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正交实验优化南极磷虾蛋白肽的纳滤脱盐工艺*

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摘要 为建立南极磷虾(*Euphausia superba*)蛋白肽纳滤脱盐工艺, 以脱盐率和蛋白损失率为评价指标, 通过单因素实验和正交实验对影响南极磷虾蛋白肽纳滤脱盐效果的主要因素(蛋白肽浓度、压力、循环次数)进行优化。结果显示, 采用纳滤技术对南极磷虾蛋白肽进行脱盐处理的最佳工艺条件: 蛋白肽浓度为 3%、纳滤压力为 1.2 MPa、循环次数 3 次, 在该条件下, 南极磷虾蛋白肽的脱盐率达到(86.35±2.11)%、蛋白损失率为(9.10±0.35)%。采用优化工艺获得的南极磷虾蛋白肽的盐分含量为(1.14±0.12)%, 蛋白质含量为(92.73±2.29)%, 相对分子质量主要分布于 3000 Da 以下, 氨基酸组成合理且符合联合国粮农组织/世界卫生组织规定的标准。研究将为高品质南极磷虾蛋白肽产品开发提供技术支撑。

关键词 南极磷虾; 蛋白肽; 正交实验; 纳滤; 脱盐

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南极磷虾(*Euphausia superba*)是地球上最大的单种生物资源, 生物量约为 6.5~10 亿 t, 具有打造成为我国第二个远洋渔业的巨大潜力(赵宪勇等, 2016; 曹荣等, 2018; 潘晓炀等, 2019)。南极磷虾蛋白含量丰富, 达到干重的 65% (Tou *et al*, 2007; Wang *et al*, 2011), 是全球最大的海洋动物蛋白资源宝库(刘志东等, 2017; 刘柯欣等, 2022)。近年来, 相关研究已证明, 南极磷虾多肽具有良好的生理活性, 包括改善骨质疏松(Wang *et al*, 2015; Han *et al*, 2018)、调节血糖(Ji *et al*, 2017)、降血压(Zhao *et al*, 2019)、抗氧化(刘小芳等, 2020; 郑景如等, 2020)、缓解疲劳(徐恺, 2012)和改善皮肤光老化(于建伟等, 2021)等。鉴于丰富的资源量和良好的营养功能, 南极磷虾蛋白肽的生产开发得到行业关注。目前, 关于南极磷虾蛋白肽制备的研究集中在酶解工艺优化方面, 主要涉及最适酶的筛选、酶解条件优化等(刘云娇等, 2019; 张华丹等, 2019; 孙如男等, 2020)。然而, 由于南极磷虾自身矿物质含量较高, 且在酶解处理时为调节最适反应条件需引入酸或碱, 极易导致酶解后获得的蛋白肽盐分含量较高。较高的盐分含量, 不仅会影响蛋白肽的口感和功能, 也会对消费者健康带来一定的潜在风险, 极大的限制产品的应用(Ras *et al*, 2000)。因此, 针对酶解后的南极磷虾蛋白肽进行脱盐处理十分必要, 但目前尚未见相关研究报道。

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针对生物活性物质的脱盐方法主要包括透析、超滤、纳滤、电渗析和大孔树脂吸附等(夏光华等, 2013)。纳滤是一种由压差驱动的膜分离技术, 作为目前膜分离研究领域的热点, 其具有基于物理分离有利于保持物质活性和风味、不发生相变节能因而运行成本低、滤膜通量高对单价离子截留率低等优点(于国才等, 2011), 近年来, 在食品行业中越来越多地被应用于蛋白肽的分离、浓缩和脱盐过程(刘亮等, 2013; Benedetti *et al*, 2016; 王长伟等, 2019)。综上所述, 本研究采用纳滤技术对南极磷虾蛋白肽进行脱盐处理, 通过单因素实验和正交实验对蛋白肽浓度、纳滤压力、循环次数等工艺条件进行优化, 以期为高品质南极磷虾蛋白肽的工业化生产应用提供技术支持。

1　材料与方法

1.1　实验材料

脱脂南极磷虾粉: 山东青岛南极维康生物科技有限公司; 碱性蛋白酶(Alcalase 2.4 L, 200 000 U/mL): 丹麦诺维信生物技术有限公司; Lowry 法蛋白浓度测定试剂盒: 北京索莱宝科技有限公司; 磷酸二氢钠、磷酸氢二钠、NaOH、HCl 等试剂: 分析纯, 国药集团化学试剂有限公司。

1.2　仪器设备

XS-Y-MINI-2 型有机膜多功能实验设备: 南京诺润机械科技有限公司; TFN-18-200 200D 型纳滤膜: 山东博纳生物科技集团有限公司; SRJX-8-13 型马弗炉: 天津泰斯特仪器有限公司; DK-98-II 型电炉: 天津泰斯特仪器有限公司; UV1-102II 型紫外/可见分光光度计: 上海天美科学仪器有限公司; BILON-6000Y 型喷雾干燥机: 上海比朗仪器制造有限公司; ST3100 型 pH 计: 奥豪斯仪器(常州)有限公司; BSA224S-CW 型电子分析天平: 赛多利斯科学仪器有限公司; SHA-B 型恒温振荡器: 常州智博瑞仪器制造有限公司; HH-2 型数显恒温水浴锅: 国华电器有限公司; LXJ-IIB 型离心机: 上海安亭科学仪器厂。

1.3　实验方法

1.3.1　*南极磷虾蛋白肽的制备*　　参考张华丹等(2019)的实验方法制备南极磷虾蛋白肽: 称取适量脱脂南极磷虾粉, 按照料液比 1∶6 (g/mL)添加 pH 为 7.5 的磷酸盐缓冲液, 混匀, 根据脱脂南极磷虾粉质量加入 2% (*v*/*m*)的碱性蛋白酶, 55℃酶解 4 h 后于 95℃加热灭酶 20 min, 冷却至室温, 5000 r/min 离心 20 min, 收集上清液, 在进风温度为 200℃、出风温度为 85℃条件下, 经喷雾干燥即得南极磷虾蛋白肽。

1.3.2　*纳滤脱盐处理工艺*　　南极磷虾蛋白肽经蒸馏水稀释配制成实验浓度, 在纳滤设备料液罐中投料 2 L, 控制过膜压力稳定在实验压力进行脱盐处理, 待压力降至 0.3 MPa 以下, 使用蒸馏水将罐内溶液补至 2 L 后继续处理相应实验循环次数。待最后 1 次循环处理压力降至 0.3 MPa 后, 将处理液经管路放出, 测定相应评价指标。

1.3.3　*单因素实验设计*　　进行蛋白肽浓度、纳滤压力、循环次数的单因素优化: 蛋白肽浓度为 3%, 循环次数为 1 次, 纳滤压力分别为 0.6、0.8、1.0、1.2 和 1.4 MPa 进行纳滤脱盐; 压力为 1.0 MPa, 循环次数为 1 次, 蛋白肽浓度分别为 1%、2%、3%、4%和 5%进行纳滤脱盐; 蛋白肽浓度为 3%, 压力为 1.0 MPa, 循环次数分别为 1、2、3、4、5 进行纳滤脱盐。以南极磷虾蛋白肽的脱盐率和蛋白损失率为评价指标, 确定各单因素的最佳条件。

1.3.4　*正交实验设计*　　根据单因素实验结果进行蛋白肽浓度、纳滤压力、循环次数等三因素的正交实验优化, 实验水平设置见表 1。根据正交表 $L_9(3^3)$进行实验, 根据南极磷虾蛋白肽的脱盐率和蛋白损失率结果, 确定最佳脱盐工艺条件。

表 1　正交实验因素水平表

Tab.1　Factors and levels used in the orthogonal tests

水平 Level	因素 Factor		
	纳滤压力 Nanofiltration pressure/MPa	蛋白肽浓度 Peptides concentration/%	循环次数 Cycle times
1	0.8	2	1
2	1.0	3	2
3	1.2	4	3

1.3.5　*脱盐率的测定*　　脱盐处理前后料液中的盐分含量按照 SC/T 3011-2001《水产品中盐分的测定》的规定执行。脱盐率按照以下公式计算:

脱盐率/%=(脱盐前料液中盐分含量–脱盐后料液中盐分含量)/脱盐前料液中盐分含量×100

1.3.6　*蛋白损失率的测定*　　按照试剂盒说明书的规定测定脱盐处理前后料液中的蛋白含量。蛋白损失率按照以下公式计算:

蛋白损失率/%=(脱盐前料液中蛋白含量–脱盐后料液中蛋白含量)/脱盐前料液中蛋白含量×100

1.3.7　*脱盐处理后南极磷虾蛋白肽的组成分析*　　按照正交实验确定的最优纳滤工艺条件对南极磷虾蛋白肽进行脱盐处理, 处理后的蛋白肽溶液经喷雾干燥

后进行组成分析：水分含量按照 GB 5009.3-2016《食品安全国家标准 食品中水分的测定》的规定执行；蛋白质含量按照 GB 5009.5-2016《食品安全国家标准 食品中蛋白质的测定》的规定执行；灰分含量按照 GB 5009.4-2016《食品安全国家标准 食品中灰分的测定》的规定执行；盐分含量按照 SC/T 3011-2001《水产品中盐分的测定》的规定执行；分子量分布按照 GB 31645-2018《食品安全国家标准 胶原蛋白肽》中附录 A 的规定执行；氨基酸组成按照 GB 5009.124-2016《食品安全国家标准 食品中氨基酸的测定》的规定执行。

1.4 数据处理

实验数据采用平均值±标准差(Mean±SD)的形式表示，采用 Excel 2016、IBM SPSS 20.0 和 Origin 2018 等软件进行数据处理分析和图表绘制。采用单因素方差分析(one-way ANOVA)进行组间比较，$P<0.05$ 为差异显著。

2 结果与讨论

2.1 单因素实验

蛋白肽浓度对南极磷虾蛋白肽的脱盐率和蛋白损失率的影响结果见图 1。当蛋白肽浓度为 1%~3%时，脱盐率随着蛋白肽浓度的增加而逐渐升高；当蛋白肽浓度为 3%时，脱盐率最高，达到(69.07±2.25)%，显著高于其他实验组($P<0.05$)；而后随着蛋白肽浓度的增加，脱盐率有所下降。蛋白肽浓度较低时，纳滤时间较短，导致脱盐效果不佳；而当蛋白浓度较高时，纳滤膜表面被截留的溶质质量浓度不断增加，浓差极化不断加强，膜的透过通量下降，进而影响脱盐效果(岳三峰, 2017)。蛋白肽浓度在 1%~5%范围内进行脱盐处理，各实验组的蛋白损失率在(7.63±0.22)%~(8.75±0.31)%之间；处理过程中，逃水现象和浓差极化现象的发生引起蛋白损失(刘亮等, 2013)，但各实验组的蛋白损失率均可控制在较低水平，达到良好的蛋白回收效果。因此，确定蛋白肽浓度 3%为最佳实验条件。

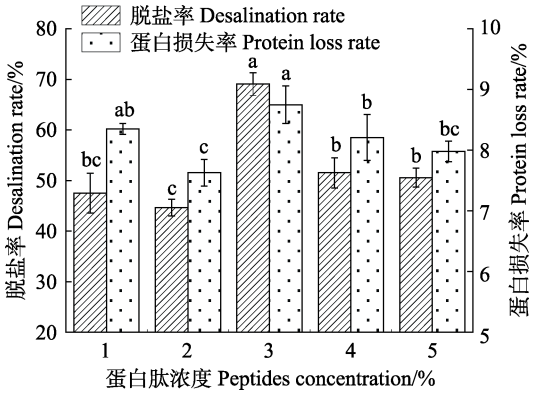

图 1 蛋白肽浓度对南极磷虾蛋白肽脱盐率和蛋白损失率的影响

Fig.1 Effect of peptides concentration on the desalination rate and protein loss rate during nanofiltration of Antarctic krill peptides

不同字母表示不同实验组间具有显著性差异($P<0.05$)。下同。

Different letters represent significant differences between different experimental groups ($P<0.05$). The same as below.

纳滤压力对南极磷虾蛋白肽的脱盐率和蛋白损失率的影响结果见图 2。在 0.6~1.0 MPa 的纳滤压力范围内，随着压力增加，蛋白肽的脱盐率不断增加；当纳滤压力为 1.0 MPa 时，脱盐率为(77.22±2.65)%，显著高于 0.6 MPa 和 0.8 MPa 实验组($P<0.05$)；而后随着纳滤压力的增加，脱盐率有所下降。在纳滤压力为 0.6~1.4 MPa 范围内进行脱盐处理，各实验组的蛋白损失率在(7.87±0.13)%~(9.01±0.33)%之间，蛋白损失均较少。在实际生产中，压力过高会使滤膜发生极限压降现象，形成水锤作用而导致滤膜衰减加剧，损坏膜组件(杨砾等, 2012)。因此，确定纳滤压力 1.0 MPa 为最佳实验条件。

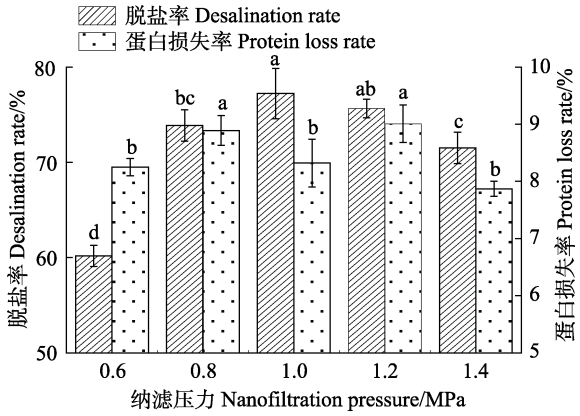

图 2 纳滤压力对南极磷虾蛋白肽脱盐率和蛋白损失率的影响

Fig.2 Effect of pressure on desalination rate and protein loss rate during nanofiltration of Antarctic krill peptides

循环次数对南极磷虾蛋白肽的脱盐率和蛋白损失率的影响结果见图 3。在循环次数为 1~3 次时，随着循环次数的增多，南极磷虾蛋白肽的脱盐率逐渐升高，而随着循环次数的增加，脱盐率趋于平稳；在循环次数在 1~5 次范围内进行脱盐处理，各实验组的蛋白损失率在(7.35±0.12)%~(8.36±0.19)%之间，蛋白损失均较少。补水循环会降低浓差极化现象，提高渗透速率，提升脱盐处理效果(孔凡丕等, 2010)。考虑到循环处理 2 次，脱盐效果已较好，达到(82.88±2.32)%，与循环处理 3 次无明显差异($P>0.05$)，且循环次数增多会增加实际生产操作难度和成本，因此，确定循环次数 2 次为最佳实验条件。

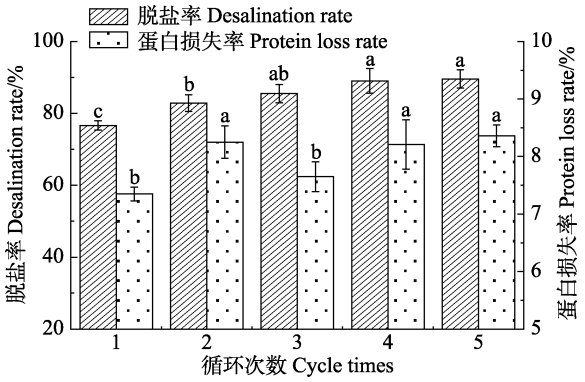

图 3　循环次数对南极磷虾蛋白肽脱盐率和蛋白损失率的影响

Fig.3　Effect of cycle times on desalination rate and protein loss rate during nanofiltration of Antarctic krill peptides

2.2　正交实验

在单因素实验基础上，采用 $L_9(3^3)$正交实验设计研究蛋白肽浓度、纳滤压力和循环次数等三因素对脱盐效果的影响，结果见表 2。由表 2 可知，极差 *R* 的波动幅度代表了实验因素对脱盐效果的影响程度，各实验因素中，蛋白肽浓度对脱盐效果的影响最大，其次为循环次数，影响最小的为纳滤压力。经 *k* 值分析得到南极磷虾蛋白肽脱盐处理最佳条件：蛋白肽浓度 *k*2、循环次数 *k*3、纳滤压力 *k*3，即蛋白肽浓度 3%，压力 1.2 MPa，循环次数 3 次。经验证，在推荐的最佳工艺条件下，南极磷虾蛋白肽的脱盐率可达到(86.35±2.11)%，蛋白损失率为(9.10±0.35)%，证明该工艺可行。

表 2　正交实验结果

Tab.2　The results of the orthogonal tests

实验编号 Experimental number	蛋白肽浓度 Peptides concentration/%	压力 Nanofiltration pressure/MPa	循环次数 Cycle times	脱盐率 Desalination rate/%	蛋白损失率 Protein loss rate/%
1	2	0.80	1.00	37.43±0.97	7.66±0.33
2	4	0.80	2.00	65.08±2.06	8.29±0.54
3	3	0.80	3.00	86.21±1.76	8.73±0.37
4	4	1.00	1.00	55.16±1.53	8.54±0.25
5	3	1.00	2.00	83.39±1.08	8.86±0.25
6	2	1.00	3.00	54.14±2.51	8.47±0.28
7	3	1.20	1.00	71.50±2.01	8.54±0.30
8	2	1.20	2.00	49.13±1.13	8.91±0.17
9	4	1.20	3.00	80.40±3.10	9.12±0.41
*k*1	47	62.90	54.70		
*k*2	80	64.23	65.86		
*k*3	67	67.01	73.58		
R	33	4.10	18.88		

2.3　脱盐处理后南极磷虾蛋白肽的品质

经脱盐处理后获得的南极磷虾蛋白肽的组成分析结果见表 3、图 4。由表 3 可知，采用优化工艺获得的南极磷虾蛋白肽，盐分含量为(1.14±0.12)%，蛋白质含量为(92.73±2.29)%；蛋白肽共检出 16 种氨基酸，含有 7 种必需氨基酸，其中，谷氨酸含量最高，天冬氨酸含量次之，必需氨基酸含量占总氨基酸含量的(40.06±0.10)%，必需氨基酸与非必需氨基酸的比值为(66.82±0.28)%，符合联合国粮农组织/世界卫生组织规定的优质蛋白标准(必需氨基酸占氨基酸总量的 40%，必需氨基酸与非必需氨基酸的比值为 60%)。由图 4 可知，蛋白肽的分子量主要在 189~6500 Da，其中，451~1450 Da 占比最高(38.45±1.37)%，而分子量在

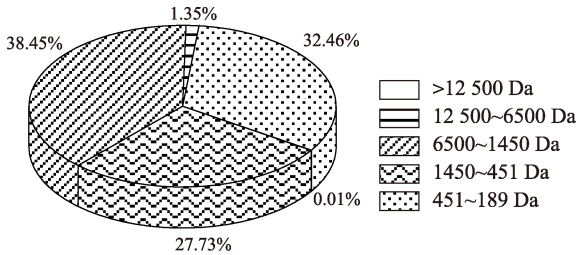

图 4　脱盐后南极磷虾蛋白肽的分子量分布

Fig.4　Molecular weight distribution of the desalinated Antarctic krill peptides

3000 Da 以下的占比达到(88.91±2.19)%，符合生物活性肽分子量分布范围(谢博等, 2021)。综上可知，采用纳滤脱盐后获得的南极磷虾蛋白肽品质良好，营养价值较高。

表 3 脱盐后南极磷虾蛋白肽的组成

Tab.3 The compositions of the desalinated Antarctic krill peptides

检测指标 Detection index	含量 Content/(g/100 g)
水分 Moisture	2.70±0.44
蛋白质 Protein	92.73±2.29
灰分 Ash	3.30±0.10
盐分 Salt	1.14±0.12
氨基酸组成 Amino acid compositions	
天冬氨酸 Aspartate	10.53±0.08
苏氨酸 Threonine	4.17±0.04
丝氨酸 Serine	3.98±0.10
谷氨酸 Glutamate	13.38±0.15
甘氨酸 Glycine	4.99±0.04
丙氨酸 Alanine	5.87±0.03
缬氨酸 Valine	4.88±0.11
甲硫氨酸 Methionine	3.37±0.02
异亮氨酸 Isoleucine	4.41±0.11
亮氨酸 Leucine	7.49±0.04
酪氨酸 Tyrosine	3.99±0.06
苯丙氨酸 Phenylalanine	4.32±0.04
赖氨酸 Lysine	7.41±0.05
组氨酸 Histidine	2.16±0.01
精氨酸 Arginine	5.06±0.03
脯氨酸 Proline	4.00±0.06
氨基酸总量 Total amino acid, TAA	90.01±0.68
必需氨基酸 Essential amino acid, EAA	36.05±0.33
非必需氨基酸 Nonessential amino acid, NEAA	53.96±0.37
必需氨基酸/氨基酸总量 EAA/TAA	40.06±0.10
必需氨基酸/非必需氨基酸 EAA/NEAA	66.82±0.28

3 结论

本研究通过单因素实验和正交实验，确定了影响南极磷虾蛋白肽纳滤脱盐效果的因素顺序：蛋白肽浓度>循环次数>纳滤压力；最佳工艺条件：蛋白肽浓度 3%，纳滤压力 1.2 MPa，循环次数 3 次；在该条件下，南极磷虾蛋白肽的脱盐率可达到(86.35±2.11)%。经优化工艺脱盐处理后获得的南极磷虾蛋白肽盐分含量低、氨基酸组成和分子量分布理想，品质良好。本研究对于南极磷虾蛋白的高效利用和高质产品开发具有重要参考价值。

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Optimization of a Nanofiltration Desalination Process for Antarctic Krill Peptides Using Orthogonal Tests

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Abstract Antarctic krill (*Euphausia superba*), an important group of marine zooplankton in the Southern Ocean, is the only fishery resource with extremely rich reserves and a low degree of development in the world. Antarctic krill is considered to be the greatest potential source of high-quality marine protein resources due to its abundant biomass and high protein content. Peptides prepared from Antarctic krill exhibit multiple physiological activities, including osteoporosis relief, glucose metabolism regulation, blood pressure amelioration, antioxidation, fatigue alleviation, and anti-aging activity. The production and development of Antarctic krill peptides has recently become an industry focus; however, existing research has been limited to the optimization of enzymatic hydrolysis processes, mainly involving the screening of suitable enzymes and the optimization of enzymatic hydrolysis conditions. Due to the high mineral content of Antarctic krill and the introduction of buffer salt in the process of enzymatic hydrolysis, current Antarctic krill peptides products have a high salt content, which leads to poor sensory experience and health risks. Hence, a process for desalination of Antarctic krill peptides is needed. Desalination methods for bioactive substances include dialysis, ultrafiltration, nanofiltration, electrodialysis, and macroporous resin adsorption. In the field of membrane separation, nanofiltration technology has been widely used in the purification, concentration, and desalination of food components owing to its advantages: low operation cost, no introduction of exogenous substances, no destruction of materials, and low rejection rate of monovalent ions. In order to improve product quality and ensure market expansion, the process of desalination of Antarctic krill peptides using nanofiltration technology was studied and optimized in this study.

Defatted Antarctic krill powder was enzymatically hydrolyzed by alkaline protease to obtain Antarctic krill peptides for further use. The main factors affecting the desalination effect of Antarctic krill peptides (peptides concentration, nanofiltration pressure, and cycle times) were optimized by single-factor and orthogonal tests, using the desalination rate and protein loss rate as evaluation indexes. The experimental optimization ranges included peptides concentration of 1%~5%, nanofiltration pressure of 0.6~1.4 MPa and cycle times of 1~5. The salt contents of the samples before and after desalination were quantified using the silver nitrate titration method; the protein contents of the experimental samples were quantified using the Lowry colorimetric method. The quality indexes of the Antarctic krill peptides after treatment (including the basic nutritional composition: moisture content, protein content, ash content, salt content; amino acid composition;

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and molecular weight distribution) were systematically evaluated by the corresponding national standard methods. All experiments were performed in triplicate, and data were expressed as mean ± standard deviation. Excel 2016, IBM SPSS 20.0, and Origin 2018 were used for data analysis and chart drawing.

Single-factor tests revealed that peptides concentration of 3%, nanofiltration pressure of 1.0 MPa and a cycle time of 2 could be selected as the design basis for the $L_9(3^3)$ orthogonal test. The range value of the orthogonal test indicated that the degree of influence of the three factors on the desalination effect was as follows: peptides concentration > cycle times > nanofiltration pressure. The optimum conditions for desalting Antarctic krill peptides obtained by *k* value analysis were as follows: peptides concentration of 3.0%, nanofiltration pressure of 1.2 MPa and a cycle time of 3. Under the optimal condition, the desalination rate of the Antarctic krill peptides reached up to (86.35±2.11)%, and the protein loss rate was controlled at (9.10±0.35)%, demonstrating the feasibility of the process. The salt content of the Antarctic krill peptides after desalination was reduced to (1.14±0.12)% and the protein content was (92.73±2.29)%. The molecular weights of the Antarctic krill peptides after desalination were mainly distributed between 189 Da and 6500 Da, of which the proportion of peptides with molecular weight less than 3000 Da was (88.91±2.19)%, conforming to the molecular weight distribution range of bioactive peptides. The amount of essential amino acids in the Antarctic krill peptides after desalination accounted for (40.06±0.10)% of the total amino acids, and the ratio of essential amino acids to nonessential amino acids was (66.82±0.28)%. The amino acid compositions of the Antarctic krill peptides after desalination were ideal and met the standard stipulated by the FAO/WHO. The established nanofiltration desalination process presented good treatment effects, and the obtained peptides were of good quality and high nutritional value.

The production of Antarctic krill protein-related products may be the next key development for the processing industry, since the sole high-value products of Antarctic krill at present are Antarctic krill oil and its derivatives. The established nanofiltration desalination process has practical application value and would provide technical support for the development of high-quality Antarctic krill peptides. This research provides scientific support for the efficient utilization of Antarctic krill resources.

Key words　Antarctic krill (*Euphausia superba*); Peptides; Orthogonal test; Nanofiltration; Desalination