Effects of superheated steam processing on common buckwheat grains: Lipase inactivation and its association with lipidomics profile during storage

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Research

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Abstract

Background: Buckwheat deteriorates easily during storage, resulting loss of nutrients, rancidity flavor and poor consumer acceptability. Superheated steam (SS) was used to inactivate lipase of common buckwheat grains in this study, in order to retard lipid hydrolytic rancidity and maintain lipid nutrition of common buckwheat.

Methods: Buckwheat grains were treated with SS at 110-200°C, for 0-7 min and SS treatment parameters were optimized by moisture content and lipase activity. The changes in free fatty acid (FFA) and lipase activity of SS-treated and untreated buckwheat during 12 weeks storage at 4°C, 25°C and 50°C were determined. Meanwhile, the effects of SS treatment on fatty acid compositions and lipidomics profile of buckwheat before and after storage were also evaluated. Moreover, the associations of hydrolytic rancidity with lipase activity and lipidomics profile were analyzed.

Results: SS processing at 170 °C for 5 min was proved to be an effective method for buckwheat stabilization. Better stabilities based on lower FFA accumulation and lipase activity were observed in SS-treated buckwheat samples during storage. Meanwhile, SS could suppress oxidation of unsaturated fatty acids (UFA) in buckwheat, significantly retard the increase of saturated fatty acids (SFA) and the decrease of polyunsaturated fatty acids (PUFA) during storage. Moreover, the lipidomics profile results indicated that SS processing could retard the increased hydrolysis and oxidation of sulfoquinovosyl diacylglycerol (SQDG), phosphatidylethanolamine (PE), phosphatidylserine (PS) and lysophosphatidic acid (LPA) during storage.

Conclusion: Thus, SS processing could effectively inactivate lipase, suppress UFA oxidation, change glycerolipids (GLs) and glycerophospholipids (GPs) subclass metabolism, and consequently retard hydrolytic rancidity and lipid nutrition loss of buckwheat during storage. This work was first time to demonstrate the application of SS processing for the effective quality control of buckwheat during storage.

Background

Consumers have been increasingly interested in buckwheat due to its unique nutritional compositions and various health benefits [1]. Although the contents of lipids are less than those of starch and protein, they play a key role in nutritional and functional significance [2]. Lipids are generally categorized into eight classes: fatty acids, glycerolipids (GLs), glycerophospholipids (GPs), sphingolipids, sterol lipids, prenol lipids, saccharolipids, and polyketides [3, 4]. GLs and GPs as the structural elements of cell membranes play the unique role in stabilizing cell membranes and protecting membrane lipids from hydrolytic enzymes [5]. Dietary GPs have beneficial effects on a range of human diseases including coronary heart disease, cancer and inflammation [6]. Evidence suggests that a high intake of saturated fatty acids (SFA) from the diet may be associated with elevated cardiovascular disease risk, the change in SFA/unsaturated fatty acid (UFA) ratio may contribute to improve human health [7]. Common

buckwheat (Fagopyrum esculentum) as one of the main cultivated buckwheat species in the world contains 8–11% phospholipids (of total lipids in buckwheat), lower SFA amounts and higher essential fatty acid (mainly linoleic acid), which may promote its positive health effects [8].

Although the lipid content in buckwheat ranges from 1.9–3.2%, it mainly contributes to easy deterioration of buckwheat during storage [8]. The content of free fatty acid (FFA) can be increased significantly and exceeded limit within short storage duration, resulting in rancidity flavor and poor consumer acceptability of buckwheat flour. UFA are easily oxidized to produce hydroperoxides and other harmful substances. It has been reported that lipid degradation and oxidation plays an important role in buckwheat quality deterioration during storage [9–11]. However, the detailed lipid degradation pathways mechanisms are unclear. Meanwhile, the lipidomics profile of buckwheat is unavailable, which limits the further study and applications of buckwheat.

The lipase activity is an important factor in grain quality deterioration, because it not only affects generation of FFA from lipids hydrolytic rancidity by enzymatic reaction of lipases [12], but also affects subsequent fatty acid oxidation and degradation, involved with the formation of conjugated hydroperoxy fatty acids by lipoxygenase or autoxidation, and then further broken down (non-enzymatic reaction) or oxygenation by peroxidase [13]. Suzuki et al. [9] suggested that lipase activity is relevant to lipid degradation in quality deterioration of buckwheat flour. Moreover, lipase is more thermally stable than lipoxygenase, and lipoxygenase exhibits little activity at the low moisture content of stored grain [14]. Therefore, inactivation of lipase is the common goal for most heat treatment to lengthen the shelf life of cereal grains [14]. However, to our best knowledge, the lipase inactivation methods of buckwheat and their effects on the buckwheat quality is still rarely studied.

To prolong the shelf life and maintain the high quality of cereal grains, various heat treatments designed to inactivate enzymes have been the primary means for cereals processing and storage, such as superheated steam (SS), hot air, infrared heating and microwave [15–19]. SS treatment has became a novel thermal stabilization technology suitable for food processing, because of its several advantages. Firstly, the higher temperature together with higher enthalpy of SS than saturated steam and hot air processing at the same pressure leads to more efficient heating [20]; secondly, high heat penetration of SS can not only increase the temperature of food rapidly, but also result in a reverse moisture transfer induced by the condensation and evaporation of moisture on the food [21, 22]; thirdly, an oxygen-free environment can significantly reduce oxidative degradation reactions during SS processing [22]. Recently, SS has been found to be effective for inactivate enzymes: peroxidase and lipolytic enzymes of wheat bran at 170 °C within 7 min [18]; peroxidase of oat groats at 110 °C for 10 and 14 min [23]; lipolytic enzymes of brown rice at a temperature above 125 °C within 2 min [24]. However, there are no detailed studies about the effects of SS processing on buckwheat, especially its effects on lipolytic enzymes inactivation and lipidomics profile.

Given that lipase activity plays an important role in the quality deterioration of buckwheat, we hypothesized that SS treated buckwheat grains prior to storage would inactivate lipase and thus stabilize

buckwheat during storage. The aim of this study were to (1) investigate the real effects of SS treatment on lipase inactivation in buckwheat by a quantification of lipase activity under different SS-treated conditions; (2) determine the changes in hydrolytic rancidity during different storage conditions of SS-treated buckwheat compared with untreated buckwheat; (3) analyze the effect of SS treatment on the lipidomics profile of buckwheat before and after storage.

Methods

Materials

Dehulled common buckwheat grains (*Chiqiao 1#*, main cultivar of common buckwheat in China) were obtained from the Chifeng Academy of Agriculture and Animal Husbandry Sciences in December, 2018 (Inner Mongolia, China). The initial moisture content of the buckwheat grain was 12.28%. High-performance liquid chromatography (HPLC)-grade acetonitrile, isopropyl alcohol, and methanol were purchased from Merck (Darmstadt, Germany). Other chemicals used were either analytical or reagent grade.

Optimization of SS treatment

The moisture content of buckwheat grains was adjusted to 20.0% (w.b.) by tempering for further SS treatment according to the reported method of Head et al. [25]. Buckwheat grains with a given mass of distilled water in a sealed bag were preserved at 20–25 °C for 12 h to guarantee the total absorption of water. Buckwheat grains were treated in an SS processing system developed by the Laboratory of Cereal Science at China Agricultural University, Beijing, China, based on our previous method and schematic diagram [21]. The tempered buckwheat grains (about 300 g) were scattered on the metal mesh sample tray in a uniform thin layer (2–3 mm) and conveyed into the processing chamber. The steam velocity of SS was 15.0 m^3/h, and the temperature was set at 110 °C, 140 °C, 170 °C and 200 °C. The processing time ranged from 1 to 7 min at 2 min intervals. The buckwheat grains were cooled to room temperature by spreading out after SS treatments, and then were used to analyze moisture and lipase activity immediately. The buckwheat grains without SS treatment were considered as the control. All experiments were conducted in triplicate.

Moisture content

Buckwheat grains were ground by a high-speed grinding mill (HY-04A, Beijing Huanyatianyuan Instrument Co., Ltd, Beijing, China) and passed through a 60 mesh sieve. The moisture content of ground buckwheat was determined using a standard method (approved method 44-19.01; AACC International, 2017). All measured response variables were reported on a dry weight basis (d.b.).

Determination of lipase activity

The lipase activity of ground buckwheat was determined according to the previous study of Jha et al. [26] by using glycerol trioleate as a substrate with phosphate buffer of pH 7.4 and expressed as mg of 0.01 M

KOH/g of buckwheat.

Storage studies

After SS treatment at 170 °C for 5 min as a optimized results, the buckwheat grains were sealed in plastic bags and stored at 4 °C, 25 °C, 50 °C in temperature controlled incubators for 12 weeks. After 2, 4, 8 and 12 weeks, samples were withdraw from the storages, ground and sieved as described in section of moisture content. The ground buckwheat flour were re-packaged in plastic bags and stored at -20 °C until further FFA, lipase activity, fatty acid compositions and lipidomics profile analysis. The untreated buckwheat grains were also stored and analyzed as the control.

Free fatty acids value

FFA values were determined according to the method of Zhao et al. [27]. The value was expressed as mg KOH which was consumed by neutralizing FFA in 100 g ground buckwheat on a dry weight basis (mg KOH/100 g d.b.). These measurements were carried out in triplicate.

Fatty acid composition

Buckwheat flour (0.5 g) were mixed with 1 mL toluene, 2 mL sulfuric acid in methanol (v/v = 1:99), then left overnight in a water bath at 50 °C. After cooling to room temperature, the mixture was further mixed with sodium chloride (5 mL, 5% w/v) and extracted with hexane twice. The hexane layer was collected and washed with 4 mL of 2% (w/v) $KHCO_3$-water solution. The upper layer of hexane containing the fatty acid methyl esters was evaporated with N_2, and dissolved in 1 mL hexane for the determination of fatty acids composition using an Agilent 7890A Gas Chromatography/Agilent 5975C Mass Spectroscopic (GC-MS) system (Agilent Technologies Inc., Beijing, China) equipped with an Agilent HP-5-MS capillary column (30 m × 0.25 mm × 0.25 μm). The oven temperature was programmed using the method of Liang et al. [28]. Fatty acids were identified by comparison of the retention times to those of a standard fatty acid methyl esters mixture, and the results were expressed as percentage of total fatty acids. These measurements were carried out in triplicate.

Lipidomics profiling

The lipid extraction was performed according to Folch and Bligh methods [29, 30] with minor modification. Ground buckwheat (60 mg) and chloroform-methanol-water (v/v/v = 2:1:1, 600 μL) were sonicated for 10 min and kept in freezer (-20 °C) for 20 min, followed by frozen centrifugation at 14,000 g for 10 min. The lipid-containing chloroform layer was collected and the residue was extracted twice more. The chloroform layer was combined and evaporated with N_2. The dried lipid residue was redissolved in 200 μL of methanol-isopropanol (v/v = 1:1) and filtered through 0.22 μm organic filter before analysis.

The lipidomics profiling was accomplished using a Nexera UPLC system (Shimadzu, Kyoto, Japan) tandem Q-Exactive Orbitrap mass spectrometer (Thermo Fisher, CA, USA) equipped with a heated electrospray ionization (HESI) probe, and separated on an ACQUITY UPLC BEH C18 column (1.7 μm, 2.1 ×

100 mm) (Waters) in both negative and positive ionization modes. The binary solvent system consisted of mobile phase (A) acetonitrile-water = 6:4 and mobile phase (B) acetonitrile-isopropanol = 1: 9 (both mobile phase containing 0.1% formic acid and 10 mM ammonium formate, v/v), and separation was achieved using the following gradient at a flow rate of 0.35 mL/min: 30% B hold 3 min, 30–62% B over 3–5 min, 62–82% B over 5–15 min, 82–99% B over 15–16.5 min, the composition was held at 99% B for 1.5 min, then 18–18.1 min, 99% to 30% B, and 18.1–22 min holding at 30% B. The injection volume was 5 μL and the mass range was set at m/z 120–1800. The HESI source parameters were set as follows: heater temperature of 300 °C; sheath gas flow rate of 45 Arb; auxiliary gas flow rate of 15 Arb; sweep gas flow rate of 1Arb; spray voltage of 3.5KV; capillary temperature of 320 °C. Lipid identification were performed using the software Lipidsearch 4.0 (Thermo Fisher, CA). According to the information for retention time and characteristic product ions, all lipids were identified based on MS1 (mass error < 5 ppm) and MS2 (mass error < 8 ppm).

Statistical analysis

Data were statistically analyzed by one-way analysis of variance (ANOVA) using SPSS version 18.0 software (SSPS Institute, Chicago, USA). Duncan's multiple range test ($P < 0.05$) was performed to determine the significant differences. The correlation among the studied variables (lipase activity, FFA and content of each lipid subclass) in the samples was determined by two-tailed Pearson correlation analysis ($P < 0.01$). The linear regression of FFA vs. lipase activity was performed using Origin software (version 8.5, Microcal Inc., Northampton, MA, USA).

Results

Effect of superheated steam on moisture content

Figure 1a shows the effect of SS processing temperature and time on moisture content of buckwheat grains. The moisture content of SS-treated buckwheat grains decreased with the extended time and increased temperature of SS. When buckwheat grains were treated by 110 °C SS,140 °C SS,170 °C SS and 200 °C SS for 7 min, their moisture contents were decreased from 20.26–14.82%, 12.10%, 8.89% and 5.81% respectively, namely, higher temperature led to higher rate of moisture removal of buckwheat grains. In particular, after treated with SS at 170 °C for 5 min, the moisture content decreased from 20.26–12.09%, which was not significantly different from the original moisture content of untreated buckwheat (12.28%).

Effect of superheated steam on lipase inactivation

Lipase activity of buckwheat grains decreased with an increase in processing time and temperature of SS (Fig. 1b). Higher temperatures (170 °C and 200 °C) of SS treatment showed a better inactivate effect than 110 °C or 140 °C. An average level of lipase activity of buckwheat grains decreased by 26.24%, 28.98%, 55.53% and 62.70%, respectively, after the SS treatment at 110 °C, 140 °C, 170 °C and 200 °C for 7 min.

Meanwhile, it was observed that when SS treatment time was shorter than 1 min, the steam temperature had no significant effect on the improvement of lipase inactivated efficiency. More than 50% of the lipase activity could be inactivated at 170 °C when the SS treatment was more than 5 min, but there was no significant difference between 170 °C-5 min and 170 °C -7 min in the reduction of lipase activity.

Free fatty acid accumulation in buckwheat during storage

The effect of SS on FFA accumulation during storage was shown in Fig. 2a-c. There was an increasing trend of FFA in untreated buckwheat at 4 and 25 °C during storage, and the highest FFA was observed after 12 weeks of storage. The FFA of untreated buckwheat stored at 50 °C increased rapidly at the beginning of storage, reached maximum levels (134.03 mg KOH/100 g d.b.) at 8 weeks, and then showed a slight decline afterward. Compared with untreated buckwheat, SS-treated buckwheat exhibited significantly fewer accumulation in FFA during storage at each storage temperature. The FFA of SS-treated buckwheat did not change significantly when stored at 4 °C and 25 °C for 12 weeks, even for 50 °C storage, which was always lower than 100 mg KOH/100 g d.b.

Changes in lipase of buckwheat during storage

Changes in lipase of untreated and SS-treated buckwheat during storage were summarized in Fig. 2e-f. In general, the lipase activity of SS-treated samples was significantly ($P < 0.05$) lower than that of untreated samples at each temperature. The lipase of all untreated buckwheat samples initially decreased from 7.57 KOH/g d.b and reached minimum levels of 3.16–5.84 KOH/g d.b after 8 weeks of storage. The lipase activity of untreated buckwheat decreased most by 57.85% at storage temperature of 50 °C for 8 weeks, but that of SS-treated buckwheat decreased slightly lower by 44.44%. Moreover, there was no significant difference in lipase activity of SS-treated buckwheat among different temperature conditions at the end of storage.

Fatty acid compositions of buckwheat during storage

Fatty acid compositions of untreated and SS-treated buckwheat during storage were summarized in Table 1. Before storage, the predominant fatty acids in untreated buckwheat were palmitic acid (18.04 ± 0.35%), oleic acid (34.31 ± 0.43%) and linoleic acid (35.00 ± 0.58%), which together constituted 87.35% of the total fatty acids. The percentages of stearic acid, behenic acid and docosenoic acid were significantly ($P < 0.05$) increased after SS treatment. There was no significant difference ($P > 0.05$) in percentage distribution of total SFA, total monounsaturated fatty acids (MUFA), and total polyunsaturated fatty acids (PUFA) between untreated and SS-treated buckwheat. After 12 weeks storage, the percentage of total SFA in untreated buckwheat increased significantly and that of total PUFA decreased significantly. The biggest reduction of PUFA was found in linoleic acid. However, SS treatment significantly retard the increase of SFA (mainly palmitic acid, stearic acid, arachidic acid and behenic acid) and the reduction of

PUFA (mainly linoleic acid) during storage. The fatty acid compositions of stored SS-treated buckwheat were similar to those of fresh untreated and SS-treated buckwheat.

Table 1
Fatty acid compositions (% of total fatty acids) of buckwheat grains during storage

Fatty acids	0 week		12 week	
	Untreated	SS-treated	Untreated	SS-treated
C14:0 Myristic	0.16 ± 0.14a	0.17 ± 0.07a	0.16 ± 0.06a	0.18 ± 0.02a
C15:0 Pentadecanoic	0.16 ± 0.01a	0.13 ± 0.02b	0.16 ± 0.02a	0.14 ± 0.00ab
C16:0 Palmitic	18.04 ± 0.35b	17.51 ± 0.14b	19.04 ± 0.65a	17.85 ± 0.79b
C16:1 Palmitoleic	0.21 ± 0.06b	0.17 ± 0.00b	0.17 ± 0.00b	0.28 ± 0.01a
C18:0 Stearic	2.06 ± 0.04c	2.14 ± 0.01b	2.49 ± 0.05a	2.00 ± 0.02c
C18:1 Oleic	34.31 ± 0.43a	33.96 ± 0.28a	34.02 ± 0.35a	34.62 ± 0.37a
C18:2 Linoleic (ω-6)	35.00 ± 0.58a	34.91 ± 0.30a	33.04 ± 0.12b	34.83 ± 0.29a
C18:3 Linolenic (ω-3)	2.85 ± 0.04a	2.72 ± 0.04a	2.43 ± 0.03b	2.47 ± 0.17b
C20:0 Arachidic	1.32 ± 0.15b	1.50 ± 0.13b	1.86 ± 0.05a	1.41 ± 0.06b
C20:1 Gadoleic	2.84 ± 0.12ab	3.07 ± 0.06a	2.64 ± 0.09b	2.73 ± 0.20b
C22:0 Behenic	1.69 ± 0.11c	1.97 ± 0.01b	2.41 ± 0.09a	1.94 ± 0.11b
C22:1 Docosenoic	0.07 ± 0.12b	0.25 ± 0.01a	0.14 ± 0.12ab	0.23 ± 0.01ab
C24:0 Lignoceric	1.29 ± 0.19a	1.49 ± 0.08a	1.44 ± 0.05a	1.32 ± 0.10a
∑SFA	24.72 ± 0.15b	24.92 ± 0.18b	27.56 ± 0.49a	24.84 ± 0.59b
∑MUFA	37.43 ± 0.45a	37.45 ± 0.35a	36.97 ± 0.56a	37.86 ± 0.55a
∑PUFA	37.85 ± 0.58a	37.63 ± 0.34a	35.47 ± 0.09b	37.31 ± 0.29a

Values are mean ± standard deviation (n = 3). Mean in a row with different lowercase letters indicate statistical difference ($P < 0.05$). SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

Lipidomics profile differences of buckwheat samples

Figure S1 (Supplementary Material 1) shows representative UPLC-Q-Exactive Orbitrap MS spectra of lipid extract from different buckwheat samples. The signals in positive ionization mode were mainly attributed to sphingoid (So), phosphatidylcholine (PC), phosphatidylethanolamine (PE), triacylglycerol (TG), lysophosphatidylcholine (LPC), diacylglycerol (DG), and in negative ionization mode were mainly

attributed to ceramide (Cer), cardiolipin (CL), phosphatidic acid (PA), phosphatidylglycerol (PG), phosphatidylserine (PS), phosphatidylinositol (PI), monogalactosyl diacylcerol (MGDG), digalactosyl diacylglycerol (DGDG). The different molecular species of lipids in buckwheat were shown in Tables S1-S4 (Supplementary Material 2). A total of 457 lipid molecular species belonging to 23 lipid classes were detected. The lipids in buckwheat were dominantly composed of GPs and GLs, which accounted for 67.57–87.59% of the total lipids. The number of lipid molecular species of each lipid subclass in buckwheat was shown in Table 2. The sum of lipid molecular species of the buckwheat were: stored SS-treated buckwheat > fresh SS-treated buckwheat > stored untreated buckwheat > fresh untreated buckwheat. Before storage, the total species of GPs decreased and that of GLs increased after SS processing. Storage significantly increased the GLs and GPs species in untreated buckwheat, and SS treatment slow down this change in GPs species during storage. The lipids categories were analyzed by summing the relative abundance of individual lipids in the same class (Fig. 3). The relative abundance of PC and PE decreased significantly after SS treatment before storage, and that of PS, PG, So, phosphatidylinositol monophosphate (PIP), lysophosphatidic acid (LPA), TG, DG and monoglyceride (MG) increased significantly ($P < 0.05$). Meanwhile, the relative abundance of PA, PC, LPA, digalactosyl monoacylglycerol (DGMG) and TG significantly decreased after storage, and that of CL, DGDG, sulfoquinovosyl diacylglycerol (SQDG), phosphatidylinositol diphosphate (PIP2), PE, PG and PS increased. However, SS treatment could retard the changes of SQDG, PE, PS and LPA during storage. It was noteworthy that the relative abundance of TG, DG and MG in stored SS-treated buckwheat decreased significantly, while that of monogalactosyl monoacylglycerol (MGMG), MGDG, DGMG and DGDG significantly increased, compared with the fresh untreated buckwheat.

Table 2
The number of lipid molecular species of each lipid subclass in buckwheat grains during storage

Lipid classes	RT(time)	0 week		12 week	
		Untreated	SS-treated	Untreated	SS-treated
LPA	2.16–3.08	3	3	ND	3
LPC	2.01–4.37	3	3	2	8
LPE	4.42–6.83	3	3	ND	1
PA	7.50-12.47	5	5	6	6
PC	6.74–12.58	26	8	25	19
PE	2.54–12.85	25	5	23	13
PG	4.57–13.16	10	14	20	11
PS	6.19–12.66	11	16	17	13
PI	6.43–12.99	7	11	9	12
CL	7.22–13.71	10	12	18	17
PIP	6.62–12.08	6	9	5	9
PIP2	9.65–9.85	ND	ND	2	ND
PIP3	8.67	1	1	1	1
Total GPs	2.01–13.71	109	89	127	112
DGDG	6.27–11.75	5	6	7	10
DGMG	1.62–3.19	3	3	1	3
MGDG	4.09–11.65	6	7	6	13
MGMG	2.23–3.80	1	1	2	3
SQDG	2.35–11.63	7	9	14	7
MG	11.75–12.09	ND	2	ND	2
DG	7.21–15.71	19	25	19	24
TG	6.57–16.08	90	135	95	123
Total GLs	1.62–16.08	131	188	144	185

ND means not detected.

Lipid classes	RT(time)	0 week		12 week	
		Untreated	SS-treated	Untreated	SS-treated
Cer	1.79–13.35	19	16	15	17
So	0.71–4.29	5	6	7	8
Total SPs	0.71–13.35	24	22	22	25
SUM		264	299	293	322
ND means not detected.					

Association of hydrolytic rancidity with lipase activity and lipidomics profile

The scatter plots and linear regression of FFA value vs. lipase activity in untreated and SS-treated buckwheat are presented in Fig. 4a and b. The association of FFA value with lipase activity in untreated and SS-treated buckwheat had a R^2 values of 0.8221 and 0.6777, respectively. The slopes of SS-treated buckwheat (-8.28) was lower than that of untreated buckwheat (-10.27). The correlations of hydrolytic rancidity and lipid profiles were analyzed using Pearson correlation analysis and the correlation coefficients were given in Fig. 4c. The results showed that FFA value was significantly positively ($P < 0.01$) correlated with the relative abundance of SQDG, PE and PIP2, while had negative correlation with LPE ($P < 0.01$), TG, DG and MG. In addition, lipase activity was negatively correlated with the relative abundance of So, CL, MGMG, MGDG DGDG, SQDG, PA, PG, PI, PIP and PIP3.

Discussion

The aim of this study were to investigate the real effects of SS treatment on lipase inactivation, hydrolytic rancidity and lipidomics profile of buckwheat, and to verify the hypothesis that SS treated buckwheat grains prior to storage would inactivate lipase and thus stabilize buckwheat during storage. The study firstly optimize the best stabilization condition by SS treatment as 170 °C-5 min, based on the retaining moisture and relative high lipase inactivation in buckwheat grains, for the following storage experiments. Tempering prior to SS treatment was observed as an efficient way to eliminate enzymes in buckwheat grains, because it increased the moisture content and protected buckwheat from drying to very low final moisture content after SS processing. SS processing at 170 °C-5 min was better for retaining moisture of buckwheat grains at 12.09%. A moisture content of 16% is considered stable for avoiding buckwheat deterioration during storage [31], accordingly, buckwheat grains treated with 170 °C-5 min SS could be stored directly without drying or other additional moisture adjustment. Meanwhile, higher temperature and longer treatment of SS could improve the efficiency of lipase inactivation, and more than 50% lipase activity could be inactivated at 170 °C for 5 min. Although the efficiency of 200 °C SS was more significant than 170 °C, when the treatment time was extended to 5 min or more, the color of buckwheat grains changed greatly due to a mass loss of water within short processing time and thus partial scorch, which seriously affected the quality and acceptability. Moreover, lipase could not be completely

inactivated in the present conditions, which might because the lipase in buckwheat was relatively stable compared with other grains, and the lipase activity in buckwheat was too low to reach a lower level. The lipase activity of oat (30 mg/g) was inactivated 78% by 160 °C-2 min SS treatment and inactivated completely at 170 °C-5 min [32]. The lipase activity of brown rice (5.25-12 mg/g) became constant at about 5% when the SS treatment time was extended to 2 min, regardless of its temperature [24]. The present lipase results of buckwheat were in accordance to the previous study in highland barley that the lipase content in barley was too low to inactivated totally, and 160 °C-8 min SS treatment could inactivated only 50% of lipase [33]. Whether SS could stabilize the quality of buckwheat by inactivating more than 50% lipase was determined in subsequent storage experiments.

Lipase hydrolysis results in the release of FFA and glycerol. Thus, an increase of the amount of FFA released from buckwheat grains during storage provides necessary information on potential development of hydrolytic rancidity. In this study, the lipidic acidity rates of untreated buckwheat significantly increased with an increase in storage period. The time to reach maximum level of FFA in the lipid hydrolysis process was related to enzyme activity and storage condition. Lipid oxidation and the inhibition of mould growth at extremely high storage temperature were key for the FFA inflection point of buckwheat at 50 °C storage [34, 35]. Similar results were reported by Li et al. [36] study that FFA value of brown rice initially increased at the beginning of storage and reached maximum levels after 225 d of storage, then followed by a slight decline. According to the GB/T35028-2018 [37], the FFA value of buckwheat flour with fresh and good quality must be below 120 mg KOH/100 g d.b.. The FFA value in untreated buckwheat exceeded this prescribed limit seriously after stored at 50 °C for 8 weeks, accompanied with rancidity flavor. However, the SS-treated buckwheat could maintain good quality after 12 weeks of storage. SS treatment could retard FFA accumulation and inhibit lipid hydrolytic rancidity in buckwheat during storage, even SS processing was more effective than low temperature (4 °C) storage.

The inhibitory effect of SS treatment on lipid hydrolytic rancidity was also observed based on the lipase activity change during storage. The significantly lower lipase activity in SS-treated samples suggested that SS treatment could inactivate lipase and slow down the conversion rate of lipid, thus reduce the hydrolytic rancidity of lipid during storage. Meanwhile, the lower level of lipase variation in SS-treated buckwheat during storage, further indicated that inactivation of more than 50% lipase by SS could effectively stabilize the quality of buckwheat. SS could change the structures or conformations of lipase [38], so as to change the active site of the enzyme and the interaction with the substrate, and finally passivate the lipase activity and stabilize the quality of buckwheat.

The fatty acid composition was in accordance with FFA and lipase activity, supporting that SS treatment could suppress lipid hydrolysis and oxidation during storage. UFA accounted for a large proportion of fatty acids (75.28%) in buckwheat, which was in accordance with the studies of lightly milled rice [15] and wheat bran [18]. Compared with SFA, UFA are important for functional properties of buckwheat and considered more beneficial to human health. However, UFA are highly susceptible to thermo-oxidation during heating due to the presence of π bonds with high reactivity [15]. SS treatment was an oxygen-free heating medium, which protected UFA from oxidation. This is the reason why SS treatment significantly

retard the increase of SFA (palmitic acid, stearic acid, arachidic acid, behenic acid) and the reduction of PUFA (linoleic acid) induced by storage. Maximum reduction in linoleic acid induced by storage indicated it might be the preferential substrate for oxidative rancidity [15]. These findings supported that SS processing was effective in suppressing lipid hydrolytic and oxidative rancidity while maintaining/improving buckwheat nutritional attributes and membrane stability during storage. Even though, SS treatment still caused slight lipid hydrolysis and changed lipid composition to some extent, according to the changes in fatty acid composition in fresh SS-treated buckwheat. It might be because heating treatment could result in disintegration of membranous structures or inactivation of heat labile antioxidants in cereals [39]. This was subsequently confirmed by the lipidomics profile results.

SS processing led to the decrease of total GPs species and the increase of total GLs species in buckwheat before storage, which was consistent with the previous results about changes in lipid molecule species by other thermal processing [40]. GPs and galactolipids are major structural constituents of cellular membranes and they play a key role in maintaining cellular homeostasis [41]. During the storage, the significant hydrolysis and oxidation of GPs and GLs occurred, based on the results of increased GLs and GPs species in stored untreated buckwheat, which caused the changes in components and structure of the phospholipid membrane, and further led to the release of TG [2]. However, SS treatment could slow down the change of GPs (mainly PE, PS and LPA) during storage, which might be because of the phospholipase inactivation. The decrease in TG, DG and MG of stored SS-treated buckwheat indicated that GLs partially hydrolyzed, probably due to the destruction of phospholipid membrane by SS treatment prior to storage. The destruction of phospholipid membrane resulted in the TG leak which could contact with lipases in the aleurone and germ tissues [2]. Although SS-treated samples contained sufficient lipase substrates (e.g. TG), the degree of lipid hydrolysis and rancidity was much lower than that of untreated samples. Therefore, the enzyme activity, action sites and environment were particularly important for the stabilization of SS-treated buckwheat during storage. It was necessary to balance the inactivation of lipase and the destruction of phospholipid membrane by heat treatment to achieve better storage effect. This was also supported by our above results of FFA value and lipase activity that SS treatment altered the lipase site where the interaction with the lipase substrate were changed so that reducing lipase activity to low enough to prevent excessive accumulation of fatty acids.

Maintaining membrane integrity of cereals by reducing the saturation of fatty acids and the synthesis of galactolipids (e.g. DGDG, MGDG, SQDG) plays a key role in their growth adaptation [41, 42]. The increase in MGMG, MGDG, DGMG and DGDG of SS-treated buckwheat, suggested that SS treatment affected lipid metabolism of buckwheat during storage by modifying lipid composition, thus inhibiting the destruction of phospholipid membrane, maintaining the integrity and fluidity of cell membrane, and finally improving the storage quality of buckwheat. In terms of the nutritional application, the increase content of galactolipids improved the nutritional value of lipids in buckwheat. Given no scientific studies have been conducted directly to confirm the lipidomics profile of buckwheat after SS processing and/or storage, the further research on lipid metabolism pathway of buckwheat is still needed to be illustrated in more details.

Liner regression analysis of FFA value in response to lipase activity showed that SS suppressed the rate of hydrolytic rancidity as compared to untreated buckwheat. With each unit decrease of lipase activity, a lower unit of FFA response were found for these SS-treated buckwheat. Correlation analysis showed that the increase of FFA mainly comes from the hydrolysis of GL, while the increase of SQDG, PE and PIP2 could be used as a marker of lipid hydrolytic rancidity. These results suggested that the inactivation of lipase by SS could effectively reduce the lipid hydrolytic rancidity of buckwheat during storage, and the change in lipidomics profile was effective to characterize the change of buckwheat quality.

Conclusion

The present study focused on the effects of SS processing on lipase activity, lipid hydrolytic rancidity, and lipidomics profile of buckwheat. SS processing at 170 °C for 5 min was proved to be an effective method for buckwheat stabilization. Inactivation of more than 50% lipase by SS could effectively inhibit lipid hydrolytic rancidity of buckwheat during storage. SS processing considerably suppressed FFA accumulation, lipase activity variation, UFA oxidation, and GPs hydrolysis (mainly PE, PS and LPA) during storage. SS processing could improve membrane integrity and fluidity of buckwheat during storage by regulating the UFA/SFA ratio and the content of galactolipids (mainly SQDG, MGMG, MGDG, DGMG and DGDG). Moreover, the correlation analysis indicated that the lipase inactivation had an important effect on lipid metabolism. Therefore, SS processing should be a new efficient technology that could inactivate lipase of buckwheat, change its GLs and GPs subclass metabolism during storage while maintain its lipid nutrition at the same time, so as to improve the storage quality of buckwheat. In addition, it is also demonstrated that lipidomics analysis is a workable approach to monitor the dynamic changes in lipid characteristics of buckwheat during storage, which provides scientific support for buckwheat quality control.

Abbreviations

SS:superheated steam; FFA:free fatty acid; UFA:unsaturated fatty acids; SFA:saturated fatty acids; PUFA:polyunsaturated fatty acids; MUFA:monounsaturated fatty acids; Cer:ceramide; CL:cardiolipin; DG:diacylglycerol; DGDG:digalactosyl diacylglycerol; DGMG:digalactosyl monoacylglycerol; GL:glycerolipid; GP:glycerophospholipid; LPA:lysophosphatidic acid; LPC:lysophosphatidylcholine; MG:monoglyceride; MGDG:monogalactosyl diacylcerol; MGMG:monogalactosyl monoacylglycerol; TG:triacylglycerol; So:sphingoid; SQDG:sulfoquinovosyl diacylglycerol; PA:phosphatidic acid; PC:phosphatidylcholine; PE:phosphatidylethanolamine; PG:phosphatidylglycerol; PI:phosphatidylinositol; PS:phosphatidylserine; PIP:phosphatidylinositol monophosphate; PIP2:phosphatidylinositol diphosphate; PIP3:phosphatidylinositol triphosphate.

Declarations

Competing interests

The authors declare no competing interests.

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Authors’ contributions

Lijuan Wang, collected, elaborated the literature and drafted the manuscript. Libo Wang, provided the technical assistance. Qiu Ju, helped to draft the manuscript. Zaigui Li, conceptualized the idea. Both authors read and approved the final manuscript.

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Availability of data and material

The dataset supporting the conclusions of this article is included within the article.

Ethics approval and consent to participate

Not Applicable.

Consent for publication

Not Applicable.

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Supplementary information

Supplementary Material 1: Figure S1.

Supplementary Material 2: Table S1-S4.

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Figures

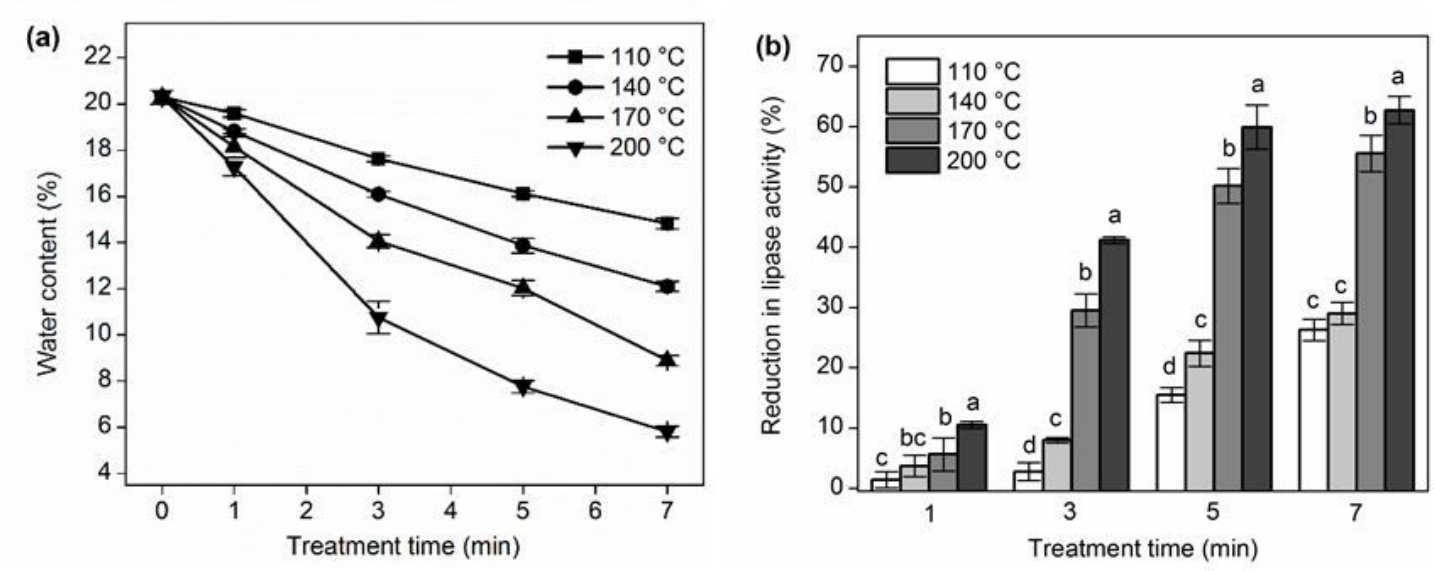

Figure 1

Effect of SS on moisture content (a) and lipase activity (b) of buckwheat grains. Values are expressed as mean ± standard deviation (n = 3). Different lowercase letters marked above the bar indicate statistical difference ($P < 0.05$).

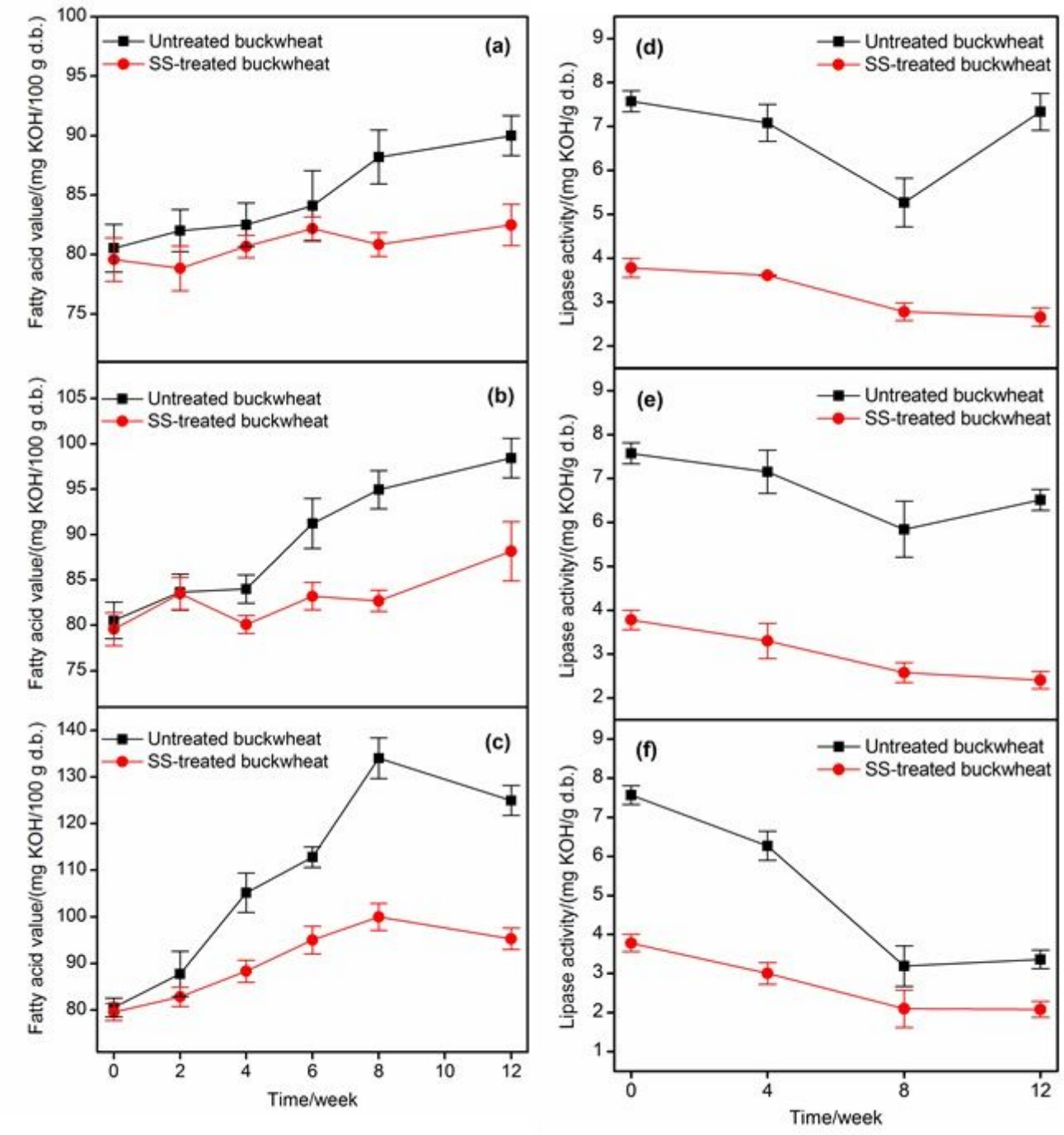

Figure 2

Changes of FFA value (a-c) and lipase activity (d-f) in buckwheat grains during storage. FFA at (a) 4, (b) 25, and (c) 50 °C. Lipase activity at (d) 4, (e) 25, and (f) 50 °C. Values are expressed as mean ± standard deviation (n = 3).

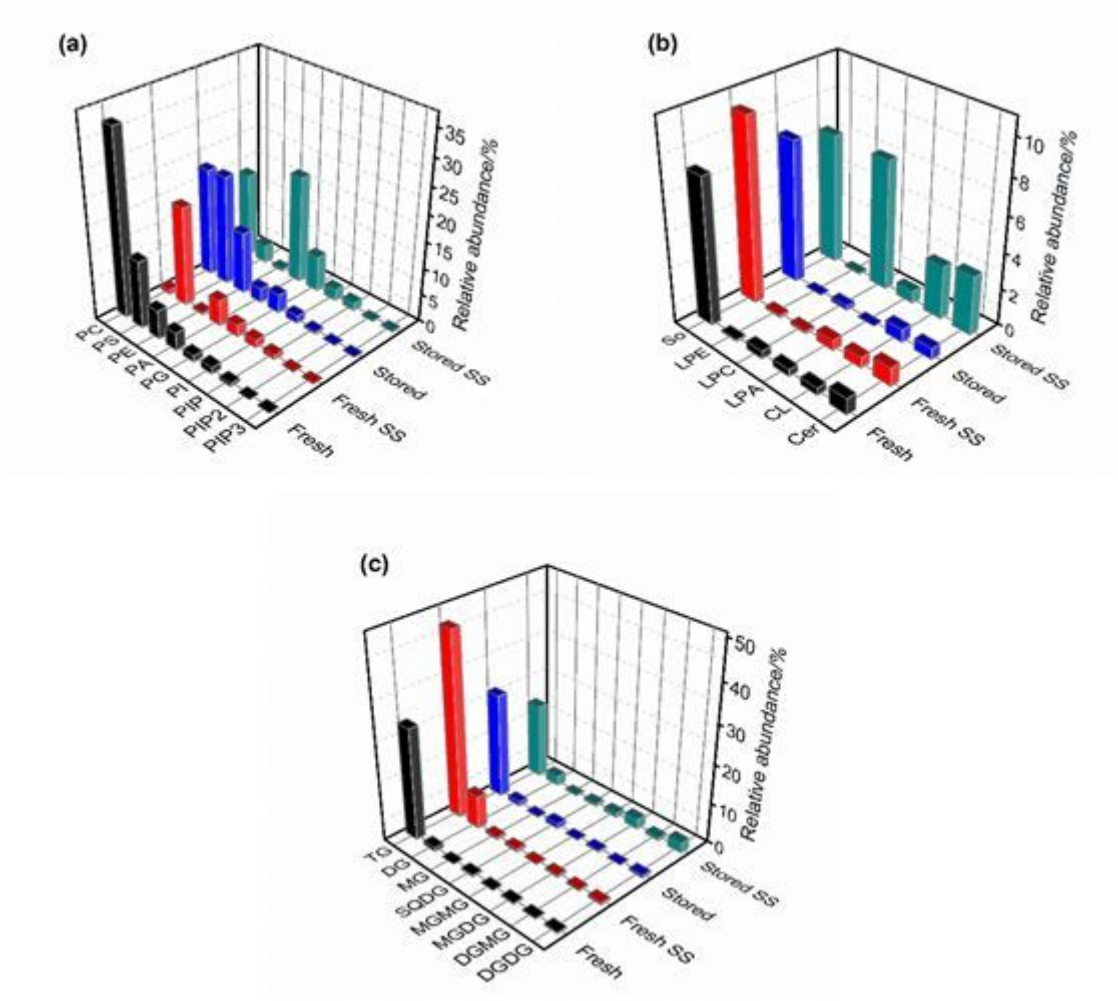

Figure 3

Relative abundance (%) of each lipid subclass in buckwheat grains during storage. Fresh: untreated buckwheat at 0 week; Fresh SS: SS-treated buckwheat at 0 week; Stored: untreated buckwheat at 12 week; Stored SS: SS-treated buckwheat at 12 week.

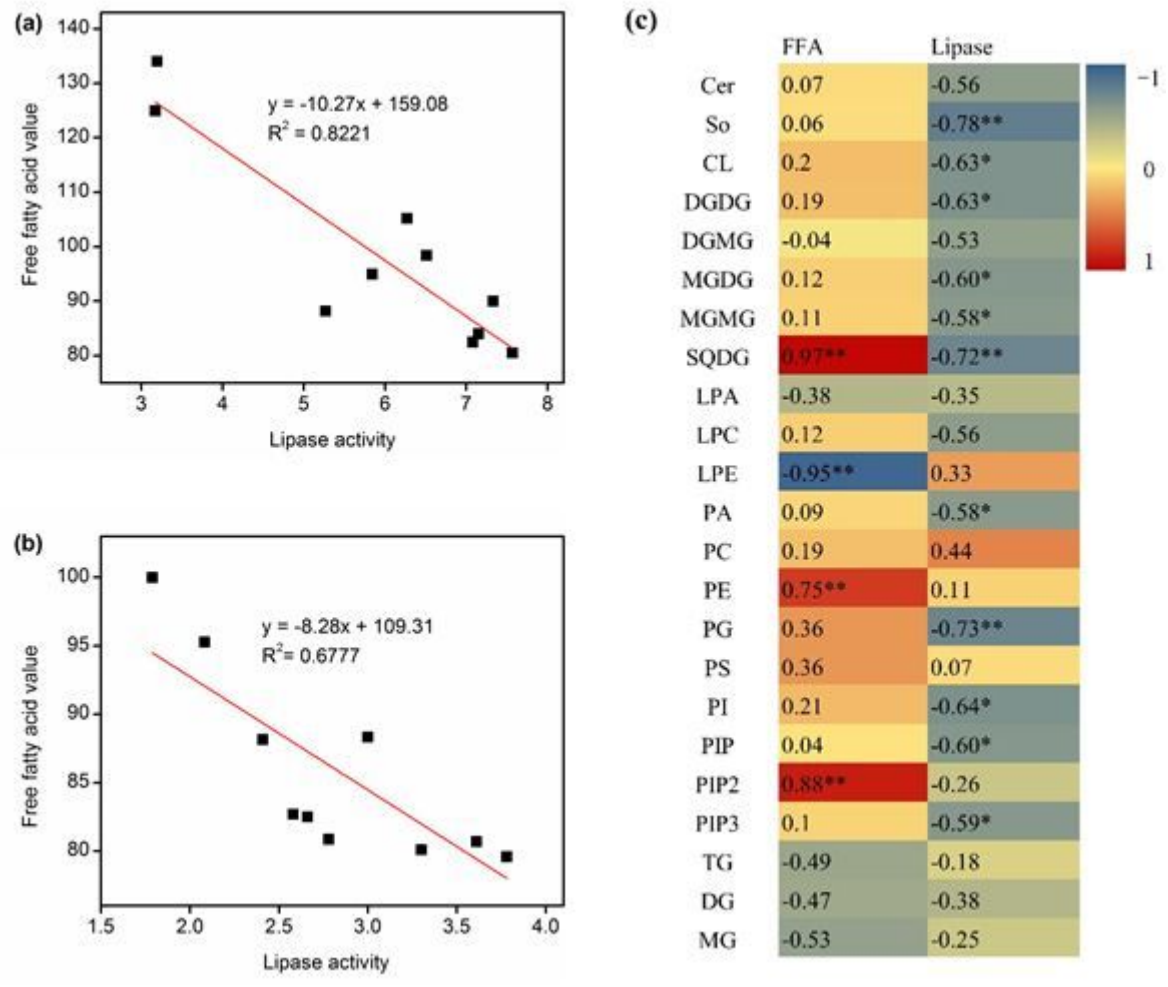

Figure 4

Association of hydrolytic rancidity with lipase activity and lipidomics profile in buckwheat. Linear regression of FFA value and lipase activity in untreated buckwheat (a) and SS-treated buckwheat (b); Pearson correlation coefficients of hydrolytic rancidity and different lipid species (c). Data with single asterisk (*) and double asterisk (**) are statistically significant at $P < 0.05$ and $P < 0.01$, respectively.

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