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Proteomic analysis of japonica sorghum following microwave intermittent drying based on label-free technology

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Abstract

The aim of this study was to investigate the influence of microwave drying on the protein quality of japonica sorghum following an intermittent drying test. Using label-free technology and liquid chromatography-tandem mass spectrometry for proteomic analysis, the effects of microwave drying on sorghum differential protein expression, functional classification, and metabolic pathways were analyzed at the molecular level. After sorghum was dried using a microwave, 85 differential proteins were identified. Among them, 51 showed up-regulated expression while 34 had down-regulated expression. The up-regulation and down-regulation of differential protein expressions significantly changed them, and proteins with larger up-regulated and down-regulated expressions were postulated to affect biological and metabolic processes of sorghum during subsequent processing. Differential proteins were significantly ($P < 0.01$) involved in metabolic pathways, such as carbon metabolism, glycolysis/gluconeogenesis, carbon fixation in photosynthetic organisms, biosynthesis of amino acids, amino sugar and nucleotide sugar metabolism, and the TCA cycle. For the protein interaction network, glyceraldehyde-3-phosphate dehydrogenase of the down-regulated proteins was postulated to be the key factor affecting the entire metabolic system or signal transduction pathway. Up-regulated proteins, including phosphoglycerate mutase and phosphopyruvate hydratase, as well as down-regulated proteins such as glyceraldehyde-3-phosphate dehydrogenase and fructose-bisphosphate aldolase, not only directly or indirectly affected a variety of metabolic processes, but were specifically closely related to glycolysis and glycometabolism. Overall, this study showed that among the related metabolic pathways, differential protein changes in glycometabolism pathways may have the greatest impact on metabolic processes. The research results discussed herein can provide theoretical support for the industrial application of microwave drying and deep processing of sorghum.

Keywords: japonica sorghum; microwave intermittent drying; proteomics; differential protein metabolism.

Practical Application: Sorghum is the best raw material for brewing Chinese Baijiu, and also an important coarse cereals.The results of this study show that the amounts of differentially expressed proteins in sorghum grain are changed significantly after microwave drying,it will have a positive impact on the brewing quality and coarse cereals quality of sorghum.

1 Introduction

As one of the ancient dryland food crops in China, sorghum has been cultivated for thousands of years (Duan et al., 2013), and was once known as the "valley of life" (Lu & Sun, 2005). Sorghum is the fifth most important and gluten-free cereal that belongs to family Poaceae(Shahzad et al., 2021).In addition to providing food and feed (Orrico et al., 2015), sorghum grains can also be used to make products such as wine, starch, and vinegar. Following the adjustment of China's agricultural planting structure and the rapid development of the coarse cereals industry, sorghum planting and processing has become a huge development in the agricultural sector.

In northeast China, especially in the Heilongjiang province, the weather during the sorghum harvest season has dramatically changed, leading to a higher moisture content than usual in harvested sorghum. Owing to this, drying is a widely applied method which aims reducing water activity of crops to storage for long-term(Aydar, 2021).Sorghum is prone to insect pests during storage; therefore, sterilization of the crop prior to storage is essential for increased storage life. The microwave is an electromagnetic wave (Qu et al., 2021).Microwave drying is an energy saving, efficient, and green drying technology that has good germicidal and insecticidal effects (Chen et al., 2017; Guo et al., 2017; Syamaladevi et al., 2016).

Drying sorghum by microwave technology does not only achieve efficient moisture removal, but also plays the role of sterilization and killing insects. Currently, research into the processing of agricultural products, including sorghum, by microwave technology is mainly focused on the quality of the final agricultural product after microwave radiation, microwave pretreatment, and microwave modification (Wang et al., 2016;

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Shi et al., 2015). However, from the point of view of grain drying, the effect of microwave drying on sorghum protein quality has not been reported. Therefore, a microwave intermittent drying experiment was conducted on the Northern Japonica sorghum, and the changes in differential proteins in the plant were analyzed after microwave drying using proteomic technology. Consequently, the effect of microwave drying on sorghum protein was analyzed at the molecular level, providing theoretical data to support the industrial application of microwave drying and the subsequent processing of sorghum.

2 Materials and methods

2.1 Materials and reagents

The original sorghum (hereinafter referred to as natural sorghum) was longza 10, and was produced in the Duerbert County of Daqing city. It belongs to the typical northern japonica sorghum. Glycerol and bromophenol blue were obtained from Sangon Biotech . SDS, urea, tris-hydroxy-methy-amino-methane, dithiothreitol, iodoacetamide were purchased from Bio-rad . Acetonitrile was acquired from Merck , while NH_4HCO_3 and formic acid were obtained from Sigma-Aldrich.

2.2 Instrumentation and equipment

The following instruments and equipment were used for these studies: GWM-80B microwave dryer (Gansu Tianshui Huayuan Pharmaceutical Equipment Limited Company), MB25 moisture analyzer (Ohouse Changzhou Instrument Limited Company), LS6200C precision electronic balance (Swiss Precisa company), ST20XB portable infrared thermometer (Beijing Leitai Photoelectric Technology Limited Company), Q Exactive mass spectrometer, EASY-nLC 1000 liquid chromatograph, and EASY SC200 150 μm × 100 mm chromatographic column (Thermo Finnigan Company), 5430R low-temperature high-speed centrifuge and Concentrator plus vacuum centrifugal concentrator (Eppendorf Company), WFZ UV-2100 visible ultraviolet spectrophotometer (Unico Company), and EPS601 electrophoresis apparatus (GE Healthcare Company).

2.3 Methods

Preparation of sorghum samples by microwave intermittent drying

Prior to the drying process, impurities in the sorghum were removed, and then sorghum plants with full grains were selected. A special drying box was used to quantitatively weigh the sorghum, and microwave drying was performed in a microwave dryer. The drying power per unit mass was 3W/g and the air velocity was 0.5 m/s. The microwave dryer was set at single cycle microwave action times of 1.02, 2.08, 3.13, 4.17, and 5.00 min. An intermittent drying method was adopted, that is, microwaves were emitted from every odd number drying chamber of the dryer but not from even numbered drying chambers. Once a drying cycle was complete, the temperature and quality of the sorghum were instantly determined, and the next drying cycle was started. This process was repeated until the moisture content of sorghum was within the required limits. At a single cycle microwave action time of 5.00 min, the microwave action intensity was the highest; therefore, sorghum samples that were dried using the 5.00 min action time and natural sorghum samples were used as the sample groups for proteomic analysis in this study.

Proteomic analysis of japonica sorghum

Based on non-labeled quantitative proteomic technology, a label-free algorithm in MaxQuant was used for quantitative calculation of proteomics data, and a comparison of the natural sorghum and microwave dried sorghum groups was carried out using mass spectrometry quantitative analysis.

(1) Extraction of proteins and enzymatic hydrolysis of peptides

Protein was extracted by the SDT splitting method (4% [w/v] SDS, 100 mM Tris/HCl pH 7.6, 0.1 M DTT), and then quantified using the BCA method. Subsequently, an appropriate amount of protein from each sample was used and trypsin was hydrolyzed by filter-aided proteome preparation. Following this, a C18 cartridge was used to desalt the peptide, and after lyophilization, the peptide was reconstituted in 4 μL of dissolution buffer for peptide quantification.

(2) LC-MS/MS data acquisition

Each grading sample was separated by a liquid phase system Easy-nLC buffer solution. Solution A was 0.1% formic acid aqueous solution and solution B was 0.1% formic acid and acetonitrile aqueous solution. The chromatographic column was equilibrated with 95% solution A prior to the sample being loaded into the loading column using an automatic injector (Thermo Scientific EASY C18 5 μm column, 100 μm × 2 cm), and then separated using an analytical column (Thermo Scientific EASY C18 3 μm column, 75 μm × 10 cm). The flow velocity was set at 250 NL/min.

Samples were separated by chromatography and analyzed using mass spectrometry; the analysis time was 60 min, the detection method was positive ion, the scanning range of the parent ion was 300–1800 m/z, and the resolution of the primary mass spectrum was 70,000 at m/z 200. Automatic gain control target was 3E6, maximum IT was 10 ms, and dynamic exclusion time was 40 s. The mass charge ratio of the polypeptide and polypeptide fragments was determined based on to the following method and settings: ten fragment maps were collected after each full scan, while MS2 activation type was HCD, isolation window was 2 m/z, resolution of secondary mass spectrometry was 17500 at 200 m/z, normalized collision energy was 30 eV, and the underfill ratio was 0.1%.

(3) Protein identification and quantitative analysis

The original MS test files were retrieved from the corresponding database using MaxQuant software (version 1.5.5.1), and the results of protein identification and quantitative analysis were obtained.

(4) Bioinformatics analysis

The Omicsbean software was used to carry out KEGG pathway annotation for the target protein set. Omicsbean software was used to compare the distribution of each KEGG pathway in the target protein set and the total protein set, and enrichment analysis of KEGG pathway annotation was performed on the target protein set. The bubble diagram of KEGG enrichment analysis was generated by software R, version 3.5.1. Based on the information from the STRING database, direct and indirect interactions between target proteins were searched, and an interaction network was generated and analyzed.

3 Results and analysis

3.1 Screening and classification results of differentially expressed proteins

Screening results of differentially expressed proteins

For natural sorghum samples and sorghum samples dried with a single cycle microwave action time of 5.00 min, the differentially expressed proteins were screened according to the criteria of $P < 0.05$ and multiple changes ≥ 1.5, or ≤ 0.667. A total of 391 proteins were identified with a total number of 604 peptides being found. The total number of differentially expressed proteins was 85 in the natural and microwave dried sorghum groups.

As shown in Figure1, the results of differentially expressed proteins screening were displayed in the form of a volcano map. The abscissa represents the multiple of the difference and the ordinate indicates the significance of the difference. In the figure, the red dots indicate 51 up-regulated differential proteins, the blue dots indicate 34 down-regulated differential proteins, and the grey dots indicate the proteins with no significant change.

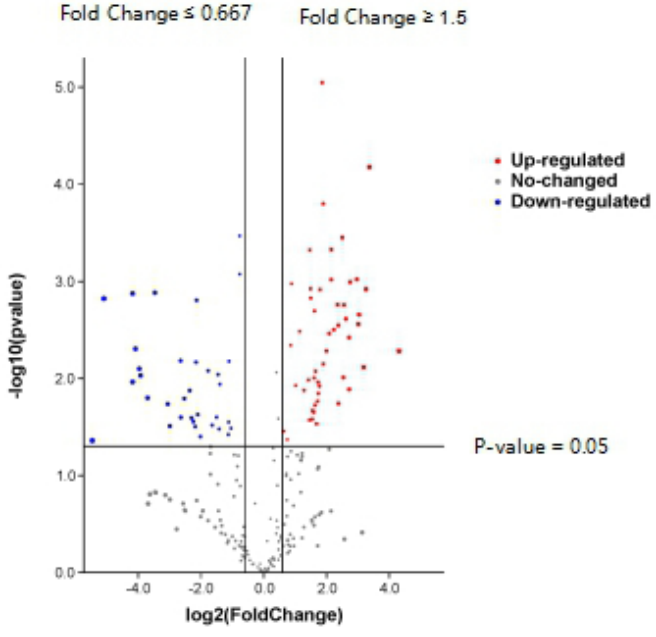


Figure 1. Volcano map of differentially expressed proteins of natural sorghum and microwave dried sorghum.

The results of differential protein screening showed that among 85 differentially expressed proteins in natural sorghum samples, 51 proteins had lower content and 34 proteins had higher content. However, after microwave drying, the 51 proteins with lower content were upregulated, and the corresponding protein content increased. Furthermore, the 34 proteins with higher content were downregulated, and the corresponding protein content decreased. These results showed that differential protein expression in natural sorghum was significantly changed after microwave intermittent drying, and protein composition and content were significantly affected.

Functional classification of differentially expressed proteins

Among the 85 differentially expressed proteins from microwave dried sorghum samples, a total of 30 proteins or enzymes with certain functions were obtained by removing unidentified proteins and proteins with unknown functions.According to biological functions (Li et al., 2020), these 30 proteins or enzymes were classified into 6 categories, including material and energy metabolism, amino acid biosynthesis, starch metabolism, antioxidation, transportation and regulation,and other functional proteins, as shown in Table 1.

As shown in Table 1, for the functional classification of material and energy metabolism, differentially expressed proteins that were significantly upregulated the most included starch branching enzyme IIb, cytochrome b5 domain-containing protein, purple acid phosphatase, and succinate dehydrogenase; yet, differentially expressed proteins that showed the most significant downregulation included chitinase and malate dehydrogenase. Starch branching enzyme (SBE) is a glycosyltransferase that integrates hydrolysis, transfer, and synthesis. Most of SBEs can simultaneously act on amylopectin and amylose, and a small portion of the SBE only acts on amylose. The mechanism includes hydrolysis and release of the α-1,4-glycosidic bond, the transfer of the cut non-reducing end to the C-6 hydroxyl 11 receptor chain, production of the α- 1,6-glycosidic bond in α- glucan, the shortening of amylose, and the increase in the degree of starch branching (Guan et al., 2020; He et al., 2020). Cytochrome b5 (Cyt-b5) is a heme protein widely distributed in the outer membrane of microsomes and mitochondria of various organisms. It performs an electronic transfer function and participates in a series of important redox reactions in biological tissues (Yanase et al., 2020). Cyt-b5 is an integral part of the microelectronic transfer chain of the endoplasmic reticulum membrane (Zhan et al.,2020). Purple acid phosphatases (PAPS) are a large family of enzymes that are widely found in animals and plants. PAPS can regulate the metabolism of phosphorus and carbon, participate in plant response to stress and biological functions such as cell wall synthesis (Zhu et al., 2020). Succinate dehydrogenase is a mitochondrial endomembrane protein complex that connects the Krebs cycle and electron transport system, and is a key membrane complex in the TCA cycle that participates in respiration and release of large amounts of energy from oxidative phosphorylation in organisms (Zhao et al., 2020).

Table 1. Mass spectrometry identification of differential proteins in natural sorghum after microwave drying.

Protein ID	Differential protein or enzyme	Sequence coverage (%)	Mol. weight (kDa)	Relative expression value	P-value
Material and energy metabolism					
C5XBD4	Phosphopyruvate hydratase	1.9	50.557	3.00 ↑	0.0223
A5Y3Z3	Glucose-1-phosphate adenylyltransferase	5.4	57.096	3.41 ↑	0.0120
A0A1B6Q8Y2	Succinate dehydrogenase	1.7	62.476	5.20 ↑	0.0181
Q7XZK8	Starch branching enzyme IIb	5.3	20.205	9.56 ↑	0.0012
C5WWD4	Cytochrome b5 domain-containing protein	3.5	24.987	8.08 ↑	0.0027
C5WRL4	Purple acid phosphatase	2	38.572	6.73 ↑	0.0010
Q93WT2	Chitinase	27.9	27.462	0.055 ↓	0.0108
C5WYF2	Malate dehydrogenase	16.6	35.464	0.058 ↓	0.0049
A0A1B6QHW3	Alpha-1,4 glucan phosphorylase	2.1	106.84	0.233 ↓	0.0235
C5XD70	rRNA N-glycosidase	19	32.282	0.292 ↓	0.0083
A0A194YMV2	Phosphoglycerate kinase	4.7	42.449	0.319 ↓	0.0303
C5XFH6	Fructose-bisphosphate aldolase	7	38.558	0.373 ↓	0.0331
A0A1B6QE06	UTP--glucose-1-phosphate uridylyltransferase	7.3	47.302	0.461 ↓	0.0282
Amino acid biosynthesis					
C5XX52	Glyceraldehyde-3-phosphate dehydrogenase	17.2	36.345	0.077 ↓	0.0158
C5XBD4	Phosphopyruvate hydratase	1.9	50.557	3.00 ↑	0.0223
A0A194YMV2	Phosphoglycerate kinase	4.7	42.449	0.319 ↓	0.0303
C5XFH6	Fructose-bisphosphate aldolase	7	38.558	0.373 ↓	0.0331
C5X1N6	Phosphoglycerate mutase(iPGAM)	1.8	61.861	1.81 ↑	0.0045
A0A1B6Q673	Aspartate aminotransferase	10	43.869	0.064 ↓	0.0079
Starch metabolism					
C5XDC4	Alpha-amylase inhibitor	6.8	12.499	0.220 ↓	0.0313
A0A1B6QHW3	Alpha-1,4 glucan phosphorylase	2.1	106.84	0.233 ↓	0.0235
A5Y3Z3	Glucose-1-phosphate adenylyltransferase	5.4	57.096	3.41 ↑	0.0120
Q7XZK8	Starch branching enzyme IIb	5.3	20.205	9.56 ↑	0.0012
Transportation and regulation					
C5XNN9	Protein transport protein Sec61 subunit beta	11.1	8.2494	3.10 ↑	0.0188
A0A1B6PU65	40S ribosomal protein	3.3	32.773	5.10 ↑	0.0017
A0A1B6Q8Y2	Succinate dehydrogenase	1.7	62.476	5.20 ↑	0.0181
C5XIN6	Ubiquitin-fold modifier 1	12.9	10.381	5.66 ↑	0.0003
A0A1B6PQQ6	Protein disulfide-isomerase	5.1	54.531	0.352 ↓	0.0251
A0A1B6QHV2	Eukaryotic translation initiation factor 5A	11.2	17.475	0.365 ↓	0.0091
Antioxidation					
C5X6H6	L-ascorbate peroxidase	3.6	27.159	0.247 ↓	0.0397
P84516	Cationic peroxidase SPC4	8.3	38.451	0.484 ↓	0.0325
A0A1B6QN96	Superoxide dismutase	14.5	15.087	0.588 ↓	0.0008
C5Z469	Peroxidase	3.2	33.206	3.27 ↑	0.0172
Other functional proteins and enzymes					
J7FIP5	Late embryogenesis abundant protein 3	17.6	18.248	3.46 ↑	0.0012
C5WQX5	Ribosomal S10 domain-containing protein	7.9	13.845	3.06 ↑	0.0020
C5YDE5	Oleosin	25	16.184	1.682 ↑	0.0426
C5XVU9	S-(hydroxymethyl)glutathione dehydrogenase	3.1	40.754	3.72 ↑	0.0001

In the table, the arrows indicate upregulated or downregulated expression.

Owing to this, differentially expressed upregulated proteins may significantly promote starch metabolism, carbon and phosphorus metabolism, redox reactions, respiration, and other energy metabolic processes of sorghum during subsequent processing.

Chitinase is an enzyme that degrades chitin into chitin oligosaccharides or monosaccharides, and plays an important role in the carbon and nitrogen cycles, microbial infection of plant tissue, body immunity, and natural biological defense (Alsina et al., 2021). Malate dehydrogenase, an important oxidoreductase in the TCA cycle, catalyzes the dehydrogenation of L-malic acid to oxaloacetic acid, which is also involved in the C4 cycle, gluconeogenesis, fatty acid oxidation, nitrogen assimilation, and other metabolic activities (Chen et al., 2020). The two differentially expressed proteins that were downregulated more significantly may delay the degradation of chitin or the C4 and TCA cycles in sorghum.

In the functional classification of amino acid biosynthesis, differentially expressed proteins which were significantly upregulated the most included phosphopyruvate hydratase and phosphoglycerate mutase (iPGAM), while those that were more significantly downregulated included aspartate aminotransferase and glyceraldehyde-3-phosphate dehydrogenase. Phosphopyruvate hydratase is a key enzyme in the pathway of glucose metabolism. Its function is to convert 2-phosphate-glyceric acid to phosphoenolpyruvic acid (Li et al., 2017). iPGAM is a key enzyme in glycolysis and gluconeogenesis, and can catalyze the conversion between 3-PGA and 2-PGA. iPGAM catalyzes the transfer of intermolecular phosphates to glyceryl monophosphate *via* interactions between serine phosphates (Lin et al., 2019). Aspartate aminotransferase catalyzes the corresponding transformation of acidic amino acids and corresponding keto acids, and plays a very important role in amino acid metabolism (Banik et al., 2020; Wu et al., 2020). Glyceraldehyde-3-phosphate dehydrogenase is a key enzyme involved in glycolysis, and catalyzes 3-glyceraldehyde phosphate to glyceric acid 1,3-diphosphate. In addition, it transfers energy to the high-energy phosphate bond, and is thus considered the central link of glucose metabolism. Furthermore, it has many other physiological functions, such as RNA binding, catalytic microtubule polymerization, regulation of protein expression and phosphorylation, participation in autophagy, nitrosylated nuclear protein, and recruitment of transferrin (Zhang et al., 2020). Significant changes in these proteins or enzymes may have consequential impacts on glucose and amino acid metabolism.

In the functional classification of starch metabolism, differentially expressed proteins that were more significantly upregulated and downregulated included starch branching enzyme IIb and glucose-1-phosphate adenylyltransferase, and alpha-amylase inhibitor and alpha-1,4 glucan phosphorylase, respectively. Upregulated expression of starch branching enzyme showed that microwave drying may shorten the amylose polysaccharide and increase the degree of branching in sorghum starch. In addition, the content and distribution of amylose and amylopectin starch in sorghum may be changed significantly, and this can be beneficial to the fermentation of sorghum. Glucose-1-phosphate adenylyltransferase is an important enzyme in starch synthesis (Sun et al., 2017). Following microwave drying of sorghum, the expression of the enzyme was upregulated, which was beneficial to the synthesis of sorghum starch. Alpha-amylase inhibitor belongs to one of the inhibitors of sugar hydrolase, and can effectively inhibit the activity of salivary and pancreatic amylase in the intestines, essentially preventing the hydrolysis and digestion of carbohydrates in foods, thus reducing sugar uptake (Udani et al., 2018). Its expression was downregulated, and the results showed that microwave drying could inhibit its expression. In this situation, hydrolysis and digestion of carbohydrates will be promoted, leading to a conducive environment for the metabolism of material and production of energy. The α- glucan phosphorylase in plants is usually called starch phosphorylase, and plays a dynamic regulatory role in starch biosynthesis and degradation of higher plants (Satoh et al., 2008). Therefore, the downregulated expression of alpha-1,4 glucan phosphorylase can have an effect on the starch metabolism process.

In the functional classification of transportation and regulation, differentially expressed proteins that were significantly upregulated the most included ubiquitin-fold modifier, succinate dehydrogenase, and 40S ribosomal protein. Differentially expressed proteins that were more significantly downregulated included protein disulfide-isomerase and eukaryotic translation initiation factor 5A. Ubiquitin-like proteins have functional modifications similar to ubiquitination of ubiquitin proteins and participate in many cell activities, such as endoplasmic reticulum regulation, DNA repair, and stress response (Fang & Pan, 2019). UFM modification plays an important role in maintaining the homeostasis of multicellular eukaryotic cells (Zhang et al., 2019). 40S ribosomal proteins play an important role in DNA repair, apoptosis, transcriptional regulation, and translation regulation(Hassan et al., 2016). Protein disulfide-isomerase participates in protein biosynthesis and mediates protein folding and modification (Okumura et al., 2021). The eukaryotic translation initiation factor 5A plays a key role in the regulation of many cellular processes, including prolonged translation, cell proliferation, mRNA conversion, and abiotic stress response (Bian et al., 2018).

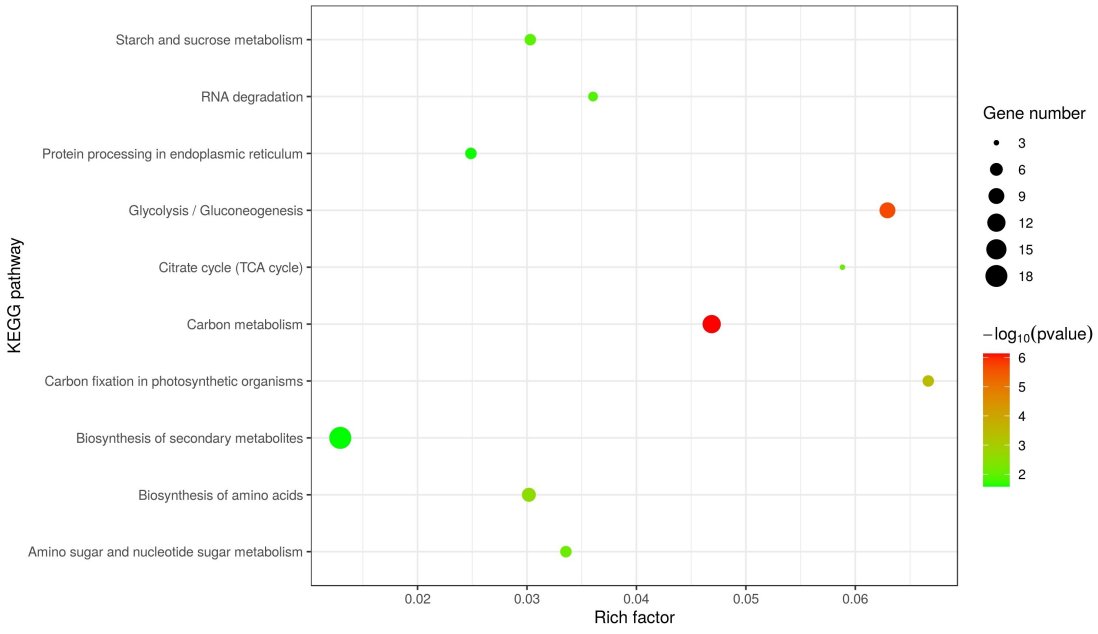
In the functional classification of antioxidation, differentially expressed proteins that were significantly upregulated and downregulated the most included peroxidase and L-ascorbate peroxidase, respectively. Peroxidase is an enzyme with heme as an auxiliary group, which mainly oxidizes substrates by catalytic decomposition of H_2O_2 or other peroxides thus can clear reactive oxygen species and participate in metabolic activities. It is therefore an important antioxidant enzyme in organisms (Kim et al., 2021; Xiao et al., 2020). L-ascorbate peroxidase (APX) is a ferrous heme protein that removes H_2O_2 produced *in vivo* using ascorbic acid as an electron donor. APX in sorghum is a hydrophilic protein located in the cytoplasm (Chen et al., 2011). After sorghum was dried by microwave technology, peroxidase expression was significantly upregulated and APX was downregulated. The observed intensity of the downregulation expression was lower than that of the upregulation. Therefore, in general, the antioxidant capacity of grain protein was enabled and improved following microwave drying.

Overall, following microwave intermittent drying of natural sorghum, significant changes in differential proteins were observed. In the functional classification of material and energy metabolism, amino acid biosynthesis, starch metabolism, antioxidation, and transportation and regulation, significant upregulation and downregulation variations of differential proteins, which have a great impact on the biological processes and functions of sorghum in subsequent processing and applications, were observed. The reason for the significant up or down regulation of differential protein expression may be attributed to the dual effects of microwave dielectric heating and electromagnetic polarization, which consequently affect biological function. During the microwave drying process of sorghum, polar water molecules in sorghum grains produce a lot of heat energy through high-speed friction and collision, causing the grain temperature of sorghum to rapidly increase. Furthermore, the existence of microwave photon energy affects the intramolecular chemical bonds of protein in the grain and the arrangement of electron

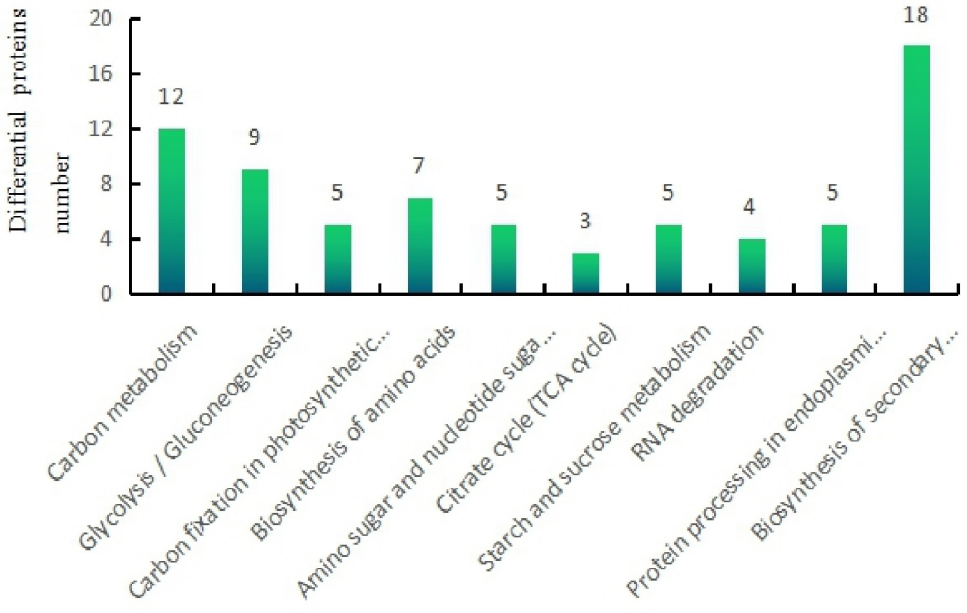
clouds around the groups, thus the conformation of protein molecules can be altered (Cheng et al., 2018).

3.2 KEGG pathway analysis of differentially expressed proteins

In living organisms, a series of biochemical molecules complete a specific biological process through a variety of cascade reactions, constituting a biological pathway (Hu et al., 2020). By means of KEGG pathway annotation of differentially expressed proteins, the metabolic or signaling pathways involved in these proteins can be understood, and a series of protein changes from cell surface to nucleus are deduced, and thus a series of biological events and factors involved in the process can be revealed (Zhang et al., 2016). Therefore, to further understand the pathway information of metabolic processes of natural sorghum after microwave drying, KEGG pathway enrichment analysis of differential expressed proteins was performed by the Fisher exact test. Figure 2 shows the enrichment analysis of the KEGG pathway involving differentially expressed proteins.



(a) Bubble diagram of the KEGG pathway



(b) Differential proteins number of the pathways

Figure 2. Enrichment analysis of the KEGG pathway involving differentially expressed proteins.

The top 10 metabolic pathways included carbon metabolism, glycolysis/gluconeogenesis, carbon fixation in photosynthetic organisms, biosynthesis of amino acids, amino sugar and nucleotide sugar metabolism, the TCA cycle, starch and sucrose metabolism, RNA degradation, protein processing in endoplasmic reticulum, and biosynthesis of secondary metabolites. The corresponding significant P-values were 0.000000730, 0.00000204, 0.000369, 0.00287, 0.00774, 0.00871, 0.0117, 0.0135, 0.0255, and 0.0262, respectively. From these results, it can be seen that the differential proteins were significantly involved largely in carbon metabolism, glycolysis/gluconeogenesis, carbon fixation in photosynthetic organisms, biosynthesis of amino acids, amino sugar and nucleotide sugar metabolism, and the TCA cycle. In addition, the metabolic pathways involved in starch and sucrose metabolism, RNA degradation, protein processing in endoplasmic reticulum, and biosynthesis of secondary metabolites were also significant.

As shown in Figure 2(a), the metabolic pathways with the highest rich factors included carbon fixation in photosynthetic organisms, glycolysis/gluconeogenesis, the TCA cycle, carbon metabolism, and RNA degradation, which suggests that the enriched significance of differential proteins in these pathways is more reliable. As shown in Figure 2(b), it can be seen from the number of differentially expressed proteins involved in metabolic pathways that the number of proteins involved in the secondary metabolite biosynthesis pathway was the largest, reaching 18; second was the number of proteins involved in the carbon metabolism pathway at 12, and third was the number of proteins involved in the glycolysis/gluconeogenesis pathway (9). Those involved in biosynthesis of amino acids were fourth (7); moreover, the number of proteins involved in other metabolic pathways ranged between 3 and 5.

3.3 Analysis of differential protein interactions

In organisms, proteins do not exist independently and their functions must be regulated and mediated by other proteins (Dong et al., 2020).The realization of this regulation or mediation firstly requires protein binding or interaction. Usually, in the interactive network, the greater the protein connectivity, the greater the disturbance to the whole system when the protein changes, which may be the key factor affecting the whole metabolic system or signal transduction pathway (Tan et al., 2020).Therefore, the interaction analysis of major differentially expressed proteins in the top 10 metabolic pathways and having high significance was carried out, and the results are depicted in Figure 3.

As seen in Figure 3, differentially expressed proteins of natural sorghum after microwave drying (upregulated and downregulated expression proteins) interact with each other. Therefore, they can be directly or indirectly involved in various metabolic pathways. Among them, upregulated phosphoglycerate mutase (SORBI_001G384100), phosphopyruvate hydratase (SORBI_002G186900), and an uncharacterized protein (SORBI_009G183700) showed obvious effects on other differentially expressed proteins. Downregulated glyceraldehyde-3-phosphate dehydrogenase (SORBI_004G205100), an uncharacterized protein (SORBI_003G072300), and fructose-bisphosphate aldolase (SORBI_003G393900) have obvious effects on other differentially expressed proteins, such as SORBI_003G072300 (unidentified protein).

Glyceraldehyde-3-phosphate dehydrogenase (SORBI_004G205100) is a key enzyme involved in glycolysis. In the interactive network, GAPDH shows the highest connectivity, and its downregulated expression not only affects the catalytic process of 3-phosphoglyceraldehyde to produce 1,3-diphosphoglyceric acid, but also directly or indirectly affects other major differential proteins in the interaction network. This protein may be the key factor affecting the whole system or signal transduction pathway. Phosphoglycerate mutase (SORBI_001G384100) is a key enzyme in glycolysis and gluconeogenesis. iPGAM shows high connectivity, and its upregulated expression not only affects the mutual transformation process between 3-PGA and 2-PGA, but also directly or indirectly affects other major differential

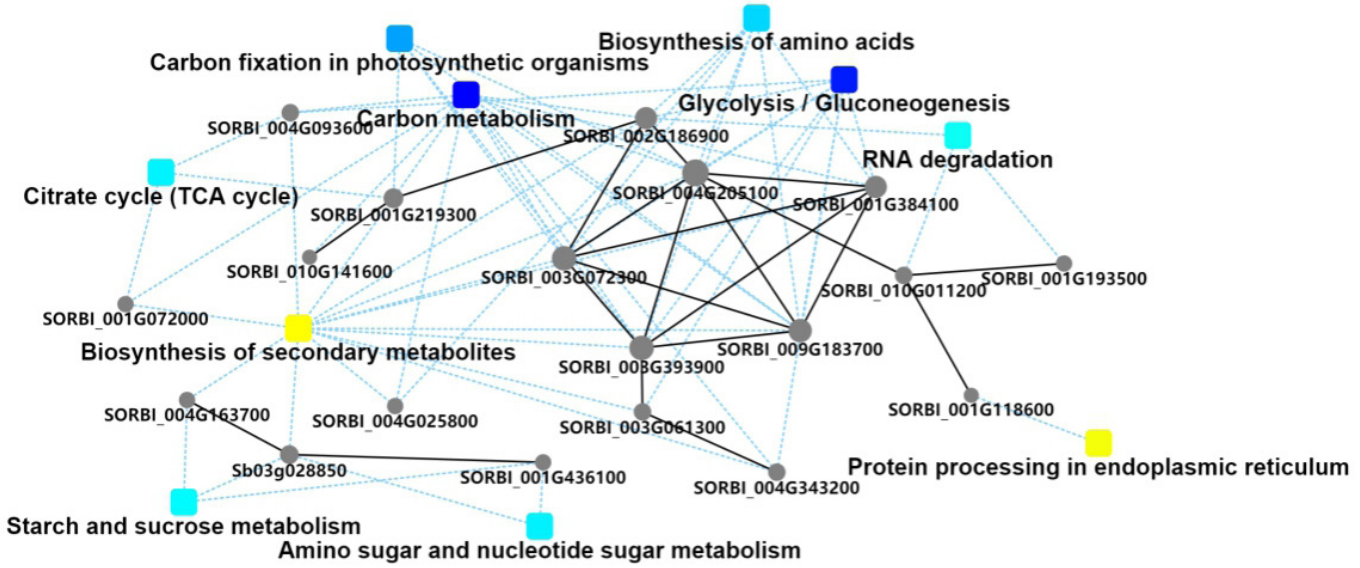


Figure 3. Interaction network of differentially expressed proteins.

proteins in the interaction network. Phosphopyruvate hydratase (SORBI_002G186900) is a key enzyme in the pathway of glucose metabolism. It shows high connectivity, and its upregulated expression not only affects the transformation of 2-phosphate glyceric acid to phosphoenolpyruvate, but also directly or indirectly affects other major differential proteins in the interaction network. Fructose-bisphosphate aldolase (SORBI_003G393900) belongs to glycolytic enzymes and is responsible for catalyzing the reversible reaction of fructose-1,6-diphosphate to glyceraldehyde triphosphate and dihydroxyacetone phosphate. It is one of the major antigenic proteins associated with cell walls (Amorim et al., 2018), and shows high connectivity. Its downregulated expression not only affects the reversible reaction of fructose 1,6-diphosphate to glyceraldehyde triphosphate and dihydroxyacetone phosphate, but also directly or indirectly affects other differential proteins.

Through the analysis of differential protein interactions, it can be seen that among the known proteins, upregulated proteins with the higher connectivity include phosphoglycerate mutase and phosphopyruvate hydratase, while downregulated proteins with the higher connectivity include glyceraldehyde-3-phosphate dehydrogenase and fructose-bisphosphate aldolase. GAPDH shows the highest connectivity in the interaction network. This protein may be the key factor affecting the whole metabolic system or signal transduction pathway. Furthermore, upregulation or downregulation of the aforementioned four proteins not only has direct or indirect effects on carbon metabolism, carbon fixation in photosynthetic organisms, biosynthesis of amino acids, amino sugar and nucleotide sugar metabolism, the TCA cycle, starch and sucrose metabolism, RNA degradation, protein processing in endoplasmic reticulum, and biosynthesis of secondary metabolites, but also has high correlation with glycolysis and glucose metabolism, which have an important impact on the metabolic pathway. The results of this analysis showed that when natural sorghum is dried by microwave technology, among the related metabolic pathways, differential protein changes in glycometabolism pathways may have the greatest impact in metabolic processes.

4 Conclusion

After natural sorghum was dried by microwave technology, among the 85 differentially expressed proteins screened, 51 proteins with low content of natural sorghum were upregulated and the corresponding protein content increased. In addition, 34 proteins with high natural sorghum content were downregulated and the corresponding protein content decreased. These results showed that the differential protein expression in natural sorghum was significantly altered following microwave drying.

Among the screened 85 differentially expressed proteins following microwave drying of sorghum, 30 proteins or enzymes with certain functions were obtained by removing unidentified proteins and proteins with unknown functions. In the functional classification of material and energy metabolism, amino acid biosynthesis, starch metabolism, antioxidation, transportation and regulation, significant upregulation and downregulation variations of differential proteins were observed. In the functional classification of material and energy metabolism, significant upregulated expression of cytochrome b5 domain-containing protein and succinate dehydrogenase, is likely to affect the redox reaction and TCA cycle, respectively, during subsequent processing of sorghum. In addition, significant downregulated expression of chitinase will delay the degradation of chitin in sorghum. In the functional classification of amino acid biosynthesis, significant upregulated expression of phosphopyruvate hydratase and iPGAM has an effect on the sugar metabolism and glycolysis process of sorghum, while significant downregulated expression of aspartate aminotransferase will have an important effect on the metabolism process of amino acids. In the functional classification of starch metabolism, significant upregulated expression of starch branching enzyme IIb and glucose-1-phosphate adenylyltransferase is likely to have favorable effects on the content and distribution of amylose and amylopectin in sorghum, and the starch synthesis process, respectively, while significant downregulated expression of alpha-1,4 glucan phosphorylase will affect the biosynthesis and degradation process of sorghum starch. Furthermore, significant downregulated expression of alpha-amylase inhibitor can promote the hydrolysis and digestion of carbohydrates, and is conducive for the metabolism of material to produce energy. In the functional classification of transportation and regulation, significant upregulated expression of ubiquitin-fold modifier will likely affect processes such as the regulation of the endoplasmic reticulum, DNA repair, and stress response. In the functional classification of antioxidation, significant upregulated expression of peroxidase is likely to improve the antioxidant capacity of sorghum in biological processes.

KEGG pathway analysis of differentially expressed proteins showed that after natural sorghum was dried by microwave technology, the differential proteins were significantly ($P < 0.01$) involved in carbon metabolism, glycolysis/gluconeogenesis, carbon fixation in photosynthetic organisms, biosynthesis of amino acids, amino sugar and nucleotide sugar metabolism, and the TCA cycle. The metabolic pathways involved in starch and sucrose metabolism, RNA degradation, protein processing in the endoplasmic reticulum, and biosynthesis of secondary metabolites reached significant levels ($0.01 < P < 0.05$). Through the interaction analysis of differential proteins, it was determined that among the known proteins, upregulated and downregulated proteins with high connectivity included phosphoglycerate mutase and phosphopyruvate hydratase, and glyceraldehyde-3-phosphate dehydrogenase and fructose-bisphosphate aldolase, respectively. GAPDH had the highest connectivity in the interaction network, implying that this protein may be the key factor affecting the entire metabolic system or signal transduction pathway. Up-regulation or down-regulation of these four proteins not only has direct or indirect effects on carbon metabolism, carbon fixation in photosynthetic organisms, biosynthesis of amino acids, amino sugar and nucleotide sugar metabolism, the TCA cycle, starch and sucrose metabolism, RNA degradation, protein processing in the endoplasmic reticulum, and biosynthesis of secondary metabolites, but has a significant correlation with glycolysis and glucose metabolism, which have an important impact on the metabolic pathway. Overall, the study showed that after natural sorghum is dried by microwave technology, among the related metabolic pathways, differential protein changes in glycolysis and glucose metabolism pathways may have the greatest impact

in metabolic processes, and can affect the biological processes and nutritional properties of microwave dried sorghum.

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