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Targeting Flavone Deglycosylation Improves Formulation of Whole Celery-Based Apigenin Rich Food

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ABSTRACT

Flavone apigenin is a plant bioactive compound abundantly found in human diets. Apigenin has antioxidant, anti-inflammatory and anti-carcinogenic activities, thereby presenting promising yet unexploited prospects for the prevention and treatment of various inflammatory diseases. Apigenin, like other flavones, naturally occurs in plants in its glycoside form. However, apigenin glycosides are less absorbed compared to their aglycone counterparts leading to diminished anti-inflammatory and anti-cancer activities in *in vitro* and *in vivo* models. Additionally, apigenin aglycones have poor aqueous solubility. Consequently, limited absorption and poor solubility pose major challenges in delivering medically effective concentrations of apigenin. To overcome these limitations, our group developed a targeted designed functional whole food from celery leaves, a rich source of apigenin, named **CEBAR (CElery Based Apigenin Rich)**. We demonstrated that CEBAR efficiently increased the intake of apigenin enabled by the removal of glycosides. Here, we describe a method that optimized the process by reducing the saltiness without affecting the efficiency of apigenin deglycosylation resulting in an improved CEBAR

formulation. We show that decreasing the concentration of phosphoric acid during acid hydrolysis aided the lowered use of potassium hydroxide needed for neutralization. These studies resulted in lessened saltiness while acquiring adequate levels of apigenin aglycone. Together, these findings would contribute to the implementation of targeted designed whole foods to deliver bioactive concentrations of apigenin and similar flavonoids and thus expanding the opportunities to prevent and treat inflammatory diseases.

INTRODUCTION

Flavonoids, one of the most abundantly found phytochemicals, are well-recognized medicinally active compounds or nutraceuticals known for their health-beneficial activities (Sudhakaran et al., 2019). Flavonoids are classified into different groups, including flavones, based on their structural and chemical modifications (Jiang et al., 2016). Apigenin, a flavone present at high levels in components of the Mediterranean diet, is a bioactive compound exerting antioxidant, anti-inflammatory and anti-cancer activities in cellular and *in vivo* models (Cardenas et al., 2016; Gao et al., 2013; Li et al., 2018; Nicholas et al., 2007). We recently showed that apigenin can be used as an adjuvant therapy to

enhance the efficacy of the chemotherapeutic drug doxorubicin in inducing apoptosis in three-dimensional cultures of triple-negative breast cancer cells (Sudhakaran et al., 2020). Meta-analyses have reported a significant correlation between flavone intake and reduced breast cancer in both pre- and post-menopausal women (Bosetti et al., 2005; Hui et al., 2013). Hence, flavones represent an untapped clinical arsenal for the prevention and treatment of inflammatory diseases. However, poor aqueous solubility of flavones presents a major obstacle to efficiently delivering effective concentrations of these active compounds in a clinical setup. Thus, delivering bioactive concentrations of flavones through functional or medical foods has gained great attention.

In plants, apigenin, like other flavonoids, is generally found as glycosides (Manach et al., 2004). Studies using mammalian cell culture and *in vivo* animal models revealed that flavone glycosides are less bioavailable compared to their aglycone counterparts (Ahn-Jarvis et al., 2019; Liu and Hu, 2002). Previously, we showed that the lack of anti-inflammatory activity of apigenin-7-*O*-glucoside in macrophages is due to its limited absorption compared with its aglycone counterpart (Hostetler et al., 2012). We also found that the reduced absorption of apigenin-7-*O*-glucoside resulted in diminished anti-cancer activity (Sudhakaran et al., 2020). In order to increase the delivery of apigenin at bioactive concentrations, we developed a targeted designed whole food from celery leaves, a rich source of apigenin, named **CEBAR (CElery Based Apigenin Rich)** (Hostetler et al., 2012). We demonstrated efficient bioactivity of the CEBAR diet, capable of delivering up to 1 μ M apigenin, as an anti-inflammatory agent *in vivo* in a mouse model by reducing the levels of proinflammatory cytokines and microRNAs at specific inflammatory sites (Arango et al., 2015; Cardenas et al., 2016; Hostetler et al., 2012). The targeted design of CEBAR successfully increased the intake of apigenin by relying on the removal of glycosides (Hostetler et al., 2012). Here, we demonstrate a process that diminished CEBAR saltiness while maintaining the efficiency of apigenin deglycosylation, thereby

improving the formulation. Our findings show that lowering the concentration of phosphoric acid (H_3PO_4) during the acid hydrolysis step facilitated the reduced use of potassium hydroxide (KOH), thus resulting in less saltiness while reaching equal success in the levels of apigenin aglycone obtained. These findings will have a beneficial impact by improving palatability, food tolerability, and broadening the inclusion of participants in future clinical studies using whole food flavone-rich diets.

MATERIALS AND METHODS

Deglycosylation of flavones. Apigenin-7-*O*-glucoside (Sigma, USA) 80 μ M was treated with 0.04 U/mL almond β -glucosidase (Sigma, USA) in 0.08 M sodium acetate at varying pH and incubated at 37°C for 60 min. Samples were then extracted three times with diethyl ether and subsequently pooled. To obtain apigenin aglycone from celery, fresh celery leaves (*Apium graveolens* L., Apiaceae) were juiced, lyophilized and ground to a powder as we previously described (Hostetler et al., 2012). One gram of celery powder was incubated at 95-100°C in the presence of H_3PO_4 (Duda Energy, USA) at varying concentrations for 90 min. After acid hydrolysis, samples were cooled and neutralized to the desired pH with 20% KOH (Duda Energy, USA). The samples were then incubated with 170 mg of ground raw almonds (*Prunus dulcis* Mill., Rosaceae) at 50°C for 2 h to induce deglycosylation. Samples were then frozen overnight and subsequently lyophilized and ground to a fine powder with a mortar and pestle. At this point, the celery is referred to as the **Celery Finished Product (CFP)**. CFP was extracted three times with 70% methanol for 30 min, centrifuged, and the supernatants were pooled. The pooled extractions were subsequently dried under N_2 stream and resuspended in 0.5 mL of 70% (v/v) methanol for HPLC analyses.

Chemical analysis of flavones. Flavone concentration in each sample was quantified using an Acquity UPLC BEH C_{18} column (2.1 \times 50 mm, 1.7 μ m), and the specific flavonoids were detected using an Acquity triple quadrupole mass spectrometer (Waters, USA) with a dwell time of 36 ms. The analyses were done with 0.1% formic acid (solvent

A) and 0.1% formic acid in acetonitrile (solvent B) at a flow rate of 0.4 mL/min with the following gradient: time $t=0$, 99% solvent A; $t=0.5$ min, 99% solvent A; $t=2.5$ min, 50% solvent A; $t=3.0$ min, 0% solvent A; $t=4.0$ min, 0% solvent A; $t=4.01$ min, 99% solvent A; $t=5.0$ min, 99% solvent A. Source parameters included: capillary voltage of 1.0 kV in negative mode, cone 50 V, source block 130°C, desolvation 350°C, desolvation gas 800 L/h, cone gas 20 L/h, and collision gas 0.16 mL/min. The concentrations of flavones were calculated against a standard curve generated using commercially available pure apigenin, luteolin, chrysoeriol, apigenin-7-*O*-glucoside, luteolin-7-*O*-glucoside and apiin (Sigma, USA). The data was analyzed and integrated using MassLynx 4.1 software and the chromatograms were plotted using GraphPad Prism software. The percentage of apigenin derived from apigenin-7-*O*-glucoside is calculated as the amount of apigenin to that of apigenin-7-*O*-glucoside. Apigenin derivatives account for apigenin, apiin and apigenin-7-*O*-glucoside.

Statistical analyses. Graph and chromatogram preparation and statistical analyses were performed using GraphPad Prism software. Statistical differences between data were measured using a one-way or two-way ANOVA and Turkey's post-test for multiple comparisons. Data is presented mean \pm SEM. p value greater than 0.05 is considered statistically insignificant.

RESULTS

Deglycosylation of apigenin-7-*O*-glucoside in celery extracts is limited by pH conditions. We previously showed that celery leaves contain high levels of apigenin glycosides occurring as apigenin-7-apisoylglucoside or apiin (Hostetler et al., 2012) (Figure 1). We demonstrated that converting flavone glycosides to aglycones increased their absorption in cells and *in vivo* in mouse models (Hostetler et al., 2012). For the preparation of CEBAR, apiin is first converted to apigenin-7-*O*-glucoside by heating the celery leaves at acidic pH conditions in the presence of 1.5N H₃PO₄ (Figure 1). The removal of glycoside groups from apigenin-7-*O*-glucoside through the process deglycosylation is then achieved by

treatment with the enzyme β -glucosidase at pH 5 (Hostetler et al., 2012).

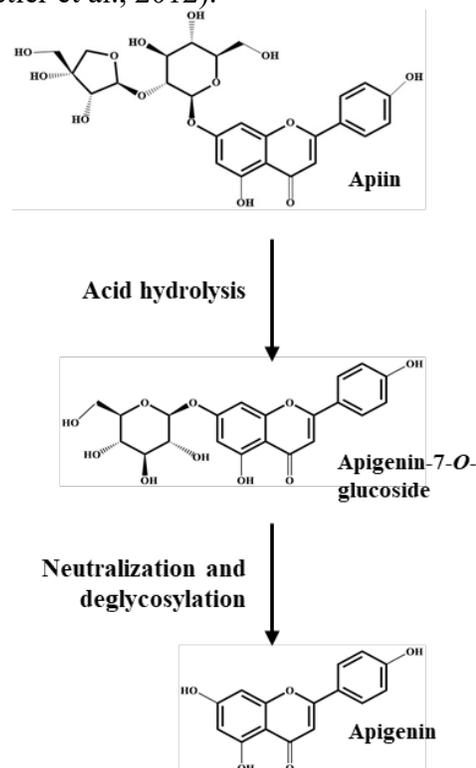


Figure 1. Conversion of celery apiin to apigenin. Apiin present in celery leaves is subjected to acid hydrolysis to generate apigenin-7-*O*-glucoside. After neutralization, apigenin-7-*O*-glucoside is deglycosylated to apigenin using ground raw almond powder containing β -glucosidase.

Intending to reduce the salt necessary to reach an optimal pH for the deglycosylation step, we first evaluated the optimal range of pH required for converting apigenin-7-*O*-glucoside to its aglycone counterpart, apigenin. For this purpose, commercially available pure apigenin-7-*O*-glucoside was incubated in the presence of β -glucosidase enzyme at various pH ranging from 5 to 4. No significant changes in the percentage of apigenin obtained were observed at all the pH tested, as shown by the quantitative chemical analyses performed (Figure 2).

We next tested the possibility to use a pH lower than 5 to deglycosylate apigenin-7-*O*-glucoside to apigenin in celery extracts. In agreement with our previous findings (Hostetler et al., 2012), apigenin-7-*O*-glucoside found in celery leaves extracts (Figure 3C) can be deglycosylated by incubating β -glucosidase found naturally in raw almond powder at

pH 5 for 2 h (Figure 3D). Increasing the incubation times to 4 or 6 h had no significant effect on the efficiency of deglycosylation at pH 5 (Figure 3E and F).

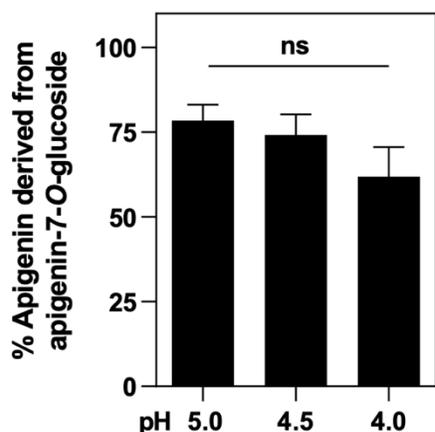


Figure 2. Effect of pH on deglycosylation of apigenin-7-O-glucoside using purified β -glucosidase. Pure apigenin-7-O-glucoside was deglycosylated using purified almond β -glucosidase at indicated pH at 37°C for 60 min. Extracted apigenin and apigenin-7-O-glucoside were detected by HPLC analyses as described in Material and Methods. All data represent mean \pm SEM, N = 3. ns indicates no statistical significance.

In order to decrease the amount of salt needed to adjust the pH required for the deglycosylation step, we next evaluated the effectiveness of performing the deglycosylation at pH 4. The chemical analyses of apigenin flavones showed that the amount of apigenin-7-O-glucoside converted to apigenin decreased at pH 4 as compared to the deglycosylation at pH 5 at incubations times of either 4 or 6 h, as depicted in the chromatograms (Figure 3G and H). There was a 50% decrease in the amount of apigenin aglycone generated at pH 4 as compared to pH 5. Overall, these results indicate that in contrast to the ability of the pure β -glucosidase enzyme to effectively deglycosylate apigenin-7-O-glucoside at a broader pH range, the deglycosylation of naturally occurring apigenin-7-O-glucoside in celery leaves extracts is favored by more restrictive pH conditions.

Reduced concentration of phosphoric acid results in improved salt conditions. Since regulating pH conditions proved to have less effect on deglycosylation, we decided to evaluate next if changes in the acid hydrolysis, wherein apiin is

converted to apigenin-7-O-glucoside (Figure 4A), will facilitate the reduction of saltiness.

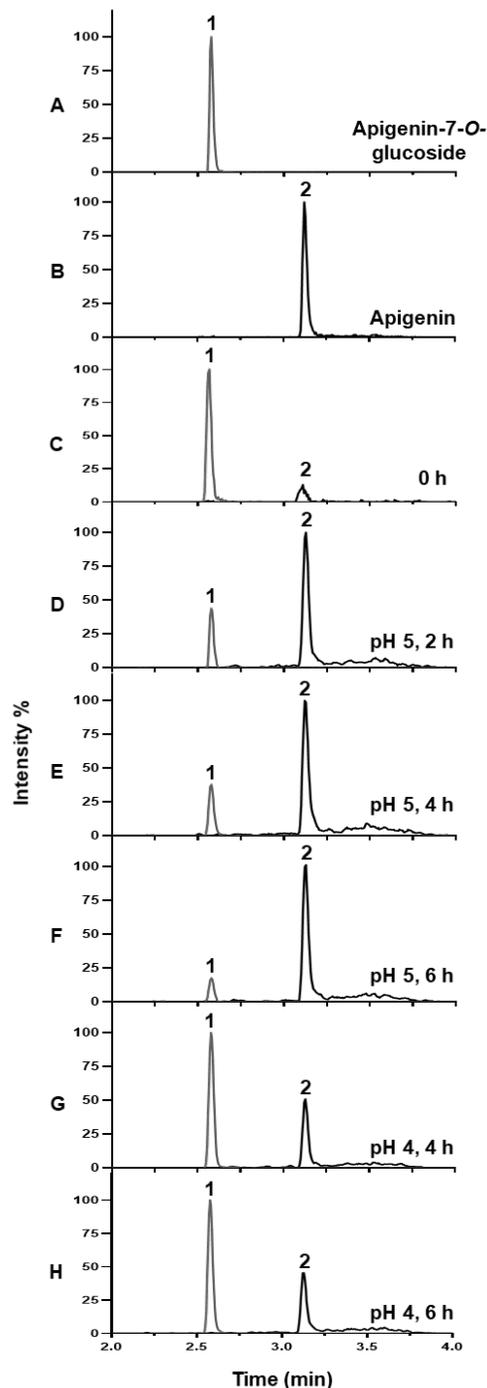


Figure 3. Effect of pH on deglycosylation of celery apigenin-7-O-glucoside using raw almond powder a source of β -glucosidase. HPLC chromatograms of (A and B) flavone standards, celery extracts (C) before deglycosylation, post-deglycosylation using almond β -glucosidase at (D-F) pH 5 and (G and H) pH 4 at indicated times. Peaks represent: 1. Apigenin-7-O-glucoside and 2. Apigenin.

To this end, we evaluated the effect of different concentrations of H_3PO_4 on the efficacy of acid hydrolysis and subsequent deglycosylation by evaluating first the levels of apigenin derivatives (apiin and apigenin-7-*O*-glucoside and apigenin) at two concentrations of H_3PO_4 . We found that decreasing the concentration of H_3PO_4 to 0.75N from 1.5N did not have any significant effect on the acid hydrolysis, as indicated by the similar levels of apigenin-7-*O*-glucoside obtained from the hydrolysis of apiin at both the concentrations tested (Figure 4B). The decreased concentration of H_3PO_4

lowered the amounts of KOH required to neutralize and reach the pH 5 optimal for deglycosylation. There was almost a decrease of 50% in the amount of KOH used for neutralization when the concentration of H_3PO_4 was reduced to 0.75N. Importantly, we found that the deglycosylation following these new conditions generated similar levels of apigenin aglycone (Figure 4B). These results demonstrate that the reduction of H_3PO_4 concentration combined with less salt content resulted in a favorable deglycosylation of apigenin in CFP.

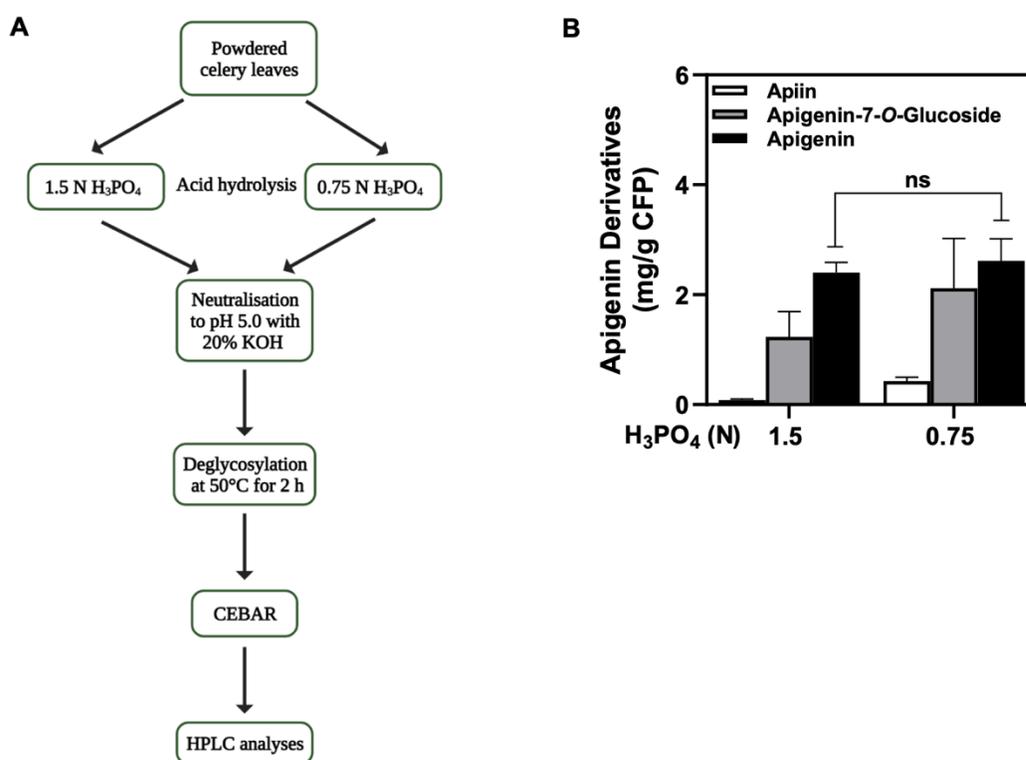


Figure 4. Effect of phosphoric acid concentration on acid hydrolysis and deglycosylation of flavones in celery leaf extracts. (A) Flowchart of experimental design. Celery extracts were subjected to acid hydrolysis at the indicated phosphoric acid concentrations and deglycosylated using almond β -glucosidase at pH 5 for 2 h. (B) Extracted apigenin derivatives were detected by HPLC analyses. All data represent mean \pm SEM, N = 3. ns indicates no statistical significance.

DISCUSSION

Bioactive compounds such as flavonoids are gaining immense interests as nutraceuticals for the prevention and treatment of inflammatory diseases.

The delivery of flavonoids at medically effective concentrations *in vivo* is critical for its efficacy. However, flavones are associated with poor aqueous solubility, thereby affecting efficient delivery *in vivo*. Additionally, plant flavones are commonly

found in plants as glycosides which have restricted cellular absorption compared to their aglycones counterparts. Therefore, poor solubility and absorption of flavonoids present a major obstacle for their biomedical applications. The use of flavonoid-rich whole foods for effective delivery presents favorable opportunities (Ahn-Jarvis et al., 2019). We previously developed a targeted designed whole food from celery leaves rich in flavone apigenin, named CEBAR, which exhibited effective bioactivity as an anti-inflammatory agent in mouse models (Arango et al., 2015; Cardenas et al., 2016; Hostetler et al., 2012). Here, we reveal an efficient method to improve CEBAR formulations by reducing the saltiness of the preparation without affecting the efficacy of the deglycosylation of apigenin, thereby potentially improving its delivery and bioavailability.

Apigenin is a bioactive flavone exhibiting anti-cancer, anti-inflammatory and antioxidant activities (Salehi et al., 2019; Sudhakaran et al., 2019). Its ability to specifically target primary human cancer cells, without affecting cells from adjacent normal tissues, presents unique opportunities for clinical applications (Voss et al., 2021). We recently reported that apigenin sensitizes human breast cancer cells to chemotherapy by inducing apoptosis, thereby emphasizing its promising role as a chemotherapeutic adjuvant in a clinical set up (Sudhakaran et al., 2020). While the reported studies have confirmed health beneficial impacts of pure apigenin both *in vitro* and *in vivo*, it has been shown to have limited solubility, characteristic of flavonoids (Hollman, 2004). In most cellular models, the effective apigenin concentrations range between 10 to 100 μM , amounts that are hard to achieve *in vivo*. Apigenin has limited bioavailability owing to its natural existence as a glycoside in plants, such as apiin and apigenin-7-*O*-glucosides (Jiang et al., 2016). We and others have reported that flavone glycosides are less absorbed by cells than their aglycone counterparts. We demonstrated that apigenin-7-*O*-glucoside showed less cellular absorption compared to its aglycone counterpart using immunofluorescence, thereby exerting diminished anti-inflammatory and anti-cancer

activity (Arango et al., 2015; Hostetler et al., 2012; Sudhakaran et al., 2020; Vargo et al., 2006). Additionally, apigenin in colorectal adenocarcinoma cells was highly permeable as compared to apigenin-7-*O*-glucoside as indicated by HPLC analytical analyses (Liu and Hu, 2002). The flavone baicalin induces apoptosis in human breast cancer cells more effectively than its glycoside counterpart (Yu et al., 2013). These differences in cellular absorption may be attributed to the planar structure of apigenin aglycone, which is conferred by the presence of a double bond between carbon 2 and 3 on the C ring. This planar structure contrasts with the change in the conformation of the structure due to the addition of the sugar in apigenin-7-*O*-glycoside. Underlining the impact of the structure on bioavailability *in vivo*, our previous studies showed that glycosylation diminished intestinal absorption of apigenin into systemic circulation in mice fed with celery-derived apigenin rich foods (Hostetler et al., 2012). These observations emphasize the need for designing food formulation that improve the delivery, solubility and bioavailability of apigenin and similar flavones to enhance their efficacy.

Our apigenin rich celery food formulation CEBAR relies on hydrolysis of celery apiin to apigenin-7-*O*-glucoside followed by subsequent deglycosylation to apigenin to improve bioavailability (Hostetler et al., 2012). We previously showed that deglycosylation enhanced systemic circulation of apigenin in mice fed with CEBAR (Hostetler et al., 2012). In this study, we improved CEBAR formulations to reduce saltiness while maintaining the levels of deglycosylated apigenin. We initially chose to reduce the saltiness by optimizing pH conditions for deglycosylation using almond derived β -glucosidase. However, deglycosylation of apigenin-7-*O*-glucoside in celery leaves was found to be favored by a restricted pH range (Figure 4). Lowering H_3PO_4 concentration during acid hydrolysis of celery extract facilitated the decreased use of KOH. This enabled reduction in saltiness while generating adequate levels of apigenin aglycone through deglycosylation (Figure 4B). This will help in enhancing apigenin absorption and confer higher bioavailability.

Several other studies have reported the use of whole food enriched with flavonoids to treat cancer in *in vivo* mice models. A whole food-based approach using freeze-dried berries and berry extracts inhibited rodent esophagus and colon cancer by more than 50% through DNA damage and apoptosis (Stoner et al., 2007; Wang et al., 2011). Dietary grape powder attenuated UVB-mediated skin carcinogenesis in SKH-1 hairless mouse model (Singh et al., 2019). There is a limited number of clinical studies for cancer and inflammatory diseases using flavonoid rich whole foods. Dietary black freeze-dried black raspberry intervention in 28 colorectal cancer patients showed beneficial anti-cancer activities by inducing metabolic changes (Pan et al., 2015). A pilot study involving patients with biopsy-confirmed oral squamous cell carcinomas administered with oral troches containing freeze-dried black raspberry powder reported that the bioactive compounds were efficiently delivered to the affected sites and reduced the expression of anti-apoptotic and anti-inflammatory markers. However, the study reported an adverse experience in three participants (Knobloch et al., 2016). Nonetheless, these studies highlight the importance of targeted whole foods to overcome the limitations of delivering pure flavonoids and reaching effective bioactive concentrations.

In conclusion, CEBAR offers a promising targeted designed whole food formulation that can deliver effective biomedical concentrations of medicinally active apigenin. Our improved food formulations have efficient deglycosylation of celery apigenin while reducing the salt content. These findings may have profound implications by improving food tolerability and enhancing the potential use of CEBAR as whole food flavone-rich adjuvants to currently available standard of care.

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REFERENCES

- Ahn-Jarvis, J.H., Parihar, A., and Doseff, A.I. 2019. Dietary flavonoids for immunoregulation and cancer: food design for targeting disease. *Antioxidants (Basel)* 8, 202.
- Arango, D., Diosa-Toro, M., Rojas-Hernandez, L.S., Cooperstone, J.L., Schwartz, S.J., Mo, X., Jiang, J., Schmittgen, T.D., and Doseff, A.I. 2015. Dietary apigenin reduces LPS-induced expression of miR-155 restoring immune balance during inflammation. *Mol. Nutr. Food Res.* 59, 763-772.
- Bosetti, C., Spertini, L., Parpinel, M., Gnagnarella, P., Laggiou, P., Negri, E., Franceschi, S., Montella, M., Peterson, J., Dwyer, Giacosa, A., and La Vecchia, C. 2005. Flavonoids and breast cancer risk in Italy. *Cancer Epidemiol. Biomark. Prev.* 14, 805-808.
- Cardenas, H., Arango, D., Nicholas, C., Duarte, S., Nuovo, G.J., He, W., Voss, O.H., Gonzalez-Mejia, M.E., Guttridge, D.C., Grotewold, E., and Doseff, A.I. 2016. Dietary apigenin exerts immune-regulatory activity *in vivo* by reducing NF- κ B activity, halting leukocyte infiltration and restoring normal metabolic function. *Int. J. Mol. Sci.* 17, 323.
- Gao, A.-M., Ke, Z.-P., Wang, J.-N., Yang, J.-Y., Chen, S.-Y., and Chen, H. 2013. Apigenin sensitizes doxorubicin-resistant hepatocellular carcinoma BEL-7402/ADM cells to doxorubicin via inhibiting PI3K/Akt/Nrf2 pathway. *Carcinogenesis* 34, 1806-1814.
- Hollman, P.C.H. 2004. Absorption, bioavailability, and metabolism of flavonoids. *Pharm. Biol.* 42, 74-83.
- Hostetler, G., Riedl, K., Cardenas, H., Diosa-Toro, M., Arango, D., Schwartz, S., and Doseff, A.I. 2012. Flavone deglycosylation increases their anti-inflammatory activity and absorption. *Mol. Nutr. Food Res.* 56, 558-569.
- Hui, C., Qi, X., Qianyong, Z., Xiaoli, P., Jundong, Z., and Mantian, M. 2013. Flavonoids,

- flavonoid subclasses and breast cancer risk: a meta-analysis of epidemiologic studies. *PLoS One* 8, e54318.
- Jiang, N., Doseff, A.I., and Grotewold, E. 2016. Flavones: from biosynthesis to health benefits. *Plants (Basel, Switzerland)* 5, 27.
- Knobloch, T.J., Uhrig, L.K., Pearl, D.K., Casto, B.C., Warner, B.M., Clinton, S.K., Sardo-Molmenti, C.L., Ferguson, J.M., Daly, B.T., Riedl, K., Schwartz, S.J., Vodovotz, Y., Buchta, A.J., Sr., Schuller, D.E., Ozer, E., Agrawal, A., and Weghorst, C.M. 2016. Suppression of proinflammatory and prosurvival biomarkers in oral cancer patients consuming a black raspberry phytochemical-rich troche. *Cancer Prev. Res. (Phila)* 9, 159-171.
- Li, Y.W., Xu, J., Zhu, G.Y., Huang, Z.J., Lu, Y., Li, X.Q., Wang, N., and Zhang, F.X. 2018. Apigenin suppresses the stem cell-like properties of triple-negative breast cancer cells by inhibiting YAP/TAZ activity. *Cell Death Discov.* 4, 105.
- Liu, Y., and Hu, M. 2002. Absorption and metabolism of flavonoids in the caco-2 cell culture model and a perused rat intestinal model. *Drug Metab. Dispos.* 30, 370-377.
- Manach, C., Scalbert, A., Morand, C., Rémésy, C., and Jiménez, L. 2004. Polyphenols: food sources and bioavailability. *Am. J. Clin. Nutr.* 79, 727-747.
- Nicholas, C., Batra, S., Vargo, M.A., Voss, O.H., Gavrilin, M.A., Wewers, M.D., Guttridge, D.C., Grotewold, E., and Doseff, A.I. 2007. Apigenin blocks lipopolysaccharide-induced lethality in vivo and proinflammatory cytokines expression by inactivating NF- κ B through the suppression of p65 phosphorylation. *J. Immunol.* 179, 7121-7127.
- Pan, P., Skaer, C.W., Stirdivant, S.M., Young, M.R., Stoner, G.D., Lechner, J.F., Huang, Y.-W., and Wang, L.-S. 2015. Beneficial regulation of metabolic profiles by black raspberries in human colorectal cancer patients. *Cancer Prev. Res. (Phila)* 8, 743-750.
- Salehi, B., Venditti, A., Sharifi-Rad, M., Kręgiel, D., Sharifi-Rad, J., Durazzo, A., Lucarini, M., Santini, A., Souto, E.B., Novellino, E., Antolak, H., Azzini, E., Setzer, W.N., and Martins, N. 2019. The therapeutic potential of apigenin. *Int. J. Mol. Sci.* 20, 1305.
- Singh, C.K., Mintie, C.A., Ndiaye, M.A., Chhabra, G., Dakup, P.P., Ye, T., Yu, M., and Ahmad, N. 2019. Chemoprotective effects of dietary grape powder on UVB radiation-mediated skin carcinogenesis in SKH-1 hairless mice. *J. Invest. Dermatol.* 139, 552-561.
- Stoner, G.D., Wang, L.-S., Zikri, N., Chen, T., Hecht, S.S., Huang, C., Sardo, C., and Lechner, J.F. 2007. Cancer prevention with freeze-dried berries and berry components. *Semin. Cancer Biol.* 17, 403-410.
- Sudhakaran, M., Parra, M.R., Stoub, H., Gallo, K.A., and Doseff, A.I. 2020. Apigenin by targeting hnRNPA2 sensitizes triple-negative breast cancer spheroids to doxorubicin-induced apoptosis and regulates expression of ABCG2 and ABCG4 drug efflux transporters. *Biochem. Pharmacol.* 182, 114259.
- Sudhakaran, M., Sardesai, S., and Doseff, A.I. 2019. Flavonoids: new frontier for immunoregulation and breast cancer control. *Antioxidants (Basel)* 8, 103.
- Vargo, M.A., Voss, O.H., Poustka, F., Cardounel, A.J., Grotewold, E., and Doseff, A.I. 2006. Apigenin-induced-apoptosis is mediated by the activation of PKC δ and caspases in leukemia cells. *Biochem. Pharmacol.* 72, 681-692.
- Voss, O.H., Arango, D., Tossey, J.C., Villalona Calero, M.A., and Doseff, A.I. 2021. Splicing reprogramming of TRAIL/DISC-components sensitizes lung cancer cells to TRAIL-mediated apoptosis. *Cell Death. Dis.* 12, 287.
- Wang, L.S., Dombkowski, A.A., Seguin, C., Rocha, C., Cukovic, D., Mukundan, A., Henry, C., and Stoner, G.D. 2011. Mechanistic basis for the chemopreventive effects of black raspberries at a late stage of rat esophageal carcinogenesis. *Mol. Carcinog.* 50, 291-300.
- Yu, C., Zhang, Z., Zhang, H., Zhen, Z., Calway, T., Wang, Y., Yuan, C.-S., and Wang, C.-Z. 2013. Pretreatment of baicalin and wogonoside with glycoside hydrolase: a promising approach to enhance anticancer potential. *Oncol. Rep.* 30, 2411-2418.