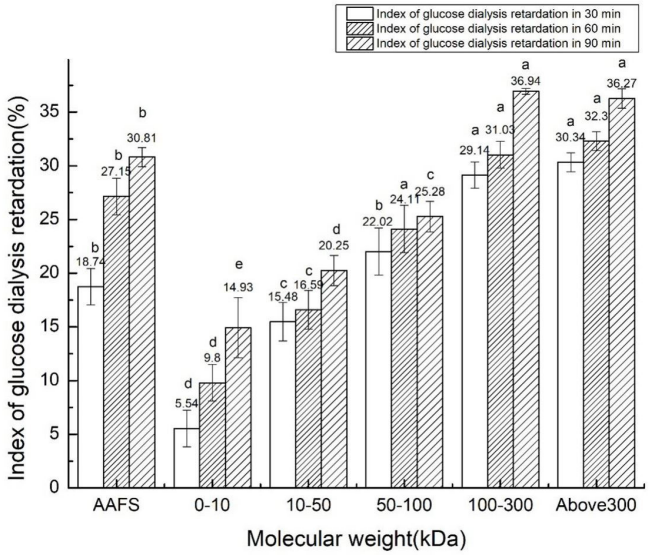

Figure 3. The index of glucose dialysis retardation of different molecular weight AAFS. The results were statistically compared by Duncan's multiple-range test. Means with different letters in the figure indicate significant differences ($P<0.05$).

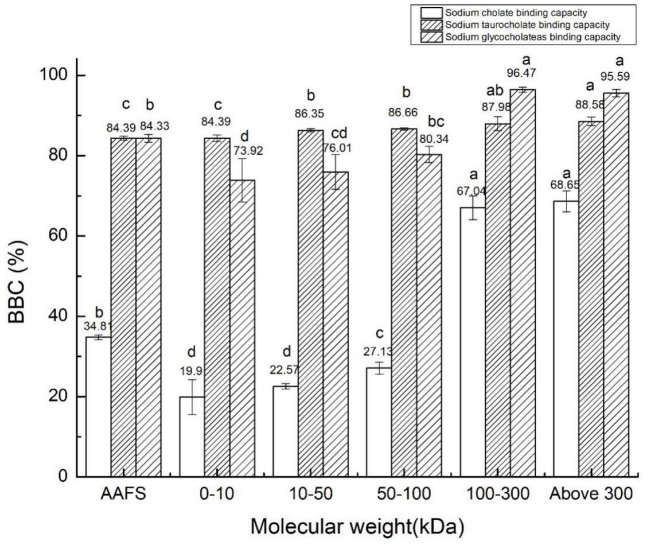

Figure 4. The binding capacity of the different molecular weight AAFS on bile salts. The results were statistically compared by Duncan's multiple-range test. Means with different letters in the figure indicate significant differences ($P<0.05$).

As shown in Table 1, with the fermentation of *Saccharomyces* cerevisiae and *Bifidobacterium* that the content of substances in the *A. auricula* has also changed slightly. Compared with *A. auricula* before fermentation, the total sugar content of AAS increased significantly after fermentation and the total sugar content of the fermentation broth of different molecular weight fractions after fermentation was increased compared to the respective molecular weight fragments before fermentation. The AAFS of above 300 kDa has the highest total sugar content and can reach 1297.43 mg/L. The total sugar content of AAFS at above 300 kDa was significantly different from the total sugar content of other molecular weight AAFS ($p<0.05$). It is clear from Table.1 that the total sugar was increased during fermentation. In previous research, the total sugar content of *A. auricula* was attested that had increased after fermentation. This means that the substance content of the medium based on *A. auricula* was changed by the microorganism's effect.

3.6 Reducing sugar content

As shown in Table 2, Compared with AAFS, the reducing sugar content of AAS has no changed significantly. But the AAS of 100-300 kDa has the highest reducing sugar content and can reach 4.78 mg/L. The reducing sugar content of AAS at 100-300 kDa was significantly different from the reducing sugar content of other molecular weight AAS ($p<0.05$). Aplin et al. (2019) showed that *Saccharomyces* yeasts consumed the reducing sugar in wines, this is similar to the results of our study.

3.7 Polysaccharide content

As shown in Table 3, the polysaccharide content of AAS increased significantly after fermentation and the polysaccharide content of 100-300 kDa was increased more compared to the respective molecular weight fragments before fermentation. The AAFS of 100-300 kDa has the highest polysaccharide content and can reach 823.09 mg/L. The polysaccharide content of AAFS at 100-300 kDa was significantly different from the polysaccharide content of other molecular weight AAFS ($p<0.05$). Some researches by González-Royo et al. (2017) reported that the yeasts can increase the concentration of polysaccharides in red wine. Because the yeasts can releas polysaccharides and oligosaccharides. The increase of polysaccharides concentration detected in red wine supplemented with Optired was mainly due to the intermediate molecular weight fraction (40-144 kDa) and low molecular weight fraction (5-40 kDa). The previous study by Zhu et al. (2016) found that polysaccharide isolated from Ganoderma atrum (PSG-1) exerted hypoglycemic and hypolipidemic effects through inhibiting expression of Bax and improving the expression of Bcl-2 protein in pancreatic tissues of type 2 diabetic rats.

3.8 Polyphenol content

As shown in Table 4, as the molecular weight increases, the polyphenol content gradually reduces. The AAS of 0-10 kDa has the highest polyphenol content and can reach 3.85 mg/mL. The polyphenol content of AAS at 0-10 kDa was significantly

Table 1. The total sugar content of the different molecular weight AAFS and AAS.

	Total sugar(mg/L)					
Molecular weight (kDa)	AAS/AAFS	0-10	10-50	50-100	100-300	> 300
Before fermentation	1090.33±45.06^{b}	982.03±46.04^{c}	1074.23±34.51^{b}	1099.11±61.52^{b}	1122.53±16.46^{b}	1271.82±10.98^{a}
After fermentation	1164.97±13.41^{b}	1001.42±50.84^{c}	1170.10±18.41^{b}	1163.51±51.69^{b}	1206.69±22.77^{b}	1297.43±8.94^{a}

Table 2. The reducing sugar content of the different molecular weight AAFS and AAS.

	Reducing sugar content (mg/L)					
Molecular weight (kDa)	AAS/AAFS	0-10	10-50	50-100	100-300	> 300
Before fermentation	3.68±0.29^{b}	3.71±0.18^{b}	3.20±0.13^{b}	3.23±0.21^{b}	4.78±16.5^{a}	3.28±0.71^{b}
After fermentation	3.40±0.27^{b}	3.37±0.27^{b}	3.11±0.27^{bc}	2.58±0.08^{d}	4.19±0.39^{a}	2.60±0.35^{cd}

The results were statistically compared by Duncan's multiple-range test. Means with different letters in the table indicate significant differences ($P<0.05$).

Table 3. The polysaccharide content of the different molecular weight AAFS and AAS.

	Polysaccharide content (mg/L)					
Molecular weight (kDa)	AAS/AAFS	0-10	10-50	50-100	100-300	>300
Before fermentation	613.06±18.75^{b}	489.39±43.98^{c}	484.10±42.19^{c}	621.11±67.50^{b}	670.87±77.87^{ab}	749.18±15.83^{a}
After fermentation	658.43±10.38^{c}	599.16±17.61^{cd}	620.38±6.71^{cd}	570.62±53.16^{d}	823.09±51.86^{a}	754.30±40.50^{b}

The results were statistically compared by Duncan's multiple-range test. Means with different letters in the table indicate significant differences ($P<0.05$).

Table 4. The polyphenol content of the different molecular weight AAFS and AAS.

	Polyphenol content (mg/mL)					
Molecular weight (kDa)	AAS/AAFS	0-10	10-50	50-100	100-300	> 300
Before fermentation	3.43±0.16^{ab}	3.85±0.09^{a}	3.40±0.43^{ab}	2.92±0.16^{b}	1.68±0.10^{c}	1.45±0.46^{c}
After fermentation	2.86±0.21^{ab}	3.48±0.59^{a}	2.69±0.15^{bc}	2.27±0.28^{bc}	2.05±0.49^{c}	1.36±0.14^{d}

The results were statistically compared by Duncan's multiple-range test. Means with different letters in the table indicate significant differences ($P<0.05$).

Table 5. The flavonoids content of the different molecular weight AAFS and AAS.

	Flavonoids content (mg/L)					
Molecular weight (kDa)	AAS/AAFS	0-10	10-50	50-100	100-300	> 300
Before fermentation	131.36±1.75^{b}	183.26±10.09^{a}	136.03±2.02^{b}	110.96±5.62^{c}	88.22±11.25^{d}	73.06±6.62^{e}
After fermentation	172.88±5.34^{b}	218.36±15.87^{a}	177.54±27.49^{b}	144.31±17.49^{c}	130.31±17.49^{c}	128.56±3.03^{c}

The results were statistically compared by Duncan's multiple-range test. Means with different letters in the table indicate significant differences (P <0.05).

different from the polyphenol content of other molecular weight AAFS ($p<0.05$) and AAS has higher polyphenol content than AAFS. Zieliński et al. (2019) showed that fermented material by L. plantarum caused increase in contents of total phenolic compounds compared to untreated raw material. Because the molecular weight of the total phenolic compounds is small, and it becomes less and less as the molecular weight increases.

3.9 Flavonoids content

As shown in Table 5, as the molecular weight increases, the flavonoids content gradually reduces. The AAFS of 0-10 kDa has the highest flavonoids content and can reach 218.36 mg/L. The flavonoid content of AAFS is higher than that of AAS. The flavonoids content of AAFS at 0-10 kDa was significantly different from the flavonoids content of other molecular weight AAFS ($p<0.05$) and AAFS has higher flavonoids content than AAS. Adebo et al. (2018) showed that fermented samples obtained at 27 °C for 24h had significantly ($p<0.05$) higher flavonoids content values for these parameters. The molecular weight of the flavonoids content is small, so it becomes less and less as the molecular weight increases.

4 Conclusions

This study demonstrated that there are different function on hypolipidemic and hypoglycemic of different molecular weight AAFS. And the content of substances has changed compared with AAS. In the function of hypolipidemic and hypoglycemic, according to the experiment of the inhibition of α-amylase activity and α-glucosidase activity, the AAFS of 100-300 kDa and above 300 kDa have stronger functional activity. In the substance contents, the content of AAFS's total sugar, reducing sugar, polysaccharide, polyphenol and flavonoids has changed compared with AAS, and the content of substances are different with different molecular weight.

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