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THE DEVELOPMENT OF OYSTER SPORTS BEVERAGE AND ITS ANTI-FATIGUE ACTIVITY ON ATHLETES AFTER TRAINING

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ABSTRACT

Sports beverage can effectively supplement the energy and nutrient substances consumed during sports and relieve fatigue for athletes. Taking oyster enzymatic hydrolysate as the raw material, we developed a good-taste and nutritional oyster sports beverage by adding some auxiliary materials according to the formulation requirements for sports beverage based on the international standard and figured out the best sterilization method. Finally, an animal experiment was carried out to evaluate the anti-fatigue activity of the beverage.

1. Introduction

Oyster is the largest cultivated shellfish in the world as well as one of the four largest cultivated shellfishes in China. Pacific oyster (Jouaux et al., 2013; Böhm and Gudehus, 2014) has drawn extensive attention for its high output and good benefit. Zymolytic oyster protein can be more easily absorbed by human body and has antihypertensive effect and oxidation resistance (Jiapei et al., 2008; Umayaparvathi et al., 2014). Therefore, more importance has been attached to preparing hydrolysate with zymolytic oyster in recent years. However, the fishy smell of oyster and the peculiar smell produced in hydrolysis process severely affect the application of oyster zymolyte.

Sports beverage can effectively supplement the energy and nutritional substances consumed during sports and help people to rapidly recover from fatigue. Sports beverages sold in the current market are electrolyte beverages added merely with saccharides, vitamins and mineral substances which cannot rapidly relieve thirst and fatigue; besides, electrolyte is difficult to be absorbed by human body due to the interference and rejection between some factors (Tomlin et al., 2013; Byars et al., 2010; Jason et al., 2011). Oyster protein enzymatic hydrolyzate contains active substances such as taurine, bioactive peptide and glycogen. It has been found that, those substances with activities of antioxidation, immunity strengthening and anti-virus can be rapidly absorbed and utilized by human body to relieve fatigue (Yang et al., 2014; Jiang et al., 2013; Shi et al., 2013). Oyster as a kind of marine organism contains a large amount of mineral substances. But if the product of oyster is eaten without desalination, it may influence the taste, flavor as well as the health of people. But those adverse factors are natural favourable factors for sports beverages, because saline ions are needed by people after sports. A new generation of oyster sports beverage which can be rapidly absorbed and effectively relieve fatigue can be developed if we add some other auxiliary materials into oyster enzymatic

hydrolysate. By doing that, the sports beverage market can be diversified and the raw material of oyster can be better developed.

Based on the study of flavor changes during the enzymolysis of oyster protein, we explored a method to reduce nutritional loss and improve flavor and developed a kind of nutritional and good-taste oyster sports beverage with strong anti-fatigue activity taking oyster protein enzymatic hydrolyzate as the raw material. The development of the product provided a theoretical basis and a practical guidance for the development of oyster product, which has economic benefits and social benefits.

2. Materials and methods

2.1 Experimental materials

The materials used included self-made oyster protein enzymatic hydrolyzate, citric acid, orange juice powder, apple juice powder, mint powder, green tea powder, mango juice powder, blackcurrant juice powder, vitamin B6, vitamin C, vitamin B12, glucose, food-grade otassium chloride, sodium chloride, sucrose, maltodextrin and high methoxyl pectin. The experimental animal was specific pathogen male (SPF) Kunming mice weighed from 17 to 23 g. Test boxes used included glycogen test box, blood urea nitrogen (BUN) test box, malonaldehyde test box, lactic acid test box and protein quantification test box. Instruments used included precision electronic balance, constant temperature vibrator, vertical type automobile pressure steam sterilizer, high-speed tissue stamping machine, vertical type refrigerated centrifuge, high-pressure homogenizer, high performance liquid chromatography, ultraviolet spectrophotometer, rotary evaporator, mini-shaker and minitype freezing point osmometer.

2.2 Experimental methods

2.2.1 Detection of nutritional components

Water was detected using direct drying method; ash was detected using dry cineration method; protein was detected with Kjeldahl determination; crude fat was detected using method of chloroform-methanol, and glycogen was detected using anthrone-sulfuric acid method (Liu et al., 2015). Calcium, iron, zinc, sodium, potassium, magnesium, aluminum and copper were detected using inductive coupling plasma-atomic emission spectroscopy.

2.2.2. Debugging of the taste of oyster sports beverage

Orange juice powder, apple juice powder, mint powder, green tea power, mango juice powder and blackcurrant juice powder of proper quantity were added. Then the best powder and the corresponding amount were confirmed by sensory evaluation.

2.2.3. The effect of sterilization method on the quality of oyster sports beverage

Oyster sports beverages were subpackaged into glass bottles. After capping, they were sterilized by water bath at 70, 90 and 120 °C for 30 min, 20 min and 4 min respectively. The best seasoning powder and sterilization method was confirmed based on organoleptic score, loss rate of vitamin C and total count of bacterial colony.

2.3. Design of experiment

The mice purchased were put into a raising room to adapt to the environment for one week and they ate and drank freely in that period. The temperature of the raising room was set as 25 °C and the humidity was set as 60%; day (12 h) alternated with night (12 h). All mice were given two-day adaptive swimming (water depth: 30 cm; water temperature: 25 °C) after one week. Mice which were unable to swim were excluded. Then the remaining mice were grouped. Mice in the normal saline group were fed with normal saline; mice in the Gatorad group were fed with Gatorad sports beverage; mice in the oyster sports beverage group were given oyster sports beverage. The volume was 0.1 mL/10g in all groups. The gavage lasted for two weeks; mice could freely eat and drink in the process. Grouping of mice is shown in Table 1.

Table 1. Grouping of mice

Group	Group A: anti-fatigue group	Group B: fatigue relief group	Group C: weight carrying swimming group	Group D: control group
Category	Normal saline group/10 mice Gatorad group/10 mice Oyster sports beverage group/10 mice	Normal saline group/10 mice Gatorad group/10 mice Oyster sports beverage group/10 mice	Normal saline group/10 mice Gatorad group/10 mice Oyster sports beverage group/10 mice	Sedentary group/10 mice

2.3.1. Detection of duration of weight carrying swimming

Mice in group C were given test substances every day. After 30-min rest, they received 39 min of swimming training after every 30-min rest, lasting for 14 days. Thirty minutes after the last gavage, the mice carrying 4% iron wire were put into a 25 °C swimming box. The time from the beginning of swimming to the immersing of head in water for 8 s was recorded as the swimming duration.

2.3.2. Evaluation of anti-fatigue activity of oyster sports beverage

Mice in group A were given 0.1 mL/10 g test substances every day. After 30-min rest, they were trained to swim for 30 min, for 14 days. Thirty minutes after the last gavage, the mice carrying 4% iron wire were put into a 25 °C swimming box. Ninety minutes later, the mice were taken out of water. Eyeballs, blood, liver and muscle were collected. Serum and tissue homogenate were prepared. Then the content of blood lactic acid, glycogen, muscle glycogen, BUN and malonaldehyde in the test substances were detected according to the instruction of kit.

2.3.3. Evaluation of anti-fatigue activity of oyster sports beverage

Mice in group B were trained for 30 min every day. Then they were taken out of the water, dried and given 0.1 mL/10g test substances. After 14-day swimming, the mice swam for 1.5 h without carrying weight. Then they were dried and given oyster sports beverage, normal saline and commercially available sports beverage respectively. After 30-min rest, eyeballs, blood, liver and muscle were collected from the mice. Serum and tissue homogenate were prepared. Then the content of blood lactic acid, glycogen, muscle glycogen, BUN and malonaldehyde in the test substances were detected according to the instruction of kit.

3. Results and discussions

3.1 Nutritional components of oyster protein enzymatic hydrolysate

Through analyzing the nutritional components of oyster protein enzymatic hydrolysate, we found that it contained many nutritional components including 1.02 g/100 mL protein, 0.76 g/100 mL sugar and 0.2 g/100 mL taurine. Sugar and protein can provide human body with energy rapidly and effectively, and taurine can effectively relieve fatigue. In addition, oyster protein enzymatic hydrolysate contains a large number of microelements and saline ions including 2.74 μg/mL Al, 39.49 2.74 μg/mL Ca, 1.15 μg/mL Cu, 49.87 μg/mL Fe, 268.51 μg/mL K, 30.04 μg/mL Mg, 768.45 μg/ml Na and 19.32 μg/ml Zn. It can effectively supplement electrolyte missing during sports and maintain the balance of osmotic pressure of body fluid. Therefore, oyster sports beverage developed based on

oyster enzymatic hydrolysate has good nutritional value and physiological property.

3.2. Allocation of oyster sports beverage

3.2.1. Basis of the addition of electrolyte

Best sports beverage should provide people with electrolyte which are lost in sweat during exercise(Morgan et al., 2004). However, the taste of sports beverages needs to be considered during allocation; hence sports beverages cannot contain all the electrolytes in actual application. Too much intake of electrolytes would affect taste and result in strong feeling of thirst. Therefore, on the premise of not influencing taste, electrolytes need to be added as more as possible by referring to the composition and content of electrolytes in human body and sweat. It has been found that, water can be absorbed well when the ratio of glucose to sodium is close to 2 (Guo et al., 2004). Therefore, the content of Na could be confirmed as 40 mg and the content of k as 10 mg.

3.2.2. Basis of the addition of saccharides

The content of carbohydrate in sports beverage can be influenced by the absorption speed of the stomach and intestine to carbohydrate and water absorption. Saccharides in low content cannot supplement human body with energy timely; and saccharides in high content can increase the burden of the stomach and intestine. Research results demonstrate that, the absorption speed of the stomach and intestine to carbohydrate is 112 g/min; and athletes need to supplement 50 ~ 1042 J/h heat if doing exercise for more than one hour. Therefore, a supplement of 40 ~ 80 g/h saccharides can achieve a relatively good effect. In addition, applying compound glycogen can not only maintain blood glucose at a proper level, but can also regulate the osmotic pressure and taste of sports taste and promote the absorption of saccharides and water. Therefore, the content of glucose, sucrose and maltodextrin could be confirmed as 4 g, 1 g and 1 g.

3.2.3. Adjustment of osmotic pressure of sports beverage

Osmotic pressure represents the quantity of particles in solution. Osmotic pressure of normal blood (or body fluids) is 280 ~ 330 mOsm/kg. The intake of a large quantity of low infiltrated drink during sports, water, for example, can lower osmotic pressure of plasma and promote the precipitation of particles; as a result, the desire of drinking water is inhibited immediately. In contrast, the intake of too much beverage with high osmotic pressure can result in gastrointestinal discomfort such as satiety and abdominal distension, increasing burden. Osmotic pressure of isotonic beverages is 280 ~ 330 mOsm/kg, which is balanced with body fluid; therefore, it is more beneficial for the supplement of nutritional substances and energy after sports. Hence the osmotic pressure of sports beverage needs to be considered. In this experiment, osmotic pressure of the oyster enzymatic hydrolysate was detected as 154 mOsm/kg by a freezing point osmometer.

Oyster sports beverage was made referring to the international standard and the components of commercially available sports beverages such as Red Bull, Mizone and Gatorad. Assume that every athlete drinks 500 ~ 1000 mL of sports beverage every day, then per 100 mL of sports beverage should contain 2 g of oyster enzymatic hydrolysate, 40 mg of Na, 10 mg of K, 0.3 mg of VB6, 12 μg of VB and 40 mg of VC. To improve the flavor of oyster liquid, orange juice powder (0.2%) and mint powder (0.1%) were added. Finally the osmotic pressure of oyster sports beverage was measured to be 315 mOsm/kg.

3.3. Influence of sterilization method on the quality of oyster sports beverage

Table 2 shows the influence of different sterilization methods on the quality of oyster sports beverage. Though pasteurization has little influence on the color, smell and vitamin C of beverage, its effect of sterilization is not satisfactory and the microbiological indicator cannot meet the international standard (cfu < 100/mL). The flavor of beverage sterilized at

90 °C for 20 min is acceptable, though the quality is affected slightly; besides, the sterilization method can achieve good sterilization effect. Therefore, 90 °C and 20 min were selected as the optimal sterilization conditions for oyster sports beverage.

Table 2. Influence of difference sterilization methods on oyster sports beverage

	Color	Smell	Flavor	Loss rate of VC	The number of colony
70 °C, 30 min	No obvious change	The previous flavor was completely remained; free from extraneous odour	Good flavor and no peculiar smell	2.14±0.05%	1466
90°C, 20 min	Slightly dark	The previous flavor was fairly completely remained; but the delight had slight reduction.	Relatively good flavor with slight burning small, but acceptable	7.77±0.11%	58
120 °C, 4 min	Very dark	Relatively strong peculiar smell; no delight	Relatively strong burning small and fishy smell, unacceptable	10.18±0.25%	None

3.4. Quality index of oyster sports beverage

Oyster sports beverage was made according to the above requirements. Then indexes of the product were detected, and the results are shown Tables 3 and 4.

Table 3. Sensory index of oyster sports beverage

Item	Color	Fragrance	Taste	Texture status
Index	Gentle color, light yellow, even	Special fragrance of oyster and no peculiar smell	Special fragrance of oyster; sour and sweet; delicate taste; no peculiar smell	Transparent, stable and even; little sediment after long-time placement; no visible foreign matters

Table 4. Physicochemical and microbiological indexes of oyster sports beverage

Item	Actual content	International requirement
Soluble solid %	7.8	3.0-8.0
Protein (g/100mL)	1.15	—
Na (g/mL)	450	50-1200
K (g/mL)	150	50-250
Ascorbic acid (g/mL)	45	≤120
VB6 (g/mL)	3	—
VB12 (ug/100mL)	2	—
Taurine (g/100mL)	0.1	—
Total bacterial count	30	≤100
Coli group	0	≤3
Mycete and saccharomycetes (cfu/mL)	0	≤20
Other pathogenic bacteria	Not detected	None

3.5. Influence of oyster sports beverage of exhaustive swimming time of mice

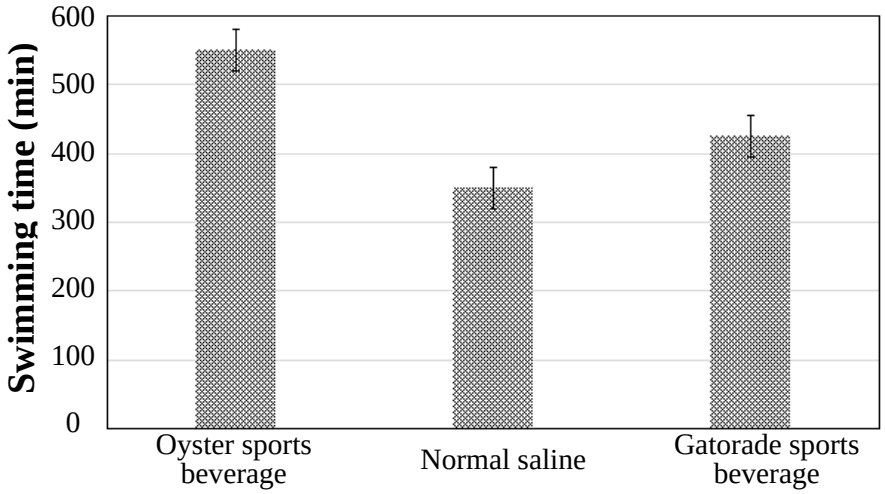

Figure 1. Influence of oyster sports beverage on exhaustive swimming time of mice

Loaded exhaustive swimming experiment is usually used for evaluating anti-fatigue performance of drugs. The swimming duration is correlated to exercise tolerance. Long swimming time indicates good anti-fatigue effect of drugs. Figure 1 shows that, the swimming time of three groups had significant difference. The swimming time of oyster group was 551 min, significantly longer than that of the other two groups ($p < 0.05$); the swimming time of Gatorade group was 422 min, which was longer than that of normal saline group. The above findings suggested that, oyster sports beverage could significantly prolong the exhaustive swimming time of mice and had certain anti-fatigue performance.

3.6. Evaluation of anti-fatigue performance of oyster sports beverage

Table 5. Influence of oyster sports beverage on biochemical indexes of mice

	Content of lactic acid (mmol/L)	BUN (mmol/L)	Hepatic glycogen (mg/g, liver tissue)	Muscle glycogen (mg/g, muscle)	Malonaldehyde (nmol/mgprot)
Oyster sports beverage	8.04±0.37	8.66±1.05	16.25±1.07	3.36±0.62	47.14±10.07
Normal saline	11.85±1.44	9.74±1.36	11.92±2.17	1.92±0.74	106.33±18.74
Gatorade sports beverage	8.97±1.47	9.51±0.85	12.78±1.25	2.56±0.44	118.25±21.07

A large amount of energy can be consumed in sports. Glycolysis reaction provides energy for muscle, but also generates a large amount of lactic acid. H+ isolated from lactic acid can result in the increase of pH in muscle, break the balance of internal environment and lead to fatigue. The content of lactic acid in blood increases when lactic acid in muscle penetrates into blood. After sports, the content of blood lactic acid gradually decreases and fatigue is relieved due to the termination of glycolysis and removal of lactic acid. Therefore, anti-fatigue activity of the test substance can be determined by detecting the content of lactic acid in serum.

Table 5 shows that, the content of lactic acid in the serum of mice in oyster sports beverage group was much lower than that in normal saline group ($p < 0.05$); but there was no significant difference between oyster sports beverage group and Gatorade sports beverage ($p > 0.05$). The above findings suggest that, oyster sports beverage with strong effect in removing lactic acid could effectively relieve fatigue.

The content of serum BUN represents the metabolic status of nitrogen substances in human body and can be used for evaluating the loaded tolerance capability of human body under special condition. When glycogen and blood glucose supply are insufficient, proteins decompose to provide partial energy.

Urea generated from the decomposition of protein in ornithine cycle can result in the increase of BUN. Lower content of BUN indicates less decomposition of nitrogen substance and stronger adaption capability of human body to load (Yu et al., 2006).

Table 5 suggests that, the content of BUN of oyster sports beverage significantly decreased (8.66 mmol/L) compared to the other two groups ($p < 0.05$), but the difference of normal saline group and commercially available beverage group was insignificant ($p > 0.05$).

As BUN is the metabolite of protein, protein rarely involves in energy supply when the sports time is too short, resulting in the insignificant change of BUN, which may be due to the failure of detection of regulatory effect of oyster sports beverage on nitrogen metabolism of serum blood urea.

Glycogen can maintain blood glucose at a normal level and it is also the source of energy in muscle fiber shrinkage. As glycogen with a highly branched structure can lead to the distribution of a large quantity of glucose at the non-reducing end of glycogen molecule, glycogen can be rapidly decomposed to supply energy. Higher storage quantity of glycogen can improve exercise tolerance.

Hepatic glycogen can supplement the blood glucose consumed in motor process, which is of great significance to maintain the balance of glucose. When the storage of glycogen is insufficient, fatigue can be induced (Jia and Wu, 2008). Though muscle glycogen cannot directly provide blood glucose for human body, it can provide the energy which is needed in muscle contraction. Table 5 suggests that, the content of hepatic glycogen of oyster sports beverage (16.25 mg/g) was much higher than that of the other two groups ($p < 0.05$); the content of hepatic glycogen of Gatorade group and normal saline group had no significant difference ($p > 0.05$); the content of muscle glycogen of oyster sports beverage group was 3.36 mg/g, 1.44 mg/g higher than normal saline group ($p < 0.05$) and 0.8 mg/g higher than Gatorade group respectively ($p > 0.05$).

Acute exercises and exhaustive exercises can increase the content of endogenous free radicals in human body and strengthen lipid peroxidation, thus damaging cellular membrane system. Free radicals and lipid peroxidization injury are in a relatively obvious correlation to exercise induced fatigue.

Malonaldehyde, the product of lipid peroxide, is an important index for evaluating the metabolism of free radicals. The content of malonaldehyde can be used for measuring the level of free radicals. In this experiment, the content of malonaldehyde of oyster sports beverage was 47.14 nmol/mgprot, which was much lower than that of the other two groups ($p < 0.05$). It indicated that, oyster sports beverage could reduce the generation of malonaldehyde during intensive exercises and improve antioxidant ability.

3.7 Evaluation of refection activity of oyster sports beverage

Table 6. Influence of oyster sports beverage on biochemical indexes

	Lactic acid (mmol/L)	BUN (mmol/L)	Hepatic glycogen (mg/g, liver tissue)	Muscle glycogen (mg/g, muscle)	Malonaldehyde (nmol/mgprot)
Oyster sports beverage	11.65±1.87	8.67±0.65	18.97±1.82	2.09±00.48	53.78±18.25
Normal saline	10.58±0.99	9.56±0.82	12.23±2.15	2.41±0.77	111.47±22.48
Gatorade	11.68±1.96	8.85±0.87	17.44±2.25	2.18±0.74	105.78±22.47
Control	16.77±1.84	9.97±1.45	18.95±2.48	2.95±0.38	66.47±19.36

Table 6 shows the content of different components including lactic acid, BUN, hepatic glycogen and muscle glycogen in different sports beverages. It can be known that, the content of lactic acid of sports groups was much less than that of the sedentary group, which suggested sports could remarkably improve the activity of lactic dehydrogenase and thus reduce the content of lactic acid. However, no significant difference of the content of lactic acid was observed between oyster sports beverage group, normal saline group and commercially available group ($p > 0.05$). That might be because the decline of the content of lactic acid was insignificant in such a short rest time.

The content of BUN of oyster sports beverage group was lower than that of normal saline group and Gatorade sports beverage group. However, we found the level of BUN of three groups had no significant difference ($p > 0.05$) and the level of BUN was normal in three groups in static state. That might be because few proteins are consumed in such a short swimming time. Therefore, the inhibition effect of oyster sports beverage on BUN was not observed.

The content of malonaldehyde in serum of mice in oyster sports beverage (53.78 nmol/mg) was much lower than that of normal saline group and Gatorade sports beverage group ($p < 0.05$); no significant difference was found between Gatorade sports beverage group and normal saline group. In addition, the content of malonaldehyde of mice in oyster sports beverage group recovered to the normal level after 30-min rest, and there was no significant difference with mice in sedentary group ($p > 0.05$). It indicated that, oyster sports beverage group could rapidly eliminate lipid oxidation product, relieve fatigue and restore physical power.

By detecting hepatic glycogen of mice in different group, we found oyster sports beverage group was significantly different with normal saline group ($p < 0.05$), but insignificant with sedentary group ($p > 0.05$), suggesting the storage quantity of hepatic glycogen could be rapidly recovered after 30-min exercises. The comparison of the content of muscle glycogen between different groups suggested that, there was no significant difference between three experimental groups and sedentary group. Hepatic glycogen not only can be synthesized with glucose, but also can be converted from nonsugar substances such as pyruvic acid, glycerin, lactic acid and amino acid through gluconeogenesis. However, muscle glycogen can only be synthesized with glucose; as a result, it cannot be synthesized in a short time.

We developed a kind of oyster sports beverage taking oyster enzymatic hydrolysate as the raw material according to the international standard as well as the formula of commercially available sports beverage. Every 100 mL of sports beverage contains 2 g of oyster protein enzymatic hydrolysate, 4 g of glucose, 1 g of sucrose, 1 g of maltodextrin, 4 mg of Na, 10 mg of K, 60.3 mg of VB6, 121 μg

of VB, 40 mg of VC, 0.2% orange juice powder and 0.1% mint powder. Different sterilization methods were compared taking sense, loss rate of VC and the total bacterial count as the evaluation indexes. Finally, water bath (90 °C and 20 min) were confirmed as the optimal sterilization conditions. The loaded swimming time of mice given oyster sports beverage 30 min before exercises was much longer than that of normal saline group and Gatorade group ($p < 0.05$); besides, oyster sports beverage could significantly reduce the generation of lactic acid and malonaldehyde and increase the content of hepatic glycogen and muscle glycogen. It indicated that, oyster sports beverage could effectively strengthen physical functions, regulate the balance of internal environment, relieve fatigue and improve athletic ability. The content of lactic acid, BUN and muscle glycogen of mice in oyster sports beverage group (oyster sports beverage was taken immediately after sports) had no significant difference with that of mice in normal saline group and Gatorade group within 30 min; besides, the content of malonaldehyde and hepatic glycogen recovered to the normal level rapidly. It indicated that, drinking oyster sports beverage could rapidly eliminate lipid oxidation product produced during exercises and effectively restore the storage quantity of hepatic glycogen, but was ineffective in rapidly eliminating lactic acid and increasing the storage quantity of muscle glycogen.

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