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Research Article
Comparison of Grain Processing Techniques on Saponin Content and Nutritional Value of Quinoa (*Chenopodium quinoa* Cv. Yellow Pang-da) Grain

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Abstract

Background and Objective: Quinoa grain contained saponin in pericarp, which causes bitter flavor. After harvesting, quinoa grain is required to remove saponin before being consumed. Thus, this study aimed to study post harvest management of grain processing on the saponin and nutrition value of quinoa grain. **Materials and Methods:** The experiment was arranged in a Completely Randomized Design (CRD) with three replications and saponin removal technique with milling process (T_1-T_2) and reagent washing (T_3-T_8) were used as experimental treatments comparing with non-process grains as a control (T_9). Nutrition analysis was an indication of quality in post-process quinoa grain. **Results:** The experiment found that T_1, T_3, T_4, T_5, T_6 and T_7 could reduce saponin content significantly different from T_9, while T_2 and T_8 still show high saponin content when compared with T_9. T_2 and T_4 techniques could maintain most of the nutritional value of quinoa grain when compared with control (T_9). **Conclusion:** Finally, this experiment could be concluded that quinoa was washed by alkaline solution (pH 8) for 8 min by three times (T_4) could be an optimum of saponin removal technique. This technique not only removed saponin but could also maintain quinoa grain qualities. Meanwhile, it potentially reduced for 66.03 percent of saponins content when compared with control treatment, which did not change in protein content, flavonoid content, moisture content, starch content, phenolic content and color (L*) of quinoa grain.

Key words: Quinoa grain, processing grain, saponin remove, alkaline wash, grain qualities, post harvest management, spectrophotometric method

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Quinoa (*Chenopodium quinoa* Willd) is a dicotyledonous plant in the Amaranthaceae family originated in the Andean regions of South America[1,2]. It had different varieties or ecotypes. It could be grown in diverse climate zones and at varying altitudes which made quinoa an excellent alternative crop and its potential contribution to ensuring global food security[3].

Quinoa was called a pseudo cereal because it had similar consumed characteristics as a cereal. Besides, quinoa was considered one of the super foods because quinoa grain was highly nutritious, especially proteins and essential amino acids[4,5]. The proteins in quinoa were considered gluten-free or very small amounts of gluten due to very little or no prolamin. Quinoa grains contained 8-22% protein, which was higher than cereal grains but less than legumes. They were also rich in essential amino acids, especially lysine, which were higher than rice, corn and wheat. Furthermore, quinoa grain contained more methionine than legumes. As for dietary fiber, they were high contained in fiber, especially insoluble fiber, up to 12-14 grams per 100 grams of dry weight. Besides, they are rich in unsaturated fats, vitamins and minerals[3,6,7]. Therefore, quinoa grain was suitable as a health supplement and a replacement protein source for people who are allergic to meat proteins or as an alternative to people who are allergic to some kind of cereals[8].

Quinoa synthesized saponins, which was primarily contained in the papillose cells of the outer seed hull as a defense against pestpredation[9]. This external coat is rough, brittle and dry[10]. Saponins were a drawback for quinoa as a food and feed application because saponins possessed a bitter taste and exhibit toxic effects[11]. After harvesting, quinoa grain is necessary to remove the bitter seed coat of saponin before being consumed. Saponins in quinoa were triterpenoid saponin, they were large molecules containing a hydrophilic part at one end separated from a lipophilic or hydrophobic part at the other[10,12,13]. Therefore, they could be removed either by abrasive or washing methods[10]. Besides, water, alcohols (methanol, ethanol) and aqueous alcohols were the most common extraction solvents for saponins, the solubility of some saponins in ether, chloroform, benzene, ethyl acetate,orglacialaceticacid[14]. Furthermore, the Solubility of saponins is also affected by the properties of the solvent as affected by temperature, composition and pH[15]. For general saponin removal methods, there are 3 types, which were dry method, wet method and combined method. Bacigalupo and Tapia[16] compared the advantages and disadvantages of wet, dry and combined methods to the effects on nutritional quality of the processed grain, effective saponin removal, water and energy consumption and the cost of these processes. They found that the dry method was comfortable to manage but the processed grain still contained high saponin content and reduced the nutritional value of the grain (protein and lipid). As for wet and combined methods, they were effective in removed saponin and maintained the nutritional value of grain but the wet method required large amounts of water and had a high cost of drying. Therefore, if having equipment and machinery, the combined method would be appropriate for removed saponin. In industry, most processing companies currently preferred the combined method, because it efficiently removes saponins and maintains grain quality[10]. Quinoa could thus be considered sweet if it contains 0.11% of saponins or less on a fresh weight basic[17]. Recently, Codex Alimentarius issued the standard of saponin level for accept consumption in quinoa[18]. Thus, the standard value at 0.12% was the maximum limit which be considered convenient for consuming in quinoa.

In the country that has produced quinoa grain in an industrial scale, various appropriate technologies have been developed for removing saponins to levels within the acceptable limits, without affecting the grain's nutritional properties[10]. Furthermore, in the north part of Thailand, quinoa cultivation has the potential to grow and yield especially Yellow Pang-da variety which could produce 12-16 tons a year. However, commercial quinoa grain production in Thailand is still limited because quinoa is a new potential introduced plant for the Thai market which was high consumer demand. Therefore, managing the post-harvest technology of quinoa grains required further education and development[8]. Thus, the objective of this research was aimed to study and compare the effects of saponin removal methods on the ability to eliminate saponin and the change in the nutritional value of quinoa grains of the Yellow Pang-da variety and also to enable to respond the needs of the production process of quinoa grains for commercial consumption demand in Thailand.

MATERIALS AND METHODS

Quinoa grains: The quinoa (*Chenopodium quinoa* cv. Yellow Pang-da) was planted in KaNoi Royal Project Development Center, Chiang Mai Province from November, 2018 to February, 2019. For the post-harvest process, quinoa grains were pre-cleaned by the grain blower method and dried until 10-12% of grain moisture content. Finally, grains were stored in a plastic storage box under room temperature before being process.

Processing of quinoa grains: The quinoa grains were submitted to the saponin removal processes which was consist of milling process and washing with many patterns. Different pH of alkaline solutions, water temperatures and different methanol concentrations (Table 1). The milling process was using 30 g of grain and processed with the rice milling machine that adjusted the rubber-roller distance and milling cycles. For washing processes, 50 g of grain was used for saponin removal processed according to Table 1. Finally, the washed grains were dried until 10-12 percentage of grain moisture content and store in the plastic bag at room temperature.

Analytical determinations

Moisture content: Moisture content was determination according to Sluiter *et al.*[19].

Saponin content: Determination of saponin content was described in Nickel *et al.*[20] and Ingkavanich *et al.*[21] (modified). For the extraction, 1.25 g of processed grains were added 5 mL of 80% (v/v) methanol and left at room temperature for 72 hrs. The extracts were filtered and adjusted volume to 5 mL with 80% (v/v) methanol. The analysis was using the combination of 0.25 mL methanolic extracts or standard to reacted with 0.25 mL of 4% (w/v) vanillin (Sigma-Aldrich, MO, USA) and 2.5 mL of 72% (v/v) H_2SO_4, then boiled at 60°C for 10 min, finally analyzed with Gene Quant 1300 spectrophotometer (GE Healthcare, CHI, USA) at 544 nm. Standardization was used diosgenin (Sigma-Aldrich, MO, USA).

Protein content: For protein content determination was using elemental analyzer LECO CHN628 (LECO Corp., MI, USA), which described by Beljkas *et al.*[22].

Lipid content: Lipid content was rapidly extracted according to Lam and Proctor[23].

Starch content: The starch content was evaluated by modified hydrochloric acid dissolution method according to Koch *et al.*[24]. Weighed 2.5 g of milled grain was added 25 mL of HCl, shook well and added a further 25 mL of HCl. Then, boiled the extraction for 15 min and adjusted final volume to 90 mL with cold water. Added 5 mL of wolfram phosphoric acid (Roth, Germany). Then, made up to volume with water and filtered into 100 mL Erlenmeyer flasks. Measured the optical rotation of the filtrate with the Kreipo 0.05 polarimeter (Zeiss, Germany) at 589 nm (= D-line of sodium).

Phenolic content: For extraction, 0.2 g milled grain was added the 5 mL 80% ethanol and then centrifuged by Centrifuge 5804 R (Eppendorf, Hamburg, Germany) at 5000 rpm min^{-1} for 10 min. Collected the supernatant then repeat the extraction and combine the supernatant. Fill with 10 mL 80% ethanol. Phenolic content was measured according to the Folin-Ciocalteu method of Mohammed *et al.*[25] (modified). Added 2.4 mL of distilled water to extracted samples and combined with 1 mL of 0.5 mol L^{-1} NaOH and 100 µL of Folin-Ciocalteu reagent (Sigma-Aldrich, MO, USA). Then, incubated with water bath at 37°C for 15 min. Measured at 735.8 nm with HP 8453 UV-Visible Spectrophotometer (Agilent Technologies, CA, USA). Gallic acid (Sigma-Aldrich, MO, USA) was used for standardization.

Flavonoid content: The extraction method was described in Hassan *et al.*[26]. Then, analyzed flavonoid content by used aluminum chloride colorimetric method according to Al-Saeedi and Hossain[27].

Total ash: For Total ash determination was using a muffle-furnace (Nabertherm, Germany), which described by Singh *et al.*[28].

Grain color: Grain color evaluation was analyzed by a colorimeter (Konica Minolta, USA). Color calibration was using white calibration plate (Y = 92.1, x = 0.3154, y = 0.3210) as reference. 10 g of milled grains were spread in a petri dish and random measurement on each sample.

Statistical analysis: Analysis of variance (ANOVA) was using a Completely Randomized Experimental Design (CRD) with 3 replications and mean comparisons were accomplished by Least Significant Difference (LSD) with alpha = 0.05. Statistix 8.0 software was required for all statistical analysis.

RESULTS AND DISCUSSION

The analyses of variance of grain processing on quinoa grain qualities showed the grain processing had no significantly different on flavonoid content, moisture content, starch content, phenolic content and L* value of grain color. Unfortunately, other qualities which were lipid content, total ash, saponin content, a* value and b* value of grain color were statistically significant differences (Table 2). The experimental results indicated that the techniques had efficiency on saponin removed which affected on some grain qualities changed.

Table 1: Experimental treatments which were saponin removal techniques

Treatment	Milling	Washing	Duration	Times
T_1	Rubber-roller 0.4 mm	-	-	2
T_2	Rubber-roller 0.4 mm	-	-	3
T_3	-	5% (w/v) NaOH at pH 7.5	6	2
T_4	-	5% (w/v) NaOH at pH 8.0	8	3
T_5	-	60°C of water	8	2
T_6	-	80°C of water	8	2
T_7	-	40% (v/v) methanol	6	3
T_8	-	40% (v/v) methanol	8	3
T_9*	-	-	-	-

*Remarked: Non-processed as control

Table 2: Analysis of variance of quinoa grain processing on grain qualities changed

										Grain color		
Source of variance	DF	Protein content	Flavonoid content	Moisture content	Starch content	Phenolic content	Lipid content	Total ash	Saponin content	L*	a*	b*
Treatment	8	2.22	2.68	3.06	2.05	1.34	3.95*	5.44*	4.64*	2.55	4.28*	42***
Error	18											
Total	26											
CV		4.26	27.74	6.34	3.08	38.34	11.49	8.27	28.18	1.13	12.97	1.20

DF: Degree of freedom, CV: Coefficient of variation and *,**,***Significant at p<0.05, 0.01, 0

As observed in Fig. 1, the result of the saponin removal technique showed T_1, T_3, T_4, T_5, T_6 and T_7 it's not significantly different, whileT_2 and T_8 still show high saponin content when compared with the control which was non-processed grain (T_9). T_3 could reduce saponin content to 381.23 mg 100 g^{-1} DW, while non-processed grain contained 1253.07 mg 100 g^{-1} DW. The recommend techniques to reduce saponin were T_1, T_3, T_4, T_5, T_6 and T_7. According to Gómez-Caravaca *et al.*[29] indicated that the pearling process could decrease saponin content to 50.88 mg 100 g^{-1} DW from non-processed grain which contains 244.3 mg 100 g^{-1} DW. In part of washing process, Vega-Galvez *et al.*[30] showed that the saponin content in raw grain (6.34 g 100 g^{-1} DW) diminished to 0.25 g 100 g^{-1} DW after washing for half an hour. Furthermore, Irigoyen and Giner[18], Liu *et al.*[31] and Yuliana *et al.*[32] reported that alkaline solution, the function of time, water temperatures and methanol solution had the efficiency to reduced saponin content from non-processed grain.

According to saponin removal techniques, they had could remove the pericarp fraction. Therefore, some grain qualities were changed. Figure 2 showed that the total ash of T_1 to T_8 was significantly decreased when compared with control. This might decrease some of the minerals content in quinoa is particularly rich in calcium, magnesium, iron and zinc. Minerals, such as calcium and phosphorus are associated with pectic compounds of the pericarp cell wall[33].

The grain color as a* value was the red/green coordinate when the a* value increased, the red color also became darker. The result showed that T_1 to T_8 significantly decreased a* value when compared with control (T_9) (Fig. 3). b* value was the yellow/blue coordinate when the b* value increased, the yellow color also became darker. b* value of T_1 to T_8 also significantly decreased especially T_1, T_2 and T_4 when compared with control (T_9) (Fig. 4). The a* and b* value of grain color was decreased because the outer layer of quinoa grain pericarp was removed, which determined the color of the grain. Quinoa grain after grain processing showed lighter. Furthermore, quinoa grain contained the largest amount of neutral lipids. Free fatty acids were detected in the seed hulls, accounting for 15.4 g 100 g^{-1} of total lipids[34]. T_1 and T_7 significantly decreased lipid content when compared with control (T_9), while T_2, T_3, T_4, T_5, T_6 and T_8 were not significantly different with control (T_9) (Fig. 5).

Effect of saponin removal process to grain quality would be depends on the location of accumulated nutrient which deposited in the grain. In fact, most of nutrients were in the inner section of grains, such as protein accumulated in embryo while the starch accumulated in perisperm[11,35,36]. Therefore, saponin removal process would not affect to another nutrient which not located at the surface or pericarp.

According to experimental results indicated that T_4 technique not only showed high saponin removed efficiency but also maintain the most of quinoa grain qualities. However, when compared the residual saponin content in the quinoa grain after the saponin removal process with the Codex Alimentarius standard, which

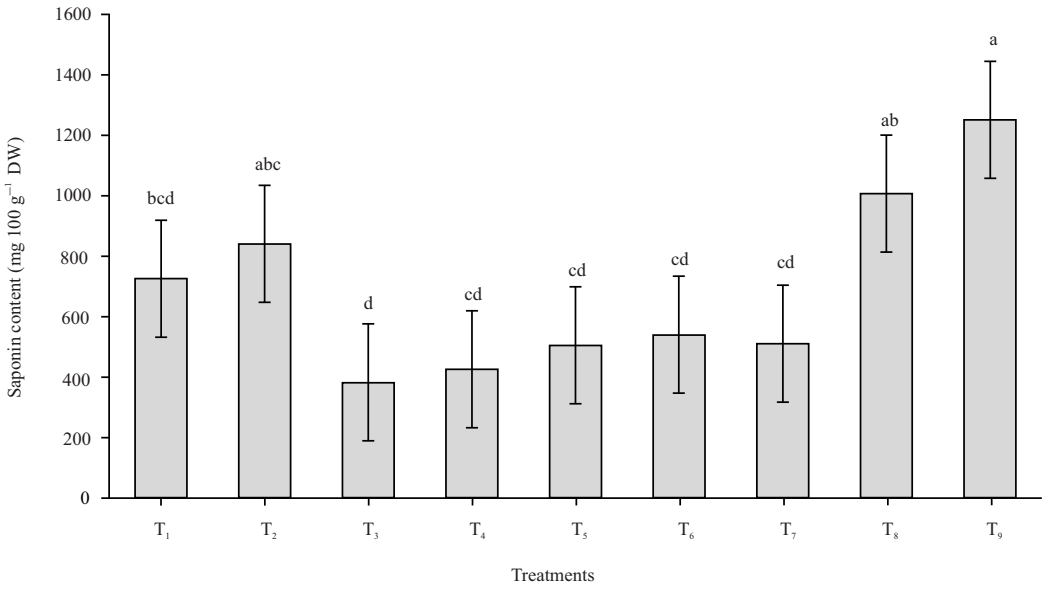

Fig. 1: Effect of grain processing treatments on saponin content (mg100 g^{-1} DW)

Experimental treatments were the distance of rubber-roller 0.4 mm with milling 2 times (T_1), the distance of rubber-roller 0.4 mm with milling 3 times (T_2), washing with an alkaline solution at pH 7.5 for 6 min and washing 2 times (T_3), washing with an alkaline solution at pH 8 for 8 min and washing 3 times (T_4), washing with 60°C of water for 8 min, washing 2 times (T_5), washing with 80°C of water for 8 min and washing 2 times (T_6), washing with 40% (v/v) methanol for 6 min and washing 3 times (T_7), washing with 40% (v/v) methanol for 8 min and washing 3 times (T_8) and non-processed (T_9*). Results are expressed as mean and SE. Different letters indicate statistical differences by the LSD's at $p<0.05$

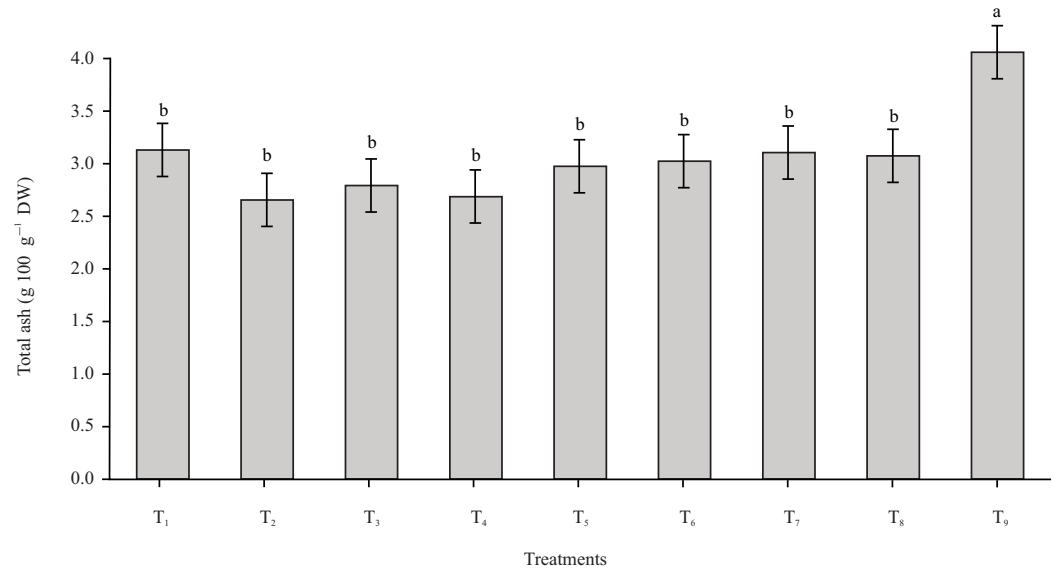

Fig. 2: Effect of grain processing treatments on total ash (g100 g^{-1} DW)

Experimental treatments were the distance of rubber-roller 0.4 mm with milling 2 times (T_1), a distance of rubber-roller 0.4 mm with milling 3 times (T_2), washing with an alkaline solution at pH 7.5 for 6 min and washing 2 times (T_3), washing with an alkaline solution at pH 8 for 8 min and washing 3 times (T_4), washing with 60°C of water for 8 min, washing 2 times (T_5), washing with 80°C of water for 8 min and washing 2 times (T_6), washing with 40% (v/v) methanol for 6 min and washing 3 times (T_7), washing with 40% (v/v) methanol for 8 min and washing 3 times (T_8) and non-processed (T_9*). Results are expressed as mean and SE. Different letters indicate statistical differences by the LSD's at $p<0.05$

determined the acceptable saponin value for consumption in quinoa at 0.12%[18]. All 8 treatments still had exceeded the standard saponin content caused by a small rice milling machine might not be suitable for quinoa morphology[10], resulting in poor abrasive performance. Meanwhile the washing treatments showed the required

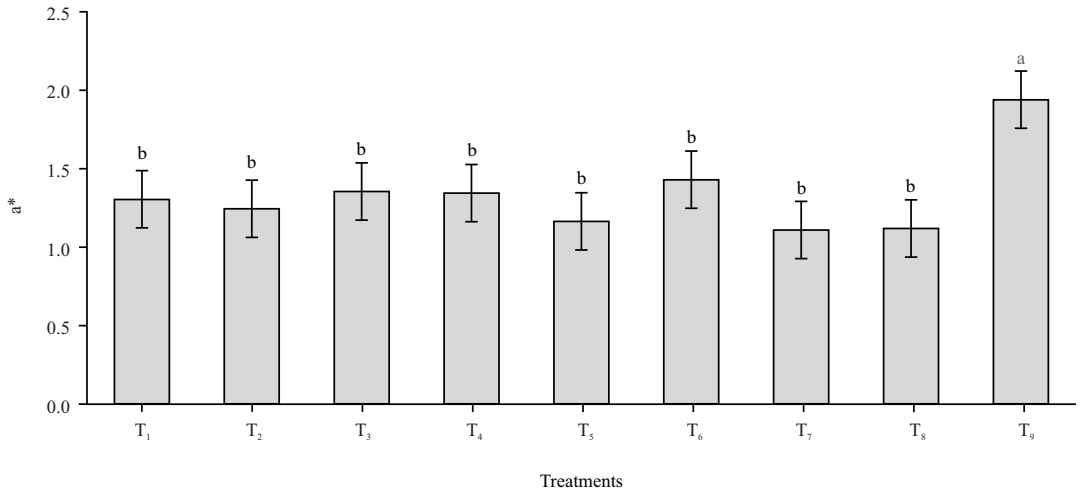

Fig. 3: Effect of grain processing treatments on grain color (a*)

Experimental treatments were the distance of rubber-roller 0.4 mm with milling 2 times (T_1), a distance of rubber-roller 0.4 mm with milling 3 times (T_2), washing with an alkaline solution at pH 7.5 for 6 min and washing 2 times (T_3), washing with an alkaline solution at pH 8 for 8 min and washing 3 times (T_4), washing with 60°C of water for 8 min, washing 2 times (T_5), washing with 80°C of water for 8 min and washing 2 times (T_6), washing with 40% (v/v) methanol for 6 min and washing 3 times (T_7), washing with 40% (v/v) methanol for 8 min and washing 3 times (T_8) and non-processed (T_9*). Results are expressed as mean and SE. Different letters indicate statistical differences by the LSD's at $p<0.05$

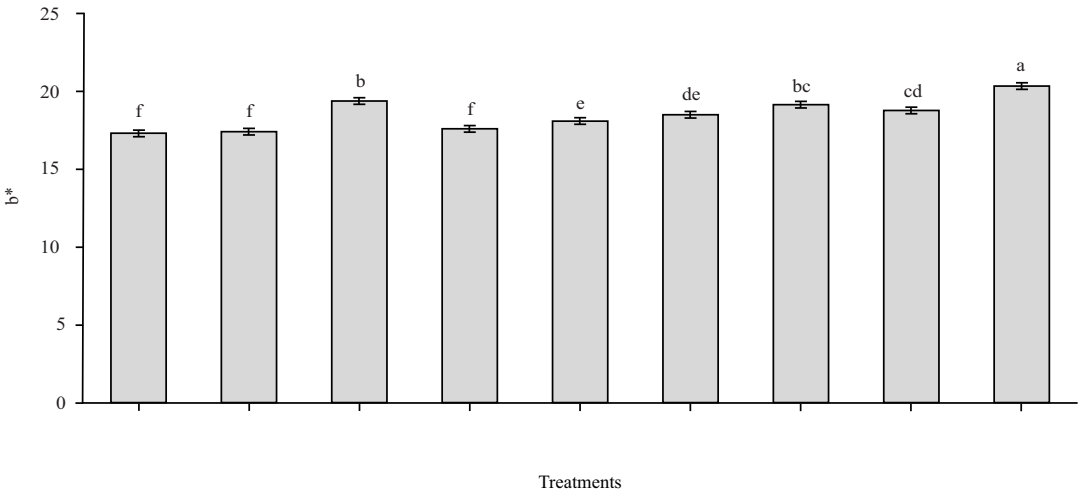

Fig. 4: Effect of grain processing treatments on grain color (b*)

Experimental treatments were the distance of rubber-roller 0.4 mm with milling 2 times (T_1), a distance of rubber-roller 0.4 mm with milling 3 times (T_2), washing with an alkaline solution at pH 7.5 for 6 min and washing 2 times (T_3), washing with an alkaline solution at pH 8 for 8 min and washing 3 times (T_4), washing with 60°C of water for 8 min, washing 2 times (T_5), washing with 80°C of water for 8 min and washing 2 times (T_6), washing with 40% (v/v) methanol for 6 min and washing 3 times (T_7), washing with 40% (v/v) methanol for 8 min and washing 3 times (T_8) and non-processed (T_9*). Results are expressed as mean and SE. Different letters indicate statistical differences by the LSD's at $p<0.05$

to add more soaking step before start washing to made it easier leaching of saponin[37]. For methanol's experiments, needed to suggest that should be performed with caution because the toxicity of methanol which remain in the grain[38,39]. In the real case for consuming the seeds, using ethanol as a solvent instead might be more recommended. Therefore, to increase the efficiency of the removal of saponins for the acceptable standard and food secure promising. Further research should develop the methods and equipment that had more efficiency to remove saponins which pass the standard of food safety.

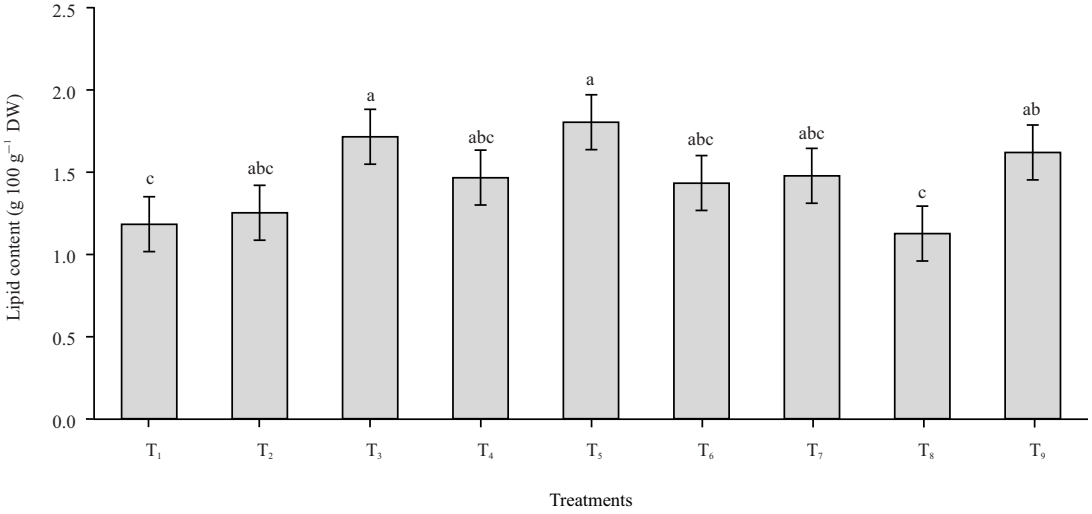

Fig. 5: Effect of grain processing treatments on lipid content (g100 g^{-1} DW)

Experimental treatments were the distance of rubber-roller 0.4 mm with milling 2 times (T_1), a distance of rubber-roller 0.4 mm with milling 3 times (T_2), washing with an alkaline solution at pH 7.5 for 6 min and washing 2 times (T_3), washing with an alkaline solution at pH 8 for 8 min and washing 3 times (T_4), washing with 60°C of water for 8 min, washing 2 times (T_5), washing with 80°C of water for 8 min and washing 2 times (T_6), washing with 40% (v/v) methanol for 6 min and washing 3 times (T_7), washing with 40% (v/v) methanol for 8 min and washing 3 times (T_8) and non-processed (T_9*). Results are expressed as mean and SE. Different letters indicate statistical differences by the LSD's at $p<0.05$

CONCLUSION

The experiment could be concluded that the T_4 technique, which was washed by the alkaline solution at pH 8 for 8 min and washed 3 times was the best suitable for the saponin removal technique. Moreover, this technique could also maintain grain qualities. However, this experiment was only doing on a lab scale for further studies should be conducted to be more effective in reducing saponins, more safety and had a reasonable cost, which will lead to being used for commercial purposes.

SIGNIFICANCE STATEMENT

This study discovered a new alternative saponin removal methods of quinoa grain with considered on the nutritional preservation of quinoa grown in Thailand. In Thailand still had no reports about the research studies on this issue. Therefore, this study would be useful for further development of an effective saponin removal method for commercial quinoa grain production in Thailand.

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REFERENCES

1. Jacobsen, S.E., A. Mujica and C.R. Jensen, 2003. The resistance of quinoa (*Chenopodium quinoa* Willd.) to adverse abiotic factors. Food Rev. Int., 19: 99-109.
2. Matiacevich, S.B., M.L. Castellion, S.B. Maldonado and M.P. Buera, 2006. Water-dependent thermal transitions in quinoa embryos. Thermochimica Acta, 448: 117-122.
3. Cauda, C., C. Micheletti, B. Minerdo, C. Scaffidi and E. Signoroni, 2013. Quinoa in the Kitchen. Slow Food, Rome, Pages: 95.
4. Jancurova, M., L. Minarovicova and A. Dandar, 2009. Quinoa-a review. Czech J. Food Sci., 27: 71-79.
5. Gordon, T., 2013. Quinoa, The Unexplored Superfood: Quinoa Recipes and Weight Loss Help. Speedy Title Management LLC, US.
6. Valencia-Chamorro, S.A., 2003. Quinoa. In: Encyclopedia of Food Sciences and Nutrition, Caballero, B. (Ed.)., Academic Press, Amsterdam, pp: 4895-4902.
7. Repo-Carrasco-Valencia, R.A.M. and L.A. Serna, 2011. Quinoa (*Chenopodium quinoa*, *Willd.*) as a source of dietary fiber and other functional components. Food Sci. Technol., 31: 225-230.

8. Kansomjet, P., P. Thobunluepop, S. Lertmongkol, E. Sarobol and P. Kaewsuwan *et al*., 2017. Response of physiological characteristics, seed yield and seed quality of quinoa under difference of nitrogen fertilizer management. Am. J. Plant Physiol., 12: 20-27.
9. van Raamsdonk, L.W.D., V.G.Z. Pinckaers, J.S. Ossenkoppele, R. Houben, M. Lotgering and M.J. Groot, 2010. Quality assessments of untreated and washed quinoa (*Chenopodium quinoa*) seeds based on histlogical and foaming capacity investigations. Microsc.: Sci. Technol. Appl. Edu., 2: 1033-1038.
10. Quiroga, C., R. Escalera, G. Aroni, A. Bonifacio and J.A. González *et al*., 2015. Traditional processes and Technological Innovations in Quinoa Harvesting, Processing and Industrialization. In: State of the Art Report on Quinoa Around the World in 2013, Bazile, D. (Ed.)., FAO & CIRAD, Rome, pp: 218-249.
11. Bhargava, A. and S. Srivastava, 2013. Quinoa: Botany, Production and Uses. CABI, Oxforshire, UK, ISBN-13: 9781780642260, Pages: 264.
12. Bone, K. and S. Mills, 2013. Principles and Practice of Phytotherapy-Modern Herbal Medicine. 1st Edn., Churchill Livingstone, New York.
13. Moghimipour, E. and S. Handali, 2015. Saponin: Properties, methods of evaluation and applications. Annu. Res. Rev. Biol., 5: 207-220.
14. Hostettmann, V.K. and A. Marston, 1996. Saponins. Pharmazie Unserer Zeit, 25: 219-220.
15. Guclu-Ustundag, O. and G. Mazza, 2007. Saponins: Properties, applications and processing. Crit. Rev. Food Sci. Nutr., 47: 231-258.
16. Bacigalupo, A. and M. Tapia, 2000. Agroindustrial Potential of Underexploited Andean Crops. In: Underexploited Andean Crops and Their Nutritional Contribution, Tapia, M. (Ed.)., FAO, Rome, Italy, pp: 136-163.
17. Koziol, M.J., 1991. Afrosimetric estimation of threshold saponin concentration for bitterness in quinoa (*Chenopodium quinoa* Willd). J. Sci. Food Agric., 54: 211-219.
18. Irigoyen, R.M.T. and S.A. Giner, 2018. Extraction kinetics of saponins from quinoa seed (*Chenopodium quinoa* Willd). Int. J. Food Stud., 7: 76-88.
19. Sluiter, A., B. Hames, D. Hyman, C. Payne and R. Ruiz *et al*., 2008. Determination of total solids in biomass and total dissolved solids in liquid process samples: Laboratory analytical procedure. Technical Report No, NREL/TP-510-42621.
20. Nickel, J., L.P. Spanier, F.T. Botelho, M.A. Gularte and E. Helbig, 2016. Effect of different types of processing on the total phenolic compound content, antioxidant capacity and saponin content of *Chenopodium quinoa* Willd grains. Food Chem., 209: 139-143.
21. Ingkachotivanich, P., Y. Chanbang, B. Chuttong, P. Page and S. Sommano, 2017. Phytochemical composition and larvicidal activity of *Litsea glutinosa* crude leaf extract against the virus-transmitting mosquito, *Aedes aegypti* (L.). Med. Plants Int. J. Phytomed. Relat. Ind., 9: 88-94.
22. Beljkaš, B., J. Matić, I. Milovanović, P. Jovanov, A. Mišan and L. Šarić, 2010. Rapid method for determination of protein content in cereals and oilseeds: Validation, measurement uncertainty and comparison with the Kjeldahl method. Accredit. Qual. Assur., 15: 555-561.
23. Lam, H.S. and A. Proctor, 2001. Rapid methods for milled rice total lipid and free fatty acid determination. AACC Int., 78: 494-498.
24. Koch, M., M. Naumann and E. Pawelzik, 2019. Cracking and fracture properties of potato (*Solanum tuberosum* L.) tubers and their relation to dry matter, starch and mineral distribution. J. Sci. Food Agric., 99: 3149-3156.
25. Mohammed, A.E., I. Smit, E. Pawelzik, A.J. Keutgen and B. Horneburg, 2012. Organically grown tomato (*Lycopersicon esculentum* Mill.): Bioactive compounds in the fruit and infection with *Phytophthora infestans*. J. Sci. Food Agric., 92: 1424-1431.
26. Hassan, S.M., A.A. Al Aqil and M. Attimarad, 2013. Determination of crude saponin and total flavonoids content in guar meal. Adv. Med. Plant Res., 1: 24-28.
27. Al-Saeedi, A.H. and M.A. Hossain, 2015. Total phenols, total flavonoids contents and free radical scavenging activity of seeds crude extracts of pigeon pea traditionally used in Oman for the treatment of several chronic diseases. Asian Pac. J. Trop. Dis., 5: 316-321.
28. Singh, Y.D., P. Mahanta and U. Bora, 2017. Comprehensive characterization of lignocellulosic biomass through proximate, ultimate and compositional analysis for bioenergy production. Renewable Energy, 103: 490-500.
29. Gomez-Caravaca, A.M., G. Iafelice, V. Verardo, E. Marconi and M.F. Caboni, 2014. Influence of pearling process on phenolic and saponin content in quinoa (*Chenopodium quinoa* Willd). Food Chem., 157: 174-178.
30. Vega-Gálvez, A., R.S. Martín, M. Sanders, M. Miranda and E. Lara, 2010. Characteristics and mathematical modeling of convective drying of quinoa (*Chenopodium quinoa* Willd.): Influence of temperature on the kinetic parameters. J. Food Process. Preserv., 34: 945-963.
31. Liu, Y., Z. Li, H. Xu and Y. Han, 2016. Extraction of saponin from *Camellia oleifera* abel cake by a combination method of alkali solution and acid isolation. J. Chem., Vol. 2016. 10.1155/2016/6903524.
32. Yuliana, P., E.B. Laconi, E. Wina and A. Jayanegara, 2014. Extraction of tannins and saponins from plant sources and their effects on *in vitro* methanogenesis and rumen fermentation. J. Indonesian Trop. Anim. Agric., 39: 91-97.

33. Vega-Galvez, A., M. Miranda, J. Vergara, E. Uribe, L. Puente and E.A. Martinez, 2010. Nutrition facts and functional potential of quinoa (*Chenopodium quinoa* willd.), an ancient andean grain: A review. J. Sci. Food Agric., 90: 2541-2547.
34. Przybylski, R. G.S. Chauhan and N.A.M. Eskin, 1994. Characterization of quinoa (*Chenopodium quinoa*) lipids. Food Chem., 51: 187-192.
35. Jacobsen, S.E. and O. Stølen, 1993. Quinoa-morphology, phenology and prospects for its production as a new crop in Europe. Eur. J. Agron., 2: 19-29.
36. Prego, I., S. Maldonado and M. Otegui, 1998. Seed structure and localization of reserves in *Chenopodium quinoa*. Ann. Bot., 82: 481-488.
37. Xue, P., L. Zhao, Y. Wang, Z. Hou, F. Zhang and X. Yang, 2019. Reducing the damage of quinoa saponins on human gastric mucosal cells by a heating process. Food Sci. Nutr., 8: 500-510.
38. Hovda, K.E., K. McMartin and D. Jacobsen, 2017. Methanol and Formaldehyde Poisoning. In: Critical Care Toxicology, Brent, J., K. Burkhart, P. Dargan, B. Hatten, B. Mégarbane, R. Palmer and J. White (Eds.)., Springer, Cham, pp: 1-18.
39. Loza, R. and D. Rodriguez, 2014. A case of methanol poisoning in a child. Case Rep. Nephrol., Vol. 2014. 10.1155/2014/652129.