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Comparison of Nutritional Components of *Allium Mongolicum* Regel Before and After Marination with Whey Liquid

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Abstract *Allium Mongolicum* Regel (AMR) possesses an exceptionally high nutritional value due to its high levels of biominerals, essential trace elements, and amino acids. However, fresh AMR is challenging to store. Local Mongolian residents have been observed to favor AMR marinated in whey liquid, which develops a unique flavor and is less prone to spoilage. This study aims to explore the potential enhancement in the nutritional value and health benefits of pickled AMR in whey by examining its amino acids, fatty acids, minerals, and other nutritional components. The results indicated that, although some nutrients, such as Vitamin C, were partially lost after pickling, the fermented AMR samples had a proportion of essential amino acids that were close to or exceeded the reference amino acids patterns. There was an increase in the content of fatty acids associated with milk fat components, while the content of major saturated fatty acids, such as palmitic acid and stearic acid, significantly decreased. The lactose content, which contributes to product flavor, was significantly increased, along with the levels of iron (Fe), phosphorus (P), zinc (Zn), calcium (Ca), and B vitamins, which are inherent to the nutritional components of whey. Considering all factors, marinating AMR in whey solution addresses the challenge of storing fresh AMR, in addition, providing an effective way to use whey resources. This process enhances the nutritional value, health benefits, and flavor of the product. The findings offer a theoretical foundation for the efficient development and use of whey resources and support the future growth of the AMR fermentation industry.

Keywords: *Allium Mongolicum Regel, Whey liquid, lactic acid bacteria, nutrients, health benefits*

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1. Introduction

Allium mongolicum Regel (AMR) is a perennial herb of the *Allium* genus in the Liliaceae family. It typically grows in deserts, sandy areas, or arid slopes at altitudes between 800 and 2,800 meters [1]. Wild AMR is predominantly found in Inner Mongolia, China. The leaves and flowers of AMR are edible and are traditionally consumed by Chinese Mongolians. AMR is rich in volatile sulfides, steroidal compounds, saponins, flavonoids, polysaccharides, and other bioactive substances, which have demonstrated anti-tumor, antibacterial, anti-inflammatory, antiviral, anti-allergic, antioxidant, and cardiovascular protective properties [2,3]. It also contains high levels of unsaturated fatty acids and complete proteins, with a ratio of essential to total amino acids that match the ideal amino acids model proposed by the Food and Agriculture Organization (FAO) and the World Health Organization (WHO). AMR is also a rich source of essential minerals, particularly potassium and iron, with iron levels 7.7 and 5.0 times higher than those found in celery and spinach, respectively. Among 80 vegetables compared, AMR ranks high in calcium, magnesium, and phosphorus content [4]. Due to its comprehensive nutrient profile, including elevated levels of biominerals, essential trace elements, and amino acids, AMR possesses an exceptionally high nutritional value.

Whey is a semi-transparent yellow-green liquid that remains after the coagulation and filtration of casein from milk, treated with acid, heat, or rennet. It is a byproduct of industrial cheese production, with a cheese-to-whey production ratio of 1:9. The global annual production of whey is approximately 200 million tons [5]. Whey is highly nutritious, containing bioactive whey proteins, lactose, various vitamins, and minerals, which provide several health benefits [6]. However, in China, a significant amount of whey is discharged as wastewater, leading to resource waste, reduced economic benefits for enterprises, and environmental pollution due to the depletion of dissolved oxygen in water caused by whey

lactose [7,8]. This highlights the urgent need for the rational development and use of whey resources.

In recent years, AMR has gained attention from Chinese researchers as a green wild vegetable with both medicinal and nutritional properties. However, there have been no studies on AMR conducted outside of China. Most of the existing research in China focuses on two main areas: the analysis of AMR's flavor and nutritional components and the functional identification of its bioactive compounds [2,4,9]. Researches on the fermentation of AMR has not been reported to date. Freshly picked AMR are challenging to store, often wilting and becoming sticky within 48 hours (h). Local herders typically store them by freezing them. Recent investigations into the living habits of Mongolian residents in Inner Mongolia revealed that many locals prefer to pickle AMR in whey liquid, resulting in a unique flavor. This pickled AMR is a prized dish for entertaining guests, is resistant to spoilage, and can be stored for extended periods [10]. It is well known that lactic acid bacteria are the dominant microorganisms in vegetable fermentation processes. Therefore, this study aimed to evaluate if pickling AMR in whey liquid, as preferred by local herders, improves its taste and nutritional value through lactic acid bacteria fermentation, while also extending its shelf life. The study, also, evaluates whether such quality enhancements could significantly advance the AMR fermentation industry in Inner Mongolia.

2. Materials and Methods

2.1. The Pickling of AMR

The AMR and whey used in this study were sourced from herder families in Saihantala Town, Sunite Right Banner, Xilingol League, Inner Mongolia Autonomous Region, China. Following the traditional method of local herdsmen, fresh scallions are washed, dried, and then layered with a thin coating of salt in a container. Once the container is nearly full, the contents are compacted, whey is added, and the mixture is sealed and marinated at room temperature for a minimum of 7 days before consumption.

2.2 Detection of free amino acids

The extraction procedure for free amino acids was modified from the method described in [11]. Free amino acids were detected using an amino acid analyzer (Biochrom 30+, UK). A 2 g sample was weighed into a 50 mL volumetric flask, dissolved thoroughly, and water was added to reach the mark. The mixture was then allowed to stand for 24 h. Next, 20 mL of the supernatant was transferred to a 50 mL centrifuge tube, followed by the addition of 20 mL of 5% sulfosalicylic acid solution and thorough mixing. The solution was centrifuged at 6000 g for 10 minutes (min) at room temperature, and 20 mL of the supernatant was evaporated using a rotary evaporator. The residue was dissolved in 1 mL of sodium citrate buffer (pH 2.2) and filtered through a 0.45 μm membrane. The amino acid analyzer conditions were as follows: buffer flow rate of 20 mL/h, reaction flow rate of 10 mL/h, Na-type cation resin chromatography column with dimensions of 200 mm × 4.6 mm, UV detection wavelengths of 570 nm and 440 nm, programmed heating with a column temperature range of 55-65-77 °C, reaction tank temperature of 138 °C, and an injection volume of 50 μL.

2.3. Detection of Medium and Long-chain Fatty Acids

An appropriate amount of the sample (0.1 – 1 g) was weighed into a clean imported EP tube, and 4 mL of chloroform was added, followed by mixing through vortexing for 30 seconds (sec). Then, 0.9% NaCl solution was added, and the mixture was vortexed again for 30 sec. The sample was centrifuged at 3325 g for 15 min at room temperature and then allowed to stand. The lower layer of liquid was pipetted and transferred to another test tube, where 2 mL of dichloromethane was added. The mixture was vortexed for 30 sec, centrifuged for 15 min, and the bottom layer was removed. The lower liquid was combined and dried with nitrogen. After drying, 2 mL of methanol (containing 5% sulfuric acid) was added, followed by vortexing for 30 secs, and then the sample was heated in a water bath at 80 °C for 2 h. After cooling, 2 mL of n-hexane and 1 mL of water were added, and the mixture was vortexed for 30 sec and centrifuged at 1,900 g for 5 min. The supernatant was collected, an additional 1 mL of water was added, vortexed for 30 sec, and centrifuged at 1,900 g for 5 min. The supernatant was collected again and dried with nitrogen. An appropriate volume of isooctane was added according to the sample concentration, vortexed for 30 sec, and allowed to stand for 5 min, and the solution was then transferred to the sample vial for detection.

The chromatographic analysis was conducted using an Agilent gas chromatography system (Agilent 7820; Agilent Technologies, USA). A CP Sil 88 gas chromatography column (100 m × 0.25 mm × 0.25 μm, Agilent, USA) was selected based on the properties of the compound. The samples injection volume was 1 μL, with a split ratio of 10:1, high-purity helium as the carrier gas, and a flow rate of 1.0 mL/min. The initial column temperature was set to 100°C and held for 5 min, then increased to 240°C at a rate of 4 °C/min and maintained for 15 min.

For mass spectrometry, a quadrupole mass spectrometry detection system (Agilent 7820; Agilent Technologies, USA) was used, equipped with an electron impact ion source (EI) and operated with a MassHunter workstation.

Using EI, the analyte was detected in a single ion monitoring (SIM) mode. The optimized mass spectrometry conditions were as follows: injection port temperature of 260 °C and quadrupole temperature of 150 °C. The scanning method employed was SIM, with a mass-to-charge ratio (m/z) range of 30 - 550. Data acquisition was performed using MassHunter GC/MS Acquisition (Agilent Technologies), and data processing was carried out with Quant-My-Way (Agilent Technologies).

2.4. Detection of Soluble Sugars

Sugar components were analyzed and detected using a Thermo ICS5000 ion chromatography system (ICS5000, Thermo Fisher Scientific, USA) equipped with

electrochemical detectors. Soluble sugars were extracted following the modified method described as [12]. A 10 - 100 mg sample was placed into a 2.0 mL tube, and 700 μL of 80% ethanol was added. The mixture was shaken at 50 °C for 2 h, then diluted with 700 μL of water and centrifuged at 9,500 g for 3 min. The supernatant was transferred to a new centrifuge tube, and the dilution ratio was determined based on preliminary results before formal testing. The analysis was performed using a CarboPac™ PA1 (250 × 4.0 mm) liquid chromatography column. The mobile phase consisted of A: water and B: 100 mM NaOH. The injection volume was 10 μL, the flow rate was 1.0 mL/min, and the column temperature was set to 30 °C. The elution gradient was as follows: 0 min with A/B (95:5 v/v), 12 min with A/B (90:10 v/v), 15 min with A/B (0:100 v/v), 25 min with A/B (0:100 v/v), 40 min with A/B (95:5 v/v), and 60 min with A/B (95:5 v/v).

2.5. Determination of Vitamins

2.5.1. Estimation of Vitamin B

Accurately weighed 100 - 500 mg of the sample and added 500 μL of 80% methanol (containing 1% acetic acid) for extraction of Vitamin B. Ultrasonic extraction was performed for 15 min, followed by centrifugation at 11,400 g for 10 min to collect the supernatant. This extraction process was repeated three times, and the supernatants from all extractions were combined into a single tube, as described by [13].

Data collection was performed using high-performance liquid chromatography (Vanquish, UPLC, Thermo, USA) and high-resolution mass spectrometry (Q Exactive, Thermo, USA, https://www.thermofisher.com). The chromatography column used was a Waters HSS T3 (50 × 2.1 mm, 1.8 μm). The mobile phases were as follows: Phase A consisted of ultrapure water with 0.1% acetic acid, and Phase B was acetonitrile with 0.1% acetic acid. The flow rate was set to 0.3 mL/min, and the column temperature was maintained at 40 °C. The injection volume was 2 μL. The elution gradient was: 0.0 min, water/acetonitrile (90:10 V/V); 1.0 min, water/acetonitrile (90:10 V/V); 5.0 min, water/acetonitrile (10:90 V/V); 7.0 min, water/acetonitrile (10:90 V/V); 7.1 min, water/acetonitrile (90:10 V/V); 9.0 min, water/acetonitrile (90:10 V/V). The sample was kept in a 4 °C automatic sampler throughout the analysis.

For the electrospray ionization (ESI) source, the conditions were: sheath gas at 40 arb, auxiliary gas at 10 arb, ion spray voltage at 3000 V, and a temperature of 350 °C. The ion transport tube was maintained at 320 °C. The scanning mode used was SIM in positive ion mode, with a first-level scanning range of 80 - 450 m/z.

2.5.2. Determination of Vitamin C

Samples were treated with 1.5 mL of 3% (w/v) metaphosphoric acid (HPO_3) [14]. The mixture was shaken for 30 min at 25 °C and then centrifuged at 11,400 g for 10 min. The supernatant was collected and filtered through 0.22 μm nylon membrane filters. The UPLC conditions were the same as for vitamin B analysis. For the electrospray ionization (ESI) source, the settings were: sheath gas at 40 arb, auxiliary gas at 10 arb, ion spray voltage at -2800 V, and temperature at 350 °C. The ion transport tube temperature was set to 320 °C. The scanning mode was SIM with negative ion mode, and the first-level scanning range was 95 - 1400 m/z.

2.5.3. Determination of Vitamin E

The extraction of tocopherols was performed using a modified method based on a well-homogenized pooled sample (0.1 – 0.5 g) placed in a 15 mL tube containing 2 mL of absolute ethanol and 0.1% BHT. The tube was vortexed and then heated in an 80 °C water bath for 5 min. After heating, 100 μL of potassium hydroxide solution (80% w/v in water) was added, and the sample was vortexed again before being returned to the water bath for 15 min. Following this, 1 mL of purified water and 1 mL of n-hexane were added. The mixture was vortexed and centrifuged at 2,850 g for 5 min, and the supernatant was transferred to a new tube. The residue was extracted again with 1 mL of n-hexane, and the two supernatants were combined. The combined extracts were evaporated to dryness under a gentle nitrogen flow at 30 °C. The dried extract was then re-dissolved in 0.2 mL of methanol for further analysis.

The chromatographic system used was a Thermo DGLC dual ternary UPLC system equipped with a fluorescence detector, with an excitation wavelength of 294 nm and an emission wavelength of 328 nm. The UPLC analytical conditions were as follows: YMC Carotenoid column (150 × 4.6 mm, 3 μm), injection volume of 2 μL, column temperature of 40 °C, and a flow rate of 1.0 mL/min. The mobile phases consisted of water (phase A) and acetonitrile (phase B). The elution gradient was set as follows: 0 min at water/acetonitrile (30:70, v/v), 15 min at water/acetonitrile (30:70, v/v), 25 min at water/acetonitrile (80:20, v/v), and 30 min at water/acetonitrile (30:70, v/v).

2.6. Determination of MInerals

Mineral analysis was conducted using an inductively coupled plasma optical emission spectrometer (ICP-OES, Thermo Fisher iCAP PRO). The sample was digested in a microwave digestion vessel at 140°C for 10 min, followed by 180°C for 20 min. The sample was then heated on an electric heating plate at 170°C to evaporate the acid until approximately 2 mL remained. After adjusting to a constant volume, the sample was ready for testing. The instrument settings were as follows: RF power of 1150 W, plasma flow rate of 0.5 L/min, auxiliary flow rate of 0.5 L/min, nebulizer flow rate of 12.5 L/min, and a sample uptake delay of 30 sec.

2.7. Determination of Other Substances

The quantification of total flavonoids, total phenols, carotenoids, and total anthocyanins was performed using a Multiskan GO Multifunctional Microplate Reader (Thermo Fisher, USA). The tests were conducted using Corning microtiter plates, which ensured minimal variation between wells at the target wavelength. Each measurement was performed three times, and the results were reported as the mean ± standard deviation.

2.8. Statistical Analysis

Data were analyzed by independent-sample *t*-test or non-parametric test using SPSS 19.0. All assays were performed in triplicate, and the results were expressed as mean ± standard deviation. *P*-values < 0.05 were considered significant.

3. Results

3.1. Free Amino Acid Analysis

The free amino acid analysis of AMR samples is summarized in Table 1. In terms of absolute content, the total amino acid content decreased after fermentation, except glycine, which increased. The levels of lysine, methionine, and cystine remained unchanged before and after fermentation, while all other amino acids showed a decrease model. The relative contents of fresh amino acids were 26.06% before fermentation and 22.76% after fermentation; sweet amino acids were 27.13% before and 28.88% after; bitter amino acids were 28.80% before and 27.93% after; and aromatic amino acids were 10.34% before and 10.17% after fermentation.

The seven essential amino acids detected in the AMR samples were compared with the essential amino acid contents in ideal proteins based on the FAO/WHO standard model (Table 2). The results showed that the levels of valine, isoleucine, leucine, phenylalanine + tyrosine, and lysine in the AMR samples were higher than those in the standard model both before and after fermentation. The lysine content was significantly higher in the post-fermentation samples compared to the pre-fermentation samples. The contents of methionine and cystine were lower than the FAO/WHO standard levels before fermentation but significantly exceeded these levels after fermentation. The amino acid ratioses in the post-fermentation samples were close to or higher than the reference protein pattern ratios of E/T 40% and E/N 60% recommended by WHO [15].

Table 1. Comparison of the amino acid content of AMR before and after pickling

amino acid types	Before pickling ($mg \cdot g^{-1}$)	After pickling ($mg \cdot g^{-1}$)	Before pickling (%)	After pickling (%)	Flavor characteristic
Aspartic acid (Asp)	0.99±0.02	0.73±0.02**	13.84±0.17	13.49±0.24	Fresh taste
Threonine (Thr)[1]	0.29±0.01	0.22±0.01**	4.06±0.07	4.16±0.09	Sweet taste
Serine (Ser)	0.66±0.03	0.44±0.02**	9.19±0.11	8.12±0.26**	Sweet taste
Glutamate (Glu)	0.88±0.01	0.50±0.02**	12.24±0.40	9.23±0.30**	Fresh taste
Glycine (Gly)	0.10±0.01	0.11±0.01	1.33±0.13	1.95±0.09**	Sweet taste
Alanine (Ala)	0.31±0.02	0.28±0.01*	4.38±0.09	5.13±0.11**	Sweet taste
Cysteine (Cys)	0.09±0.01	0.09±0.01	1.21±0.06	1.60±0.11**	Aromatic taste
Valine (Val)[1]	0.44±0.02	0.34±0.01*	6.09±0.12	6.27±0.09	Bitter taste
Methionine (Met)[1]	0.12±0.01	0.12±0.01	1.70±0.07	2.14±0.10**	Bitter taste
Isoleucine (Ile)[1]	0.38±0.02	0.31±0.01**	5.35±0.07	5.77±0.18*	Bitter taste
Leucine (Leu)[1]	0.53±0.01	0.42±0.01**	7.39±0.09	7.81±0.21*	Bitter taste
Tyrosine (Tyr)	0.24±0.02	0.21±0.00*	3.40±0.12	3.86±0.08**	Aromatic taste
Phenylalanine (Phe)[1]	0.49±0.01	0.34±0.01*	6.89±0.25	6.41±0.08*	Aromatic taste
Histidine (His)	0.21±0.02	0.18±0.01*	2.99±0.13	3.26±0.10*	Sweet taste
Lysine (Lys)[1]	0.46±0.03	0.46±0.01	6.39±0.25	8.64±0.12**	Fresh taste
Arginine (Arg)	0.38±0.01	0.14±0.01*	5.22±0.09	2.67±0.10**	Bitter taste
Proline (Pro)	0.60±0.01	0.51±0.01**	8.33±0.08	9.49±0.16**	Sweet taste
Total amino acids (T)	7.18±0.20	5.38±0.02**	100±0.00	100±0.00	——
essential amino acid (E)	2.72±0.08	2.22±0.01**	37.88±0.14	41.20±0.21**	——
Non-essential amino acids (N)	4.46±0.12	3.16±0.02**	62.13±0.14	58.80±0.21**	——
E/T (%)	37.87±0.14	41.20±0.21**	37.87±0.14	41.20±0.21**	——
E/N (%)	60.95±0.35	70.08±0.60**	60.95±0.35	70.08±0.60**	——

Note: The symbol "[1]" represents essential amino acids. Data are expressed in mean ± standard deviation. Significant difference of each parameter between fresh and pickled samples was evaluated by independent-sample *t*-test or non parametric test. *, $p < 0.05$; **, $p < 0.01$.

Table 2. Comparison of the proportion of essential amino acids in AMR and the amino acid pattern spectrum

AA	Threonine	Valine	Methionine+cystine	Isoleucine	Leucine	Phenylalanine+tyrosine	Lysine
amino acid pattern spectrum	4.00	5.00	3.50	4.00	7.00	6.00	5.50
before pickling	4.06±0.07	6.09±0.12	2.90±0.06	5.35±0.07	7.39±0.09	10.29±0.14	6.39±0.25
after pickling	4.16±0.09	6.27±0.09	3.74±0.20	5.77±0.18*	7.81±0.21*	10.27±0.11	8.64±0.12**

Note: Data are expressed in mean ± standard deviation. Significant difference of each parameter between fresh and pickled samples was evaluated by independent-sample *t*-test or non parametric test. *, $p < 0.05$; **, $p < 0.01$.

3.2. Fatty Acid Analysis

This study analyzed the medium- and long-chain fatty acids in AMR samples, with the results presented in Table 3. A total of 31 fatty acids were identified, including 16 saturated fatty acids, 7 monounsaturated fatty acids, and 8 polyunsaturated fatty acids. The total fatty acid content decreased after pickling. Palmitic acid was the most abundant fatty acid in the samples, followed by stearic acid, linolenic acid, linoleic acid, and erucic acid. These five fatty acids together made up 93.88% to 95.72% of the total fatty acid content, while the remaining 26 fatty acids accounted for only 4.28% to 6.12%. In terms of absolute content, the levels of capric acid, lauric acid, myristic acid, pentadecanoic acid, oleic acid, palmitoleic acid, and myristoleic acid increased after pickling, while the rest of the fatty acids decreased.

3.3. Soluble Sugar Analysis

This study identified 10 soluble sugars in AMR samples, as detailed in Table 4. In fresh AMR, the primary soluble sugars were fructose (44.58%), glucose (31.91%), sucrose (19.72%), and raffinose (2.50%). In pickled AMR, the main sugars were lactose (72.11%), glucose (9.99%), galactose (8.74%), fructose (5.57%), and sucrose (2.64%). Overall, the total soluble sugar content increased in pickled AMR compared to fresh AMR. There was a significant decrease in fructose and sucrose, a significant increase in lactose and galactose, and a slight increase in glucose and raffinose.

3.4. Mineral Element Analysis

This study measured 9 mineral elements in AMR samples, with the results presented in Table 5. In pickled AMR, the contents of Iron (Fe), Phosphorus (P), zinc (Zn), Calcium (Ca), and Sodium (Na) were significantly higher compared to fresh AMR, whereas the levels of Copper (Cu), Magnesium (Mg), and Potassium (K) were significantly lower.

3.5. Vitamins

This study assessed the levels of B vitamins, vitamin C, and vitamin E in AMR samples, with results shown in Table 6. The content of B vitamins in pickled AMR increased significantly compared to fresh AMR, while vitamin C content decreased significantly, and vitamin E content showed a slight increase. Specifically, the levels of VB2, VB6, γ-VE, and δ-VE increased significantly.

Table 3. Comparison of fatty acids in AMR before and after pickling

Fatty acids	Chain	μg/mg		%	
		Before pickling	After pickling	Before pickling	After pickling
Methyl Hexanoate	C6：0	0.0000±0.0000	0.0001±0.0000*	0.0000±0.0000	0.0057±0.0006*
Methyl Octanoate	C8：0	0.0000±0.0000	0.0004±0.0000*	0.0018±0.0000	0.0244±0.0002*
Methyl Decanoate	C10：0	0.0001±0.0000	0.0014±0.0001*	0.0055±0.0001	0.0781±0.0026*
Methyl Undecanoate	C11：0	0.0001±0.0000	0.0001±0.0000	0.0028±0.0000	0.0069±0.0001*
Methyl Laurate	C12：0	0.0031±0.0001	0.0051±0.0001**	0.1517±0.0057	0.2908±0.0091**
Methyl Tridecanoate	C13：0	0.0001±0.0001	0.0002±0.0001*	0.0039±0.0034	0.0125±0.0042*
Methyl Myristate	C14：0	0.0129±0.0001	0.0205±0.0004**	0.6338±0.0039	1.1718±0.0355*
Methyl Myristoleate	C14：1	0.0015±0.0001	0.0019±0.0001*	0.0722±0.0037	0.1072±0.0030**
Methyl Pentadecanoate	C15：0	0.0018±0.0001	0.0025±0.0002*	0.0874±0.0014	0.1409±0.0133*
Methyl Palmitate	C16：0	0.7901±0.0100	0.7557±0.0051**	38.7679±0.2537	43.19±0.1333**
Methyl Palmitoleate	C16：1	0.0014±0.0001	0.0017±0.0001*	0.0701±0.0027	0.0990±0.0042*
Methyl Heptadecanoate	C17：0	0.0029±0.0001	0.0029±0.0001	0.1417±0.0051	0.1640±0.0040**
Methyl Heptadecenoate	C17：1	0.0007±0.0001	0.0006±0.0001	0.0325±0.0025	0.0359±0.0039
Methyl Stearate	C18：0	0.4350±0.0050	0.4083±0.0076**	21.3444±0.1186	23.3344±0.2506**
Methyl Oleate	C18：1n9c	0.0118±0.0005	0.0284±0.0005**	0.5801±0.0213	1.6246±0.0435**
Methyl Linoleate	C18：2n6c	0.2063±0.0065	0.1366±0.0057*	10.1221±0.2861	7.8053±0.3664**
Methyl Arachidate	C20：0	0.0131±0.0001	0.0127±0.0001**	0.6435±0.0105	0.7255±0.0092**
Methyl Gamma Linolenate	C18：3n6	0.0015±0.0001	0.0011±0.0002*	0.0716±0.0032	0.0640±0.0087
Methyl 11-Eicosenoate	C20：1	0.0022±0.0002	0.0016±0.0001*	0.1060±0.0076	0.0890±0.0022*
Methyl Linolenate	C18：3n3	0.3833±0.0058	0.2471±0.0062*	18.8100±0.1176	14.1218±0.2314**
Methyl Heneicosanoate	C21：0	0.0018±0.0001	0.0017±0.0001*	0.0894±0.0037	0.0953±0.0038
Methyl 11，14-Eicosadienoate	C20：2	0.0011±0.0001	0.0007±0.0002*	0.0528±0.0048	0.0386±0.0092
Methyl Behenate	C22：0	0.0108±0.0013	0.0089±0.0004	0.5315±0.0574	0.5061±0.0241
cis-8，11，14-Eicosatrienoic acid methyl ester	C20：3n6	0.0065±0.0005	0.0047±0.0004**	0.3175±0.0242	0.2707±0.0230

Fatty acids	Chain	μg/mg		%	
		Before pickling	After pickling	Before pickling	After pickling
Methyl Erucate	C22：1n9	0.1358±0.0052	0.0926±0.0065**	6.6643±0.3099	5.2933±0.3547**
Methyl 11，14，17-Eicosatrienoate	C20：3n3	0.0008±0.0001	0.0005±0.0001*	0.0414±0.0021	0.0269±0.0036**
Methyl Tricosanoate	C23：0	0.0024±0.0003	0.0025±0.0001	0.1160±0.0166	0.1454±0.0042*
Methyl Docosadienoate	C22：2	0.0014±0.0002	0.0009±0.0001*	0.0700±0.0081	0.0531±0.0026*
Methyl Lignocerate	C24：0	0.0078±0.0005	0.0072±0.0003	0.3824±0.0199	0.4133±0.0145
Methyl Eicosapentaenoate	C20：5n3	0.0001±0.0001	0.0001±0.0001	0.0061±0.0033	0.0075±0.0034
Methyl Nervonate	C24：1	0.0016±0.0002	0.0011±0.0001**	0.1053±0.0392	0.0627±0.0065

Note: Data are expressed in mean ± standard deviation. Significant difference of each parameter between fresh and pickled samples was evaluated by independent-sample *t*-test or non parametric test. *, $p < 0.05$; **, $p < 0.01$.

Table 4. Comparison of soluble sugar content before and after AMR pickling

Name	Molecular formula	μg/mg		%	
		Before pickling	After pickling	Before pickling	After pickling
Trehalose	$C_{12}H_{22}O_{11}$	0.0771±0.0011	0.0000±0.0000*	0.7484±0.0010	0.0000±0.0000*
Fucose	$C_6H_{12}O_5$	0.0062±0.0003	0.0114±0.0006**	0.0601±0.0026	0.0345±0.0019**
Rhamnose	$C_6H_{14}O_6$	0.0275±0.0007	0.0000±0.0000*	0.2668±0.0063	0.0000±0.0000*
Arabinose	$C_5H_{10}O_5$	0.0105±0.0005	0.0190±0.0015**	0.1019±0.0044	0.0573±0.0045**
Galactose	$C_6H_{12}O_6$	0.0127±0.0005	2.9044±0.0038**	0.1232±0.0044	8.7525±0.0105**
Glucose	$C_6H_{12}O_6$	3.2870±0.0030	3.3153±0.0032**	31.8938±0.0393	9.9908±0.0086**
Fructose	$C_6H_{12}O_6$	4.5917±0.0019	1.8458±0.0033**	44.5541±0.0231	5.5623±0.0106**
Sucrose	$C_{12}H_{22}O_{11}$	2.0344±0.0046	0.8754±0.0014**	19.7400±0.0382	2.6380±0.0046
Lactose	$C_{12}H_{22}O_{11}$	0.0000±0.0000	23.9268±0.0047*	0.0000±0.0000	72.1036±0.0097*
Raffinose	$C_{18}H_{32}O_{16}$	0.2588±0.0013	0.2857±0.0029**	2.5115±0.0124	0.8611±0.0087**
Total content	—	10.3060±0.0035	33.1840±0.0039**	100.00%	100.00%

Note: Data are expressed in mean ± standard deviation. Significant difference of each parameter between fresh and pickled samples was evaluated by independent-sample *t*-test or non parametric test. *, $p < 0.05$; **, $p < 0.01$.

Table 5. Comparison of mineral elements before and after AMR pickling

Mineral elements	Element content C_x (mg/kg)		Element content W (%)	
	Before pickling	After pickling	Before pickling	After pickling
Fe	70.6486±0.4953	77.7708±0.9594**	1.0555±0.0069	0.7769±0.0117**
P	492.5002±3.0923	744.0459±4.8703**	7.3577±0.0422	7.4322±0.0218
Zn	5.4328±0.0729	12.3382±0.3430**	0.0812±0.0009	0.1232±0.0030**
Mn	2.8872±0.0997	3.3220±0.0660**	0.0431±0.0015	0.0332±0.0007**
Cu	77.4541±0.9828	0.2451±0.0407**	1.1571±0.0117	0.0024±0.0004**
Mg	453.0380±5.6202	319.4949±1.0224**	6.7682±0.0771	3.1915±0.0158
Ca	769.2464±1.2085	1377.5900±5.5180**	11.4922±0.0154	13.7608±0.0339
Na	125.0304±2.0441	3737.9534±2.1052**	1.8680±0.0356	37.3389±0.1502**
K	4697.3922±16.0398	3738.2274±29.0082**	70.1770±0.1110	37.3409±0.1533**

Note: Data are expressed in mean ± standard deviation. Significant difference of each parameter between fresh and pickled samples was evaluated by independent-sample *t*-test or non parametric test. *, $p < 0.05$; **, $p < 0.01$.

Table 6. Comparison of vitamin content before and after AMR pickling

Name	Unit	Molecular formula	Before pickling	After pickling
Thiamine (VB1)	ng/100 mg, FW	$C_{12}H_{17}N_4OS$	15.7398±0.0113	10.1660±0.0361**
Nicotinamide (VB3)	ng/100 mg, FW	$C_6H_6N_2O$	47.7009±0.7295	26.1645±0.1498**
Pyridoxine hydrochloride (VB6)	ng/100 mg, FW	$C_8H_{11}NO_3$	17.6001±0.0657	65.3206±0.5812**
Riboflavin (VB2)	ng/100 mg, FW	$C_{17}H_{20}N_4O_6$	53.6647±0.7238	148.7489±1.2931**
Ascorbic acid (VC)	ug/100 mg, FW	$C_6H_8O_6$	14.9647±0.0351	8.1835±0.2334**
α-Tocopherol (α-VE)	ug/100 mg, FW	$C_{29}H_{50}O_2$	1.0132±0.0139	0.9492±0.0160**
β- Tocopherol (β-VE)	ug/100 mg, FW	$C_{28}H_{48}O_2$	0.0789±0.0010	0.0461±0.0034**
γ- Tocopherol (γ-VE)	ug/100 mg,	$C_{28}H_{48}O_2$	0.2824±0.0021	0.3709±0.0017**

Name	Unit	Molecular formula	Before pickling	After pickling
	FW			
δ-Tocopherol (δ-VE)	ug/100 mg, FW	$C_{27}H_{46}O_2$	0.0749±0.0023	0.1049±0.0023**

Note: Data are expressed in mean ± standard deviation. Significant difference of each parameter between fresh and pickled samples was evaluated by independent-sample *t*-test or non parametric test. *, $p < 0.05$; **, $p < 0.01$.

Table 7. Other analyses of AMR before and after pickling

Detection indicators	Before pickling	After pickling
Total flavonoids (mg/g)	1.1577 ± 0.0025	0.7717 ± 0.0117**
Total phenols (mg/g)	1.2763 ± 0.0097	1.1370 ± 0.0142**
Carotenoids (mg/100g)	9.6432 ± 0.1431	6.6919 ± 0.0852**
Total anthocyanins (mg/g)	0.1062 ± 0.0014	0.1002 ± 0.0015**

Note: Data are expressed in mean ± standard deviation. Significant difference of each parameter between fresh and pickled samples was evaluated by independent-sample *t*-test or non parametric test. *, $p < 0.05$; **, $p < 0.01$.

3.6. Other Analyses

This study also compared the levels of total flavonoids, total phenols, carotenoids, and total anthocyanins in AMR before and after pickling. As shown in Table 7, all three substances—total flavonoids, total phenols, and carotenoids—decreased to varying extents in pickled AMR, while the total anthocyanins content remained unchanged.

4. Discussion

During the pickling and ripening of vegetables, proteins are gradually broken down into amino acids by microorganisms, and the protein-degrading enzymes are naturally present in the vegetables. This process is a key factor in the development of the luster, aroma, and flavor of pickled products. Presently, the observed decrease in the total amino acid content of AMR after fermentation aligns with the findings of [16], which suggest that prolonged fermentation leads to the proliferation of lactic acid bacteria and the accumulation of lactic acid and other metabolites. As the available sugars for fermentation diminish rapidly, some microorganisms begin to autolyze and hydrolyze due to the acidic environment. This deamination of amino acids helps to provide energy by breaking down carbon sources to compensate for the lack of available sugars [17]. The process leads to a reduction in the total amount of amino acids in pickled AMR. The increase in the absolute content of glycine, a common sweet amino acid, and the significant rise in the relative content of both sweet and fresh amino acids after fermentation indicate that specific microorganisms or protein-hydrolyzing enzymes in the fermentation process affect glycine. This alteration contributes to the overall flavor and nutritional profile of the pickled AMR. Furthermore, comparing the seven essential amino acids in the fermented samples with the FAO/WHO standard model revealed that the amino acid ratioses are close to or exceed the WHO reference protein model (E/T 40% vs. E/N 60%) [15], highlighting the nutritional value of the pickled product.

The reduction in fatty acid content of AMR after pickling with whey liquid is consistent with result of [18]. This decrease may be attributed to several factors: (i) lactic acid bacteria in the whey liquid may promote the breakdown of medium and long-chain fatty acids into short-chain fatty acids or other metabolites through their metabolic activities during pickling; (ii) inherent enzymes in the whey liquid might contribute to the hydrolysis of fatty acids; (iii) the salt used in the pickling process could alter the permeability of cell membranes in the AMR, affecting the stability and content of fatty acids.

The observed increase in capric acid, lauric acid, myristic acid, pentadecanoic acid, oleic acid, palmitoleic acid, and myristoleic acid after pickling is likely due to the milk fat content in the whey liquid. Saturated fatty acids are associated with various health issues, such as cardiovascular disease, diabetes, obesity, and inflammation-related conditions. The content of palmitic acid and stearic acid-key saturated fatty acids in AMR-was significantly reduced after pickling, which is considered more favorable for human health.

The level of soluble sugars significantly influences fruit flavor and is an important factor in consumer evaluation this study, the total soluble sugar content in pickled scallions increased due to the high lactose content in the whey liquid, which comprises over 70 - 80% of the total solids. The increase in sugars after pickling was primarily due to lactose and galactose, consistent with research of [18] using yogurt for pickling scallions. Lactose not only provides energy and aids in metabolism and calcium absorption but also has a strong affinity for key volatile flavor compounds like aldehydes, ketones, and esters, enhancing the flavor of the final product [19,20], thus improving the flavor of the final product. Research indicates that the soluble sugar content in vegetables pickled with low-concentration salt typically decreases initially before stabilizing [21,22]. This trend is because low salt concentrations do not sufficiently inhibit microbial activity. Microorganisms such as lactic acid bacteria and yeast convert soluble sugars like fructose and sucrose into various organic acids, leading to a decrease in soluble sugar content [23], which is consistent with the results of this study. The significant increase in Fe, P, Zn, Ca, and B vitamins in the fermented AMR is also attributed to the nutritional components inherent in the whey liquid [7,8,24].

5. Conclusion

In conclusion, while pickling scallions may result in some loss of nutrients such as vitamin C, flavonoids, phenols, and carotenoids, overall, pickling with whey solution offers several benefits. It not only addresses the

issue of poor storage tolerance of fresh scallions but also enhances the nutritional value, health benefits, taste, and flavor of the fermented product. This study provides a theoretical foundation for the rational development and use of whey resources and supports the future promotion of the fermentation industry for wild AMR.

Abbreviations

AMR = *Allium Mongolicum* Regel; E = essential amino acid; ESI = electric spray ion source; FAO = the Food and Agriculture Organization of the United Nations; HPAEC = high-performance anion-exchange chromatography; MS = mass spectrometry; N = non essential amino acids; SIM = single ion detection; T = Total amino acids; UPLC = ultra performance liquid chromatography; WHO = the World Health Organization.

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Competing Interests

The authors have not stated any conflicts of interest.

Authors’ Contributions

Chenxia Cao conceived, designed the experiments and wrote the original manuscript; Rong A, Chang Liu and Dawei Gao performend the experiments; Rina Su and Haifang Zhang analyzed the data; Wenbiao Zhang revised the manuscript. All authors read and approved the final manuscript.

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