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ORIGINAL ARTICLE

Anti-fatigue effects of pea (*Pisum sativum* L.) peptides prepared by compound protease

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Abstract In this study, the anti-fatigue effect of pea peptides in mice was explored. Mice were administrated with pea peptides for 30 days and then anti-fatigue related experiments and assays were performed. Swimming times of mice fed with pea peptides were very significantly longer than those of mice from control group in weight-loaded swimming test. Pea peptides showed very significant effect on decreasing level of blood urea nitrogen and blood lactic acid, increasing content of muscle glycogen and hepatic glycogen. Insulin level and lactate dehydrogenase activity was also improved by pea peptides treatment. Pea peptides demonstrated strong antioxidant activity in vivo test. Moreover, supplementation of pea peptides could improve immunity by increasing phagocyte activity, stimulating sIgA secretion and decreasing the pro-inflammatory cytokines in mice. These findings indicated that pea peptides had strong anti-fatigue effect in mice.

Keywords Pea peptides · Anti-fatigue effect · Antioxidant activity · Immune improvement

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Introduction

Fatigue is used to describe an overall feeling of tiredness or lack of energy. As the symptom which indicated decline of exercise ability, fatigue reminds people to reduce exercise intensity or stop exercise so as to avoid injury (Ding et al. 2011). Besides reducing body's exercise ability, fatigue is also harmful to human health (Chaudhuri and Behan 2004). If fatigue is not eliminated in time, it will lead to chronic fatigue syndrome, which seriously affects people's health. Therefore, delaying appearance of fatigue and promoting elimination of fatigue, namely anti-fatigue, is a hot topic in sports medicine and health medicine research.

For a long time, due to the lack of fatigue mechanism study, research on anti-fatigue was mainly focused on nutrient supplementation (Blomstrand and Newsholme 1992) and drug therapy by stimulants, such as caffeine (Childs and De Wit 2008), magnesium pemoline (Orzack et al. 1968), and modafinil (Niepel et al. 2013). Until now, chemical drugs are far from satisfactory because of side effects and limited efficacy (Zlott and Byrne 2010). Recently, nutritional interventions by using natural products or natural product ingredients with little side effects gradually become a research hotspot in anti-fatigue field (Luo et al. 2019). Several types of natural products have been reported for relieving fatigue, such as flavonoids (Miao et al. 2016), polysaccharides (Wang et al. 2010) and peptides. Among of them, peptides derived from natural products have attracted more and more attention owing to easy absorption, multiple functions and safety. Some animal source peptides and plant protein peptides have been used to eliminate fatigue, such as jellyfish (Ding et al. 2011), oyster (Miao et al. 2018), silk (Kim et al. 2014), wheat (Zheng et al. 2017) and American ginseng (Li et al. 2018). Compared with animal source peptides,

vegetable peptides which are widely available and inexpensive are more suitable as anti-fatigue food and supplement.

Pea (*Pisum sativum* L.), one of the most widely cultivated edible legumes in the world, is rich in protein, starch and essential amino acids. Because of the low biological value (65%) of pea protein, direct consumption of pea will waste a lot of proteins. Therefore, deep processing of pea protein to improve the protein utilization has been widely concerned in recent years. One attractive way is to hydrolyse pea protein into peptides. Pea peptides, whose main component is oligopeptides composed of 2-10 amino acids, is easy to be absorbed by the intestine (Monchi and Rérat 1993). In addition, pea peptides shows several interesting biological activities, such as ACE inhibitory effect (Jakubczyk et al. 2013), antioxidant activity (Pownall et al. 2010), anti-bacterial activities (Niehues et al. 2010), anti-inflammatory and immunomodulating properties (Ndiaye et al. 2012). As far as we know, there is no research on anti-fatigue effect of pea peptides.

This study explored the anti-fatigue, anti-oxidation and immunomodulation effect of pea peptides in mice, and analyzed the possible mechanism of anti-fatigue with pea peptide supplement. The results can provide reference for high value-added utilization of pea protein and new product development of anti-fatigue sports foods and beverages.

Materials and methods

Materials and chemicals

Isolated pea protein (protein content was 85%) was purchased from Shuangta Food Co., LTD. Kits for analysis of blood urea nitrogen (BUN), glycogen, blood lactic acid (BLA), malonaldehyde (MDA), superoxide dismutase (SOD), and glutathione peroxidase (GSH-Px) were all purchased from Nanjing Jiancheng Bioengineering Institute. Insulin (Mouse) ELISA Kit was purchased from Abnova. Lactate dehydrogenase (LDH) analysis Kit was provided by Biovision. Interleukin-2 (IL-2), interleukin-4 (IL-4), interleukin-6 (IL-6), interferon-γ (IFN-γ), tumor necrosis factor-α (TNF-α) and secretory immunoglobulin A (sIgA) ELISA kit were purchased from Cusabio. All the other chemical reagents were analytical grade and purchased from Sigma-Aldrich.

Preparation of pea peptides

The pea peptides (molecular weight < 2000 Da) were provided by Nanning Chemmatic Co. Ltd (Nanning, China). The pea peptides were produced via enzymatic hydrolysis of isolated pea protein. In brief, isolated pea protein powder was dispersed in distilled water at a concentration of 10% (w/v). After stirred evenly, pH value of the solution was adjusted to 7.5. Compound protease (90% alkaline protease and 10% papain, Nanning Pangbo Biological Engineering Co., Ltd, China) of 1000 U g^{-1} was added, and the mixture of pea protein and enzyme was hydrolyzed for 4 h at 55 °C. After enzymolysis, the mixture was heated to 100 °C for 15 min to inactivate enzyme. Then the mixture was subjected to centrifugation, ultrafiltration, purification and lyophilisation to give pea peptides powder.

Experimental animals and experimental diet

One hundred and fifty Kunming mice (20 ± 2 g) were purchased from the Experimental Animal Center of Guangxi Medical University. Mice were allowed free access to standard laboratory diet (provided by Experimental Animal Center of Guangxi Medical University) and water during the experiment. All animal test procedures were approved by Guangxi University for Nationalities.

After one week adaptation, mice were randomly assigned into five groups: Control group (Control), Isolated Pea Protein group (IPP), Pea Peptides low-dose group (PPL), Pea Peptides Middle-dose group (PPM), and Pea Peptides High-dose group (PPH). Each group was randomly divided into 3 subgroups (A, B and C), with one subgroup for the weight-loaded swimming test and the other subgroups for determining the biochemical parameters. The Control group were fed with distilled water (0.1 mL g^{-1} body weight day) for 30 days; the IPP group were fed with pea protein (200 mg Kg^{-1} body weight day) for 30 days; the PPL, PPM and PPH group were respectively fed with the pea peptides in three different doses (100, 200 and 400 mg kg^{-1} body weight day) for 30 days. The pea protein and peptides were dissolved in 2 mL distilled water and then the solutions were fed to mice via gavage administration every day.

Weight-loaded swimming test

Mice from Subgroup A were used for weight-loaded swimming test. Thirty minutes after the last gavage administration, the exhaustive swimming test was carried out. Mice were forced to swim in a swimming tank (60 cm x 60 cm x 45 cm) with 30 cm deep water. Water temperature was maintained at 25 ± 1 °C. The tail root of each mouse was loaded with 5% weight of lead wire. Mouse was considered as exhaustion when it cannot return to water surface within 10 s. Time from the beginning of swimming to exhaustion was recorded as the swimming time of mice.

Determination of biochemical parameters on fatigue

Thirty minutes after the last gastric administration, mice from subgroup B were forced to swim without loaded weight at 25 °C for 10 min. Blood samples for BLA test were drawn separately before swimming, 0 min after swimming and 20 min after swimming. After 60 min of rest, blood samples were collected to determine the level of BUN and LDH activity. Liver and hind leg muscles were removed and rinsed with saline for determining the content of hepatic glycogen and muscle glycogen. Skeletal muscles from hind legs were also used for analysis of MDA, SOD and GSH-Px (Miao et al. 2018). All of the above biochemical parameters were determined according to the procedures provided in the kits.

Analysis of insulin and immunity related parameters

Thirty minutes after the last gastric administration, mice from subgroup C were anesthetized and executed. Blood samples were collected to determine the level of insulin, IL-2, IL-4, IL-6, IFN-γ, TNF-α (Kim et al. 2014). Small intestinal mucous samples were collected for determination of sIgA (Ndiaye et al. 2012). All of the mentioned parameters were determined following the instruction provided by the kits.

Phagocytosis assay was carried out according to method reported by Perdigon et al. (1986). The peritoneal cavity of mouse was washed with 5 ml phosphate buffer saline (containing 10 U mL^{-1} of Heparin and 0.1% bovine serum albumin). The peritoneal macrophage suspension was collected via centrifugation and was adjusted to 10^6 cells mL^{-1} with the same buffer. To evaluate phagocytosis, peritoneal macrophage suspension was mixed with same volume of heat-killed Candida albicans (10^7 cells mL^{-1}) suspension and then incubated for 30 min at 37 °C. Activated macrophages were observed and counted under optical microscope. The phagocytosis percentage was calculated after counting 100 cells.

Amino acid analysis and MALDI–TOF–MS

Amino acid analysis was carried out according to method reported by Zheng et al. (Zheng et al. 2017). Pea peptides powder was dissolved in deionized water, and was hydrolysed with 6 M HCl under nitrogen at 110 °C for 24 h. After hydrolysis, total amino acid composition of hydrolyte was determined with amino acid auto-analyzer (HITACHI 835-50, Japan). For cysteine and methionine analysis, sample was hydrolysed at 110 °C for 24 h in 6 M HCl following treatment with performic acid. To determine content of tryptophan, sample was hydrolysed in 5 M NaOH at 110 °C for 22 h.

Matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI–TOF–MS, ultrafleXtreme, Bruker) was used for the identification of peptides (Zheng et al. 2017).

Statistical analysis

All the experimental data were expressed as mean ± standard deviation. Statistical analyses were performed using the SPSS software version 19.0. One-way analysis of variance (ANOVA) was used to analyze results. Differences were thought significant at $P < 0.05$, and $P < 0.01$ indicated that the difference was very significant.

Results and discussion

Body weight and weight-loaded swimming time of mice

The initial body weights and the final body weights of mice were measured. As shown in the Table 1, the weights of mice fed with pea peptides did not differ significantly from those of the control group and IPP group ($P > 0.05$). The result showed that pea peptides had no effect on the body weight of mouse.

Fatigue results in change of exercise ability. Stronger fatigue resistance will lead to longer exercise time. Herein we evaluated the anti-fatigue effect of pea peptides by the time of weight-loaded swimming in mice. The swimming times of mice in each pea peptides feeding group were longer than those in the blank control group, and the differences were very significant ($P < 0.01$) (Table 1). Pea protein had no significant effect on weight-loaded swimming time ($P > 0.05$). The swimming times of mice from the high dose group were significantly longer than those of mice from the low dose group. The results indicated that the pea peptides could prolong the swimming time and enhance the exercise endurance of mice.

Analysis of BUN, glycogen and insulin

When body's glycogen is exhausted, the organism will obtain necessary energy by consuming protein. The product of protein catabolism is urea, which is produced in liver and carried by blood to kidney and then discharged from body. Therefore, urea content in blood is a significant marker of fatigue level. According to the data of Table 2, the BUN contents of mice in the PPL, PPM and PPH group were very significantly lower than those of the control group after exercise ($P < 0.01$). There was no significant difference in the content of BUN between IPP group and the control group ($P > 0.05$). This result indicated that the

Table 1 Body weight and swimming times of mice

Group	Initial body weight (g)	Final body weight (g)	Swimming time (min)
Control	20.19 ± 0.88	29.82 ± 1.16	10.41 ± 0.72
IPP	19.92 ± 1.16	30.05 ± 1.12	11.28 ± 0.87
PPL	20.26 ± 1.08	29.95 ± 1.08	12.73 ± 1.11**##
PPM	20.02 ± 1.10	30.04 ± 1.01	14.51 ± 1.02**##
PPH	20.03 ± 1.18	29.97 ± 1.09	17.33 ± 1.14**##

Data are showed as mean ± SD (n = 10). *$P < 0.05$, **$P < 0.01$ versus Control; #$P < 0.05$, ##$P < 0.01$ versus IPP

Table 2 Levels of BUN, glycogen and insulin

Group	BUN (mmol/L)	Hepatic glycogen (mg/g)	Muscle glycogen (mg/g)	Insulin (ng/mL)
Control	8.95 ± 0.36	8.66 ± 0.73	2.13 ± 0.22	1.13 ± 0.22
IPP	8.64 ± 0.46	9.12 ± 1.08	2.33 ± 0.33	1.27 ± 0.25
PPL	7.72 ± 0.36**##	12.11 ± 1.10**##	2.93 ± 0.32**##	1.53 ± 0.30**#
PPM	7.27 ± 0.35**##	15.37 ± 1.22**##	3.21 ± 0.31**##	1.73 ± 0.31**##
PPH	6.95 ± 0.30**##	17.89 ± 1.06**##	3.66 ± 0.29**##	1.87 ± 0.24**##

Data are showed as mean ± SD (n = 10). *$P < 0.05$, **$P < 0.01$ versus Control;#$P < 0.05$, ##$P < 0.01$ versus IPP

fatigue degrees of mice fed with pea peptides were lower. Peptides did not promote urea excretion in kidney, so we suggested that much glycogen storage would be the reason why mice in the peptide feeding group consumed less protein. To validate our hypothesis, we determined contents of liver glycogen and muscle glycogen.

Sugar, mainly stored in body in the form of liver glycogen and muscle glycogen, provides necessary energy for exercise through aerobic respiration and anaerobic respiration. As exercise proceeds, glycogen will be exhausted. Exhaustion of glycogen leads to decrease of blood glucose concentration, and then results in hypoglycemia and fatigue (Dohm et al. 1983). Therefore, glycogen content plays an important role in improving exercise endurance and restoring exercise fatigue (Jin and Wei 2011; Ren et al. 2011). In Table 2, data showed that hepatic glycogen and muscle glycogen contents of mice fed with pea peptides were very significantly higher than those of mice from control group and IPP group ($P < 0.01$).

Supplementation of pea peptides composed of amino acids does not directly supply glucose for synthesis of glycogen in vivo. However, supplementation of peptides or proteins may affect efficiency of consumption and replenishment of glycogen. van Loon et al. (2000) reported amino acids and protein hydrolysates could stimulate release of insulin. Calbet and MacLean (2002) reported that ingestion of glucose and protein hydrolysates at the same time promoted insulin secretion. Morifuji et al. (2011) reported supplementation of whey protein hydrolysates and glucose significantly reduced muscle glycogen consumption. They proposed supplementary whey protein hydrolysates could stimulate release of insulin which activated glycogen synthase, Akt/PKB, and PKCζ in skeletal muscle. In our case, pea peptides may stimulate release of insulin to promote the formation of glycogen as well. In order to verify our hypothesis, we determined the insulin concentration in blood of mice. In Table 2, we observed that insulin concentrations of mice in PPL, PPM and PPH group were very significantly higher than those of the control group and IPP group ($P < 0.01$). This result indicated that pea peptides could stimulate insulin release. Therefore, we suggest that pea peptides may increase glycogen content by promoting insulin secretion.

BLA level and LDH activity of mice

Because aerobic respiration cannot meet the energy demand of living body during exercise, living body will obtain energy through anaerobic respiration. Lactic acid is the main metabolic product of sugar under anaerobic respiration. Excessive lactic acid accumulation in the body can cause muscle soreness, even lactic acid acidosis, which is a major cause of fatigue in human body (Gibson and Edwards 1985). So detection of BLA can help us to judge the degree of fatigue. We took blood from mice before exercise, after exercise and 20 min after rest for comparison. In the Table 3, we found the BLA levels of mice in every group were very close before exercise. After exercise, contents of BLA in all groups were increasing, but BLA levels of PPM and PPH group were significantly lower than those of control and IPP group ($P < 0.05$). After 20 min rest, BLA levels of all groups decreased, and those

Table 3 BLA levels and LDH activity of mice

Group	BLA before swimming (mmol/L)	BLA after swimming (mmol/L)	BLA after 20 min rest (mmol/L)	LDH (U/L)
Control	6.18 ± 0.91	10.12 ± 1.41	7.61 ± 0.91	764 ± 121
IPP	5.98 ± 0.86	10.01 ± 1.30	7.48 ± 0.94	814 ± 141
PPL	5.50 ± 0.88	9.29 ± 1.32	6.45 ± 0.78**##	1038 ± 126**##
PPM	5.46 ± 0.66	9.01 ± 1.34*#	6.04 ± 0.81**##	1169 ± 140**##
PPH	5.37 ± 0.71	8.61 ± 1.23**#	5.73 ± 0.72**##	1296 ± 151**##

Data are showed as mean ± SD (n = 10). *$P < 0.05$, **$P < 0.01$ versus Control; #$P < 0.05$, ##$P < 0.01$ versus IPP

of peptides feeding group decreased very significantly ($P < 0.01$). This result indicated that pea peptides could effectively reduce the BLA contents of mice after exercise.

To find out how pea peptides can reduce BLA concentration, we tested activity of LDH in serum. The role of LDH is to reduce the accumulation of lactic acid via catalyzing the oxidation of lactic acid to pyruvic acid (Koo et al. 2004). The activities of LDH in pea peptides feeding groups were very significantly higher than those in the control group and pea protein feeding group ($P < 0.01$) (Table 3). This result demonstrated that pea peptides could reduce BLA in blood by enhancing the activity of LDH.

Determination of anti-oxidant activity

Free radical theory claims that a large number of free radicals produced by human tissues during exercise are the important causes of exercise fatigue. Free radicals which are highly reactive molecules can damage cellular proteins, lipids or DNA via oxidation (Powers et al. 2004). Supplementation of antioxidants and improvement of antioxidant capacity in vivo are effective ways to reduce free radicals content. Herein we evaluated antioxdative ability of pea peptides in vivo through widely-used MDA, SOD and GSH-Px test (Table 4) (Villanueva and Kross 2012). MDA, is peroxidative product of membrane lipids, reflects the tissue and cell oxidative stress (Bagis et al. 2005). SOD removes oxygen radicals in body. GSH-Px catalyzes decomposition of peroxide in vivo. In Table 4, MDA level in IPP group was significantly lower than control group ($P < 0.05$). Compared with control group and IPP group, MDA levels in PPL, PPM and PPH group were very significantly lower ($P < 0.01$). When examining SOD and GSH-Px activity, we found that the markers in peptides feeding group showed very significant change compared with those of control group and IPP group ($P < 0.01$); change between control group and IPP group is no significant ($P > 0.05$). The results suggested that pea peptides could enhance activity of SOD and GSH-Px, inhibit oxidative reaction and reduce cell membrane damage.

Table 4 MDA levels, SOD activity and GSH-Px activity in skeletal muscles of mice

Group	MDA (nmol/mg)	SOD (U/mg)	GSH-Px (U/mg)
Control	3.48 ± 0.34	88.43 ± 8.75	54.53 ± 5.34
IPP	3.24 ± 0.30*	93.69 ± 9.08	58.87 ± 5.88
PPL	2.41 ± 0.27**##	103.74 ± 9.96**#	65.36 ± 6.37**#
PPM	2.04 ± 0.22**##	115.28 ± 10.96**##	75.18 ± 6.85**##
PPH	1.67 ± 0.17**##	124.59 ± 11.45**##	82.26 ± 7.19**##

Data are showed as mean ± SD (n = 10). *$P < 0.05$, **$P < 0.01$ versus Control; #$P < 0.05$, ##$P < 0.01$ versus IPP

Improving immunity

Fatigue and immunity are interrelated (Mensah et al. 2017). As we know, when people feel tired, their immunity will decrease significantly, and they will easily become infected; when human body is infected, people also easily feel fatigue. Improving immunity to keep body in best condition can enhance human body's exercise endurance, delay appearance of fatigue and accelerate the recovery of fatigue. Herein we made a preliminary study on the immune regulation of pea peptides in mouse model. We used phagocyte activity, levels of sIgA, interleukin (IL-2, IL-4 and IL-6), interferon-γ (IFN-γ) and tumor necrosis factor-α (TNF-α) to evaluate the immune regulatory effect of pea peptides. Phagocytes, which can recognize, phagocytize and destroy foreign bodies entering the living body, are major defense line of the human immune system. sIgA is a major antibody in exocrine fluid and the first immune defense line against pathogens and harmful substances in respiratory tract, digestive tract and urogenital tract. It is the most important antibody in mucosal immunity. Thus phagocyte activity and level of sIgA can directly reflect the immune activity of the body. From the Table 5, we found that phagocytosis effects of pea peptides feeding group were very significantly higher than those of the control group and protein feeding group ($P < 0.01$). The

Table 5 Immunity related parameters

Group	Control	IPP	PPL	PPM	PPH
Phagocytosis (%)	22.4 ± 1.4	23.5 ± 1.8	29.6 ± 2.2**##	31.4 ± 2.3**##	32.5 ± 1.9**##
sIgA (ug/mL)	14.32 ± 1.07	14.41 ± 0.96	16.65 ± 1.23**##	18.94 ± 1.27**##	19.64 ± 1.42**##
IL-2 (pg/mL)	24.72 ± 3.06	23.95 ± 3.23	25.31 ± 2.87	24.93 ± 3.41	25.02 ± 3.11
IL-4 (pg/mL)	26.67 ± 3.72	26.22 ± 3.45	26.01 ± 3.66	26.39 ± 3.07	25.78 ± 2.94
IL-6 (pg/mL)	64.63 ± 8.18	66.99 ± 8.36	55.01 ± 6.57*##	52.84 ± 8.57**##	49.71 ± 8.40**##
IFN-γ (pg/mL)	202.31 ± 17.82	206.85 ± 19.33	205.19 ± 20.22	212.65 ± 19.62	210.23 ± 18.04
TNF-α (ng/mL)	10.52 ± 2.26	10.70 ± 2.47	9.41 ± 1.78	9.28 ± 1.99	8.47 ± 1.96*#

Data are showed as mean ± SD (n = 10). *$P < 0.05$, **$P < 0.01$ versus Control; #$P < 0.05$, ##$P < 0.01$ versus IPP

concentrations of sIgA in mice fed with pea peptides were also very significantly higher than those in control group and IPP group ($P < 0.01$). Cytokine, including interleukin, IFN-γ and TNF-α, is a kind of small molecular protein which is synthesized and secreted by immune cells or some non-immune cells through stimulation. It plays an important role in immune response. IL-6 level of pea peptides feeding groups were very significantly lower ($P < 0.01$). TNF-α level also decreased significantly in PPH group ($P < 0.05$). IL-6 and TNF-α had been considered as the pro-inflammatory cytokines (Hirano 2010). These results indicated that to supplement pea peptides could obviously eliminate the inflammatory reaction. Levels of IL-2, IL-4 and IFN-γ did not affected by pea peptides significantly ($P > 0.05$).

Composition of amino acid and MALDI–TOF–MS analysis

The composition of amino acids influences bioactivity of pea peptides as well. It has been reported that single hydrogen atoms in the side chain of Glutamic acid (Glu) residues can react with free radicals, thus playing an important role in antioxidation (Castell 2002). Histidine (His), Proline (Pro) and Tyrosine (Tyr) have also been reported to have high free radical scavenging capacity (Saito et al. 2003). The amino acid composition of pea peptides is shown in the Table 6. Glu is the most abundant amino acid of pea peptides (24.19%). His, Pro and Tyr also account for a considerable portion of the total (10.97%). This may be the reason why pea peptides show strong antioxidant activity. In addition, pea peptides are also rich in branched chain amino acids (13.88%) and Aspartic acid (Asp) (11.28%), which can improve exercise ability, significantly alleviate the catabolism of muscle proteins, and reduce the accumulation of lactic acid in blood (Blomstrand and Newsholme 1992; Marquezi et al. 2003).

Table 6 Amino acids composition of pea peptides

Amino acid	Composition (g/100 g)
Isoleucine (Ile)	2.98
Leucine (Leu)	6.47
Valine (Val)	4.43
Branched-chain amino acids (BCAAs)	13.88
Phenylalanine (Phe)	5.66
Methionine (Met)	1.06
L-Threonine (Thr)	2.17
Lysine (Lys)	7.13
Tryptophan (Trp)	0.87
Histidine (His)	3.64
Arginine (Arg)	9.21
Glutamic acid (Glu)	24.19
Tyrosine (Tyr)	2.41
Cysteine (Cys)	2.89
Serine (Ser)	3.17
Aspartic acid (Asp)	11.28
Glycine (Gly)	3.10
Proline (Pro)	4.92
Alanine (Ala)	1.73
Total amino acids	97.31

Three major peptide fragments (MW: 845.45, 992.52 and 1252.71 Da) were identified by MALDI–TOF–MS. Their sequences are Gln–Leu–Glu–Glu–Leu–Ser–Lys (QLEELSK), Lys–Gly–Asp–Phe–Glu–Leu–Val–Gly–Gln (KGDFELVGQ), and Phe–Phe–Glu–Leu–Thr–Pro–Glu–Lys–Asn–Gln (FFELTPEKNQ). The biological activities of these peptides depend on their amino acid composition. These peptides contain Glu and Pro residues which show free radical scavenging capacity (Saito et al. 2003). Leu and Asp residues which have the best effect on anti-fatigue (Marquezi et al. 2003) are also included in the peptide sequences.

Conclusion

In summary, our study revealed anti-fatigue effects of pea peptides for the first time. The anti-fatigue effect of pea peptides is mainly achieved by increasing the glycogen content in muscle and liver, eliminating accumulated lactic acid, inhibiting in vivo oxidation induced by free radicals and improving the immunity function of the body. Further research should focus on exploring the anti-fatigue mechanism, including how pea peptide enhances enzyme (LDH, SOD and GSH-Px) activity and promotes insulin secretion in vivo. In addition, the regulation effect of pea peptides on human immunity still needs to be further explored.

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