Advances in the production and functional properties of corn protein peptides

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Abstract: The corn peptides are a low molecular polypeptide produced by hydrolysis of corn protein. It has many physiological effects such as anti-alcoholism, liver-protection, antihypertension, antitumor, and antioxidation. The recent researches of corn peptides from domestic and foreign literature were integrated in this paper. The production methods and functional properties of corn peptides were summarized. The related researches of enzymatic hydrolysis and microbial fermentation of corn protein peptides were discussed in detail, and a variety of physiological functions of corn protein peptides were analysed. This paper could provide reference for the development and utilization of corn protein peptides.

1. Introduction
Corn (Zea mays L.) is an annual herb of the gramineae family. Corn originate from Latin America and grow world-wide in many regions such as Africa and Asia, where it is consumed as “corn on the cob”.[1] Compared to other cereal crops, such as rice and wheat, corn has better cold tolerance, drought endurance and environmental adaptivity, which makes it as one of the world leading crops. Therefore, it is widely applied in feed and food industry. Corn has high nutrient value, except starch, Cron still contains about 11% fiber, 7% protein, prolamin and gluten are the primary ingredients, taking up approximately 40% of the corn protein, globulin and albumin are 8-9%. The components of corn protein powder processed by industries are protein (65%), water (10%), starch (15%), fat (7%), fiber (2%), ash (1%), and carotenoid (100-200mg/kg).[2-3] The corn protein has quite poor water solubility, only has better solubility in ethyl alcohol or isopropyl-ketone, therefore, corn protein’s poor solubility in water is an important reason of limiting its development and utilization. In addition, the components of industrially processed corn protein powder are intricate, directly using it as feed is a huge resource waste. The corn protein powder is also not suitable to be food material, because many standards to judge whether a protein can be the source of high-quality edible protein, and the protein content is only one of them, still need to analyze whether the proportion of amino acid in that protein is coordinated. According to the reference standard is the proportion of amino acids required by human body, if a protein contains more complete kinds of amino acids, and the proportion of amino acids closer to that needed by human body, then it is a high-quality protein. As shown in Table 1, the proportions of amino acids of corn protein include leucine (15.2%), isoleucine (3.4%), methionine (3.6%), lysine (1.9%), and tyrosine (4.1%), tryptophan (0.8%), phenylalanine (4.1%), valine (4.8%), but the proportion of amino acids required for human body for leucine (7.0%), isoleucine (4.0%), methionine (5.5%), lysine (3.5%), and tyrosine (4.1%), tryptophan (1.0%), phenylalanine (3.4%), valine (5.0%)[4]. In conclusion, the contents of lysine and tryptophan in corn protein are relatively low, and those two amino acids cannot be synthesized by human body, therefore, comparing to the proteins from meat, milk, bird, egg etc., corn protein is not an ideal protein source. Additionally, the final product of corn protein has a rough taste,
unique bitter taste, light color and poor solubility, it would cause a great waste of food resource by merely using it as the production of protein roughage or directly disposing it as production waste[5]. For the comprehensive utilization and sustainable development of resource, it is vital to seek solutions to produce high-value protein products from corn protein.

Table 1. The proportions of amino acids

| Amino acids     | Corn protein (%) | Human needs (%) |
|-----------------|------------------|-----------------|
| leucine         | 15.2             | 7.0             |
| isoleucine      | 3.4              | 4.0             |
| methionine      | 3.6              | 5.5             |
| lysine          | 1.9              | 3.5             |
| tyrosine        | 4.1              | 4.1             |
| tryptophan      | 0.8              | 1.0             |
| phenylalanine   | 4.1              | 3.4             |
| valine          | 4.8              | 5.0             |

It may provide a new solution for all the problems mentioned above to use physical, chemical and biological technologies to turn the corn protein into small peptides. Corn peptides are the low molecular weight peptides obtained from the corn protein by enzymes hydrolyzing and purification technology[6]. By comparison, corn peptides have more advantages than its raw materials. Firstly, the peptides have better water solubility, high water retention, and low viscosity in high concentration, etc. And it is not easily to condense in the acid condition or sediment near isoelectric point. Secondly, the corn peptides also get strong emulsifying ability, better foaming capability, and better stability. In addition, the corn peptides still have good effect in digestive, nutrition absorption and many physiological activities, such as anti-oxidant activities, anti-fatigue, improving human immunity, lowering blood glucose concentration, reducing blood pressure, promoting ethanol metabolism, protecting liver and other benefits[7-10].

2. Production of corn protein peptides

2.1 Enzymatic hydrolysis

Enzymatic hydrolysis is a method to produce the corn protein peptides by hydrolysis of corn protein powder utilizing protease under certain circumstances. This method has many advantages, such as mild reaction condition, strong hydrolysis action specificity, high selectivity, generally without side reaction, and easily controllable reaction process. The enzymes applied in producing corn peptides can be divided into three types: alkaline protease, neutral protease and acidic protease. The optimum conditions for catalytic reactions are generally selected according to the enzyme used.

According to the types of the enzymes, the enzymatic hydrolysis also can be divided into single enzymatic method and complex enzymatic method. The single enzymatic method refers to only utilize one type enzyme during the production of corn protein peptides. As shown in Figure 1, normally, single enzymatic method is applied to prepare corn peptides in the following sequences: pretreatment of corn
protein, enzymatic hydrolysis, enzyme inactivation, collection of supernatants, dehydrate and refine the supernatant and finished product of corn peptides. Many studies of the preparation of corn peptides by single enzymatic hydrolysis have been reported in recent years. For example, Liu et al. [11] used alkaline protease to hydrolyze corn protein. Their results showed that under the optimal conditions of: concentration of substrate 3%, enzyme to substrate ratio 3.0%, temperature 45 ℃, hydrolysis time 1 h and pH 9.0, the degree of hydrolysis is 38%. And Zhao et al. [12] used neutral protease to hydrolyze corn protein, under the conditions of concentration of substrate 5%, enzyme to substrate ratio 2.0%, temperature 50℃, hydrolysis time 4h and pH 7.5, the degree of hydrolysis and nitrophen of the reaction reach 23.74% and 24.02%, respectively. In general, the hydrolysis of corn protein by alkaline protease is higher than that by neutral protease and acidic protease. Even if using the same protease, the quality of corn protein peptides still varies due to the different raw materials and processing conditions.

Using the single enzymatic method, the degree of hydrolysis usually reaches the limit and no more increasing. Having various enzymes used together, the sites of them are different and depending on the different types of peptides chain, which resulted in the increase of the cutting point of the peptides chain and the number of broken peptides chain [13-14]. Compared to the single enzymatic method, the complex enzymatic method equips with many advantages, such as strong enzyme activity, fast hydrolysis rate, high efficiency and the high yield of the prepared corn protein peptides. The hydrolysis effect of the combination alkaline enzyme and neutral enzyme is better than that of any single enzyme[14]. The studies of Zhang et al. [15] showed corn protein powder was hydrolyzed by Alcalase AF 2.4L and Flavourzyme under the optimum condition for 2 hours, the degree of hydrolysis was up to 31% in two hours as well as the yield of oligopeptides was 70%. With the further research on the production of corn peptides by complex enzymatic method, more progress and achievements will be done in the future.

2.2 Microbial fermentation

Microbial fermentation is using the specific types of microorganisms in the process of metabolism, relying on their own internal enzyme system to produce peptides from corn protein. The general processes include: strain screening, strain culture, strain identification and optimization, inoculation culture, aerobic fermentation culture, and finished product of corn protein peptides [16]. Compared to the enzymatic hydrolysis method, the microbial fermentation can reduce the production of bitter peptides while preparing corn peptides, improving their tastes and flavor, and lower the production cost. Wu et al.[17] obtained a bacillus subtilis which can hydrolyze protein with high ability by casein plate process, and identified its physiological and biochemistry function. After cultivation and optimization, it can ferment corn protein powder to produce corn protein peptides, the result showed its enzyme activity is 1468.7 U/mL, and the corn protein degree of hydrolysis is 9.64%. For now, fewer researches on production of corn protein peptides by microbial fermentation have been done compared to the enzyme hydrolysis method. Although the development prospect is promising, there are still many technical problems to be solved.

3. Functional properties of corn protein peptides

3.1 Anti-alcoholism and liver-protection

Drinking is a traditional culture, moderate drinking can promote blood circulation and bring certain health benefits, but excessive drinking will cause drunkenness, severe coma and death. At present, a lot of researches of the anti-alcoholism effect of corn protein peptides have been reported, proving that the corn peptides can promote the ethanol metabolism to achieve the anti-alcoholism effect. Yamaguchi et al. [18] studied the impact of corn peptides on the changes of blood ethanol content in rats treated with alcohol intragastric administration, and found that the blood ethanol and acetaldehyde content dropped observably after corn peptides intragastric administration, which indicates the corn peptides can promote ethanol metabolism. Further research shows that corn peptides may have the effect on the reduction of increase in blood ethanol level after alcohol intake by the marked elevation of plasma alanine and leucine to produce oxidized coenzyme I, which speeds up the metabolism of ethanol in the liver[19]. For the
advanced study of understanding the mechanism of anti-alcoholism, Guo et al.\textsuperscript{[20]} used Alcalase to hydrolyze corn protein and obtained corn peptides fractions with different molecular mass (Mm) by ultrafiltration. Their result showed the fraction administration (200 mg/kg body weight) with Mm fewer than 5,000 Da (CP < 5,000) demonstrated maximum ability to decrease blood alcohol concentration in mice. Ma et al.\textsuperscript{[21]} evaluated the ability of corn peptides to facilitate alcohol metabolism in vivo, the peptides were separated and classified by HPLC–MS/MS and ultrafiltration, and the amino acid sequence of the peptide was determined as Q-L-L-P-F. Zhang et al.\textsuperscript{[22]} investigated that corn peptides have a significant protective effect on early alcoholic liver injury in rats, and their result indicated that the corn peptides treatment prevented the elevation of serum aminotransferase and alleviated the hepatic histological damage that was induced by alcohol. Guo et al.\textsuperscript{[23]} evidenced biochemical parameters and liver histopathological characters of mice to explore the significant protection of corn peptides against BCG/LPS-induced hepatocellular injury, and revealed that the best hepatoprotective effect of CP treatment was observed at the dose of 600 mg/kg (body weight). Yu et al.\textsuperscript{[24]} evaluated the liver-protection effects of corn peptides against carbon tetrachloride (CCl4)-induced liver injury in mice, using the male mice as laboratory animals to detect the concentration of serum transaminase, malondialdehyde, and glutathione in them and the activity of superoxide dismutase in the liver as the evaluation indexes for liver injury. Results showed that, compared with the model group, the mice with corn peptides intake had a significant decrease of the aminotransferase activities in serum and malondialdehyde level in liver, and by a significant increase of superoxide dismutase activity and glutathione level in liver. The biochemical findings supplemented by histopathological examination of liver sections demonstrated the treatment of corn peptides showed a significant protective effect to alleviate CCl4-induced hepatocellular injury. In conclusion, the corn peptides have great effect in anti-alcoholism and liver-protection with a broad market prospect and the value of development and utilization.

3.2 Antihypertension effect

Hypertension is a clinical syndrome characterized by increased systemic arterial blood pressure (systolic blood pressure ≥140 MMHG, diastolic blood pressure ≥90 MMHG) accompanied by functional or organic damage to the heart, brain, kidney and other organs. Abundant studies show that corn protein peptides have the antihypertensive function by inhibiting angiotensin-converting enzymes (ACE), the carboxyl terminus of them with hypotensive activity is proline or aromatic amino acid, and the amino terminus of that is hydrophobic amino acid. Miyoshi et al.\textsuperscript{[25]} studied the ACE produced by Thermolysin enzyme hydrolyzing corn protein, and analyzed its chemical structures. They obtained plenty ACE amino acid sequence dominated by tripeptide, most of them were found the carboxyl terminus to be proline such as Leu-Arg-Pro, Leu-Ser-Pro, and Leu-Gln-Pro. Then these peptides were synthesized by a solid phase procedure and verified the inhibitory activity of ACE. Lin et al.\textsuperscript{[26]} with complex enzymatic method, frozen centrifugation, super nanofiltration separation and purification, low-temperature drying technology for corn protein peptide pilot production, through animal experiments and ACE inhibitory activity test in vitro showed that the activity of corn protein peptides have the good antihypertensive effect, and then using high performance liquid chromatography - electrospray level 4 pole time of flight mass spectrometry technology separation and purification of the lower blood pressure peptide, get the ACE of active peptides principal component for alanine - tyrosine amino acid sequence. Overall, with the increasing number of hypertension patients in world year by year, developing the antihypertensive products with corn peptides as the main ingredient may be an effectual measure to prevent or as adjuvant therapy of hypertension.

3.3 Antitumor effect

Some studies have shown that corn peptides can cause the change of calcium concentration in cancer cells, so that cancer cell morphology changes, and then apoptosis. Yamaguchi et al.\textsuperscript{[27]} used the 7,12-dimethylbenz[a]anthracene (DMBA) to induce mammary tumor in female Sprague-Dawley rats as control groups, fed the other group rats on both DMBA and corn peptides, and then result showed the corn peptides had significant inhibitory effect on the occurrence and growth of mammary tumor induced...
by DMBA. Li et al. [28] evaluated the anti-tumor mechanism of corn peptides, the upshot demonstrated, in vitro, corn peptides significantly inhibited human hepatoma cell viability, meanwhile the correlation between growth inhibition rate of human hepatoma cell and the doses of corn peptides were used and the treatment time of corn peptides on cancer cells. The in-vivo result showed that corn peptides could not only inhibit the growth of the tumor, but also enhance the spleen function, and could prolong the survival time in rats with cancer. Therefore, corn peptides may develop to be a new resource health food to prevent and inhibit cancer, and as a drug for adjuvant treatment of cancer patients.

3.4 Antioxidative effect

Many studies have shown that corn peptides have certain antioxidant activity in-vitro or in-vivo. Zhuang et al. [29] applied the DPPH (1, 1-diphenyl-2-trinitrophenylhydrazine) free radical scavenging capacity and metal ion chelating capacity were used as evaluation indexes, optimized the hydrolysis conditions by multiple linear regression and response surface methodology alkaline protease and compound flavor protease hydrolysis, and then by Sephadex G-25 gel filtration chromatography separation classification to the strongest antioxidant activity of components, LC/MS/MS on the component separation classification, confirm the principal component of amino acid sequence is: Gly-His-Lys-Pro-Ser. Xu et al. [30] has reported the antioxidative activity of corn peptide Leu-Asp-Tyr-Glu (LDYE) in cellular level and molecular level, and their results illustrated the corn peptides, in certain level, can inhibit the Cardiomyocyte mitochondria damage caused by free radicals. And its mechanism might be that corn antioxidative peptides can effectively remove the free radicals generated by Fenton reaction (hydroxyl radicals with strong oxidizing ability generated by hydrogen peroxide in the presence of ferric ions), and prevent the structural and functional damage of Cardiomyocyte mitochondria caused by hydroxyl radicals. The study of Zhou et al. [31] found that the molecular weight distribution of corn peptides are correlated with its antioxidant activity, which means the antioxidant activity of corn peptides with small molecular weight is higher than that of corn peptides with large molecular weight. In summary, corn peptides achieve their antioxidant activity by inhibiting the oxidation of biological macromolecules or by removing free radical products in the body, including superoxide anion radicals and hydroxyl radicals.

4. Conclusion

With the development of society economy and the improvement of living standard, the consumers have more and more request for health, nature, nutrition, safety and hygiene. The corn peptides have been applied to dinks, medicine, anti-alcoholism products, special food and so on, and have huge market potential, due to its pure natural, easy to be digested and absorbed by the human body, with a variety of physiological effects, and non-toxic side effects and other excellent characteristics. At present, some studies about the corn peptides, however, still have some drawbacks, such as, the production cost of enzymatic hydrolysis is high, the preparation technology of microbial fermentation is still not mature, and the purification technology needs to be improved. We believe, with the development and improvement of the researches and applied level of corn peptides, whose industry will have a brighter prospect.

References
[1] Ai Y, Jane J. Macronutrients in corn and human. COMPR REV FOOD SCI F, 15(3), 581-598 (2016)
[2] Subedi K D, Ma B L. Corn crop production: growth, fertilization and yield. Agriculture issues and policies, (2009)
[3] Assefa Y, Roozeboom K L, Thompson C, et al. Corn and grain sorghum comparison: all things considered. Academic Press(2013)
[4] Rooney L W, Serna-Saldivar S O. Tortillas: Wheat Flour and Corn Products. Elsevier (2015)
[5] Compendium of bioenergy plants: Corn. CRC Press, (2014)
[6] Jin D, Liu X, Zheng X, et al. Preparation of antioxidative corn protein hydrolysates, purification and evaluation of three novel corn antioxidant peptides, FOOD CHEM, 204, 427-436(2016)
[7] Wang Y, Chen H, Wang J, et al. Preparation of active corn peptides from zein through double
enzymes immobilized with calcium alginate–chitosan beads, PROCESS BIOCHEM, 49(10), 1682-16(2014)

[8] Liang Q, Chalamaiah M, Ren X, et al. Identification of New Anti-inflammatory Peptides from Zein Hydrolysate after Simulated Gastrointestinal Digestion and Transport in Caco-2 Cells, J AGR FOOD CHEM, 66(5), 1114-1120(2018)

[9] Tang N, Zhuang IH. Evaluation of antioxidant activities of zein protein fractions, J FOOD SCI, 79(11), C2174-C2184(2014)

[10] Tang X, He Z, Dai Y, et al. Peptide Fractionation and Free Radical Scavenging Activity of Zein Hydrolysate. J AGR FOOD CHEM, 58(1), 587-593(2010)

[11] Liu Z, Dong Y, Wang Z. Study on alkaline protease hydrolysis of corn gluten meal, J FOOD SCI, 11, 130-133(2008)

[12] Zhao C, Liu X, Li Z, et al. Optimization of enzymolysis of corn gluten meal by neuramidase. Modern Food Science and Technology, 24(11), 1164-1166(2012)

[13] Syaftika N, Matsumura Y. Simple equation for enzymatic hydrolysis of cellulose using cellulase complex and beta-glucosidase mixture, J JPN PETROL INST, 60(6), 322-328(2017)

[14] Li Y, Cong J, Chen G. Double enzyme hydrolysis for preparation of corn peptides. J FOOD SCI, 31(1), 145-148(2010)

[15] Zhang X, Chen Q, Pang G. The preparation of the oligopeptides and the investigations of the antioxidative activity of oligopeptides from the corn gluten meal, J FOOD SCI, 26(9), 33-35(2005)

[16] Zhu Z, Xu Y, Qiu Y, et al. Optimization on liquid-state fermentation process of composite peptides from soybean and corn proteins. Science and Technology of Food Industry, 23, 46, 245-251(2015)

[17] Wu Z, Chao L. Screening of hydrolyzed corn gluten meal strains. Cereals and Oils Processing, 11, 92-97(2008)

[18] Yamaguchi, Magoichi, Takada, Masayasu, Nozaki, Osamu, et al. Preparation of corn peptide from corn gluten meal and its administration effect on alcohol metabolism in stroke-prone spontaneously hypertensive rats, J NUTR SCI VITAMINOL, 42(3), 219-231(1996)

[19] Yamaguchi M, Nishikiori F, Ito M, et al. The effects of corn peptide ingestion on facilitating alcohol metabolism in healthy men, BIOSCI BIOTECH BIOCH, 61(9), 1474-1481(1997)

[20] Yu G, Li J, He H, et al. Ultrafiltration preparation of potent bioactive corn peptide as alcohol metabolism stimulator in vivo and study on its mechanism of action. J FOOD BIOCHEM, 37(2), 161-167(2013)

[21] Ma Z, Zhang W, Yu G, et al. The primary structure identification of a corn peptide facilitating alcohol metabolism by HPLC-MS/MS. PEPTIDES, 37(1), 138-143(2012)

[22] Zhang F, Zhang J, Li Y. Corn oligopeptides protect against early alcoholic liver injury in rats, FOOD CHEM TOXICOL, 50(6), 2149-2154(2012)

[23] Guo H, Sun J, He H, et al. Antihypertotoxic effect of corn peptides against Bacillus Calmette-Guerin/lipopolysaccharide-induced liver injury in mice, FOOD CHEM TOXICOL, 47(10), 2431-2435(2009)

[24] Yu G, Lv J, He H, et al. Hepatoprotective effects of corn peptides against carbon tetrachloride-induced liver injury in mice. J FOOD BIOCHEM, 2012, 36(4), 458-464.

[25] Miyoshi S, Ishikawa H, Kaneko T, et al. Structures and activity of angiotensin-converting enzyme inhibitors in an α-zein hydrolysate. Agricultural and Biological Chemistry, 55(5), 1313-1318(1991)

[26] Lin F, Chen L, Liang R, et al. Pilot-scale production of low molecular weight peptides from corn wet milling byproducts and the antihypertensive effects in vivo and in vitro, J FOOD CHEM, 124(3), 801-807(2011)

[27] Yamaguchi M, Takeuchi M, Ebihara K. Inhibitory effect of peptide prepared from corn gluten meal on 7, 12-dimethylbenz [a] anthracene-induced mammary tumor progression in rats, NUTR RES, 17(7), 1121-1130(1997)
[28] Li J T, Zhang J L, He H, et al. Apoptosis in human hepatoma HepG2 cells induced by corn peptides and its anti-tumor efficacy in H22 tumor bearing mice, FOOD CHEM TOXICOL, 51, 297-305(2013)

[29] Zhuang H, Tang N, Dong S, et al. Optimisation of antioxidant peptide preparation from corn gluten meal, J SCI FOOD AGR, 93(13), 3264-327(2013)

[30] Xu L, Li X, Huang Y, et al. Protection of small molecule corn peptide Leu-Asp-Tyr-Glu from mitochondria against oxidative damage, CHEM J CHINESE U, 25(6), 1073-1075(2004)

[31] Zhou K, Sun S, Canning C. Production and functional characterisation of antioxidative hydrolysates from corn protein via enzymatic hydrolysis and ultrafiltration, J FOOD CHEM, 135(3), 1192-1197(2012)