RUTIN EXTRACTION AND CONTENT IN BUCKWHEAT (*FAGOPYRUM ESCULENTUM*) BRAN-FORTIFIED PASTA

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The Supervisory Committee certifies that this ***disquisition*** complies with North Dakota State University's regulations and meets the accepted standards for the degree of

MASTER OF SCIENCE

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ABSTRACT

The objectives of this study were to optimize extraction of rutin from buckwheat bran and buckwheat bran-fortified spaghetti and to determine the stability of rutin during spaghetti production and preparation. Aqueous ethanol and ethanol at 50, 60, 70, 80, and 90 % were used with Soxhlet or ultrasound-assisted extraction methods and 80 % methanol extraction was evaluated with or without papain treatment. Optimal extraction treatment (80 % methanol using ultrasound-assisted extraction without enzyme treatment) was used to determine rutin content in buckwheat bran-fortified spaghetti dried at low (40 °C) or high (90 °C) temperature. Rutin content was evaluated in raw, hydrated, extruded, dried, and cooked pasta. High temperature drying reduced rutin content more than low temperature drying, and total reduction in rutin content from raw pasta mix to cooked pasta was 25 – 30 %.

ACKNOWLEDGEMENTS

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Lastly, I thank my family for their love and support. To my brother, Dallas Hoekstra, I am particularly grateful for constant willingness to be interested in my work and for teaching me to laugh in the face of my greatest challenges. And to my husband, Brian: for everything from pep talks to programming advice, you have always been there for me. Thanks for believing in me and for being my greatest support and best friend.

DEDICATION

I dedicate the efforts put into this research paper to the Creator of life, Source of genius, and Promotor of diligence.

"And whatever you do or say, do it as a representative of the Lord Jesus, giving thanks through Him to God the Father."

Colossians 3:17

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INTRODUCTION

Buckwheat (*Fagopyrum esculentum*) is a pseudo-cereal produced chiefly for flour destined for human consumption (Oplinger, Oelke, Brinkman & Kelling, 1989). By-products of buckwheat milling include a nutrient-dense bran fraction containing the seed coat, aleurone layer, and embryo of the seed (Steadman, Burgoon, Lewis, Edwardson & Obendorf, 2001a). In addition to being rich in protein, fiber, and minerals, buckwheat bran contains a high concentration of various phytochemicals (Steadman, Burgoon, Lewis, Edwardson & Obendorf, 2001b). The flavonoid quercetin-*3*-*O*-rutinoside, also known as rutin, is one of these phytochemicals found in high concentrations in buckwheat bran. Rutin has garnered much attention for the health benefits associated with its use as a supplement (Ganeshpurkar & Saluja, 2017). Fortification of cereal-based foods with buckwheat bran could therefore not only add value to a by-product of buckwheat milling but also create a functional food offering a variety of nutritional and health benefits.

One important factor in the creation of a functional food is assurance that the added benefits can sustain the typical shelf life of a product as well as any further processing performed by the consumer prior to consumption. Consumer-level processing is of particular concern in the case of dried pasta, a good candidate for the creation of a stable functional food due to low water activity during storage and widespread consumption. However, the cooking of pasta can result in nutrient leaching (Rocchetti et al., 2017). It is important for formulators of functional pasta products to understand the effect of cooking as well as of the pre-consumer processing stages of pasta production (hydration, extruding, and drying) to ensure that the intended benefit is being received by the consumer.

The purpose of the main experiment in this study was to evaluate the stability of rutin in spaghetti fortified with buckwheat bran at each stage of pasta production and preparation. The initial null hypothesis of this objective was no effect of pasta processing stage on population values for rutin content in buckwheat bran-fortified pasta. To accurately evaluate this first research objective, an appropriate rutin extraction procedure was needed. Therefore, two additional experiments were conducted. The objective of the first extraction experiment was to determine the most effective solvent type, solvent concentration, and extraction protocol (Soxhlet versus ultrasound-assisted extraction) for the removal of rutin from buckwheat bran. The initial null hypothesis of this study was no impact of any extraction factors on population values for rutin extraction from buckwheat bran. The objective of the second extraction experiment was to determine the effect of papain treatment on the extraction of rutin from buckwheat bran-fortified pasta, with the initial null hypothesis that papain treatment has no impact on population values for rutin extracted from this pasta.

LITERATURE REVIEW

Buckwheat

Buckwheat is a part of the dicot family *Polygonaceae* (Cai, Corke & Li, 2004). It is often referred to as a pseudo-cereal because seed composition and utilization is like that of cereals, but it does not belong to the family *Poaceae* as do the true cereals. Buckwheat seeds are slightly smaller than wheat kernels, brown, and roughly pyramidal in shape (Figure 1). The two most commonly-cultivated species of buckwheat are common buckwheat (*Fagopyrum esculentum*) and bitter or tartary buckwheat (*F. tatarcum*). The latter has a high flavonoid content but a strong bitter flavor and is produced in much lower quantities than the former. Buckwheat has been used for green manure and as animal feed in the past, but the most common current use is human consumption (Oplinger et al., 1989). Buckwheat is most commonly consumed by humans in the form of flour, which is used to prepare noodles, pasta, and breads and other baked goods. Groats (hulled seeds) in whole, cracked, or coarsely-milled form are also sometimes consumed as porridges.

Figure 1. Whole buckwheat seed (left) and buckwheat groats (right)

Economic relevance

The United States is in the top ten buckwheat-producing countries, along with Russia, China, Ukraine, France, Poland, and Kazakhstan (FAO, 2017b). American average annual production of buckwheat has been 76-83 thousand metric tons, about one-third of which is exported for use in other countries (FAO, 2017a). Within the United States, buckwheat is produced mainly in the states of North Dakota and Washington (NASS, 2017).

Composition of buckwheat bran

Buckwheat consumption has been increasing in recent years due to the growing consumer interest in nutraceutical and gluten-free cereal-based products (Ahmed, Khalid, Ahmad, Abbasi, Latif & Randhawa, 2014). During milling, buckwheat groats are generally separated into endosperm and bran fractions, the latter containing the embryo, aleurone, and seed coat (Steadman et al., 2001a). The bran fraction is 18 % starch, 36 % protein, 11 % lipid, 11 % soluble fiber, 4 % insoluble fiber, and 7 % ash. The predominant components of the ash fraction are potassium (14,160 mg/kg), magnesium (5,910 mg/kg), and phosphorus (13,530 mg/kg), 73 % of which is present in the form of phytic acid (Steadman et al., 2001b). Buckwheat bran also contains most of the tannins and other polyphenols while the flavonoids are present in the groats.

Rutin

Structure and potential health benefits

Numerous flavonoids have been identified in buckwheat, including polymers of catechins, kaempferol glycosides, and quercetin glycosides (Kreft, 2016). The most common quercetin glycoside in buckwheat is quercetin-*3*-*O*-rutinoside, also known as rutin. It is composed of the flavonoid quercetin and rutinose, which is a disaccharide of L-rhamnose and D-glucose (Suzuki et al., 2015). The function of rutin in buckwheat is related to defense against UV

light, temperature, and dehydration stresses. Reported effects of rutin supplementation in humans include antihyperglycemic, anti-inflammatory, antioxidant, neuroprotective, nephroprotective, and hepatoprotective activities (Alinejad, Ghorbani & Sadeghnia, 2013; Ghorbani, 2017; Janbaz, Saeed & Gilani, 2002; Sadeghnia, Yousefsani, Rashidfar, Boroushaki, Asadpour & Ghorbani, 2013; Selloum, Bouriche, Tigrine & Boudoukha, 2003; Yang, Guo & Yuan, 2008).

Extraction methods

Previous studies on rutin extraction from biological materials has included conventional solvent extraction as well as a variety of other technologies, including ultrasound-assisted extraction (Gullon, Lu-Chau, Moreira, Lema & Eibes, 2017). Solvent extraction involves exposure of the biological material to solvent, often at elevated temperatures. In the extraction of rutin from switchgrass using the conventional solvent process, extraction was higher with 60 % methanol than with water and did not increase with temperature in the methanol extracts in the range of 50 to 80 °C (Uppugundla et al., 2009). However, in yacon (*Smallanthus sonchifolius*) leaves and flowers, Soxhlet extraction with pure methanol was less effective than extraction with water at 100 °C in removing rutin from leaf and flower tissues (de Andrade, de Souza Leone, Ellendersen & Masson, 2014). Ultrasound-assisted extraction has been effective in increasing extraction yield and decreasing solvent use in the extraction of bioactives from a variety of fruits and vegetables and has the added benefit of being a gentler extraction method than conventional Soxhlet extraction (Chernat, Rombaut, Sicaire, Meullemiestre, Fabiano-Tixier & Abert-Vian, 2017). Ultrasonic-assisted extraction of rutin from olive fruits using an 80 % methanol solvent achieved optimal results with a 30-minute extraction at 47 °C when a variety of time and temperature combinations were evaluated (Deng et al., 2017). Extraction of rutin from stinging nettle (*Urtica dioica* L.) was optimized with an ultrasound extraction of 38 min using 54 %

aqueous methanol extract, when solvent concentration and extraction time were optimized using response surface methodology (Vajic et al., 2015). Ultrasound extraction yield of rutin from *Sophora japonica* was higher than that of a Soxhlet process and was optimal when using 70 or 80 % ethanol, 56-68 kHz ultrasound treatment frequency at or above a power level of 150 W, and a solvent to solid ratio of 25-50 ml/g (Liao, Qu, Liu & Zheng, 2015). Increasing temperature above 20 °C did not increase yield of ultrasound-assisted extraction. In summary, the literature indicates that optimal solvents for rutin extraction are aqueous ethanol or methanol at 50 – 80 % concentration. Additionally, ultrasonic-assisted extraction procedures of approximately 30 minutes tend to result in superior extraction yield compared to conventional solvent extraction.

Pasta as a functional food

Functional foods are foods that can potentially confer a health benefit beyond being a simple source of energy. One way of creating a functional food enrichment with a bioactive substance. Cereal products such as pasta are good candidates for enrichment with bioactives due to widespread consumption. Pasta formulations fortified with a wide variety of bioactive substances, particularly various types of dietary fiber, have been investigated and are becoming increasingly available commercially (Brennan & Tudorica, 2008). There has already been some success in fortifying pasta with antioxidant compounds. When spaghetti was formulated with the addition of olive paste (a byproduct of the olive oil industry) at a rate of 10 % w/w, flavonoid content of the dried pasta was 15 times higher than that of the control (Padalino et al., 2018). The cooking of dried spaghetti tends to be the processing stage at which antioxidant content is reduced in pasta (Rocchetti et al., 2017). Flavonoids such as rutin were retained in pasta better than lignans, but not as well as phenolic compounds.

MATERIALS AND METHODS

Materials

Buckwheat bran flour (marketed under the name FARINETTA™) for this project was obtained from MinnDak Growers Ltd., Grand Forks, ND. Semolina was obtained from the North Dakota State Mill, Grand Forks, ND. Rutin standard was obtained from Sigma-Aldrich, St. Louis, MO.

Pasta preparation

Semolina-buckwheat bran (75/25 or 65/35 w/w) mixtures were hydrated to 32% moisture and extruded as macaroni using a Demaco semicommercial laboratory extruder (Demaco, Melbourne, FL). Extrusion occurred under the following conditions: extrusion temperature, 45 °C; mixing chamber vacuum, 46 cm of Hg; and auger extrusion speed, 25 rpm. Macaroni were dried in a laboratory pasta dryer (Standard Industry, Fargo ND) using a low temperature (40 °C) or an ultra-high temperature (90 °C) drying cycle (Yue, Rayas-Duarte & Elias, 1999).

Extraction of bran samples

Rutin was extracted from buckwheat bran with methanol or ethanol at concentrations of 60, 70, 80, 90, or 100 % (v/v) using a sample mass to solvent volume ratio of 1:12. Sample suspensions were extracted with either a 4-hour Soxhlet process or an ultrasound extraction procedure. The latter consisted of three sets of 10 minutes of sonication with a 100 W Branson 3510 Ultrasonic Cleaner, 10 minutes of settling, and decanting and filtration of the supernatant with a Whatman® #1 filter. The decanting step was skipped following the final sonication. Following either Soxhlet or ultrasound extraction, solvent was removed in a rotary evaporator at 45 °C and sample was then resuspended in the respective extraction solvent to a final volume of 100 ml. Extraction was performed in duplicate under all extraction conditions.

Papain-assisted extraction

Pasta with 25 % or 35 % buckwheat bran was ground in a Retsch Z 200 Ultra Centrifugal Mill with a 0.5 mm screen. Ground pasta was mixed with a 5 % aqueous papain solution (or plain DI water, for the control) at a sample mass to solvent volume ratio of 1:3. Suspension pH was adjusted to 6.1-6.3 and then suspensions were incubated for 30 minutes at 60 °C. Water and methanol were added to bring the solution to 80 % methanol with a sample mass to solvent ratio of 1:18. Samples were exposed to 30 min of sonication with a 100 W Branson 3510 Ultrasonic Cleaner and then centrifuged at 4000 rpm for 15 min. Solvent was removed from the supernatant with a rotary evaporator at 45 °C, after which the sample was resuspended in 80 % methanol to a final volume of 100 ml. The experiment was completed in duplicate for the 35 % buckwheat bran pasta and in triplicate for the 25 % buckwheat bran pasta.

Extraction of pasta samples

Pasta with 25 % buckwheat bran was prepared and samples collected at each of the 5 preparation stages (raw mix, hydrated mix, extruded pasta, dried pasta, and cooked pasta) in triplicate. Sample were ground in a Retsch Z 200 Ultra Centrifugal Mill with a 0.5 mm screen and then mixed with 80 % methanol at a sample mass to solvent volume ratio of 1:12. Sample suspensions were treated with sonication and filtration as in the ultrasound method of bran extraction but with the extension of the first sonication period to 30 minutes. Solvent was removed with a rotary evaporator at 45 °C and sample was then resuspended in the respective extraction solvent to a final volume of 100 ml. This extraction procedure was repeated with pasta dried at 40 °C and spiked with a known quantity of rutin to confirm method recovery rate.

Rutin analysis

Bran extracts were analyzed with a Hewlett-Packard 1090 Liquid Chromatograph and HP 3396 Series II Integrator. Pasta extracts were analyzed with a Waters 2695 Separations Module HPLC using a Waters 996 Photodiode Array Detector and the Millennium[32] version 3.20 software. An aliquot of each extract was filtered through a 0.45 µm nylon syringe filter (VWR International) before analysis. Standards were prepared with serial dilutions of rutin in 80% methanol, resulting in a curve that covered the range of 5 ppm – 100 ppm. A Licrospher® 100, RP-18 pre-packed C_{18} chromatographic column was used at 40°C with a solvent flow rate of 0.9 mL/min. A binary gradient was used with (A) 65/30/5 v/v/v water/methanol/acetic acid and (B) 95/5 v/v methanol/acetic acid executed as follows: 0-11 min, 100% A; 16-18.5 minutes, 50% A; 21-23 min, 100% A. Elution time for rutin was 9.5 minutes (Figures 2-4).

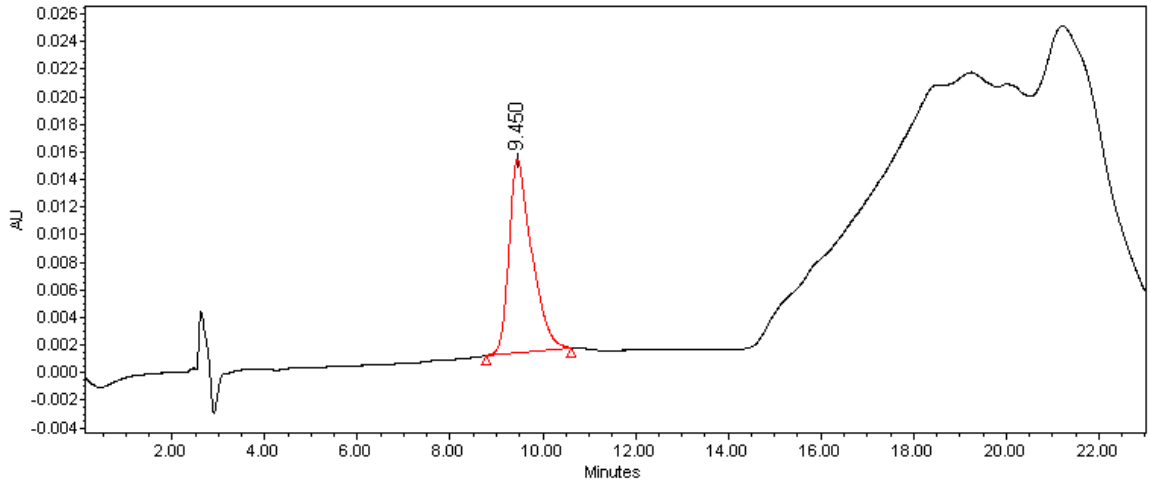

Figure 2. HPLC chromatogram of a rutin standard in 80 % methanol (rutin peak in red)

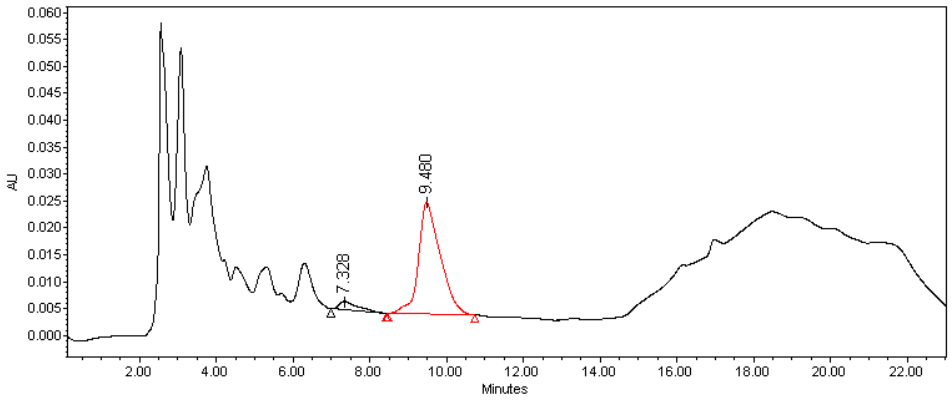

Figure 3. HPLC chromatogram of an 80 % methanol/ultrasound extraction of buckwheat bran (rutin peak in red)

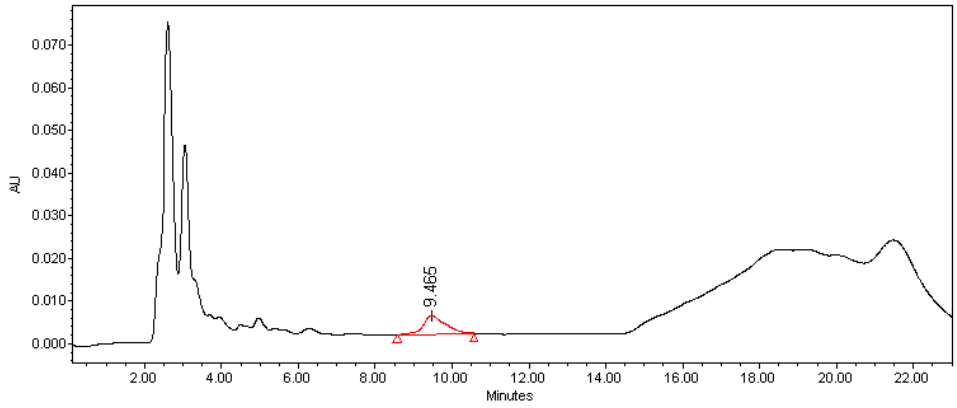

Figure 4. HPLC chromatogram of an 80 % methanol/ultrasound extraction of buckwheat bran-fortified pasta (rutin peak in red)

Statistical analysis

Data was analyzed with SAS 9.4 (SAS Institute, Cary, NC) (Appendix). For the rutin extraction experiment, duplicate application of each treatment resulted in a sample size of 40. The following fixed factorial effects model was used to model the main and interaction effects of solvent type, solvent concentration, and extraction method on rutin extraction:

$$Y_{ijkl} = \mu + \alpha_i + \beta_j + \gamma_k + \alpha\beta_{ij} + \alpha\gamma_{ik} + \beta\gamma_{jk} + \alpha\beta\gamma_{ijk} + \varepsilon_{ijkl}$$

In this model, Y_{ijkl} was the individual rutin extraction values, μ was the overall mean rutin extraction, ε_{ijkl} was the random error of the l^{th} treatment replicate (assuming independently and identically distributed error with zero mean and constant variance), α_i was the effect of the i^{th}

solvent (methanol versus ethanol), β_j was the effect of the j^{th} solvent concentration (at 5 levels from 60 to 100 %), γ_k was the effect of the k^{th} extraction method (Soxhlet versus ultrasound), $\alpha\beta_{ij}$ was the interaction effect of the i^{th} solvent at the j^{th} concentration, $\alpha\gamma_{ik}$ was the interaction effect of the i^{th} solvent with the k^{th} extraction method, $\beta\gamma_{jk}$ was the interaction effect of the j^{th} solvent concentration with the k^{th} extraction method, and $\alpha\beta\gamma_{ijk}$ was the interaction effect of the i^{th} solvent, j^{th} concentration, and k^{th} extraction method. ANOVA was used to test the null hypothesis of no effect for each main and interaction effect. Residuals were approximately normally distributed (Figure 5) and there were no patterns of concern in the plot of residuals versus predicted values, considering the small size of the dataset (Figure 6). Therefore, the assumptions related to the error term of the model were adequately met. For effects for which the null hypothesis was rejected, the Tukey test was conducted to test for differences between all possible pairs of levels for the effect.

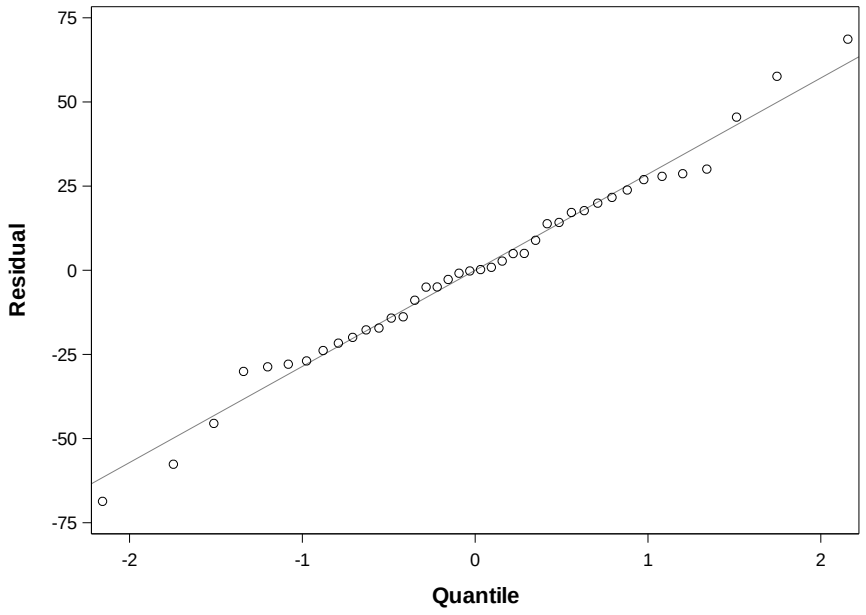

Figure 5. Quantile-quantile plot of the residuals of extracted rutin from the fixed full factorial model involving solvent type, solvent concentration, and extraction method

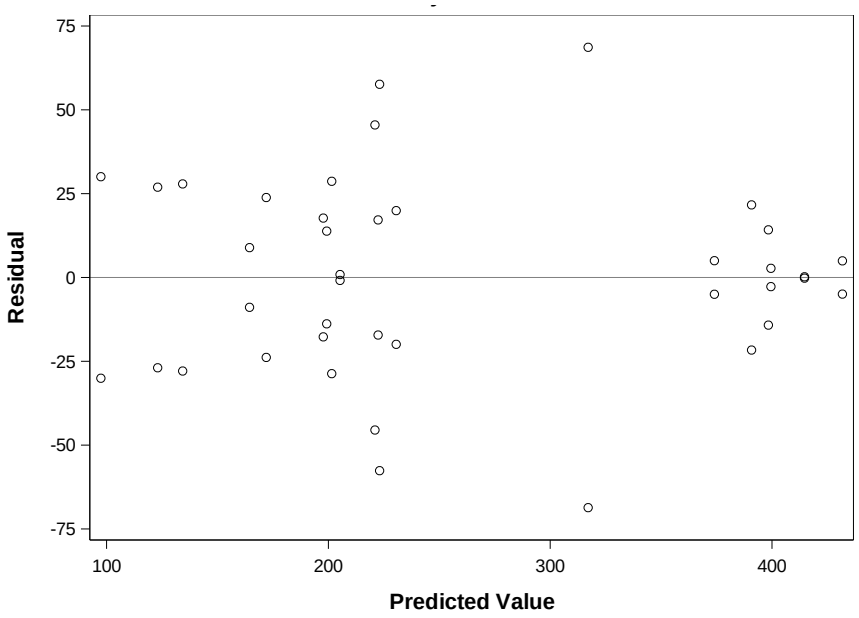

Figure 6. Plot of residuals versus predicted values of extracted rutin from the fixed full factorial model involving solvent type, solvent concentration, and extraction method

For the papain-assisted extraction experiment, duplicate application of treatments involving 25 % supplementation and triplicate application of treatments involving 35 % bran supplementation resulted in a sample size of 10. The following fixed factorial effects model was used to model the main and interaction effects of papain enzyme and buckwheat bran level on rutin extraction:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + \alpha\beta_{ij} + \varepsilon_{ijk}$$

Y_{ijk} was the individual rutin extraction values, μ the overall mean rutin extraction, ε_{ijk} the random error of the k^{th} treatment replicate (assuming independently and identically distributed random error with zero mean and constant variance), α_i the effect of the i^{th} enzyme treatment (present or absent), β_j the effect of the j^{th} buckwheat bran level (25 % or 35 %), and $\alpha\beta_{ij}$ the interaction effect of the i^{th} enzyme treatment the j^{th} buckwheat bran level. ANOVA was used to test the null hypothesis of no effect for each of the effects. Residuals were approximately normally distributed (Figure 7) with no patterns of concern in the plot of residuals versus predicted values,

considering the small sample size (Figure 8). Therefore, the assumptions of the model were adequately met. For effects for which the null hypothesis was rejected, the Tukey test was conducted to test for differences between all possible pairs of levels for the effect.

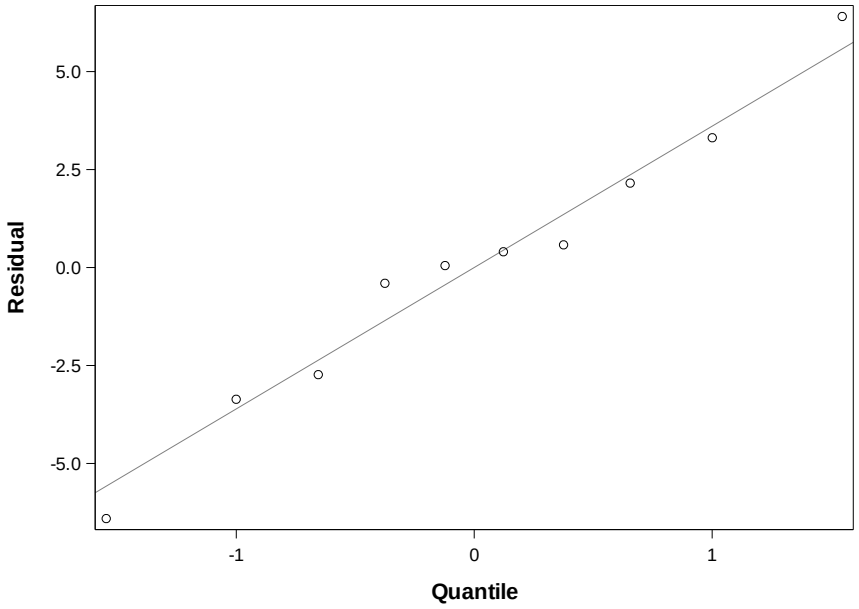

Figure 7. Quantile-quantile plot of the residuals of rutin extracted from buckwheat bran-fortified pasta, from the fixed full factorial model involving buckwheat bran level and papain treatment

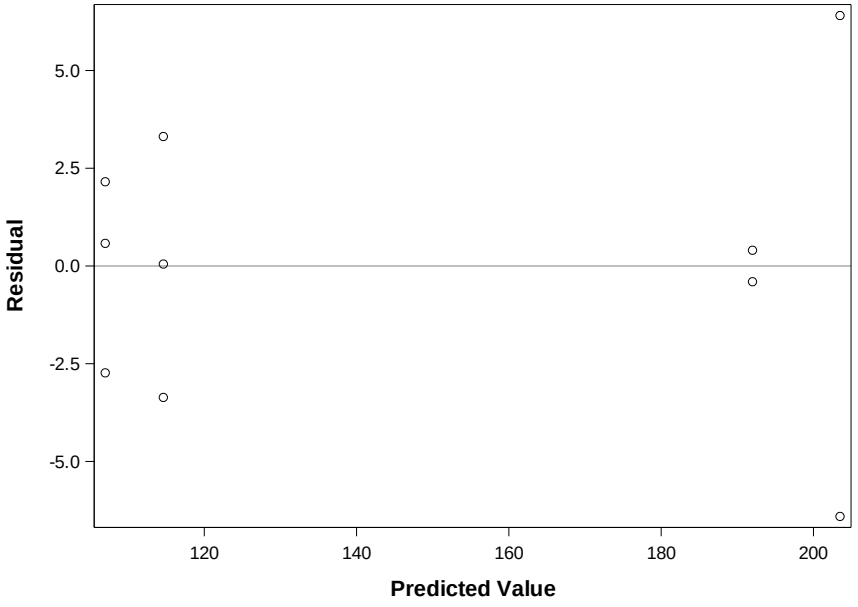

Figure 8. Plot of residuals versus predicted values of extracted rutin from the fixed full factorial model involving papain enzyme and varying buckwheat bran levels

For the rutin stability experiment, triplicate application of all treatments resulted in a sample size of 30. The following fixed factorial effects model was used to model the main and interaction effects of drying temperature and processing stage on pasta rutin content:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + \alpha\beta_{ij} + \varepsilon_{ijk}$$

Y_{ijk} was individual rutin content values, μ the overall mean rutin content, ε_{ijk} the random error of the k^{th} treatment replicate (assuming independently and identically distributed random error with zero mean and constant variance), α_i the effect of the i^{th} drying temperature (40 or 90 °C), β_j the effect of the j^{th} processing stage (5 different stages), and $\alpha\beta_{ij}$ the interaction effect of the i^{th} drying treatment and the j^{th} processing stage. ANOVA was used to test the null hypothesis of no effect for each of main and interaction effect. Since residuals were approximately normally distributed (Figure 9) with no concerning pattern in the residual plot (Figure 10), the assumptions of the model were adequately met. For effects for which the null hypothesis was rejected, the Tukey test was used to test for differences between all possible pairs of levels for the effect.

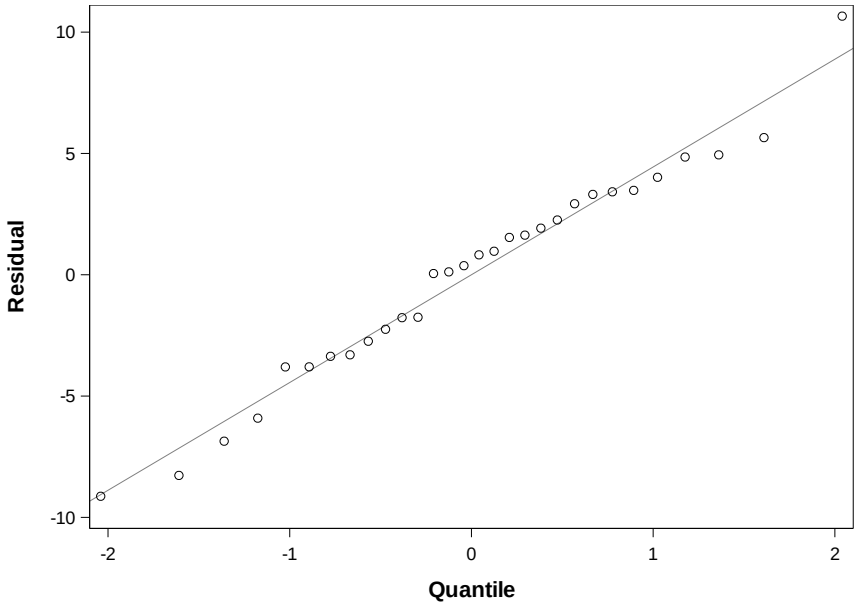

Figure 9. Quantile-quantile plot of the residuals of rutin extracted from buckwheat bran-fortified pasta, from the fixed full factorial model involving pasta drying temperature and processing stage

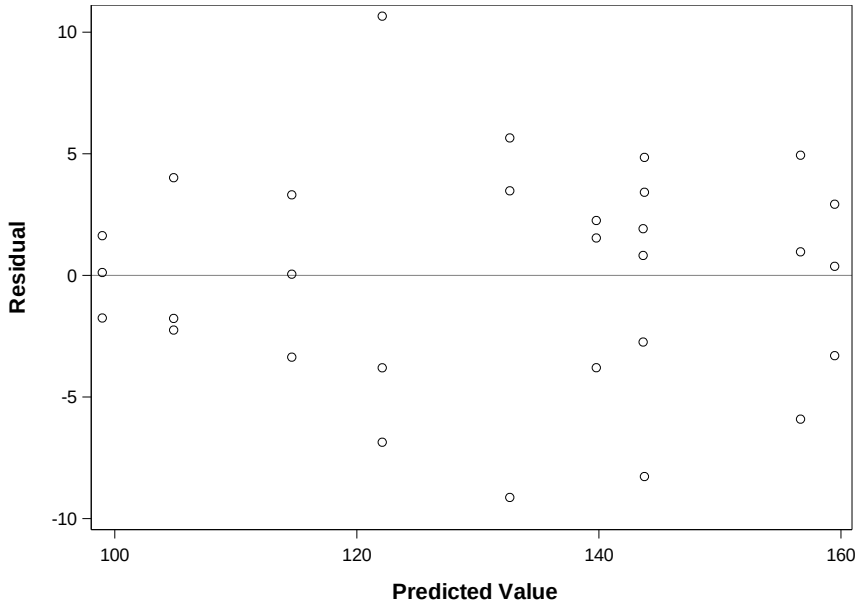

Figure 10. Plot of residuals versus predicted values of extracted rutin from the fixed full factorial model involving pasta drying time and processing stage

RESULTS AND DISCUSSION

Extraction optimization

The purpose of the extraction study was to determine the most effective method of extracting rutin from buckwheat bran flour. Mean rutin extracted from buckwheat bran using methanol (275 ppm) was higher than that extracted using ethanol (236 ppm) and this difference was significant based on the rejection of the null hypothesis for the effect of solvent type ($p < 0.05$) (Table 1). However, the interaction effect between solvent type and concentration was also statistically significant ($p < 0.05$), indicating the effect of solvent must be interpreted with respect to solvent concentration. Within each solvent concentration, there was no statistically significant difference between mean rutin extracted by methanol versus ethanol, based on the Tukey range test (Figure 11). Since the two solvents had similar effectiveness in these results, methanol was chosen due to having a lower boiling point, which would facilitate solvent removal following extraction. The impact of solvent concentration on rutin extraction was dependent on the extraction method, based on the significance ($p < 0.05$) of the interaction between the solvent concentration and extraction method factors (Table 1). Based on the Tukey test comparing all pairs of all combinations of the extraction method and solvent concentration (mean of results using ethanol or methanol at a given concentration), rutin extracted using ultrasonic extraction was not significantly different ($p > 0.05$) at solvent levels of 60, 70, or 80 % (Figure 12—shown by all three bars having the same letter). However, ultrasound extraction at these three solvent levels was significantly higher ($p < 0.05$) than extraction using any other solvent concentration and extraction method combination except for ultrasound extraction at 90 % solvent concentration. The highest extraction was observed using ultrasound at 70 % solvent concentration; however, this extraction was not significantly different from that of ultrasound

extraction at 80 % solvent. Since solvent removal would be easier at 80 % solvent than at 70 % solvent, the extraction protocol of 80 % methanol using ultrasound treatment was selected for ease of use and to facilitate rutin stability during extraction. Previous literature has also noted the effectiveness of ultrasound-assisted extraction and organic solvent concentrations in the range of 70-80 % for removing rutin from other plant materials (Deng et al., 2017; Liao, Qu, Liu & Zheng, 2015; Vajic et al., 2015).

Table 1. Results of ANOVA performed on rutin extraction data from buckwheat bran

Source	Degrees of freedom	Sum of squares	Mean squares	*F* Value	*p* > *F*
Solvent	1	15141.54	15141.54	6.47	0.0166
Conc[1]	4	139274.72	34818.68	14.87	<0.0001
Method[2]	1	200870.16	200870.16	85.79	<0.0001
Solvent*Conc	4	18528.46	4632.12	2.91	0.0475
Solvent*Method	1	4418.42	4418.42	2.78	0.1112
Conc*Method	4	52804.03	13201.01	5.64	0.0017
Solvent*Conc*Method	4	13139.07	3284.77	2.07	0.1236
Error	20	31812.21	1590.61		

[1] Conc = solvent concentration, [2] Method = extraction method

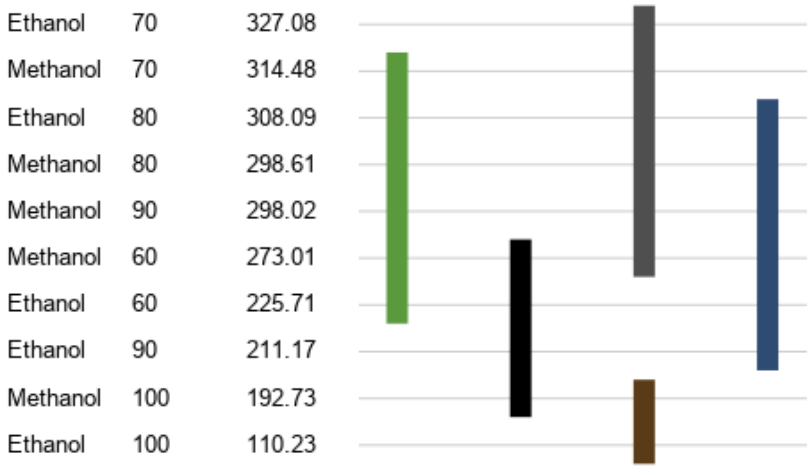

Figure 11. Tukey groupings for the LS-means of solvent concentration*extraction method effect (column 1 is solvent type, column 2 is solvent concentration, and column 3 is treatment mean)—means covered by the same bar were not significantly different ($p > 0.05$)

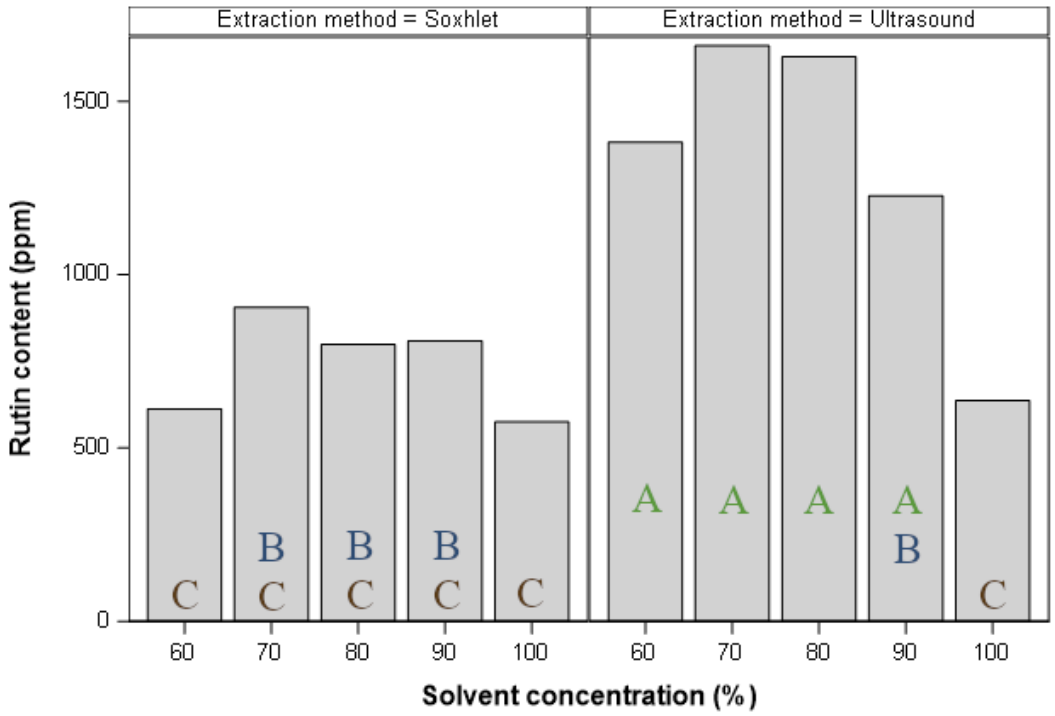

Figure 12. Relationship among solvent concentration (mean of ethanol and methanol), extraction method, and rutin extracted from buckwheat bran (bars with the same letter indicate no significant difference between means at $\alpha = 0.05$)

Papain digestion

Both papain treatment and buckwheat bran content of pasta significantly impacted rutin content ($p < 0.05$) (Table 2). However, interaction between these two factors was not significant, indicating that the effect of papain on rutin content was the same at 25 % or 35 % buckwheat bran content. Instead of rutin extraction being facilitated by papain treatment, rutin recovery was lower in the papain-assisted extraction from both 25 % and 35 % buckwheat bran-fortified pastas (Figure 13). While the negative impact of papain was quite small, these results indicated that papain was not useful in achieving the goal of enhanced rutin extraction from buckwheat bran-fortified pasta. Literature related to papain-assisted extraction of antioxidants is limited but does suggest that papain can facilitate the extraction of some polyphenols from some protein-containing matrices (Lin, Wang, Hu, Ge, Zheng & Zeng, 2018).

Table 2. Results of ANOVA performed on papain-assisted rutin extraction data from buckwheat bran-fortified pasta

Source	Degrees of freedom	Sum of squares	Mean squares	*F* Value	*p* > *F*
Papain treatment	1	210.49	210.49	11.69	0.0112
Bran content	1	18137.68	18137.68	1007.16	<0.0001
Papain treatment*Bran content	1	8.93652	8.93652	0.46	0.5239
Error	6	117.12	19.52		

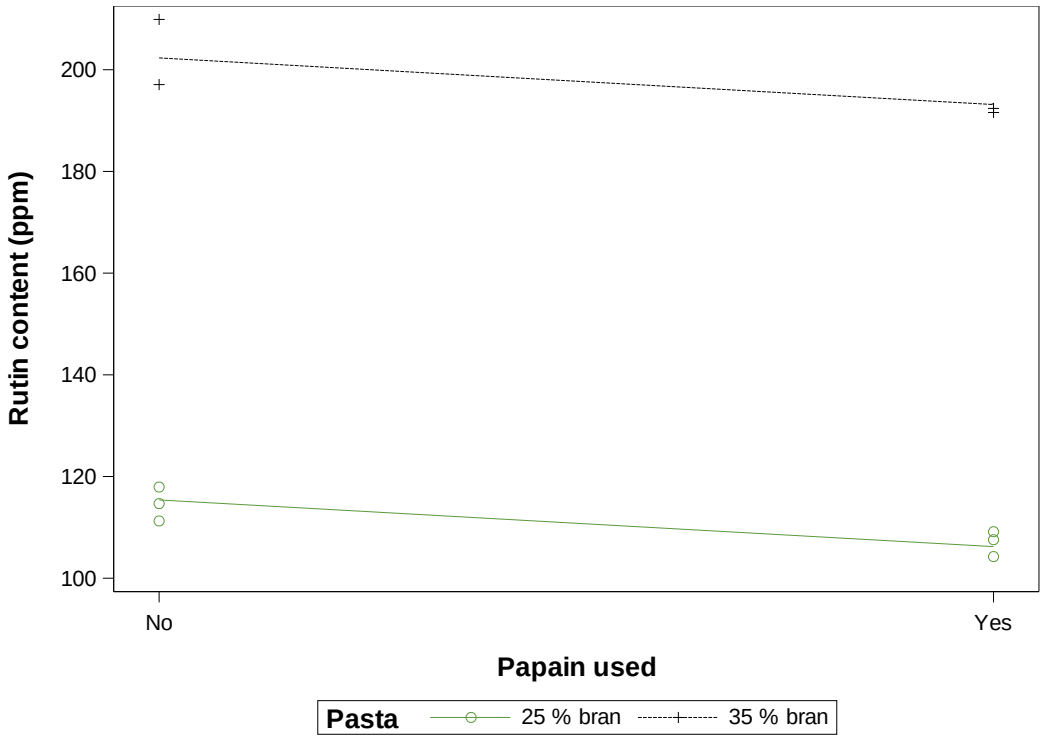

Figure 13. Plot of rutin extraction from pasta fortified with buckwheat bran, with or without papain treatment (data points represent observed values and lines connect treatment means)

Rutin stability in buckwheat bran-fortified pasta

Although the main effect of drying temperature did not significantly impact rutin content of buckwheat bran-fortified pasta, both processing stage and the interaction between drying temperature and processing stage did significantly impact rutin content (Table 3). The extracted rutin of the low temperature dried pasta was significantly higher in the hydrated pasta mix than in the raw mix or extruded pasta (Figure 14—indicated by no shared letters between the "hydrated" bar and the "raw" and "extruded" bars). However, for the high temperature dried mix

there were no significant differences (p >0.05) among these processing stages (Figure 14—indicated by a shared letter among the three bars). However, since these differences occurred prior to the pasta drying stage, this difference was not due to differences in pasta drying temperatures. Additionally, rutin content decreased significantly due to the drying step when dried under high temperature (Figure 14—indicated by no shared letter between "extruded" and "dried" bars) but did not decrease significantly during drying at low temperature (Figure 14—indicated by a shared letter between "extruded" and "dried" bars). However, within any given pasta processing step, there was no significant difference in the mean rutin contents of pasta dried at either low or high temperature. At either drying temperature, a significant reduction occurred at the drying stage compared to the raw mix, and a further significant reduction occurred at the cooking stage compared to the drying stage. These results support previous literature that indicates the cooking step of pasta processing can result in losses in total antioxidant compounds and particularly in the free antioxidant content (Khan, Yousif, Johnson & Gamlath, 2013; Rocchetti et al., 2017), and further indicated that pasta drying can reduce the quantity of flavonoids such as rutin. Heating at temperatures as low as 70 °C caused a reduction in rutin content that followed first-order degradation kinetics (Kadakal & Duman, 2018).

Table 3. Results of ANOVA performed on rutin extraction data from buckwheat bran-fortified pasta

Source	Degrees of freedom	Sum of squares	Mean squares	*F* Value	$p > F$
Temperature[1]	1	0.42917	0.42917	0.02	0.9037
Stage[2]	4	11418.30	2854.58	99.80	<0.0001
Temperature*Stage	4	355.61	88.90	3.11	0.0384
Error	20	572.08	28.60		

[1] Pasta drying temperature, [2] Pasta processing stage

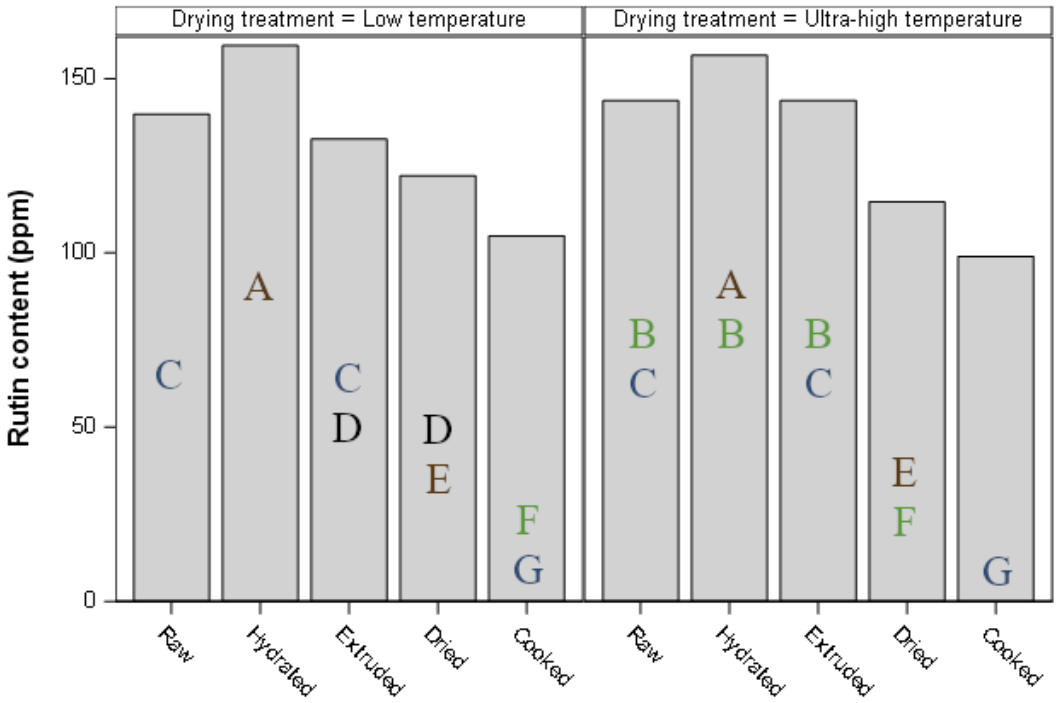

Figure 14. Relationship between processing conditions and rutin extracted from buckwheat bran-fortified pasta (bars with the same letter indicate no significant difference between means at α = 0.05)

CONCLUSIONS

Optimal rutin extraction conditions were observed using a methanol solvent of 70-80 % in conjunction with repeated exposure of the suspended sample to ultrasound treatment. Recovery of rutin with this extraction method from pasta spiked with a known amount of rutin was 76 %. High pasta drying temperature (90 °C) resulted in statistically significant losses in rutin during drying, while pasta prepared with both low (40 °C) and high drying temperatures had lower rutin content after cooking. Rutin content was reduced 25-30 % between the raw pasta mix and the cooked pasta for both low temperature and high temperature dried pasta. Future research should be done to determine the impact of buckwheat bran on the quality and sensory acceptability of pasta and what fortification level adequately balances nutritional and health benefits with sensory aspects.

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APPENDIX: SAS CODE

```
*************************************************************************
Rutin in Pasta (rutin.sas)
Directory: Directory
Data sets:
      Extraction method.csv
      Papain extraction.csv
      Stability in pasta.csv

Variables
      Extraction method
            solvent     - Solvent used for extraction (methanol or ethanol)
            conc        - Solvent concentration (60-100 %)
            method      - Extraction method (soxhlet or ultrasound)
            rutin       - Bran rutin content in ppm
      Papain extraction
            papain      - Papain used (no or yes)
            pasta       - Type of pasta (25% or 35% bran supplementation)
            rutin       - Pasta rutin content in ppm
      Stability in pasta
            temp        - Pasta drying temperature (UHT/90 C or LT/40 C)
            stage       - Stage in pasta processing (raw, dried, hydrated,
                              extruded, cooked)
            rutin       - Pasta rutin content in ppm
*************************************************************************;

data extract;
      infile "Directory\Extraction method.csv" firstobs=2 dsd;
      input solvent $ conc $ method $ rutin;
      label solvent="Solvent" conc="Solvent concentration (%)"
            method="Extraction method" rutin="Rutin content (ppm)";
run;

data extract1;
      infile " Directory \Extraction method.csv" firstobs=2 dsd;
      input solvent $ conc method $ rutin;
      label solvent="Solvent" conc="Solvent concentration (%)"
            method="Extraction method" rutin="Rutin content (ppm)";
run;

data papain;
      infile " Directory \Papain extraction.csv" firstobs=2 dsd;
      input papain $ Pasta $ rutin;
      label papain="Papain used" Pasta="Bran rate (%)"
            rutin="Rutin content (ppm)";
run;

data papain1;
      infile " Directory \Papain extraction.csv" firstobs=2 dsd;
      input papain $ pasta rutin;
      label papain="Papain used" pasta="Bran rate (%)"
            rutin="Rutin content (ppm)";
run;

data stability;
```

```
      infile " Directory \Stability in pasta.csv" firstobs=2 dsd;
      input temp $ stage $ rutin;
      label temp="Drying treatment" stage="Processing stage"
            rutin="Rutin content (ppm)";
run;

proc format;
      value $method     "Sox"="Soxhlet"
                        "Ultra"="Ultrasound";
      value $temp       "UHT"="Ultra-high temperature"
                        "LT"="Low temperature";
      value $stage      "1"="Raw"
                        "4"="Dried"
                        "2"="Hydrated"
                        "3"="Extruded"
                        "5"="Cooked";
      value $Pasta      "25"="25 % bran"
                        "35"="35 % bran";
run;

proc template;
      define style styles.rutin;
      parent=styles.journal;
      class GraphColors /
            'gcdata4' = stbr 'gcdata3' = charcoal
            'gcdata2' = black 'gcdata1' = stlg
            'gcdata5' = stgb 'gdata5' = stgb
            'gdata4' = stbr 'gdata3' = charcoal
            'gdata2' = black 'gdata1' = stlg;
       style colors /
            'headerfgemph' = cx000000 'headerbgemph' = cxFDCD6F
            'headerfgstrong' = cx000000 'headerbgstrong' = cxFDCD6F
            'headerfg' = cx000000 'headerbg' = cxFDCD6F
            'datafgemph' = cx000000 'databgemph' = cxFFFFFF
            'datafgstrong' = cx000000 'databgstrong' = cxFFFFFF
            'databorder' = cx89562D 'datafg' = cx000000
            'databg' = cxFFFFFF 'batchfg' = cx000000
            'batchbg' = cxFFFFFF 'tableborder' = cx000000
            'tablebg' = cxFFFFFF 'notefg' = cx000000
            'notebg' = cxFFFFFF 'bylinefg' = cx000000
            'bylinebg' = cxFFFFFF 'captionfg' = cx000000
            'captionbg' = cxFFFFFF 'proctitlefg' = cx000000
            'proctitlebg' = cxFFFFFF 'titlefg' = cx000000
            'titlebg' = cxFFFFFF 'systitlefg' = cx000000
            'systitlebg' = cxFFFFFF 'Conentryfg' = cx000000
            'Confolderfg' = cx000000 'Contitlefg' = cx000000
            'link2' = cx800080 'link1' = cx0000FF
            'contentfg' = cx000000 'contentbg' = cxFFFFFF
            'docfg' = cx000000 'docbg' = cxFFFFFF;
      class GraphFonts /
            'GraphDataFont' = ("<sans-serif>, <MTsans-serif>",10pt)
            'GraphUnicodeFont' = ("<MTsans-serif-unicode>",10pt)
            'GraphFootnoteFont' = ("<sans-serif>, <MTsans-serif>",12pt,bold)
            'GraphTitleFont' = ("<sans-serif>, <MTsans-serif>",12pt,bold)
            'GraphTitle1Font' = ("<sans-serif>, <MTsans-serif>",14pt,bold)
            'GraphValueFont' = ("<sans-serif>, <MTsans-serif>",10pt)
            'GraphLabel2Font' = ("<sans-serif>, <MTsans-serif>",12pt)
```

```
            'GraphLabelFont' = ("<sans-serif>, <MTsans-serif>",12pt,bold)
            'GraphAnnoFont' = ("<sans-serif>, <MTsans-serif>",12pt);
      end;
run;

ods rtf file=" Directory \rutin_results.rtf" bodytitle style=styles.rutin;

proc glm data=extract plots=all;
      class solvent conc method;
      model rutin = solvent| conc| method;
      lsmeans conc*method solvent*conc / adjust=tukey lines;
      format method $method.;
      title "Primary and secondary tests for extraction method";
run;

proc means data=extract mean noprint;
      class conc method;
      var rutin;
      ways 2;
      output out=extractmean mean=rutin;
run;

proc sgpanel data=extract1;
      panelby method;
      vbar conc / response=rutin;
      format method $method.;
      title "Graph of extraction study - rutin vs solvent concentration
            and extraction method";
run;

proc glm data=papain plots=all;
      class papain Pasta;
      model rutin = papain| Pasta;
      lsmeans papain Pasta / adjust=tukey lines;
      format Pasta $Pasta.;
      title "Primary and secondary tests for papain use in extraction";
run;

proc sgplot data=papain1;
      scatter x=pasta y=rutin / group=papain;
      title "Graph of papain study";
run;

proc glm data=stability plots=all;
      class temp stage;
      model rutin = temp | stage;
      lsmeans temp*stage / adjust=tukey lines;
      format temp $temp. stage $stage.;
      title "Primary and secondary tests for pasta rutin content";
run;

proc means data=stability mean noprint;
      class temp stage;
      var rutin;
      ways 2;
      output out=stablemean mean=rutin;
run;
```

```
proc sgpanel data=stablemean;
      panelby temp;
      vbar stage / response=rutin;
      format temp $temp. stage $stage.;
run;

ods rtf close;
```