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The effect of blanching on antioxidant activity and glycosides of white saffron (*Curcuma mangga* Val.)

[1*]Pujimulyani, D., [2]Raharjo, S., [2]Marsono, Y. and [2]Santoso, U.

[1]*Faculty of Agroindustry, Mercu Buana University, Yogyakarta, Indonesia*
[2]*Faculty of Agricultural Technology, Gadjah Mada University, Yogyakarta, Indonesia*

Abstract: White saffron (*Curcuma mangga* Val.) rhizome grows well in Indonesia. The objectives of this research were to determine antioxidant activity and to identify and quantify glycoside and aglicone substances in fresh and blanched white saffron. Sample of white saffron rhizome was extracted with methanol, rota-evaporated, and freeze dried. Dried extract was identified and quantified for its glycoside and aglicone content by High Performance Liquid Chromatography (HPLC). One of the identified glycoside compound was quercetin-3-rutinoside. The antioxidant activity and quercetin content of blanched white saffron extracts were significantly higher ($p \leq 0.05$) than that of fresh white saffron. Blanching of white saffron with citric acid and distilled water hydrolyzed quercetin-3-rutinoside into quercetin.

Keywords: White saffron, antioxidant activity, blanching, glycoside

Introduction

White saffron (*Curcuma mangga* Val.) belongs to the *Zingiberaceae* family, a perennial stalk rhizome. It grows well in Indonesia. This rhizome has round shape, and many branches. The main rhizome is relatively harder than those of the first and second branches. The colour and odor of the rhizome is similar to that of ripe mangoes.

The white saffron extract has been reported able to decrease the rate of lipid oxidation because of its tannin and curcuminoid content (Pujimulyani and Sutardi, 2003; Abas *et al*., 2005). Processed white saffron, in the form of wet and dry sweets, was well accepted by panelists and has antioxidant activity (Pujimulyani and Wazyka, 2009a,b).

White saffron syrup made from rhizome which was blanched in citric acid solution showed higher antioxidant activity than that of unblanched syrup (Pujimulyani, 2003b). Despite of the boiling process these syrups are still showing antioxidant activity. It suggests that some of the antioxidant compounds the white saffron are heat stable or it may change into different compounds having higher antioxidant activity.

Blanching is one of the most important preparation steps in processing various frozen vegetables. The primary purpose of blanching is to inactivate enzymes (Barrett and Theerakulkait, 1995). Blanching can decrease or increase antioxidant activity of agricultural products. Blanching of red *turi (Sesbania grandiflora* L. *(Pers))* flower decreased the anthocyanin and vitamin C content. This due to the fact that antioxidant substances leached in the blanching media and vitamin C was damaged during heating (Wahyuningsih, 2008). The blanching of red cabbage at 94-96ºC for 3 mins decreased 42% of FRAP value (Volden *et al*., 2008). The blanching of lentil beans at 5 psi pressure for 5 minutes decreased RSA by 29.5% and ORAC by 11.1% (Xu and Chang, 2008). The decrease of antioxidant activity was probably due to the leaching of antioxidant compounds in the blanching media.

However, other studies reported that blanching increased antioxidant activity. Pressurized blanching of wheat after being harvested at 100ºC increased the total phenolic content in the wheat powder (Cheng *et al*., 2006). Blanching of corn in autoclave increased its total phenolic content (Randhir *et al*., 2008). Blanching of beans, corn, and tomato increased its antioxidant activity as measured using DPPH method (Kwan *et al.*, 2007). Brussel sprouts (*Brassica oleracea* L.), after water blanching at 100ºC for 2 and 3 mins, showed higher antioxidant activity ~~d~~than that of fresh brussel sprouts (Viña *et al.*, 2007; Olivera *et al*., 2008). Bilberry extract which was heated at 100ºC for 10 minutes had a higher antioxidant activity than that of fresh extract. This due to anthocyanin was hydrolyzed into anthocyanidin and sugar (Yue and Xu, 2008). Zhang *et al*. (2004) and Sadilova *et al*. (2006) found that hydrolysis of anthocyanin glycoside into anthocyanidin occurred during heating in acidic condition. The objectives of this research were to determine antioxidant activity and to identify and quantify glycoside and aglicone substances in fresh and blanched white saffron.

*Corresponding author.
Email: *dwiyati2002@yahoo.com*
Tel: +62 274 6498 212; Fax: +62 274 6498 213

Materials and Methods

Materials

White saffron rhizomes (*Curcuma mangga* Val.) were harvested from a local farm in Yogyakarta. This study used quercetin-3-rutinoside and quercetin from Sigma Chemical Co., St. Louis. Methanol, HCl, acetic buffer, 2,4,6-tri-(2-pyridyl)-s-triazine (TPTZ), $FeCl_3.6H_2O$, Fe^{2+}, H_3PO_4, N_2, CH_3CN, citric acid, acetic ethyl, from E. Merck.

Extraction of white saffron samples

White saffron rhizomes were peeled, washed, and dripped. Approximately 500 g of white saffron was blanched at 100ºC with 0.05% and 0% citric acid solution as blanching media, for 0, 5, and 10 mins. Fresh and blanched white saffron were extracted using methanol. White saffron was crushed for 5 mins in methanol. White saffron was macerated at room temperature in methanol (1:1 w/v) for up to 12 hours, homogenized using homogenizer for 15 mins, then vacuum filtered to obtain extract I. Methanol (500 ml) was added to the residue, then macerated for 12 hours and homogenized for 15 mins. The homogenized residue was then vacuum filtered to obtain extract II. Both extracts I and II were mixed and then filtered again and evaporated using rotary evaporator to get concentrated extract. The concentrated extract was freeze dried. The antioxidant activity of the dried extract was examined using FRAP method. The identification and quantification of glycoside was carried out by HPLC (Monagas *et al*., 2007).

Ferric reducing antioxidant power (FRAP) assay

Antioxidant capability to reduce Fe^{3+} was determined using FRAP method (Benzie and Strain, 1996; Volden *et al*., 2008). The FRAP reagent was prepared using 300 mM acetic buffer pH 3.6, mixed with 10 mM TPTZ in 40 mM HCl and 20 mM $FeCl_3.6H_2O$ (ratio 10:1:1). Approximately 3 ml of FRAP reagent was heated at 37ºC for 10 mins. The sample (100 µL) was mixed with 300 µL distilled water and mixed with FRAP reagent using vortex for 1 min and left for 4 mins. The absorbance was determined at λ of 593 nm, FRAP value was calculated in mg ferro equivalent/g dried extract using calibration curve Fe^{2+} (from 4.3 to 137.5 mg ferro/L, r = 0.99).

Determination of quercetin-3-rutinoside and quercetin content by HPLC

Glycoside in the dried extracts was identified and quantified using HPLC column C_{18} (Monagas *et al*., 2007). HPLC KNAUER with a 3880 auto sampler smartline, a Photodiode Array Detector (PAD) spectra system UV6000LP, and software chromgate 3.1.6 data module was used. The preparation of samples before injecting to HPLC was as follows: 0.5 mL extract (from 100 mg dried extract in 3 mL methanol:HCl, ratio 1000:1) was evaporated using N_2 and added with 1 mL H_3PO_3, filtered using millex filter 0.45 µm, then injected to HPLC system. The column used was C_{18} (4.6 x 250 mm, dp 5 µm) and at 50ºC, monitored using λ of 256 nm for quercetin-3-rutinoside. The eluting solvent used was H_3PO_3:CH_3CN:acetic ethyl (ratio 84:12:4), with flow rate of 1.2 mL/ minute at 10 minutes. The volume of injection was 20 µL. This preparation was applied to all samples and this product was compared with glycoside standard. The glycoside standard used was quercetin-3-rutinoside and the aglicone standard was quercetin.

Statistical analysis

Statistical analysis was performed with the SPSS software package. The data were analyzed by random design in complete block. The variables were 2 media (0.05% and 0% citric acid) and 3 various levels of blanching time. Duncan's multiple range tests was carried out to determine any significant differences. Significant levels were defined at $p \leq 0.05$.

Result and Discussion

The FRAP value of blanched white saffron

The FRAP value of blanched white saffron in citric acid and distilled water media showed significant increase compared to that of fresh white saffron (Figure 1). This was probably due to polyphenol compounds degradation to simple phenolics during blanching. Similar study on the blanching of corn (Randhir *et al*., 2008) and wheat (Cheng *et al*., 2006) increased their total phenolic contents. Blanched white saffron in 0.05% citric acid for 5 minutes had the highest FRAP value. This might be due to the hydrolysis of quercetin-3-rutinoside into aglicone quercetin (Table 1). It was found that quercetin showed higher antioxidant activity than quersetin-3-rutinoside as measured by FRAP method. Li *et al*. (2009) reported that aglicone had higher antioxidant activity than glycoside. Other study also reported that anthocyanin of bilberry extract was hydrolyzed during heating (Yue and Xu, 2008). In addition, some degradation products of anthocyanin were also reported to have antioxidant capability (Seeram *et al*., 2001). Similar study was reported by Halvorsen *et al*. (2006) that blanching vegetables could increase the FRAP value. Kim *et al*. (2010) reported that heating tannin showed increased antioxidant activity

compared to unheated tannin.

Table 1. Quercetin-3-rutinoside content remaining and quercetin content resulted from heating 50 ppm quercetin-3- rutinoside in different blanching medium

Blanching medium	Quercetin-3-rutinoside content (ppm)	Quercetin content (ppm)
50 ppm Quercetin-3-rutinoside heated in water	42.98 ± 0.80*	2.16 ± 1.18
50 ppm Quercetin-3-rutinoside heated in Citric acid 0.05%	40.36 ± 5.70	2.23 ± 0.96

* Indicates mean ± standard deviation

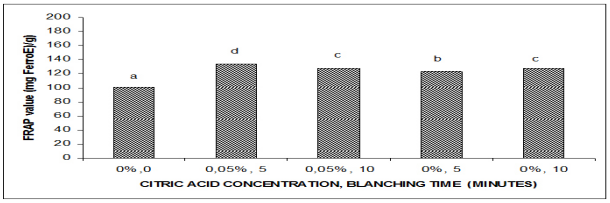

Figure 1. FRAP value of blanched white saffron (FRAP: Ferric Reducing Antioxidant Power; Ferro E : Ferro Equivalent)

Chromatograms of quercetin-3-rutinoside (rutin) and quercetin standard

Retention time of quercetin-3-rutinoside standard was 2.54 mins and quercetin was 4.97 mins. The standard curves of quercetin-3-rutinoside and quercetin were made with four different concentrations 10 and 2.5 ppm; 20 and 5 ppm; 30 and 7.5 ppm; 40 and 10 ppm, respectively. The determination of quercetin-3-rutinoside and quercetin standard as well as in the white saffron was set at λ 256 nm. Chromatograms of quercetin-3-rutinoside and quercetin standard are presented in Figure 2.

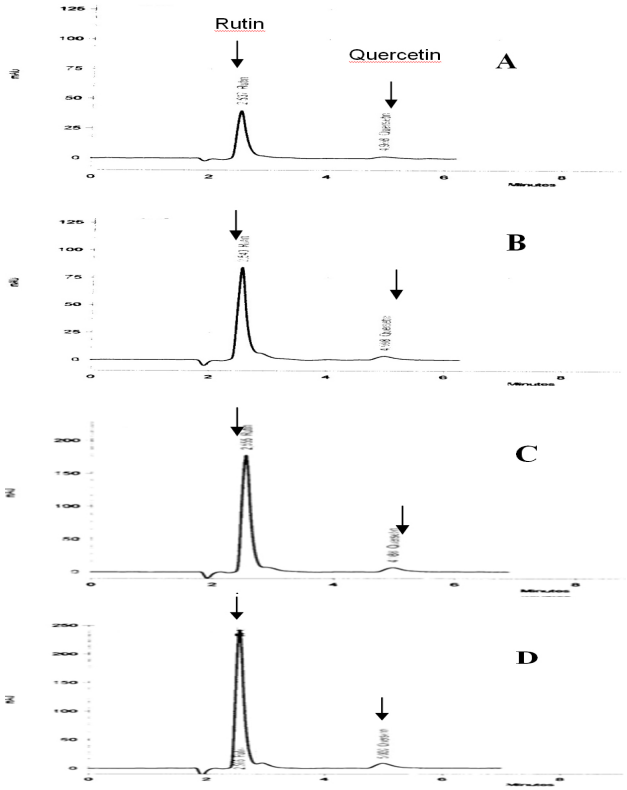

Figure 2. Chromatograms of rutin (quercetin-3-rutinoside) and quercetin standard (A= 10 and 2.5 ppm; B= 20 and 5 ppm; C= 30 and 7.5 ppm; D= 40 and 10 ppm)

Quercetin-3-rutinoside content of white saffron

Figure 3 shows that the content of quercetin-3-rutinoside of white saffron decreased after blanching, because part of glycoside compound was hydrolyzed into aglicone. Chromatograms of quercetin-3-rutinoside of white saffron are shown in Figure 4. The quercetin content of fresh white saffron was not detected. However, the blanched white saffron had 0.25-0.27 mg/ g of quercetin. This might be due to the hydrolysis of quercetin-3-rutinoside into aglicone quercetin (Table 1). A similar study reported by Yue and Xu (2008) showed that the heating of bilberry extract could increase the antioxidant activity, because glycoside changed into aglicone and sugar. Some studies reported that anthocyanidins were produced from anthocyanins using acid hydrolysis method (Zhang *et al*., 2004).

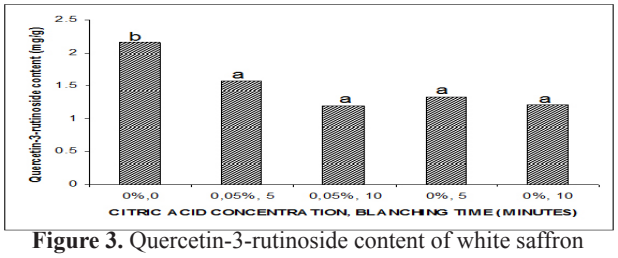

Figure 3. Quercetin-3-rutinoside content of white saffron

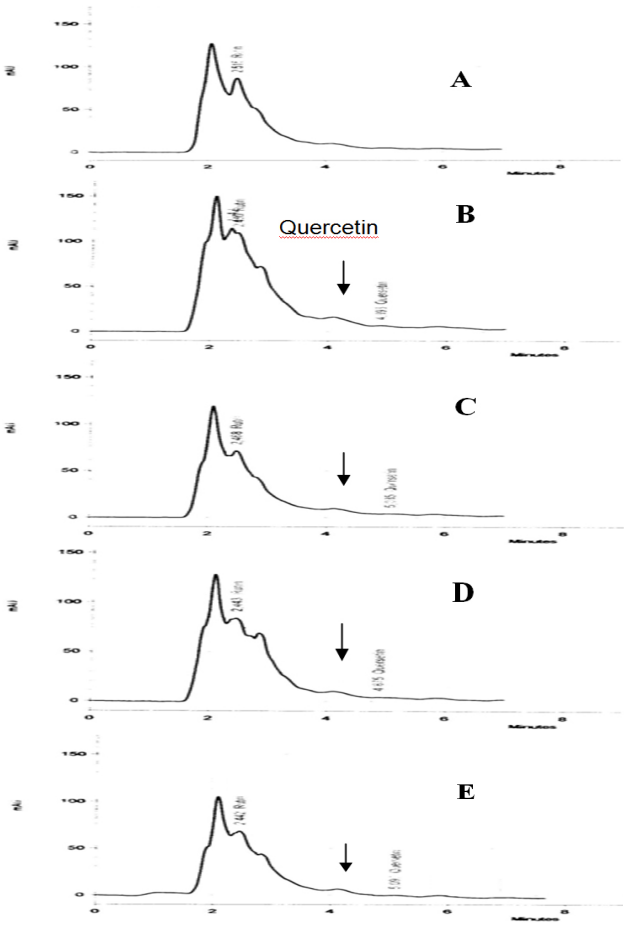

Figure 4. Chromatograms of quercetin-3-rutinoside of blanched white saffron using citric acid solution (%); blanching time (minutes) (A= 0;0 B= 0.05;5 C= 0.05;10 D=0;5 E= 0;10). Arrow indicates quercetin of blanched white saffron

Quercetin Content of White Saffron

The quercetin contents of blanched white saffron in various media and with different blanching time are shown in Figure 5. Heating of 50 ppm of quercetin-3-rutinoside produced quercetin-3-rutinoside and quercetin as shown in Table 1. The quercetin content was not detected in fresh white saffron, but in the blanched white saffron there was 0.25-0.27 mg/g of quercetin. This indicated that hydrolysis of quercetin-3-rutinoside into quercetin. A similar study was reported by Garrido *et al*. (2008), that the content of quercetin was greater for blanched almond skins from American varieties than for roasted ones. Sadilova *et al*. (2006) reported that hydrolysis of anthocyanin glycoside in acid condition resulted in anthocyanidin. Kirca *et al*. (2007) and Mishira *et al.* (2008) reported the degradation of the glycoside in fruits and vegetables during processing and storage.

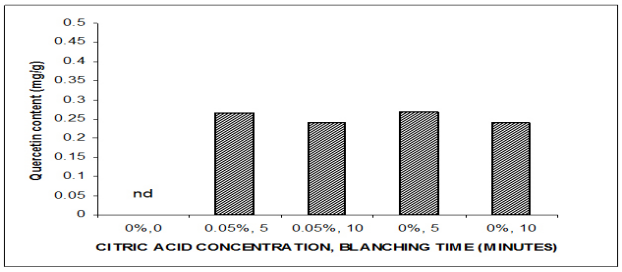

Figure 5. Quercetin content of blanched white saffron
nd: not detected

FRAP value of quercetin-3-rutinoside is presented in Table 2. It shows that the heated quercetin-3-rutinoside compound resulted in increased antioxidant activity. Heating quercetin-3-rutinoside in medium containing 0.05% citric acid showed a greater increase of antioxidant activity than that of heated glycoside without citric acid addition or the unheated glycoside.

Table 2. FRAP value of heated standard quercetin-3-rutinoside with and without citric acid

Samples	FRAP value (mg Ferro E/g)
Quercetin-3-rutinoside (unheated)	73.26 ± 0.71
Quercetin-3-rutinoside heated in water	83.82 ± 0.33*
Quercetin-3-rutinoside heated in 0.05% citric acid	107.77 ± 16.02*

*Indicates significant difference from unheated quercetin-3-Rutinoside at $p \leq 0.05$

Conclusion

Antioxidant activity and quercetin content of of blanched white saffron increased significantly ($p \leq 0.05$) compared to that of fresh white saffron. One of the identified glycoside of compounds in white saffron is quercetin-3-rutinoside. Blanching white saffron in citric acid or water media resulted in hydrolysis quercetin-3-rutinoside into quercetin.

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References

Abas, F., Lajis, N. H., Shaari, K., Israf, D. A., Stanslas, J., Yusuf, U. K. and Raof, S. M. 2005. Labdane diterpene glucoside from the rhizomes of *Curcuma mangga* Val. Journal of Natural Product 68 (7): 1090-1093.

Barrett, D. M. and Theerakulkait, C. 1995. Quality indicators in blanched, frozen, stored vegetables. Food Technology 49: 62-65.

Benzie, I. F. F. and Strain, J. J. 1996. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power" the FRAP assay. Analytical Biochemistry 239: 70-76.

Cheng, Z., Su, L., Moore, J., Zhou, K., Luther, M., Yin, J. and Yu, L. 2006. Effects of post harvest treatment and heat stress on availability of wheat antioxidants. Journal of Agricultural and Food Chemistry 54: 5623-5629.

Garrido, I., Monagas, M., Gómez-Cordovés, C. and Bartolomé, B. 2008. Polyphenols and antioxidant properties of almond skins: influence of industrial processing. Journal of Food Science 73 (2): C106-115.

Halvorsen, B. L., Carlsen, M. H., Phillips, K. M., Bohn, S. K., Holle, K. and Jacobs, D. R. 2006. Content of redox-active compounds i.e., antioxidant in foods consumed in the United States. American Journal of Clinical Nutrition 84 (1): 95-135.

Kim, T. J., Silvia, J. L., Kim, M. K. and Jung, Y. S. 2010. Enhanced antioxidant capacity and antimicrobial activity of tannic and by thermal processing. Food Chemistry 118: 740-746.

Kirca, A., Ozkan, M. and Cemeroglu, B. 2007. Effects of temperature, solid content and pH on the stability of black carrot anthocyanins. Food Chemistry 101: 212-218.

Kwan, Y. I., Apostolidis, E. and Shetty, K. 2007. Traditional diet of americans for management of diabetes and hypertension. Journal of Medicinal Food 10: 266-275.

Li, C., Du, H., Wang, L., Shu, Q., Zheng, Y., Xu, Y., Zhang, J., Yang, R. and Ge, Y. 2009. Flavonoid composition and antioxidant activity of tree peony (*Paeonia section moutan*) yellow flowers. Journal of Agricultural and Food Chemistry 57: 8496-8503.

Mishira, D. K., Dolan, K. D. and Yang, L. 2008. Confidence intervals for modeling anthocyanin retention in grape pomace during nonisolthermal heating. Journal of Food Science 73: E9-15.

Monagas, M., Garrido, I., Lebron-Aguilar, R., Bartolome, B. and Gomez-Cordoves, C. 2007. Almond (*Prunus dulcis* Mill) skins as a potential source of bioactive

polyphenols. Journal of Agricultural and Food Chemistry 55: 8498-8507.

Olivera, D. F., Viña, S. Z., Marani, C. M., Ferreyra, R. M., Mugride, A., Chaves, A. R. and Mascheroni, R. H. 2008. Effect of blanching on the quality and of brussels sprouts (*Brassica oleracea* L. *gemmifera* DC) after frozen storage. Journal of Food Engineering 84: 148-155.

Pujimulyani, D. 2003. The effect of blanching on antioxidant properties of white saffron (*Curcuma mangga* Val.) syrup. Agritech 23 (2): 137-141.

Pujimulyani, D. and Sutardi 2003. Curcuminoid content and antioxidative properties of white saffron extract *(Curcuma mangga* Val.). Proceeding International Conference, ISBN 979-95896-6-5.

Pujimulyani, D. and Wazyka, A. 2009a. Antioxidation, chemical, and physical propesties of wet sweets from white Saffron (*Curcuma mangga* Val.). Agritech 29 (3): 167-173.

Pujimulyani, D. and Wazyka, A. 2009b. An antioxidant activity and curcumin content of dry sweets from white saffron (*Curcuma mangga* Val.). Proceeding Palm Oil and Sustainable Chemistry on International Chemistry Seminar (XIX ICS), Proceeding, ISSN, No.1410-8313, 82-86.

Randhir, R., Kwan, Y. I. and Shetty, K. 2008. Effect of thermal processing on phenolics, antioxidant activity and health-relevant functionality of select grain sprouts and seedlings. Innovative Food Science and Emerging Technologies 9: 355-364.

Sadilova, E., Stintzing, E. C. and Carle, R. 2006. Thermal degradation of acylated and nonacylated anthocyanins. Journal of Food Science 71: C504-512.

Seeram, N. P., Bourquin, L. D. and Nair, M. G. 2001. Degradation products of cyanidin glycosides from tart cherries and their bioactivities. Journal of Agriculturaland Food Chemistry 49: 4924-4929.

Viña, S. Z., Olivera, D. F., Marani, C. M., Ferreyra, R. M., Mugridge, A., Chaves, A. R. and Maschereni, R. H. 2007. Quality of brussels sprouts (*Brassica oleracea* L. *gemmifera* DC) as affected by blanching method. Journal of Food Engineering 80: 218-225.

Volden, J., Borge, G. I. A., Bengtsson, G. B., Hansen, M., Thygesen, I. E. and Wicklund, T. 2008. Effect of thermal treatment on glucosinolates and antioxidant-related parameters in red cabbage (*Brassica oleracea* L. ssp. *Capitataf rubra*). Food Chemistry 109: 595-605.

Wahyuningsih, D. 2008. The Effect of Method and Blanching Time on Anthocyanin and Ascorbic Acid Content of Red *Sesbania grandiflora* L. (*Pers*) Flower. Faculty of Agroindustry, Mercu Buana University, Yogyakarta.

Xu, B. and Chang, S. K. C. 2008. Effect of soaking, boiling, and steaming on total phenolic content and antioxidant activities of cool season food legumes. Food Chemistry 110: 1-13.

Yue, X. and Xu, Z. 2008. Changes of anthocyanins, anthocyanidins, antioxidant activity in bilberry extract during dry heating. Journal of Food Science73: 494-499.

Zhang, Z., Kou, X., Fugal, K. and Mclaughlin, J. 2004. Comparison of HPLC methods for determination of anthocyanins and anthoyanidins in bilberry extracts. Journal of Agricultural and Food Chemistry 52: 688-691.