UNIVERSIDADE DO ALGARVE

"ANTIOXIDANT AND PHENOLIC COMPOUNDS IN SWEET POTATO PEELS AND LEAVES: FOOD APPLICATIONS AND HEALTH BENEFITS"

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Tese

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Declaração de autoria de trabalho

Declaro ser a autora deste trabalho, que é original e inédito. Autores e trabalhos consultados estão devidamente citados no texto e constam da listagem de referências incluída.

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Resumo

A batata-doce (*Ipomoea batatas* L.) tem sido muito utilizada ao longo dos tempos tanto na alimentação humana como na medicina tradicional. As cascas de batata-doce (*sweet potato peels*, SPP) são consideradas um resíduo na maioria dos processos de transformação do tubérculo. Apesar de consumidas em algumas partes do mundo, as folhas (*sweet potato leaves,* SPL) não são ainda utilizados na dieta de muitos países. Estes resíduos apresentam potencial para a sua conversão em alimentos funcionais com elevado teor de compostos fenólicos e alta atividade antioxidante. Neste contexto, foi desenvolvido um protótipo de bancada para uma bebida antioxidante à base de SPP com potenciais benefícios para a saúde associados à proteção do *stress* oxidativo. As etapas do processo de desenvolvimento do novo produto foram: revisão da literatura e de patentes, estudo preliminar, rastreio de fatores-chave, otimização da extração e otimização da formulação. O teor total de compostos fenólicos (*total phenolic content*, TPC) e a atividade antioxidante foram avaliados respetivamente, pelo método Folin-Ciocalteau e ensaios *in vitro*. A revisão da literatura e de patentes para o período 2003-2014 revelou lacunas na investigação, para SPL e especialmente para SPP, em aplicações alimentares com benefícios para a saúde. Da pesquisa realizada nas bases de dados *ISI Web of Knowledge* e *ESPACENET* foram selecionados e analisados 122 artigos e 170 patentes. O desenvolvimento de novos produtos e a utilização de ferramentas de *design* experimental foram hiatos identificados em estudos sobre SPP e SPL. Para SPP, foi ainda identificada a necessidade da análise da atividade antioxidante, da composição em fenólicos e a realização de ensaios pré-clínicos e clínicos. O produto alimentar mais estudado, nos artigos, foi na forma de farinha/pó enquanto que nas patentes foi a bebida. Deste modo, a valorização das SPP e SPL através do desenvolvimento de uma bebida com atividade antioxidante, recorrendo a ferramentas de desenho experimental e análise da atividade antioxidante *in vitro,* irá preencher lacunas identificadas na investigação. A mineração de textos (*texto mining*), aplicada aos resumos dos artigos e das patentes, identificou eficazmente os principais temas de investigação. No estudo preliminar foram investigados os efeitos de uma variável de produto (ramas de batata-doce) e de duas variáveis de processo (tempo e solvente de extração) em TPC e no teor de flavonoides (*total flavonoid content*, TFC) bem como na atividade antioxidante *in vitro*. Os métodos utilizados na avaliação da atividade antioxidante *in vitro* foram a atividade antioxidante

total (*total antioxidant activity*, TAA), o poder redutor (*reducing power*, RP), o poder redutor do ferro (*ferric reducing activity, FRAP*) e o método de sequestro de radicais livres de 2,2-difenil-1-picrilhidrazila (DPPH). As experiências foram realizadas de acordo com um plano fatorial completo para três fatores: parte da planta (caule ou folhas), tempo (24, 48 ou 72 h) e solvente (não acidificado e acidificado). O fator mais importante influente nas respostas foi a parte da planta seguido do fator solvente. TPC, TFC, FRAP e RP apresentaram uma elevada influência do fator parte da planta enquanto que o solvente apresentou um maior efeito no método DPPH. O tempo de extração teve unicamente influência em RP, tanto como efeito principal como em interação com o fator parte da planta. As atividades antioxidante das folhas foram 1,25 g de equivalentes de ácido ascórbico (*ascorbic acid equivalent*, AAE)/100 g (peso seco) para TAA e 50,8 g de equivalentes de ácido gálico (*galic acid equivalente*, GAE,)/100 g para RP. A atividade sequestrante do radical DPPH foi superior à atividade antioxidante medida por FRAP (.i.e., 58,6 e 29,3 mM de equivalentes de Trolox (*Trolox equivalent*, TE)/g de peso seco, respetivamente). A etapa seguinte, do processo de desenvolvimento, foi o rastreio de fatores chave na extração de compostos fenólicos de farinhas de SPP. Para tal, utilizou-se um *design* experimental de 12 ensaios de Plackett-Burman tendo como variáveis a razão solvente: sólido (10 ou 50 mL/g), o tempo (60 ou 180 min), o pH (2,3 ou 7,0), a profundidade do corte da casca (1,5 ou 3,0 mm), o tamanho das partículas (<600 ou <300 µm), a temperatura (27 ou 40 ºC), o solvente (água destilada ou água do abastecimento público), o tamanho da amostra (0,1 ou 1,0 g) e o nível de agitação (50 ou 200 rpm). Os fatores chave significativos (p <0,05) para TPC foram a razão solvente: sólido (efeito positivo) e a profundidade do corte da casca (efeito negativo). Estes dois fatores apresentaram efeitos de direção, magnitude e significância semelhante para FRAP. Os modelos de regressão obtidos para os resultados de FRAP, transformados pela função raiz quadrada, mostraram a interação entre os fatores profundidade de corte e tamanho da amostra como o terceiro efeito mais influente. A modelação e otimização da extração aquosa de compostos fenólicos, com atividade antioxidante, de farinhas de SPP foram realizadas recorrendo às metodologias de Superfície de Resposta (*Response Surface Methodology*, RSM) e de Redes Neurais Artificiais (*Artificial Neural Network*, ANN). A razão solvente: sólido (30 a 60 mL/g), o tempo (30 a 90 min) e a temperatura (25 a 75 ºC) foram combinados de acordo com um plano de experiências de composto central (*Central Composite Design*, CCD). Os fatores temperatura e a razão solvente: sólido,

individualmente e em interação, apresentaram um efeito positivo quer para TPC quer para os métodos ABTS e DPPH. Individualmente, o fator tempo,teve uma influência negativa no método ABTS e, quando combinado com a temperatura, apresentou o mesmo efeito para TPC e para o método ABTS. A direção da influência do fator tempo foi sempre negativa e de magnitude inferior aos efeitos positivos dos outros fatores. As condições de extração ótimas obtidas foram 60 mL/g, 45 min e 75 °C com valores previstos para os métodos TPC, ABTS e DPPH de 11,87 ± 0,69 mg GAE, 12,91 ± 0,42 mg TE e 46,35 ± 3,08 mg TE por g de material seco, respetivamente. O rendimento em compostos fenólicos e as respetivas condições de extração obtidas para SPP foram semelhantes aos conseguidos para as cascas de batata, as cascas de fruta do chá (*Camelia sinensis* L.) e as cascas de manga (*Mangifera pajang* Kostern). A etapa final do desenvolvimento do novo produto consistiu na otimização da formulação de uma bebida antioxidante, tendo como componentes um extrato aquoso de SPP (SPPE), um extrato aquoso de SPL (SPLE) e uma solução de mel (HonS). As formulações foram testadas de acordo com um desenho de mistura *I-optimal* sendo avaliadas pelos métodos TPC, FRAP, DPPH e pelos sólidos solúveis (SS). A modelação dos dados foi realizada pelas metodologias de regressão dos mínimos quadrados (*least squared regression*, LSR) e ANN. Os componentes apresentaram um efeito positivo para os métodos TPC, FRAP e DPPH na seguinte ordem: SPLE> SPPE> HonS. Foram observados efeitos sinergéticos entre SPPE e HonS, SPLE e HonS e SPPE e SPLE, e todos os efeitos antagónicos foram não significativos (p> 0,05). A formulação ótima da bebida foi obtida pela metodologia ANN tendo como resultado 50,0 % de SPPE, 21,5 % de SPLE e 28,5 % de HonS. Os valores previstos para os métodos TPC, FRAP, DPPH e para SS foram respetivamente 309 mg GAE/L, 476 mg TE/L, 1098 mg TE/L e 12,3 ºBrix, tendo-se obtido resultados idênticos com a utilização dos modelos LSR. O protótipo posicionou-se próximo das bebidas comerciais de frutas e vegetais quanto à sua atividade antioxidante. A transferência de conhecimento destes resultados para a indústria alimentar pode oferecer uma oportunidade para a valorização de SPP e SPL promovendo paralelamente o interesse na investigação em aplicações alimentares com benefícios para a saúde.

Palavras-chave: cascas de batata-doce, folhas de batata-doce, atividade antioxidante, compostos fenólicos, planeamento de experiências, bebida funcional.

Abstract

The valorisation of sweet potato (*Ipomoea batatas* L.) wastes into a food application with health benefits was studied using a product development framework to translate a product concept into a benchtop prototype. A functional beverage with attributes related to oxidative stress protection was developed using sweet potato peel (SPP) and sweet potato leaf (SPL) as components. The developing processes involved the following steps: literature and patent review, preliminary study, key factors screening, extraction optimization and beverage formula optimization. Total phenolic content (TPC) and antioxidant activity was measured by Folin-Ciocalteau method and antioxidant activity by *in vitro* screening assays. Literature and patent review for the period 2003-2014 identified research gaps on food applications with health benefits for SPL and especially SPP. Text mining analysis on articles abstracts could picture research main themes. A preliminary study using a factorial design revealed that plant part (stems or leaves) presented the largest main significant effect ($p < 0.001$) on TPC, total flavonoids content (TFC), total antioxidant activity (TAA), reducing power (RP) and ferric reducing activity power (FRAP) while for DPPH scavenging assay, solvent acidification was the most influential factor. Time of extraction had the only significant effect on RP assay, as main effect and interaction with plant part. Solvent: solid ratio and peel cut depth were the key factors identified by a Plackett-Burman design for the aqueous extraction of phenolics of SPP flour. The aqueous extraction conditions of phenolic from SPP flour were modelled by Response Surface Methodology (RSM) and Artificial neural Network (ANN) analysis of a Central composite Design (CCD) experimental plan. Optimized conditions were 60 mL/g for solvent: solid ratio, 30 min for time and 75 ºC for temperature. Predicted responses were 11.87 ± 0.69 mg GAE/g DM for TPC, 12.91 ± 0.42 mg TE/g DM for ABTS and 46.35 ± 3.08 mg TE/g DM for DPPH assay. Beverage formula components SPP aqueous extract (SPPE), SPL water extract (SPLE) and honey solution (HonS) were combined according to a mixture design. The optimized formula obtained by ANN models for the SPP beverage benchtop prototype was 50.0 % of SPPE, 21.5 % of SPLE and 28.5 % of HonS. TPC, FRAP, DPPH assays and SS predicted responses for the optimal formula were 309 mg GAE/L, 476 mg TE/L, 1098 mg TE/L and 12.3 ± 0.8 ºBrix, respectively. Beverage positioned next to commercial vegetable and fruit beverages in terms of antioxidant activity. Knowledge transfer of these findings to food processors

may offer an opportunity to transform SPP and SPL from a liability to an asset and promote further research on food application with health benefits.

Keywords: Sweet potato peels; sweet potato leaves; antioxidant activity; phenolic compounds; design of experiments; functional beverage.

List of Publications

Articles

Anastácio, A. and Carvalho, I. S. (2013). Spotlight on PGI sweet potato from Europe: Study of plant part, time and solvent effects on antioxidant activity. *Journal of Food Biochemistry*. 37, 628–637.

Anastácio, A. and Carvalho, I.S. (2013). Phenolics extraction from sweet potato peels: Key factors screening through a Plackett–Burman design. *Industrial Crops and Products* 43, 99-105.

Anastácio, A., Silva, R. and Carvalho I.S. Phenolics extraction from sweet potato peels: Modelling and optimization by Response Surface Modelling and Artificial Neural Network. *Journal of Food Science and Technology*. Submitted.

Anastácio, A. and Carvalho, I.S. Development of a beverage benchtop prototype based on sweet potato peels: Optimization of antioxidant activity by a mixture design. *Food Science and Nutrition*. Submitted.

Anastácio, A. and Carvalho, I.S. Food applications and health benefits related to antioxidant activity of phenolic compounds from sweet potato peels and leaves: review of literature and patents. *Critical Reviews in Food Science and Nutrition*. Submitted.

Poster communications

"SWOT analysis on the use of Sweet Potato Leaves for feed aquaculture applications" Anastácio, A. and Carvalho, I.S. IMMR 2012 - International Meeting in Marine Resources, pp. 145. School of Tourism and Maritime Technology, Polytechnic Institute of Leiria (IPL), Peniche, Portugal, May 24- 25.

"Hazard analysis during concept design of a plant based food supplement: case study on sweet potato peel". Anastácio A. and Carvalho I.S. International Congress on Environmental Health 2012, pp. 142. Escola Superior de Tecnologia da Saúde de Lisboa, Lisbon, Portugal, May29-June1.

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List of Abbreviations

AAE	Ascorbic acid equivalent
ABTS	2-2'-azino-bis (3 ethylbenzothiazoline-6-sulfonic acid)
ANN	Artificial neural network
ANOVA	Analysis of variance
CAT	Catalase
CBI	β-Carotene bleaching inhibition
CCD	Central composite design
DM	Dried material
DPPH	2,2-diphenyl-1-picrylhydrazyl
dw	dry weight
FAO	Food and Agriculture Organization of the United Nations
FRAP	Ferric reducing activity power
FTC	Ferric thiocyanate
GAE	Gallic acid equivalent
GGT	Gamma glutamyl transferase
GSH Px	Glutathione peroxidase
GSH	Glutathione
GSt:	Glutathione-S-transferase
H_2O_2	Hydrogen peroxide
HCA	Hierarquical cluster analysis
HonS	Honey solution
HPLC	High performance liquid chromatography
ICA	Iron (II) chelating activity
LC	Liquid chromatography
LDL	Low-density lipoprotein
LSR	Least squares regression
NO	Nitric oxide
OH•	Hydroxyl radical
ORAC	Oxygen radical absorbance capacity
PBD	Plackett-Burman design
PCA	Principal Components Analysis,
PGI	Protected geographic indication

PV	Peroxide value
QE	Quercetin equivalent
RP	Reducing power
RSM	Response surface methodology
SOD	Superoxide dismutase
SP	Sweet potato
SPL	Sweet potato leaves
SPLE	Sweet potato leaves extract
SPP	Sweet potato peels
SPPE	Sweet potato peels extract
SS	Soluble solids
TAA	Total antioxidant activity
TBARS	Thiobarbituric acid-reactive substances
t-BHP	*tert*-Butyl hydroperoxide
TE	Trolox equivalent
TFC	Total flavonoid content
TMA	Total monomeric anthocyanin
TPC	Total phenolic content

Chapter 1

Aims, objectives and work plan of the Thesis

Aims, objectives and work plan of the Thesis

Sweet potato is widely cultivated throughout the world, being a favourite staple of many cultures, it is a favourite ingredient in many ethnic cuisine (Huntrods, 2013). Originated on the American continent at least 5000 years ago, it was brought to Europe in1492. Portuguese explorers of the sixteenth century took it to Africa and Asia (Loebenstein, 2009), however, it seems that sweet potato has a long history cultivation in Oceania (Lebot, 2009). The tuberous root of the plant is most commonly consumed in the Western part of the world, but sweet potato leaves, consumed primarily in the islands of Pacific Ocean and in Asian and African countries, are decidedly rich in nutrients and functional compounds (Johnson & Pace, 2010). In 2013, sweet potato ranked as the 14^{th} top commodity by quantity production, 1.11 x 10^8 ton. According to 2011 proportions, the main destination of total amount produced was food supply (53 %), followed by feed (40 %) and waste (7 %) (FAOSTAT, 2014). There are no official data for sweet potato tops, however the harvested area of 8.2 million hectare can be used for the estimation of vines production. Considering an average yield of 30 ton of fresh vines per hectare from Hartemink et al. (2000), 2.46 x 10^8 tons of fresh vines could have been produced during 2013.

Sweet potato peels had also received attention for food applications as they possessed high levels of phenolics (Zhu, Cai, Yang, Ke, & Corke, 2010) and can reach almost three times more antioxidant activity than the other plant tissues (Cevallos-Casals & Cisneros-Zevallos, 2001). Peels are one of the major wastes generated during the processing of sweet potato and currently it has reduced market value (Maloney, Truong, & Allen, 2012).

Sweet potato leaves were classified as a physiologically functional food which offers protection from various diseases (Islam, 2006) and health promotion by reducing oxidative stress (Johnson & Pace, 2010). Their role is mediated by the ability to promote more favourable antioxidant status, free radical scavenging capacities, and prevent processes involved in disease pathogenesis (Johnson & Pace, 2010). Active compounds were grouped into two types of polyphenols as anthocyanins and phenolic acids. The anthocyanins were acylated cyanidin and peonidin type while the phenolic acids were composed of caffeic acid and five kinds of caffeoylquinic acid derivatives (Islam, 2006). Physiological functions related to these polyphenols as radical scavenging, antimutagenic, anticancer, antidiabetes, antibacterial, anti-inflammatory, cardioprotective and chemopreventive activities have been demonstrated *in vitro* and *in vivo*. (Islam, 2006; Johnson & Pace, 2010)

Research has been done on the recovery of various chemical substances as polyphenolics from food processing wastes as they are highly valuable compounds that may be used as functional food ingredients and as natural antioxidants as they can be readily extracted by simple methods and can then be used for the preparation of functional foods or ingredients (Barberán & Waldron, 2007). Recycling of agro wastes into value added products with potential health benefits could be achieved through extraction, (Peschel et al., 2006). The biological activity of extracts from food wastes needs more research (Barberán & Waldron, 2007).

Functional foods demand a continuous innovative approach to develop convenient and healthy foods according to consumer's expectations (Betoret, Betoret, Vidal, & Fito, 2011). It is a multistage process that requires input from commercial, academic, regulatory and consumer interests. The commencing stages involves the translation of an essential concept often originated from academic research into a prototype (Jones & Jew, 2007). A competitive innovation environment in the food industry requires a collaborative knowledge development and resource sharing activities among stakeholders academics and industry (Khan, Grigor, Winger, & Win, 2013). Teams of academics working with business interests could deliver an optimal product concept that enables forward the innovation cycle (Jones & Jew, 2007). The assessment of efficacy for a prototype can be performed using *in vitro* systems with further proof-of-concept testing animal and human trials (Jones & Jew, 2007). The information obtained with *in vitro* antioxidant assays was considered adequate for the evaluation of food quality although not of food biofunctionality. The assessment of food antioxidant functionality uniquely by this non-compositional approach contains several drawbacks and difficulties due to several factors that can affect the measurement in food matrices (Pompella et al., 2013).

This thesis was a result of several extrinsic and intrinsic motivations shaped by context factors:

- Functional foods, a category of the health and wellbeing market, are becoming a major focus of new product development in the food industry (Khan et al., 2013);

- The growth of health and wellness beverage market is set to outperform the wider soft and hot drinks industry through 2016 (Euromonitor International, 2013);

- Sustainability values are driving a growing interest in the valorisation of agro-wastes valorisation in functional products (Spatafora & Tringali, 2012);

- Although recognized as providing significant protection against the development and progression of many chronic diseases, the role of polyphenols in human health is still a fertile area of research (Pandey & Rizvi, 2009);
- Substantial evidence indicates that antioxidants may be of great benefit in improving the quality of life by preventing or postponing the onset of degenerative diseases (Alam, Bristi, & Rafiquzzaman, 2013);
- Sweet potato is being cultivated traditionally in Algarve that holds a Protected Geographical Indication (PGI) by the European Union (European Union, 2008);
- Actual trend on the revival forgotten healthy food as sweet potato leaves that were consumed in Algarve in the form of salads, soups or *sautée* in 1940's (Nascimento, 1944);
- In the beginning of the thesis, Food Science Lab had just published a study on sweet potato leaves including its polyphenolic profile (Carvalho, Cavaco, Carvalho, & Duque, 2010);
- Researcher aims to transfer research methodology/results to industry, with focus on waste valorisation through functional food product development.

1.1. Research aims

A great variability of techniques is required in order to meet the needs and expectancies in the area of functional foods. Traditional techniques as formulation have a long history in the development of successful functional foods (Khan et al., 2013) and as they constitute simple and cheap processing methods. In the past decades, the uses of sweet potato have diversified considerably, having great potential as a source of local value-added products and ingredients. Some examples include food products like noodles and desserts and some industrial products such as flour or starch. Sweet potato flour can be fermented to make products like soy sauce and alcohol, or if immediately cooked, it can be further processed into wine, vinegar and "nata de coco", a popular dessert in the Philippines and in Japan. Drying, freezing or processing sweet potatoes into a frozen prepared food may benefit producers or processors by extending product shelf life (Huntrods, 2013). Alternative food products, particularly frozen type have become commercially available and are steadily growing. Sweet potato beverages were marketed since 1997 in Japan and dried sweet potato snacks manufactured by Chinese companies are sold as a health food for weight loss (Barnes & Sanders, 2012).

Sweet potato tuber has also been profusely included in the composition of innovative applications such as ice-cream (Gurgel, Farias, Farias, & Moreira, 2011) or non-carbonated drink (Wireko-Manu, Ellis, & Oduro, 2010). Functional uses for tuber includes fries and chips, flakes, and yogurt (Barnes & Sanders, 2012). Leaf dry powder for juice, paste and ice cream was suggested applications besides tea, breads, confectioneries, and as a nutritional supplement (Islam, 2006). As the growth of health and wellness beverage market is set to outperform the wider soft and hot drinks industry through 2016 (Euromonitor International, 2013), an antioxidant beverage could be a promising valorisation of sweet potato wastes as peels and leaves.

1.2. Objectives

The general objectives defined for this Thesis were:

- Propose a framework for the valorisation of agro wastes into a food application through the development of a benchtop prototype with added value;
- Characterize sweet potato wastes as peels and leaves antioxidant activity;
- Define the optimal operational settings for the aqueous extraction of phenolics with antioxidant activity from sweet potato peels;
- Propose an antioxidant beverage benchmark prototype using as components sweet potato peel, sweet potato leaves and honey.

The specific objectives defined were:

- Use of text mining tools in the analysis of articles and patents to picture the state of the art regarding sweet potato food applications with antioxidant activities;

- Analyse the effects of vine part, time and solvent on the antioxidant activity of vines of sweet potato;
- Analyse the correlation between total phenolic content and antioxidant activities of sweet potato peels and leaves extracts;
- Screen the factors that alone and combined influence the extraction of phenolic compounds with antioxidant activity of sweet potato peels;
- Model the effect of solvent: solid ratio, time and temperature on the extraction of phenolic compounds with radical scavenging activity from sweet potato peels;
- Use of design of experiments as factorial design, Plackett-Burman design, response surface methodology and mixture design though research for higher efficiency;
- Include multivariate data analysis tools as principal component analysis (PCA), hierarquical cluster analysis (HCA) and artificial neural network (ANN) to enhance conclusion robustness.
- Develop a functional antioxidant beverage prototype using sweet potato peel aqueous extract, sweet potato leaves aqueous extract and honey solution as components and a mixture design.
- Provide tools to benchmark *in vitro* antioxidant results with published data on commercial and laboratory food products.

1.3. Outline of the Thesis

Thesis general introduction, in **Chapter 2**, aims to reveal the state of the art on antioxidant activity of phenolic compounds from sweet potato and its food applications. The review was based on the analysis of articles and patents published during 2003 to 2014, aided by text mining tools as hierarquical cluster analysis (HCA) and co-occurrence networks.

In **Chapter 3**, extraction parameters of phenolics with *in vitro* antioxidant activity from sweet potato vines were studied through a factorial design. Plant part, time and solvent acidification were the factors studied, while total phenolic content and different antioxidant activities assays were the responses for design evaluation. Statistical analyses were based on analysis of variance (ANOVA), *post-hoc* tests and principal components analysis (PCA).

In **Chapter 4**, the effect of nine variables identified as relevant in previous studies on phenolics extraction were combined in a twelve run Plackett–Burman design to screen for the key factors. Statistical tools as ANOVA and regression analysis evaluated significant main effects and two factors interactions, respectively. Total phenolic content (TPC) and ferric reducing activity power (FRAP) assays were determined to evaluate the different runs.

The aqueous extraction of phenolics with radical scavenging activity from sweet potato peels was modelled and optimized in **Chapter 5**. Solvent: solid ratio, time and temperature were varied according to a Central Composite Design, with total phenolic content and three *in vitro* antioxidant assays as responses. Response surface methodology (RSM) and artificial neural network (ANN) methodologies were used for data analysis.

In **Chapter 6**, a functional beverage benchtop prototype related to oxidative stress protection was developed. The three formula components, sweet potato peel water extract, sweet potato leaves water extract and honey solution were varied though a mixture design. Formulation modelling and optimization was performed by regression analysis and ANN,

Finally, general conclusions were presented in **Chapter 7**.

The work plan of the thesis was presented in Table 1.1.

Table 1.1. Work plan of the Thesis.

Chapter	Objective	Plant part				Type of study	Responses	Statistical analysis	Food application
		Tuber	Peel	Stem	Leaf				
Chapter 2	Definition of the state of the art on food applications with health benefits related to antioxidant activity of phenolic compounds	X	X	X	X	Review	Articles and patents for the period 2003-2014	Text mining HCA Co-occurrence networks	---
Chapter 3	Determination of the effects of vine part, time and solvent on antioxidant activity			X	X	Product and process variables effect	TPC, TFC, TAA, RP, FRAP, DPPH radical scavenging method	Factorial design ANOVA *Post-hoc tests* PCA	Four Extract
Chapter 4	Key factor screening for the extraction of phenolic compounds with antioxidant activity		X			Screening product and process variables	TPC FRAP	Plackett- Burman design ANOVA HCA Regression analysis	Flour Extract
Chapter 5	Optimization of the extraction of phenolics with radical scavenging activity		X			Optimization of process variables	TPC, ABTS and DPPH radical scavenging methods	RSM ANN	Flour Extract
Chapter 6	Functional beverage prototype development		X		X	Formula optimization	TPC FRAP DPPH radical scavenging method	Mixture design Regression analysis ANN	Beverage
Chapter 7	General conclusion	X	X	X	X	---	---	---	---

Abbreviations: ABTS: 2-2'-azino-bis (3 ethylbenzothiazoline-6-sulfonic acid); ANN: Artificial neural network; ANOVA: Analysis of variance; DPPH: 2,2-diphenyl-1-picrylhydrazyl; FRAP: Ferric reducing activity power; HCA: Hierarquical cluster analysis; PCA: Principal Components Analysis; RP: Reducing power; RSM: Response surface methodology; TAA: Total antioxidant activity; TFC: Total flavonoid content; TPC: Total phenolic content.

1.4. References

Alam, M. N., Bristi, N. J. and Rafiquzzaman, M. (2013). Review on *in vivo* and *in vitro* methods evaluation of antioxidant activity. *Saudi Pharm. J.* **21**: 143-152.

Barberán, F. and Waldron, K. (2007). High-value co-products from plant foods: nutraceuticals, micronutrients and functional ingredients. *Handbook of Waste Management And Co-Product Recovery in Food Processing, Volume 1*, 448-469.

Barnes, S. L. and Sanders, S. A. (2012). Advances in Functional Use of Sweet Potato,[*Ipomoea batatas* (L.) Lam]. *Recent Pat Food Nutr Agric.* **4**: 148-154.

Carvalho, I. S., Cavaco, T., Carvalho, L. M., & Duque, P. (2010). Effect of photoperiod on flavonoid pathway activity in sweet potato (*Ipomoea batatas* (L.) Lam.) leaves. *Food Chemistry, 118*, 384-390.

Cevallos-Casals, B. & Cisneros-Zevallos, L. (2001). Bioactive and functional properties of purple sweetpotato (Ipomoea batatas (L.) Lam). In: I International Conference on Sweetpotato. Food and Health for the Future 583. pp. 195-203.

Euromonitor International. (2013). Functionality, Naturalness and Stevia Key to Developing Beverages to Fit Today's trends. Global report, Jan 2013.

European Union. (2008) Publication of an to Article 6(2) of Council Regulation (EC) No. 510/2006 Batata Doce de Aljezur EC No: PT-PGI-0005-0517-03.01.2005 C324 C.F.R.

FAOSTAT. (2014). http://faostat.fao.org/ (visited 13 November 2014).

Gurgel, C., Farias, S., Farias, L. and Moreira, R. (2011). Sensory analysis of sweet potato ice cream. *Rev Bras Prod Agroind.* **13**: 21-26.

Hartemink, A. E., Poloma, S., Maino, M., Powell, K., Egenae, J. and O'Sullivan, J. (2000). Yield decline of sweet potato in the humid lowlands of Papua New Guinea. *Agric Ecosyst Environ.* **79**: 259-269.

Huntrods, D. (2013). Sweet potato profile. *Agricultural Marketing Resource Center. http://www. agmrc. org/commodities__products/vegetables/sweet_potato_profile/ (accessed November 7, 2014).*

Islam, S. (2006). Sweetpotato (*Ipomoea batatas* L.) leaf: its potential effect on human health and nutrition. *J Food Sci.* **71**: R13-R121.

Johnson, M. & Pace, R. D. (2010). Sweet potato leaves: properties and synergistic interactions that promote health and prevent disease. *Nutr Rev.* **68**: 604-615.

Jones, P. J. & Jew, S. (2007). Functional food development: concept to reality. *Trends Food Sci Tech.* **18**: 387-390.

Khan, R. S., Grigor, J., Winger, R. & Win, A. (2013). Functional food product development–Opportunities and challenges for food manufacturers. *Trend Food Sci Tech.* **30**: 27-37.

Lebot, V. (2009). Tropical Root and Tuber Crops: Cassava, Sweet Potato, Yams and Aroids CAB International: Oxford, UK, Crop Production Science in Horticulture Series, 17, p. 94.

Loebenstein, G. (2009). Origin, distribution and economic importance *In: Loebenstein G, Thottappilly G, Ed., The Sweetpotato* (pp. 9-12): Springer.

Maloney, K. P., Truong, V. D. & Allen, J. C. (2012). Chemical optimization of protein extraction from sweet potato (*Ipomoea batatas*) peel. *J Food Sci.* **77**: E307-E312.

Nascimento, B. S. (1944). *Cultura de Batata Doce na Província do Algarve.* (Relatório final de Curso de Engenheiro Agrónomo), Instituto Superior de Agronomia da Universidade Técnica de Lisboa.

Pandey, K. B. & Rizvi, S. I. (2009). Plant polyphenols as dietary antioxidants in human health and disease. *Oxid Med Cell Longev.* **2**: 270-278.

Peschel, W., Sánchez-Rabaneda, F., Diekmann, W., Plescher, A., Gartzía, I., Jiménez, D., Lamuela-Raventos, R., Buxaderas, S. and Codina, C. (2006). An industrial approach in the search of natural antioxidants from vegetable and fruit wastes. *Food Chem.* **97**: 137-150.

Spatafora, C. & Tringali, C. (2012). Valorization of vegetable waste: identification of bioactive compounds and their chemo-enzymatic optimization. *Open Agric J.* **6**: 9-16.

Wireko-Manu, F., Ellis, W. & Oduro, I. (2010). Production of a non-alcoholic beverage from sweet potato (*Ipomoea batatas* L.). *Afr J Food Sci.* **4**: 180-183.

Zhu, F., Cai, Y.-Z., Yang, X., Ke, J. & Corke, H. (2010). Anthocyanins, hydroxycinnamic acid derivatives, and antioxidant activity in roots of different Chinese purple-fleshed sweetpotato genotypes. *J Agric Food Chem.* **58**: 7588-7596.

Chapter 2

Food applications and health benefits related to antioxidant activity of phenolic compounds from sweet potato peels and leaves: review of literature and patents

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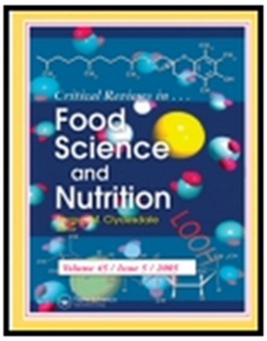

Submitted

Food applications and health benefits related to antioxidant activity of phenolic compounds from sweet potato peels and leaves: review of literature and patents

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Abstract

To define research lines for the valorisation of sweet potato (SP) wastes as peels and leaves, a literature and patent review on food applications with health benefits was performed. The documents on antioxidant activity of phenolic compounds from the different plant parts – tuber, leaf, stem, vine and also cell line and processing waste – were selected for the period 2003-2014 from Web of Science™ and ESPACENET databases. SP articles presented a demarked trend towards preclinical studies and new foods that did not include SPP or SPL. DPPH method was the *in vitro* antioxidant activity assay most used and hydroxycinnamic acids and anthocyanins were the phenolic compounds more analysed. Enhancement of antioxidant defence and anticancer were health benefits verified by clinical trials for SPL while studies on peel were absent. Among the food applications studied in articles, flour/powder was the most studied for tuber, leaf and peel. Beverage and extract were the top patented foods for tuber and leaf, respectively. Text mining applied to abstracts revealed main themes. The development of new food products as beverages using design of experiment tools will reduce the knowledge gaps on SP leaves and specially peels and their valorisation into food application with health benefits.

Keywords: Sweet potato peels; sweet potato leaves; antioxidant activity; phenolic compounds; food applications, health benefits, text mining.

2.1. Introduction

Sweet potato (*Ipomoea batatas* L.) ranked as 14th top commodity by quantity-wise production. In 2013 (FAOSTAT, 2014),being a favourite staple of many cultures it is an ingredient in many ethnic cuisine (Huntrods, 2013). Although sweet potato (SP) tuber is most commonly consumed in the Western part of the world, their leaves consumed primarily in the islands of the Pacific Ocean and in Asian and African countries, are decidedly rich in nutrients and functional compounds (Johnson & Pace, 2010). Peels are one of the major wastes generated during processing of sweet potato with currently little market value (Maloney, Truong, & Allen, 2012). The peeling step required in many SP tuber processing methods can lead to losses up to 29 % and 17 % for abrasive and manual peeling respectively (Oladejo, 2011). Thus, SP peels and leaves are residues that should be included in the drive for recycling agro wastes as peels into value added products (Peschel et al., 2006). SP peels possessed high levels of phenolics (Zhu, Cai, Yang, Ke, & Corke, 2010) that can reach almost three times more antioxidant activity than the other plant tissues (Cevallos-Casals & Cisneros-Zevallos, 2001). Similarly, polyphenolic compounds from SP leaves were biologically active and presented physiological functions which may be helpful for maintaining and promoting human health (Islam, 2006). Thus, the development of food application with health benefits related to the antioxidant activity of phenolic compounds from SP peels and leaves are valuable research line both of academic and industrial interests. There have been reviews on sweet potato tuber and leaf on during the period of study. In 2007, (Bovell-Benjamin, 2007) highlighted sweet potato utilization, and its potential as value-added products in human food systems. It pointed out that a multidisciplinary, integrated research and developmental activities, from production to processing technologies, were need to explore SP potential value-added products. Panda & Sonkamble (2012) reviewed the phytochemical constituents and pharmacological activities of SP where the peel was indicated as containing high levels of polyphenols such as anthocyanins and phenolic acids. Health benefits as antioxidant, antidiabetic, wound healing, anti-ulcer, antibacterial and anti-mutagenic were listed. Finally, Mohanraj & Sivasankar (2014) covered the health benefits, phytochemical composition and medicinal properties of SP, among other topics, to demonstrate their potential as a medicinal food. Regarding leaf, Islam (2006) in his review highlighted the presence of higher amounts of polyphenolics compared with the major commercial vegetables. Significant medicinal value was already attributed to biologically active anthocyanins and phenolic acids as they showed physiological functions which may be helpful for maintaining and promoting human health. The benefits of radical scavenging activity, antimutagenic activity, anticancer, antidiabetes, and antibacterial activity were also listed (Islam, 2006). In 2010, Johnson & Pace summarised the bioactive compounds in SP leaves that played a vital role in health promotion by improving immune function, reducing oxidative stress and free radical damage, reducing cardiovascular disease risk and suppressing cancer cell growth. The ability of antioxidants from SP leaves to reduce the risks associated with disease is mediated by their ability to promote more favourable antioxidant status, free radical scavenging capacities, and thwart processes involved in disease pathogenesis (Johnson & Pace, 2010). It clearly concluded that promoting the consumption of sweet potato leaves warrants further and more intensive research investigation related to reduce the prevalence of chronic diseases (Johnson & Pace, 2010). Several foods were enumerated as a source for beneficial polyphenolic compounds. Frozen products and beverages are available in the market in some countries for a long time. Considerable progress has been made in diversifying usage of the SP for alternative foods as fries, chips, flakes, yogurt, as well as juices or weight loss snacks (Barnes & Sanders, 2012). Innovative applications such as ice-cream (Gurgel, Farias, Farias, & Moreira, 2011) and non-carbonated drink (Wireko-Manu, Ellis, & Oduro, 2010) were studied in an academic context. Tea, noodles, breads, confectioneries and nutritional supplement were food applications previously mentioned for SP leaves (Islam, 2006). In 2006, products as dry powder for juice, paste, ice cream were in development by cooperation between universities and food companies. The development of SP alternative foods was predicted to continue its upward climb trend as consumers are more conscious about their health (Barnes & Sanders, 2012). The development of functional foods is a multistage process where the commencing stages involve the translation of an essential concept often originated from academic research into a prototype (Jones & Jew, 2007). Although, there are available some reviews on the phytochemical composition and health benefits of sweet potato tuber and leaf, the health benefits specifically related to the antioxidant activity of phenolic compounds in the different food applications were not focused. In addition, a framework for the valorisation of agro wastes through the development of functional foods requires the identification of knowledge gaps to favour early product prototypes. To gain insight on trend and state of the art on food applications with health benefits from antioxidant activity of phenolic compounds from sweet potato, a literature and patent review for the period 2003 -2013 was performed and analysed by plant part. Text mining tools as co-word clustering is a powerful method for literature-review and knowledge discovery by exploring the co-occurrence and co-absence of key words that appear in the titles or abstracts of texts (Stegmann & Grohmann, 2003). Additionally, network analysis shows the relationship among words as a visual network and therefore assists the analyser in intuitively comprehending the overall structure of a document database (Yoon & Park, 2004). The objective of this review was to analyse the type of studies, antioxidant assays, phenolic compounds, statistical analysis, health benefits and food applications for the different SP plant parts in the period 2003 -2014. Together with the analysis of the state of the art pictured by text mining tools, relevant research lines for the valorisation of SP peels and leaves into food applications with health benefits will be identified.

2.2. Materials and Methods

A qualitative approach was taken to analyse the selected documents by summarizing information in tables, graphics and applying text-mining tools.

2.2.1. Databases

A search on ISI Web of Knowledge (http://wokinfo.com/) and ESPACENET (http://worldwide.espacenet.com/#) electronic databases for the period 2003-2014 was performed to gather relevant documents on topic of this study. The databases Web of ScienceTM Core Collection, Current Contents Connect®, MEDLINE® and SciELO Citation Index were selected for articles search. For patents, previous tests presented only one and zero results in the WIPO and European collection, respectively. Therefore, the worldwide collection of published application from 90 countries was chosen as database.

2.2.2. Selection of articles and patents

For the selection of articles, the first criteria used in the Web of Knowledge database was documents with "sweet potato" or "*Ipomoea batatas*" in the title for the period 2003-2014. The following filters were included: "science and technology" for the research domain, "food science and technology" for the research area and "articles" (review were excluded) for the document type. The first collection of documents were selected by the presence of the terms "phenol*", "antho*", "flavon*", "antiox*" and/or "scaveng*" in document topic. This collection was narrowed by two reviews to the theme antioxidant activity and/or phenolic composition of sweet potato plant. Finally, when essential information was absent from the article description and could not be acquired by contact with the corresponding authors, it was not included in the review. Regarding the selection of patents, a search was performed in the ESPACENET database for documents with "sweet potato" or "*Ipomea batatas*" in the title or in abstract with publication date in the range 01/01/2003 to 31/12/2014 (20030101-20141231). Patents published in the field that presented the terms "phenol*", "antho*", "flavon*", "antiox*" and/or "scaveng*" in their abstract were selected. A patent was included in the review when the invention was related to a food application with antioxidant activity and/or phenolic composition.

2.2.3. Documents analysis

2.2.3.1. Traditional approach

The body of information gathered from the selected documents were synthetized into tables (Supplementary data). Articles were classified by SP plant part (tuber, leaf, stem, vine, peel, cell line and waste) on variety (purple and not purple), type of study, antioxidant activity assays, phenolic compounds, statistical analysis, health benefits and food applications. For patents, documents categorization by the different SP plant parts was made for variety and food applications.

2.2.3.2. Text mining

The free text mining software tool KH coder (http://sourceforge.net/projects/khc/) was used to perform an automatic text mining of articles and patents abstracts. Text pre-processing was done by breaking sentences into component parts of speech (parsing), removal of common words with little significance (stop words) and aggregation of similar words (stemming or lemming). Only nouns and adjectives were used for analysis and both term frequency and document frequency were adjusted so 27 to 31 words were used for graphical representation. A Hierarquical cluster analysis (HCA) using the Ward method and Jaccard distance with automatic detection of the number of clusters was applied to nouns and adjectives to form groups of words that summarize documents major themes. Co-occurrence networks were built to pattern high-frequency words occurring together and identify content communities. Nodes size was proportional to respective word frequency and links (edges) thickness was determined by Jaccard coefficient. Fruchterman-Reingold algorithm was used to determine the position of the nodes in the network layout while community detection was performed using the function "edge.betweenness.community" from the "igraph" package of R.

2.3. Results and discussion

Food applications and health benefits related to antioxidant activity of phenolic compounds from SP were analyzed by plant part. The plant parts considered were tuber, leaf, stem, vine and peel. Due to results from documents analysis, cell line and processing waste were also considered and named as plant part. The following features were analyzed: types of studies, antioxidant activity assays, phenolic compounds, statistical analysis, health benefits and food applications. Text mining tools applied to articles and patents abstracts intended to picture the main results for the period 2003-2014 on antioxidant activity of phenolic compounds from SP.

2.3.1. Selection of documents

A search on article titles with "sweet potato" or "*Ipomoea batatas*" in the Web of Science™ Core Collection for the period 2003-2014 in the science and technology domain and food science and technology research area delivered 443 results. When refined to the topic terms "phenol*", "antho*", "flavon*", "antiox*" or "scaveng*" a total of 432 different articles was obtained. Thus, most of the studies for the food science and technology research area addressed SP phenolic compounds or antioxidant activity. The abstract of these articles was verified for relevance, when one of the refining topic terms was present in the article abstract, it was included in the review. The final selection of articles on phenolic compounds with antioxidant activity from SP totalized 122 articles (Table 2.1.).

Table 2.1. Indication of the 122 selected articles for the period 2003-2014.

SP articles		
a1 Ahmed et al. (2009)	a41 Jeng et al. (2012)	a83 Ray et al. (2012)
a2 Ahmed et al. (2010a)	a42 Jiao et al. (2012)	a84 Redovnikovic et al.
a3 Ahmed et al. (2010b)	a43 Ju et al. (2011)	(2012)
a4 Ahmed et al. (2011a)	a44 Jung, J.-K. et al. (2011)	a85 Roy et al. (2012)
a5 Ahmed et al. (2010)	a45 Jyothi et al. (2005)	a86 Rumbaoa et al. (2009)
a6 Ahmed et al. (2011b)	a46 Kano et al. (2005)	a87 Saigusa et al (2007)
a7 Ahmed et al. (2010)	a47 Karna et al. (2011)	a88 Saigusa et al. (2005)
a8 Anastácio and Carvalho	a48 Kawano et al. (2010)	a89 Sasaki et al. (2013)..
(2013a)	a49 Kim et al. (2012)	a90 Sasaki and Ohba (2004)
a9 Anastácio and Carvalho	a50 Koncic et al. (2013)	
(2013b)		a91 San et al. (2009)
a10 Boo et al. (2005)	a51 Konczak et al. (2004)	a92 Shan et al. (2013)
	a52 Konczak-Islam et al.	a93 Shao and Huang (2008)
a11 Carvalho et al. (2010)	(2003a)	a94 Song et al. (2013)
a12 Cevallos-Casals and	a53 Konczak-Islam et al.	a95 Song et al. (2011)
Cisneros-Zevallos (2004)	(2003b)	a96 Suda et al. (2008)
a13 Chan et al. (2012)	a54 Krishnan et al. (2010)	a97 Taira et al. (2013)
a14 Chang et al. (2007)	a55 Kurata et al.(2007)	a98 Takenaka et al. (2006)
a15 Chang et al. (2010)	a56 Li and Zhang (2013)	a99 Teow et al. (2007)
a16 Chen et al. (2008)	a57 Li, F. et al. (2009)	a100 Tian et al. (2005)
a17 Cho et al. (2003)	a58 Li, C. et al. (2013)	
a18 Choi et al. (2008)	a59 Li, J.Y. et al. (2012)	a101 Tokusoglu and Yildirim
a19 Chon et al. (2005)	a60 Liao et al. (2011)	(2012)
a20 Cui et al. (2011		a102 Truong et al. (2012)
	a61 Lien et al. (2012)	a103 Wang et al. (2011)
a21 Dincer et al.(2011)	a62 Lien et al. (2010)	a104 Wang et al. (2012)
a22 Ding et al. (2010)	a63 Lim et al. (2013)	a105 Wu et al. (2012)
a23 Dini et al. (2006)	a64 Lin et al. (2006)	a106 Xu et al. (2010)
a24 Donado-Pestana et al.	a65 Lu et al. (2010)	a107 Ye et al. (2010)
(2012)	a66 Min et al. (2006)	a108 Ye et al. (2004)
a25 Fan et al. (2008a)	a67 Montilla et al. (2010)	a109 Zhang et al. (2009)
a26 Fan et al. (2008b)	a68 Nagai et al. (2011)	a110 Zhang et al. (2013)
a27 Gan et al (2012)	a69 Niwa et al. (2011)	
a28 Gundala et al. (2013)	a70 Oki et al.(2003)	a111 Zhao, J.G. et al. (2013)
a29 Han et al. (2011)		a112 Zhao et al. (2007)
a30 Harada et al. (2004)	a71 Padda and Picha (2007)	a113 Chun et al. (2014)
	a72 Panda et al. (2009a)	a114 Kim, H. Y. and Mo
a31 Harrison et al. (2003)	a73 Panda et al. (2009b)	(2014)
a32 Huang et al. (2004)	a74 Panda and Ray (2007)	a115 Li, J. et al. (2014)
a33 Huang et al. (2010)	a75 Panda et al. (2013)	a116 Ojeda et al. (2014)
a34 Huang et al. (2013)	a76 Park et al. (2010)	a117 Sasaki et al. (2014)
a35 Huang et al. (2006)	a77 Peng et al. (2013)	a118 Soison et al. (2014)
a36 Hwang et al. (2011a)	a78 Philpott et al. (2009)	a119 Sun et al. (2014a)
a37 Hwang et al. (2011b)	a79 Pochapski et al. (2011)	a120 Sun et al. (2014b)
a38 Hwang et al. (2011c)	a80 Qiu et al. (2009)	
a39 Ishiguro et al. (2007)		a121 Sun et al. (2014c)
a40 Islam et al. (2003)	a81 Rabah et al. (2005)	a122 Zhao, J.G. et al. (2014)
	a82 Rabah et al. (2004)	

Search on ESPACENET database delivered a total of 4895 patents with "sweet potato" in the title or abstract for the period 2003-2014. When the search was narrowed by including the presence of the terms "phenol*", "antho*", "flavon*", "antiox*" or "scaveng*" in the title or abstract, it delivered 9, 137, 19, 76 and 11 results, respectively. Combined, a total of 217 different patents were obtained. After verification if invention was related to a food application by analyzing abstract content, patents were reduced to 170 documents (Table 2.2.). Combined, articles and patents formed a collection of 292 documents with the following main features: a) 58 % of the documents were patents; b) 71 % of the documents were published in the period 2010-2013; c) 68 % of the documents were on the purple variety and d) tuber was the subject of 79 % of the documents, in opposition to the other forms (leaf, stem, vine, peel, cell line, or processing waste) (Figure 2.1). The time series map of articles date of publication showed a one step-profile with an average of 7 articles per year for the period 2003 to 2009 (Figure 2.2). A growth to an average of 16 articles per year was observed to the period 2010 to1013 followed by a decrease to 10 articles published in 2014. Patents presented a different time profile in comparison to articles. Until 2010, an average of 6 patents were published per year. The number of patents on sweet potato has increased since 2011, with a higher growth (70 %) from 2013 to 2014.

2.3.1.2. SP Articles

The 122 selected articles for this review were published in 58 different journals, where 4 - *Journal of Agricultural and Food Chemistry*, *Food Chemistry*, *International Journal of Food Science and Technology* and *Food Science and Technology Research* - reached 33 % in number. Articles were classified by plant part and variety as presented in Supplementary Table S2.1. As depicted in Figure 2.3, tuber presented the highest number of papers both on the purple and non-purple variety. SP leaf was the second most studied plant part with more articles than stem, vine, peel, cell line and processing waste together. Although SP leaves are considered a waste in many parts of the world, they are consumed in Asia as a leafy vegetable and therefore gaining high research attention. All the SP plant parts with exception of the tuber presented more articles on the non-purple varieties. The purple variety was mostly studied by research centers from China that presented the highest number of papers (18), followed by Korea (14).

Table 2.2. Indication of the 170 selected patents for the period 2003-2014.

SP patents		
p1 Baek et al. (2004)	p46 Li, J. et al.(2012)	p91 Xiangdong (2012)
p2 Chen and Wang (2013a)	p47 Li and Li (2013)	p92 Xianxiang (2008)
p3 Chen and Wang (2013b)	p48 Li, Z. et al. 2013 (2013)	p93 Xiaoling et al. (2009)
p4 Chengyu (2009)	p49 Lichao et al. (2009).	p94 Xiaosong (2010)
p5 Cho et al. (2013)	p50 Liming et al. (2011).	p95 Xiaoyan (2012)
p6 Chuanbin and Xianxun (2011)		p96 Xiguang et al. (2012)
	p51 Lin and Jiping (2009)	p97 Xingcang (2011)
p7 Chun et al. (2007)	p52 Liu, H. (2013)	p98 Xuesong (2012)
p8 Dechao et al. (2010)	p53 Liu, L. (2013)	p99 Yan et al. (2012)
p9 Defa (2009)	p54 Lixin et al. (2011)	p100 Yanxiang (2011)
p10 Dehua et al. (2012)	p55 Ma (2013)	
	p56 Mingna et al. (2011)	p101 Yasumoto et al. (2006)
p11 Fanzhong (2011)	p57 Minyao et al.(2011)	p102 Ye and Li (2004)
p12 Ge et al. (2013)	p58 Mu et al. (2013a)	p103 Yin et al. (2003)
p13 Gong (2013)	p59 Mu et al. (2013b)	p104 Yongbin et al. (2012b)
p14 Gongjian et al. (2008)	p60 Na et al. (2013)	p105 Yongbin et al. (2012a)
p15 Guangyou (2008)		p106 Yuan et al. (2013)
p16 Hidaka and Obayashi (2009)	p61 Ning et al. (2010)	p107 Yuanzheng et al.(2011)
	p62 Not_acessible (2007)	p108 Yubao (2010)
p17 Hiramoto et al. (2005)	p63 Not_acessible (2011)	p109 Yubo (2010)
p18 Hong and (2011)	p64 Oba and Saegusa (2003)	p110 Yunli (2008)
p19 Hong and Seonryu (2013)	p65 Oba and Sasaki (2006)	
p20 Hongmei et al. (2008)	p66 Oba and Noujiyo (2008)	p111 Zhang, L. W. (2007)
	p67 Oiwa and Oiwa (2007)	p112 Zhang, Z. (2013)
p21 Hui et al. (2012)	p68 Peng (2013)	p113 Zhao, G. (2013)
p22 In et al. (2004)	p69 Qiang (2012)	p114 Zhao, X. et al. (2013)
p23 Iwata (2006)	p70 Qiuyun (2012)	p115 Zhenchang et al (2012)
p24 Jeong (2012)		p116 Zheng et al. (2009)
p25 Ji (2013)	p71 Quanan et al. (2011)	p117 Zhenxin et al. (2012a)
p26 Ji et al. (2010)	p72 Quanan and Yubo (2012)	p118 Zhenxin et al. (2012b)
p27 Jian et al. (2011)	p73 Ryu and Lee (2013)	p119 Zhichao (2011)
p28 Jianyi (2012)	p74 Saeki et al. (2006)	p120 Chen, R. (2014)
p29 Jinglue (2011)	p75 Seo et al. (2010)	
p30 Jingyu et al. (2012)	p76 Shi (2013)	p121 Chen, X. et al. (2014)
	p77 Shuhua et al. (2012)	p122 Chen, Y. et al. (2014)
p31 Jingyu et al. (2012)	p78 Shuyong (2011)	p123 Cheng et al. (2014)
p32 Jinsong et al. (2012)	p79 Sugawara et al. (2005)	p124 Choi et al. (2014)
p33 Jinsong and Bing (2009)	p80 Sun et al. (2013)	p125 Dong (2014)
p34 Jiping and Lin (2009)		p126 Guan (2014)
p35 Jo et al. (2013)	p81 Suzuki et al. (2006)	p127 Guo, C. (2014)
p26 Juanying (2012)	p82 Takagaki and Tsubata (2009)	p128 Guo, T. (2014)
p37 Jung et al. (2007)		p129 Han et al. (2014)
p38 Jung et al. (2011b)	p83 Tang et al. (2005)	p130 Hou et al. (2014)
p39 Jung et al. (2011a)	p84 Tianyin (2011)	
p40 Kamata (2006)	p85 Tsukada et al. (2004)	p131 Jiang (2014)
p41 Kim et al. (2010)	p86 Wang, F. et al. (2013)	p132 Jing et al. (2014)
p42 Kwon et al. (2012)	p87 Wang, S. et al. (2013)	p133 Kim et al. (2014)
p43 Lee (2013)	p88 Wang, Z. W. (2006)	p134 Kwon (2014)
p44 Li, Y. (2013)	p89 Wong et al. (2013)	p135 Lee et al. (2014)
p45 Li, C. et al. (2009)	p90 Wu (2013)	p136 Li, B. (2014a)

(*cont.*)

	SP patents	
p137 Li, B. (2014b)	p148 Rao et al. (2014)	p159 Xu, G. and Xu (2014a)
p138 Li, C et al. (2014)	p149 Shao (2014)	p160 Xu, G and Xu (2014b)
p139 Liu et al. (2014)	p150 Sun, F. (2014)	
p140 Lu, J. (2014a)		p161 Yang, R. (2014a)
	p151 Tao et al. (2014)	p162 Yang, R. (2014b)
p141 Lu, J. (2014b)	p152 Tong et al. (2014)	p163 Yao et al. (2014)
p142 Lu, K. (2014)	p153 Wang, K. (2014)	p164 Zeng et al. (2014)
p143 Luo et al. (2014)	p154Wang, Q. (2014a)	p165 Zhang, J. et al. (2014)
p144 Mou et al. (2014)	p155 Wang, Q. (2014b)	p166 Zhang, X. et al. (2014)
p145 Pi and Li (2014)	p156 Wang, Y. (2014)	p167 Zhao et al (2014)
p146 Qin (2014)	p157 Wei (2014)	p168 Zhou (2014)
p147 Qiong (2014)	p158 Xu, D. (2014)	p169 Zhou et al. (2014)
		p170 Zou, G. (2014)

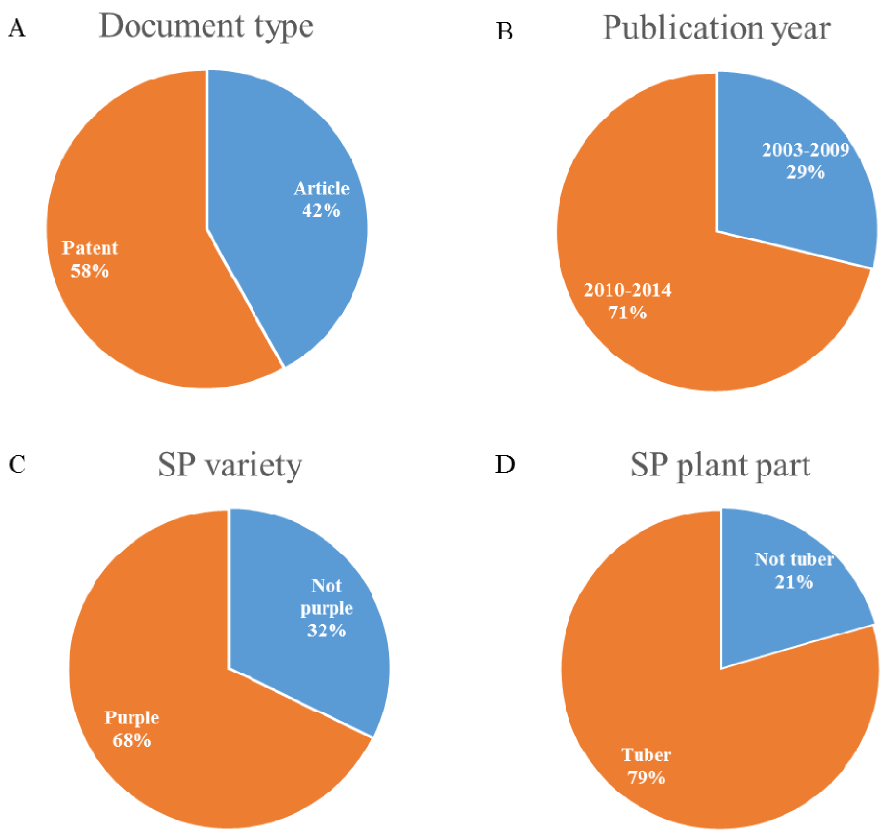

Figure 2.1. Main features of the SP documents used in the review for A) document type, B) publication year, C) variety and d) plant part.

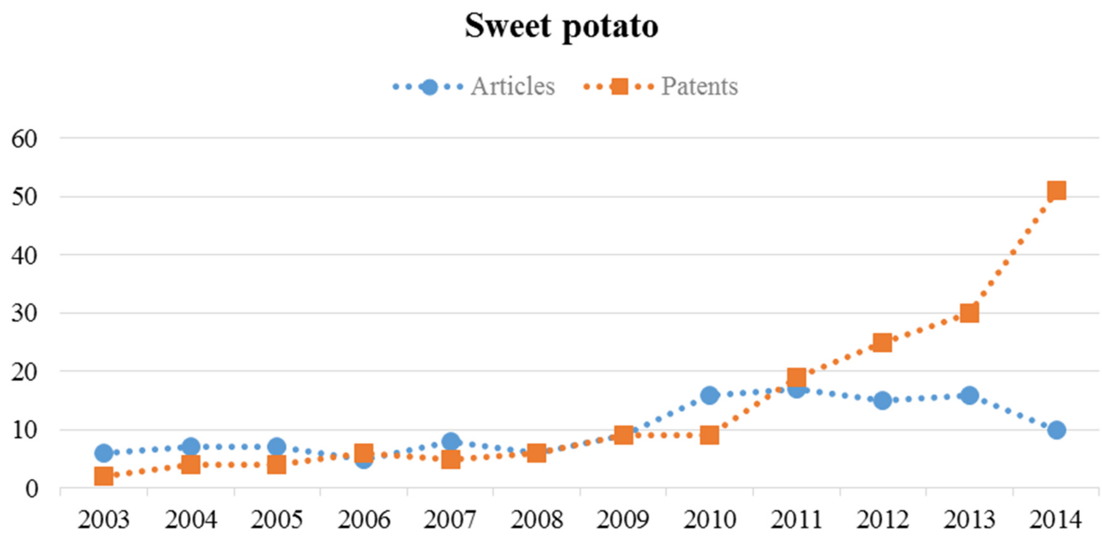

Figure 2.2. Time series map of SP articles and patents for the period 2003 to 2014.

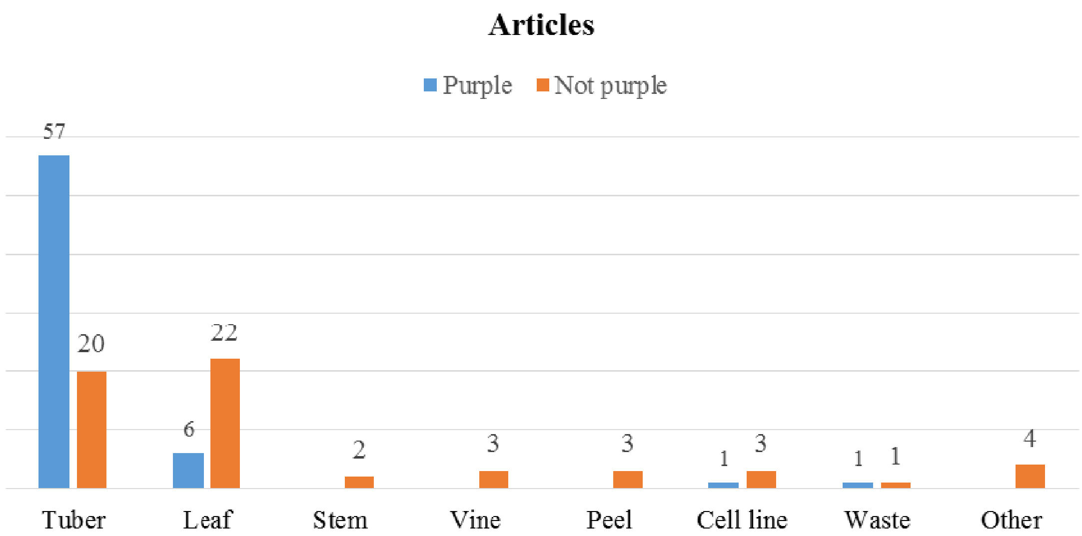

Figure 2.3. Distribution of SP articles by variety and plant parts for the period 2003 to 2014.

For other SP varieties than purple, research centers from Asia counties were still dominant but authors' affiliations were from other continents (Europe, America and Oceania) in ~ 35 % of the papers Articles on the purple and non-purple varieties presented a similar trend regarding the publication date profile over the period 2003-2014 (Figure 2.4A). The number of articles on the purple variety presented an average of 3 per year in the period 2003-2005 that increased to an average of 9 per year in the period 2010-2013. In 2014, a decrease in the number of articles on the purple variety decreased to 6. For non-purple SP, the average in the period 2003-2005 was of 4 articles per year that increased to an average of 7 per year in the period 2010-2013. As observed for articles on the purple variety, in 2014 the number of articles published for the non-purple SP decreased. Research on the other SP plant parts SP less or absent for the period 2003-2014.

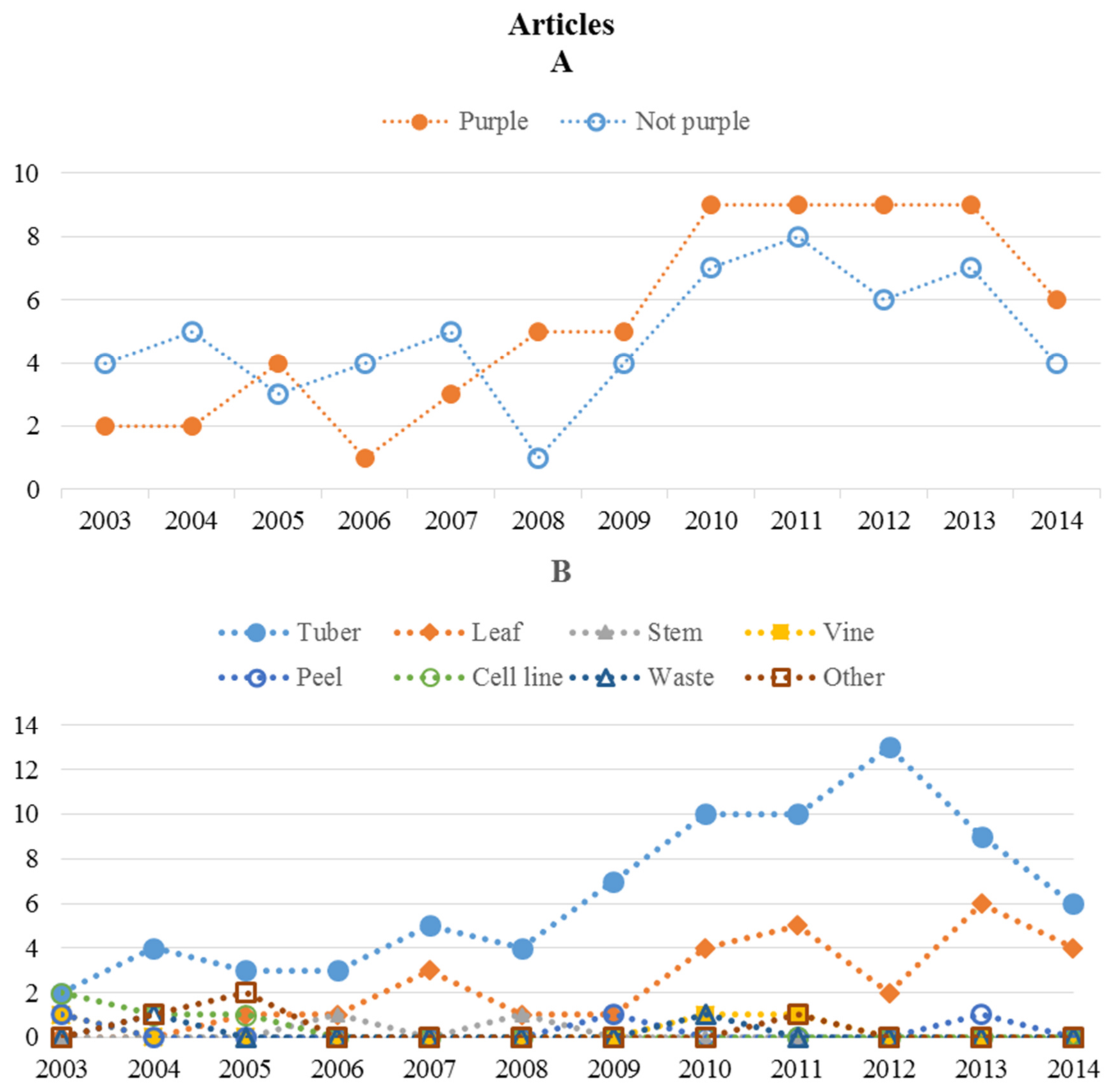

Figure 2.4. Time series map of SP articles by A) variety and B) plant parts for the period 2003 to 2014.

The time series map by plant part (Figure 2.4B) showed the number of articles on SP tuber and leaf presented similar trends and tuber presented always more publication per year than the other plant parts considered. Thus, antioxidant activity of phenolic compounds by SP plant part had the following order regarding the number or articles: tuber>>leaf>>peel. Studies on SP peel received much less attention than tuber of leaf. To respond to the growing interest of agro- and food industries in value added recycling (Peschel et al., 2006), there is a need for research on antioxidant activity of phenolic compounds from SP leaves but specially peels. This could contribute to overcome this knowledge gap and promote to the valorization of both peels and leaves into food applications with health benefits.

2.3.1.2. SP Patents

The 170 selected patents were predominantly from China (72 %) with Qingdao Agricultural University as the top applicant (11 patents). The classification of the selected patents by plant part and variety was presented in Supplementary Table S2.2. The number of patents on antioxidant activity of phenolic compounds from SP was concentrated on the purple variety (79 %) and on the tuber plant part (91 %) (Figure 2.5). Inventions with SP leaf or peel occurred in a very small scale. As occurred in articles, leaf (alone or combined with stem) was the second plant part most mentioned in patents.

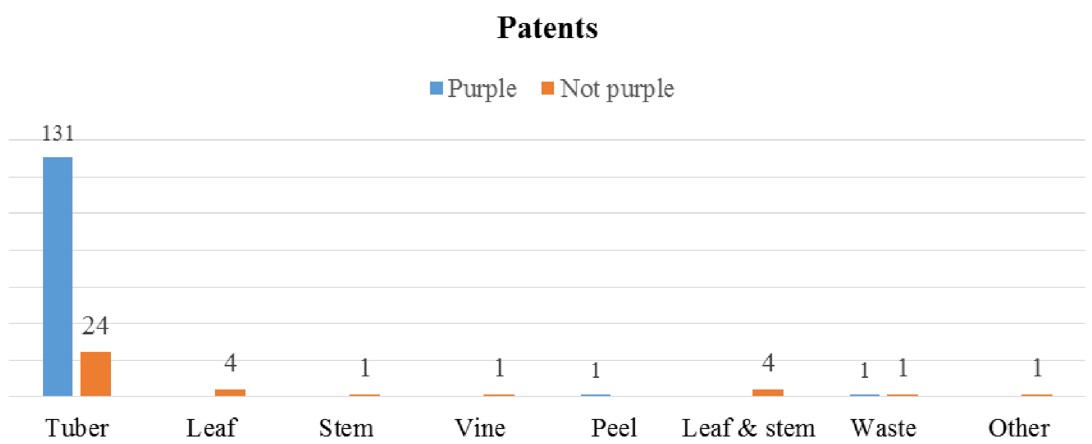

Figure 2.5. Distribution of SP patents by variety and plant parts for the period 2003-2014.

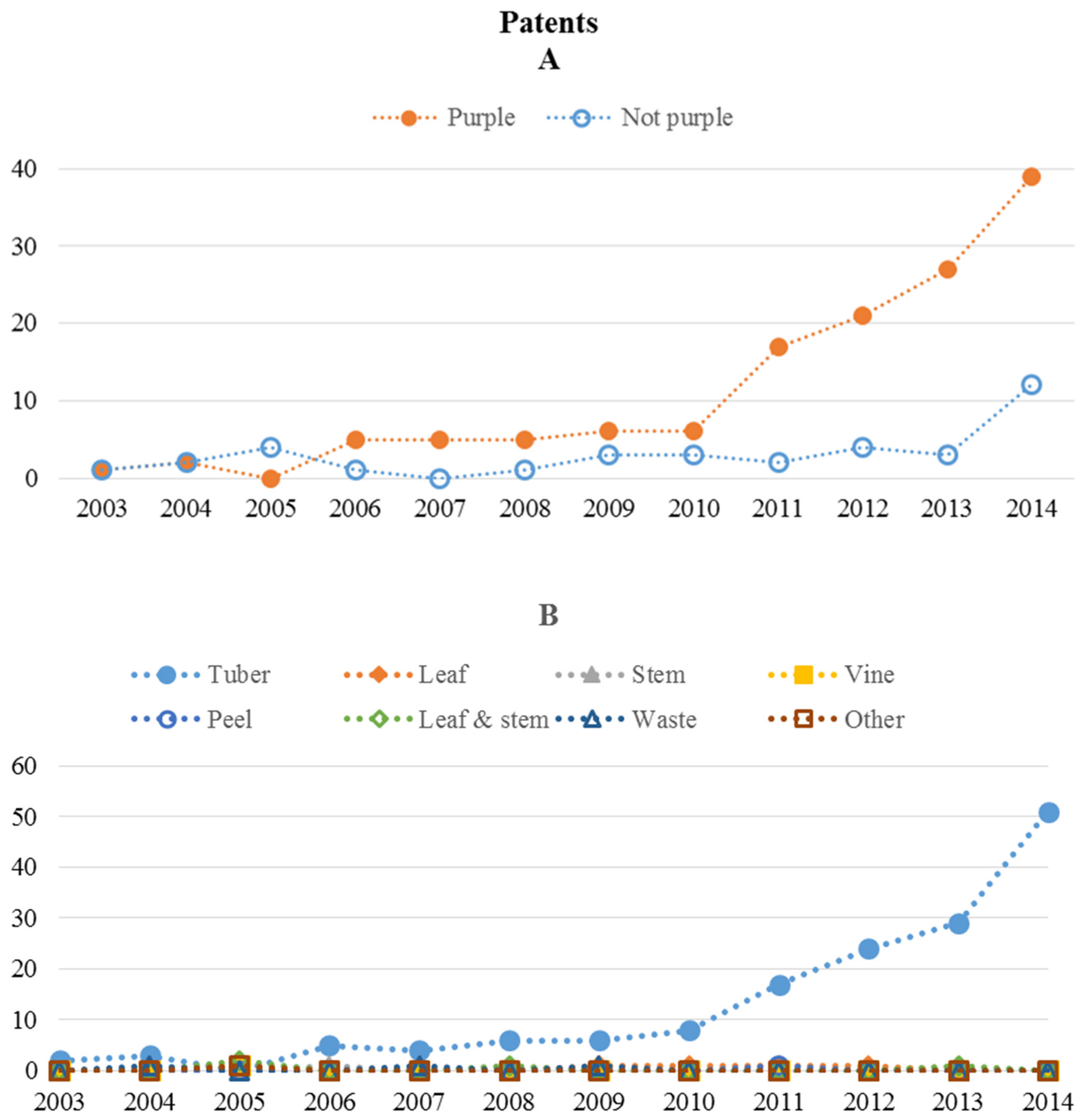

Figure 2.6. Time series map of SP patents by A) variety and b) plant parts for the period 2003 to 2014.

In addition, inventions including peels were scare for the period 2003-2014. The time series map of patent publication date showed that inventions including the purple variety supplanted the non-purple varieties in 2006, with a large growth from 2010 to 2014 (Figure 2.6A). Tuber presented a similar trend than the purple variety due to their prevalence on SP patents (Figure 2.6B). Therefore, research on SP peels and leaves for food applications have potential to be considered an invention and protected by patent registration. As the strategy of combining different SP plant parts presented a low intensity in the period of time studied, it could be undertaken in future developments of value added products.

2.3.2. Types of study of SP articles

Articles on antioxidant activity of phenolic compound from sweet potato were classified into thirteen different types of study (Supplementary Table S2.3). More than one type of study or plant part could be attributed to a paper in accordance to their objectives. For SP tuber, the top four types of study were in decreasing order: new food, preclinical (animal), processing method and cultivar/genotype/variety (Figure 2.7A). Leaf presented a different order for the type of studies, namely cultivar/genotype/variety > preclinical (animal) > plant part = clinical = production cultivation (Figure 2.7B). Tuber presented more preclinical (animal) studies than leaf and an inverse order was observed for clinical studies. Regarding peels, papers were on the phenolic composition, processing methods and processing variables effects on antioxidant activity. SP processing waste (2 papers) was the only plant part beside tuber that had articles on new food.

The distribution in time of the different types of studies showed that until 2009 there was no predominance of any type of study (Figure 2.8). However, from 2010 until 2013, the main types identified previously appeared markedly. Preclinical (animal) studies reached 6 articles both in 2010 and 2013 (Figure 2.8A). An increase in new food studies was also observed for the period 2012-2014. Studies on the effect of processing variables on SP antioxidant activity reach 5 articles in 20110 while 4 articles on formulation studies were published in 2004 (Figure 2.8B). Therefore, for the late period 2003-2014.there was a demarked trend towards preclinical studies followed by research on new foods related to antioxidant activity of phenolic compounds from SP. The effect of processing methods and phenolic compounds represented a steady or positive trend from 2013 to 2014.

The knowledge gap was higher for SP peel than for leaves. Research on new foods for peels and leaves combined with processing variables studies may benefit from the body of knowledge already built for the tuber plant part. In addition, the inclusion of optimization and formulation may accelerate the development of food application with health benefits based on SP peels and leaves.

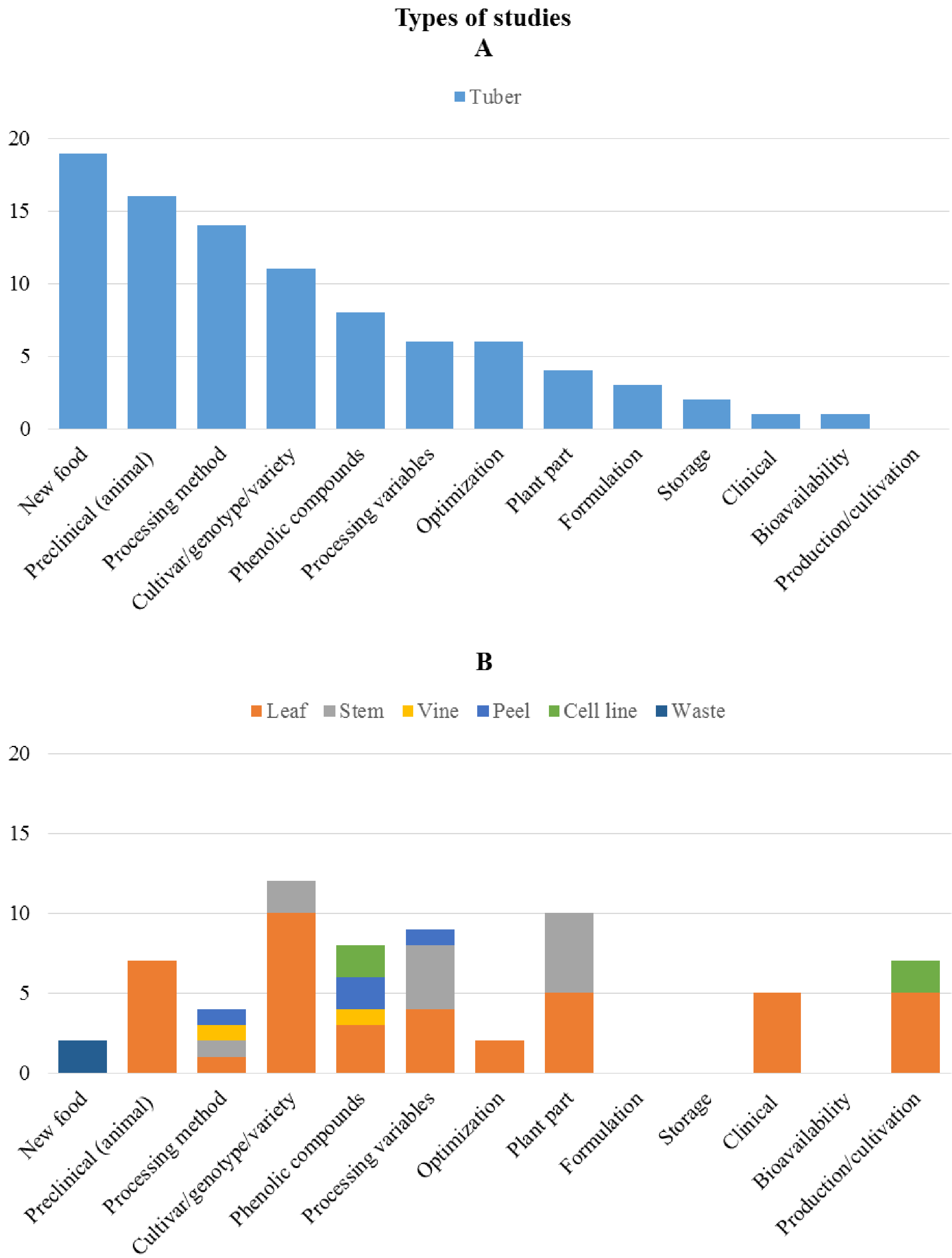

Figure 2.7. Stacked bar chart of SP articles by types of studies for the period 2003-2014 for A) tuber and B) other plant parts (leaf, stem, vine, peel, cell line and waste). More than one type of study could be attributed to an article.

2.3.3. Antioxidant activity assays used in SP articles

The antioxidant activity results from the collection of articles related to phenolic compound from sweet potato were distributed by fourteen *in vitro* antioxidant assays and nine *in vivo* assays (Supplementary Table S2.4). Frequently, more than one assay was used in a paper. For SP tuber, the *in vitro* assay 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity was predominant, with ferric reducing-antioxidant power (FRAP) assay as the second most used assay (Figure 2.9A). DPPH assay was also the most frequent used method for most of SP plant parts (Figure 2.9B) and this was also observed by Alam et al. (2013). DPPH is often included in antioxidant studies as it is more rapid, simple and inexpensive when compared to other free radical scavenging methods.

Regarding *in vivo* antioxidant estimation for the tuber plant part, lipid peroxidation assay (LPO) was the most used for tuber followed by reduced glutathione (GSH) estimation. The decreasing order for *in vitro* antioxidant methods used to study SP leaf were DPPH, RP, thiobarbituric acid method (TBA) and FRAP (Figure 2.9B). The antioxidant activity of SP peels was only published using the FRAP method so future research should include other methods as DPPH and RP for comparison purposes with other SP plant parts. The time series map of articles publication dates did not add significant information so it was not presented here. Therefore, there it was identified a knowledge gap between SP leaf and peel regarding *in vitro* antioxidant activities and also between DPPH assay and the other *in vitro* assays.

2.3.4. Phenolic compounds determined in SP articles

The phenolic composition of the different SP plant parts published in the selected articles was divided into two categories (Supplementary Table S2.5). Three spectrophotometric methods were considered for the global phenolic composition while individual compounds, obtained by methodologies as HPLC or LC, were grouped in four classes. The spectrophotometric methods considered were: total phenolic compounds (TPC), total monomeric anthocyanins (TMA) and total flavonoid content (TFC). TPC and TMA results were more frequent for SP tuber while TFC was used in lesser extent (Figure 2.10A).

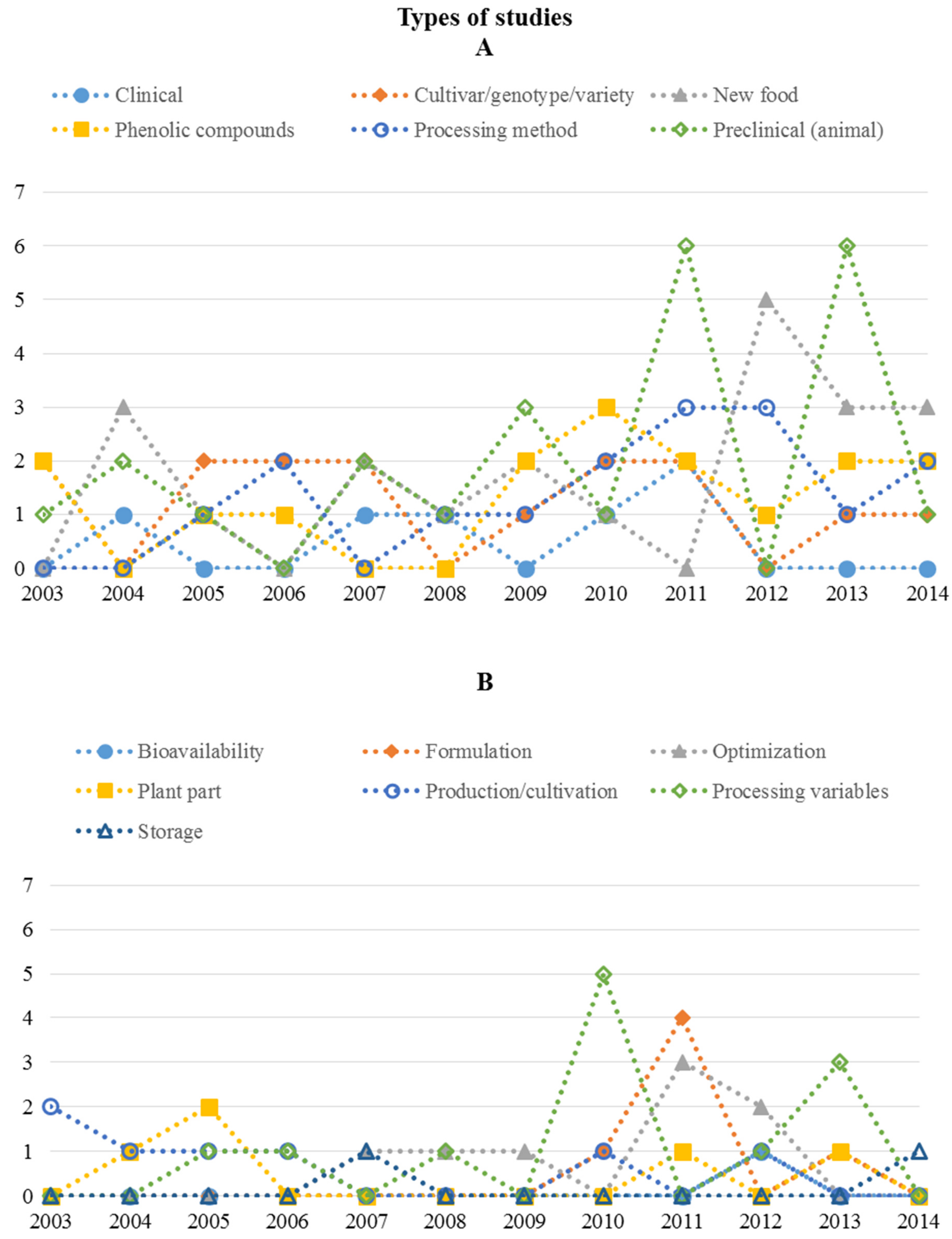

Figure 2.8. Time series map of SP articles by types of studies for the period 2003-2014 for A) tuber and B) other plant parts (leaf, stem, vine, peel, cell line and waste). More than one type of study could be attributed to an article.

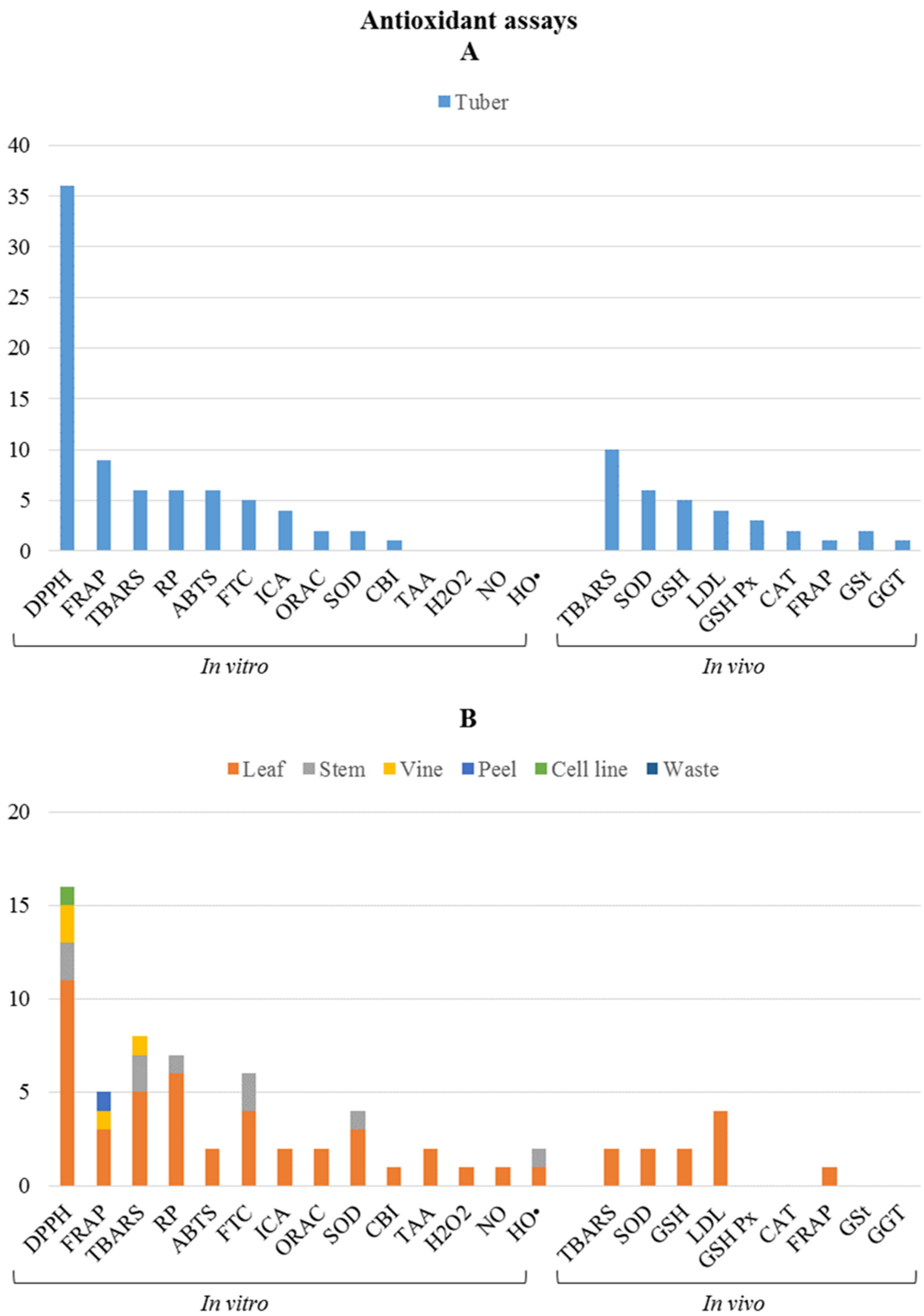

Figure 2.9. Stacked bar chart of SP articles by antioxidant assay for the period 2003-2014 for A) tuber and B) other plant parts (leaf, stem, vine, peel, cell line and waste). More than one assay could be attributed to an article. See Table S2.4 for assays abbreviations.

For leaves, the decreasing order of articles was TPC > TFC > TMA (Figure 2.10B). This order was different than the observed for the tuber as a result of the less number of articles for the purple variety for leaf so anthocyanin content was not as much determined. The individual compounds were grouped into hydroxycinnamic acids, hydroxybenzoic acids, flavonoids, flavonols and anthocyanins. Tuber presented more articles on anthocyanins composition than hydroxycinnamic acids, and no results for the other compounds groups were identified ((Figure 2.10A). The other plant parts together covered the five groups of individual compounds and hydroxycinnamic acids were analysed for all the plant parts (Figure 2.10B). There was more articles with results on hydroxycinnamic acids from SP leaf followed by flavonols. Interestingly, stem was the third plant part with more results published while peel had only articles on TPC and hydroxycinnamic acids. Time series map of articles publication dates did not add significant information so it was not considered. Thus, there was a higher knowledge gap on phenolic compounds of SP peel than leaf that should be reduced by future research on the development of food applications with health benefits.

2.3.5. Statistical analysis used in SP articles

The statistical analysis used to examine the results of articles antioxidant activity of phenolic compounds from sweet potato were summarized into fifteen tools (Supplementary Table S2.6). Analysis of variance (ANOVA), Duncan's *post hoc* test followed by Tukey’s test and correlation analysis were the most used statistical analysis for tuber plant part (Figure 2.11A). This resulted from the question of interest of articles was to evaluate whether one factor had a significant impact on the response variables. The usual procedure was to perform the analysis of variance to test whether the means of several groups are equal followed by *post hoc* tests to check the difference significance group by group. For SP leaf, analysis of variance, Student’s t test and correlation were the most used (Figure 2.11B).

Analysis tools as principal component analysis, response surface methodology of factorial designs were absent or used in a reduced number of articles. Multivariate analysis tools, based upon statistics, provide a rigorous inferences from experimental data and an efficient and effective planning of experiments for product excellence (Joglekar & May, 1987). The use of design of experiments and multivariate statistical tools for the development of food applications with health benefits form SP leaf and peels will narrow this gap on statistical analysis.

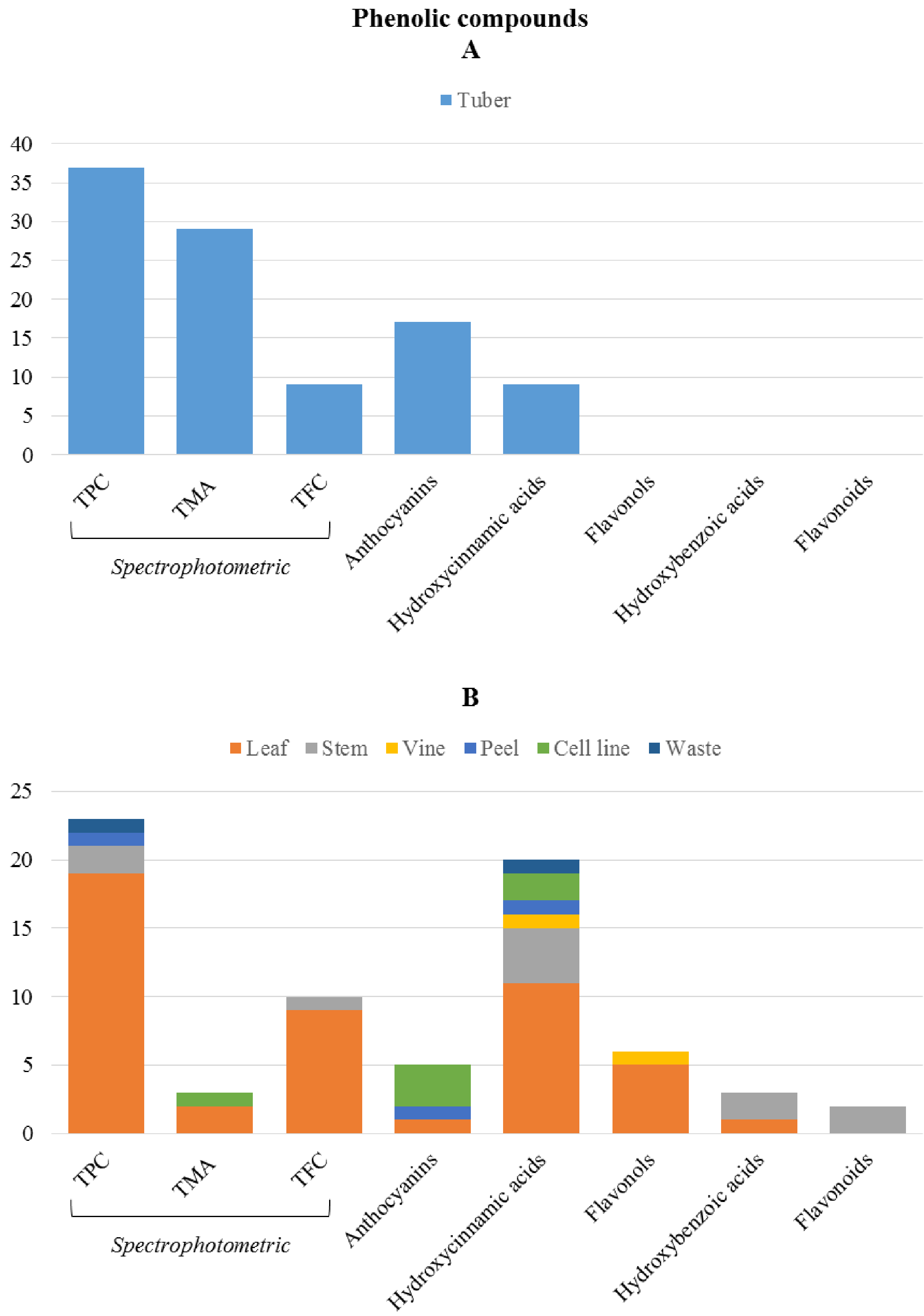

Figure 2.10. Stacked bar chart of SP articles by phenolic compounds for the period 2003-2014 for A) tuber and b) other plant parts (leaf, stem, vine, peel, cell line and waste). TPC: total phenolic compounds; TMA: total monomeric anthocyanins; TFC: total flavonoid content.

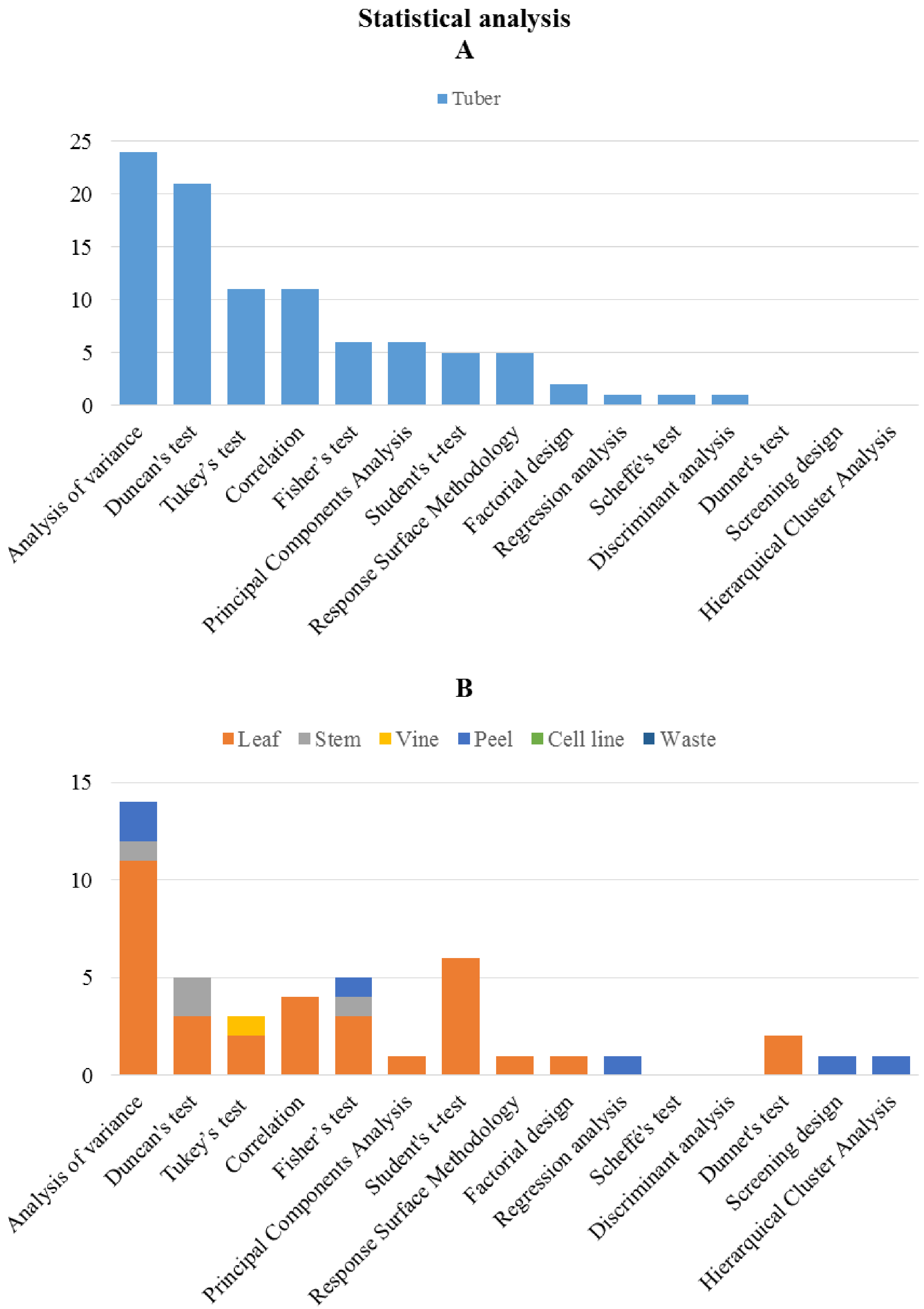

Figure 2.11. Stacked bar chart of SP articles by statistical analysis tools for the period 2003-2014 for A) tuber and b) other plant parts (leaf, stem, vine, peel, cell line and waste).

2.3.6. Health benefits listed in SP articles

The health benefits related to antioxidant activity of phenolic compounds from sweet potato were summarized from the clinical and preclinical (animal) publication (Supplementary Table S2.7). SP tuber and leaf were the only plant parts used and leaf presented more clinical studies than tuber. In general, benefits of antioxidant activity were linked to the reduction of oxidative stress, anti-diabetic and anti-cancer functions and related to the brain and liver. Phenolic compounds from SP tuber, mostly anthocyanin fractions, decreased oxidative stress, modulated antioxidative status, enhanced antioxidant defence, reduced DNA oxidation, decrease pro-inflammatory cytokine secretion and presented cytoprotective activity against γ-radiation. Anticancer benefits included effects in preventing cancers namely colorectal cancer, growth suppression of cancer cells and apoptosis in prostate cancer. Benefits for the liver include the decrease the serum levels of biomarkers, attenuation of inflammatory response, attenuation of oxidative stress induced by D-galactose, attenuation of dimethylnitrosamine-induced injury, protection against *tert*-butyl hydroperoxide (t-BHP), protection on induced lipid accumulation and attenuates hepatic insulin resistance via blocking oxidative stress. Phenolic compounds via antioxidant activity imparted neuroprotection by attenuation of oxidative damage, amelioration of cognition deficits, and amelioration of inflammation in brain and also pretended enhancing effects in memory and spatial learning. Finally, SP tuber ameliorated diabetic disorders and presented anti-fatigue activity, and was also considered a treatment of Alzheimer's disease. They were active in reducing lipid oxidation and suppressing low-density lipoprotein oxidation. The health benefits related to SP leaf were obtained by clinical studies were enhancement of antioxidant defence, anticancer activity, and reduction of lipid oxidation, of DNA oxidation and of inflammation. Health benefits as anti-fatigue, antidiabetic activity and treatment of Alzheimer's disease were established by animal testing.

Tuber presented studies distributed in the range of 2003 to 2014 while SP leaf studies were published between 2007 and 2013 (Figure 2.12). There was no health benefits established for SP peel. Although the health benefits of peels could be inferred from its antioxidant activity and phenolic composition by comparison to the tuber, validation through clinical and preclinical (animal) studies will be necessary. The information regarding the health benefits in SP leaf should be tested for new food applications.

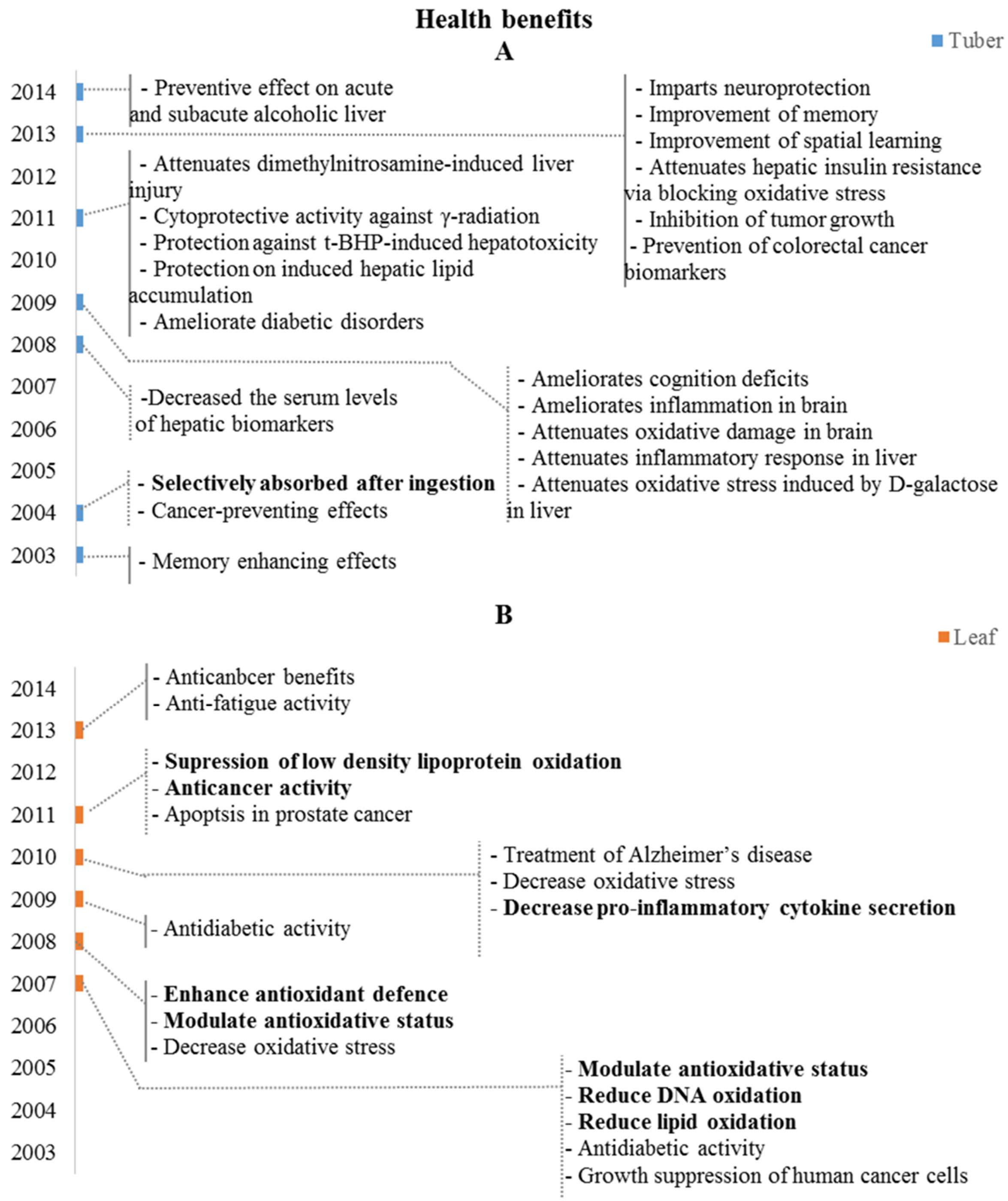

Figure 2.12. Time line of health benefits from SP articles for the period 2003-2014 for A) tuber and B) leaf. Only clinical (in bold) and preclinical studies were considered. t-BHP: *tert*-butyl hydroperoxide.

2.3.7. Food applications in SP articles

Food applications in SP articles on antioxidant activity of phenolic compounds were registered in Supplementary Table S2.8. Articles were distributed by a total of twenty five different food applications where flour/powder was in majority. For tuber, anthocyanin extracts and extracts were the second and third food applications, respectively (Figure 2.13A). Fermented (stems) and vinegar (from processing waste) were the only food applications not found for SP tuber. Articles that studied beverages based on SP tuber where in equal number as the boiling and as fresh products. Different home cooking preparations for tuber, as boiling, steaming, stir frying (*sautée*), baked, frying, microwaving or roasted were already addressed, although in a reduced number. SP tuber presented different products in the beverage category, such as alcoholic drinks (excluding wine), wine, nectar and a lactic acid beverage. Food applications for the other plant parts were much less miscellaneous than for the tuber with only eleven different products (Figure 2.13B). As for SP tuber, flour/powder was the most classified food type for leaf followed by extract.

The distribution in time of the different food applications was represented in Figure 2.14. Globally, SP flour/powder was predominant over the over food application, with more number of studies in the period 2010 to 2013. A large increase in articles about the SP flour/powder form was observed from 2009 to 2010 but in 2014 number decreased abruptly. There was no positive trend for any food application from 2013 to 2014. In 2014, the food applications that were more mentioned in articles were extract and anthocyanin extract. There is a lack of studies on food applications for other SP plant parts than tuber or leaf. Research on applications for the valorisation of SP wastes, a flour/powder or a beverage could benefits from knowledge accumulated for tuber.

2.3.8. Food applications in SP patents

Food applications of SP with antioxidant activity of phenolic compounds reported in patents were summarized in Supplementary Table S2.9. The number of different products – 43 – was higher than the one obtained for articles. The product with more patents application for tuber was a beverage followed by pasta (that including noodles products) (Figure 2.15A).

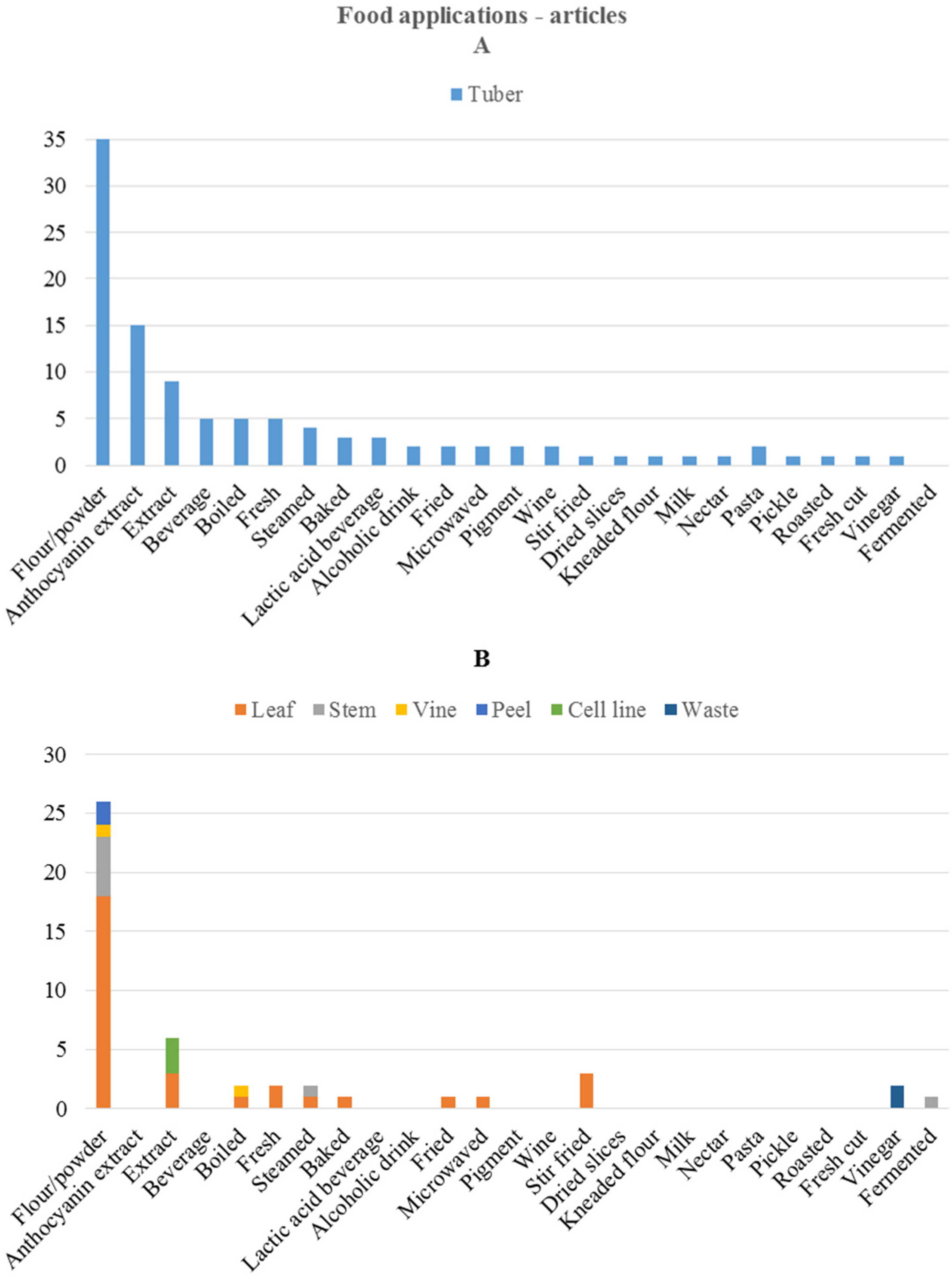

Figure 2.13. Stacked bar chart of SP articles by food applications for the period 2003-2014 for A) tuber and B) other plant parts (leaf, stem, vine, peel, cell line and waste). More than one food application could be attributed to an article.

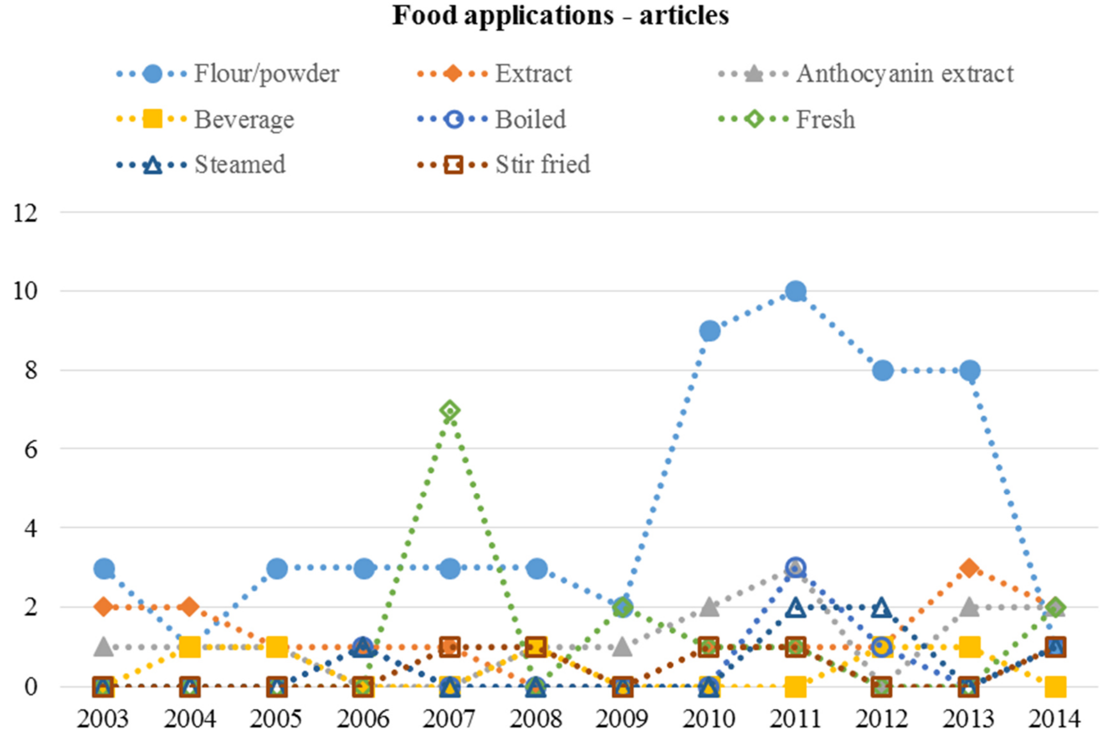

Figure 2.14. Time series map of SP articles by food applications for the period 2003-2014. Only food applications up to a total of three articles were represented.

Although flour/powder products were the main food application found in articles, it ranked 6th for patents. As expected, the traditional home cooking preparations for tuber were absent in the patent food applications. The beverage food category comprised more different products for patents than for articles as juice, beer and liquor. SP tuber was also mentioned for healthy foods, functional foods and supplements. As observed for articles, patented food applications for the other SP plant parts were less miscellaneous than for tuber with only seven different products (Figure 2.15B). For SP leaf, extracts were the most patented food application followed by beer. Three products that were patented for leaf – essence, fiber and pickle – were absent in tuber. The time series map of patents' date of publication was represented in Figure 2.16 for food applications with more than nine patents in the period 2003-2014. Until 2007 there were only published 7 patents on phenolic compounds and/or antioxidant activity of SP. Beverages patents grew in number from 2007to 2011. In 2014, the food applications that presented a positive trend were healthy foods, pasta, flour/powder and beverage.

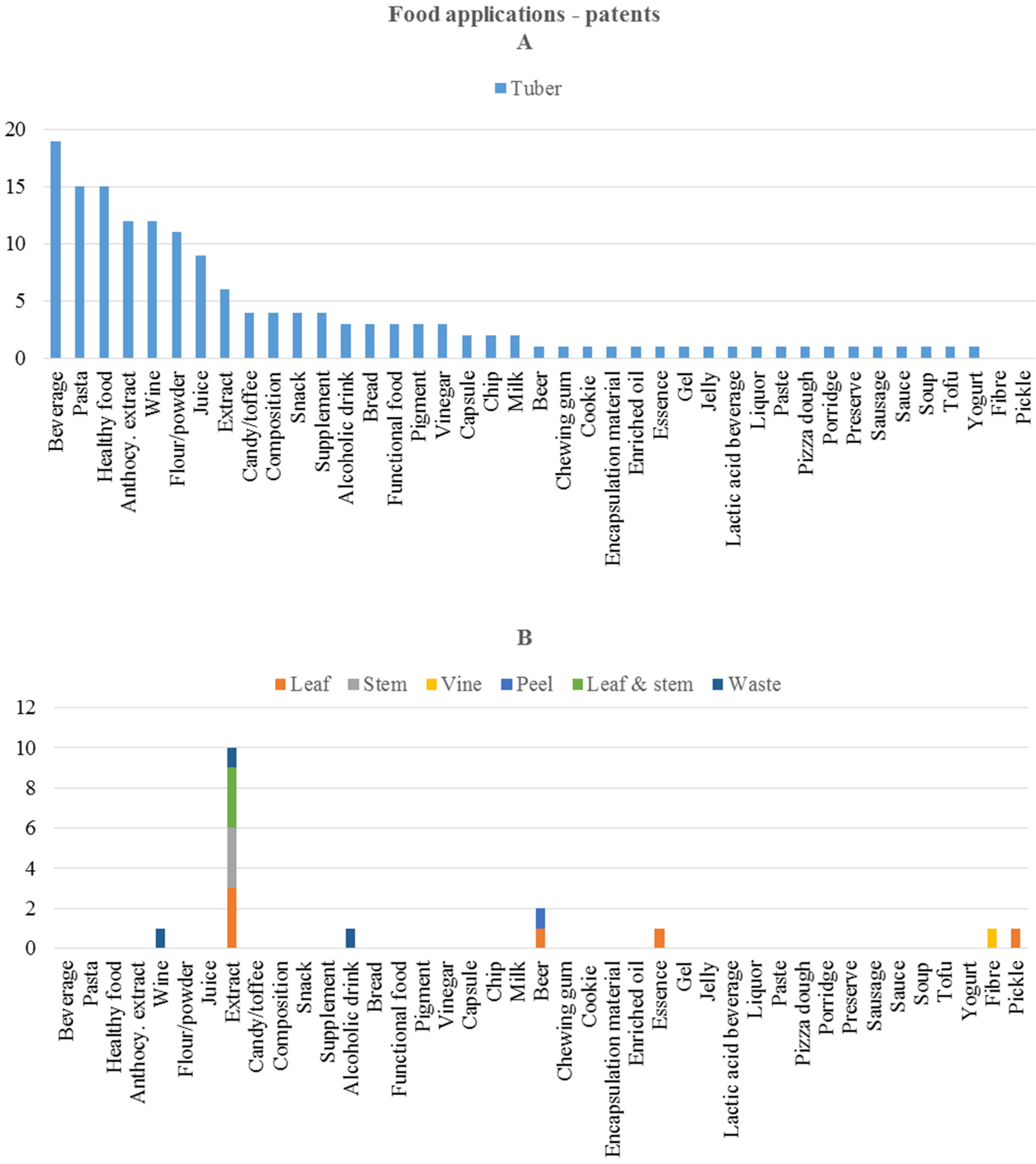

Figure 2.15. Stacked bar chart of SP patents by food applications for the period 2003-2014 for A) tuber and B) other plant parts (leaf, stem, vine, peel, cell line and waste).More than one food application could be attributed to a patent.

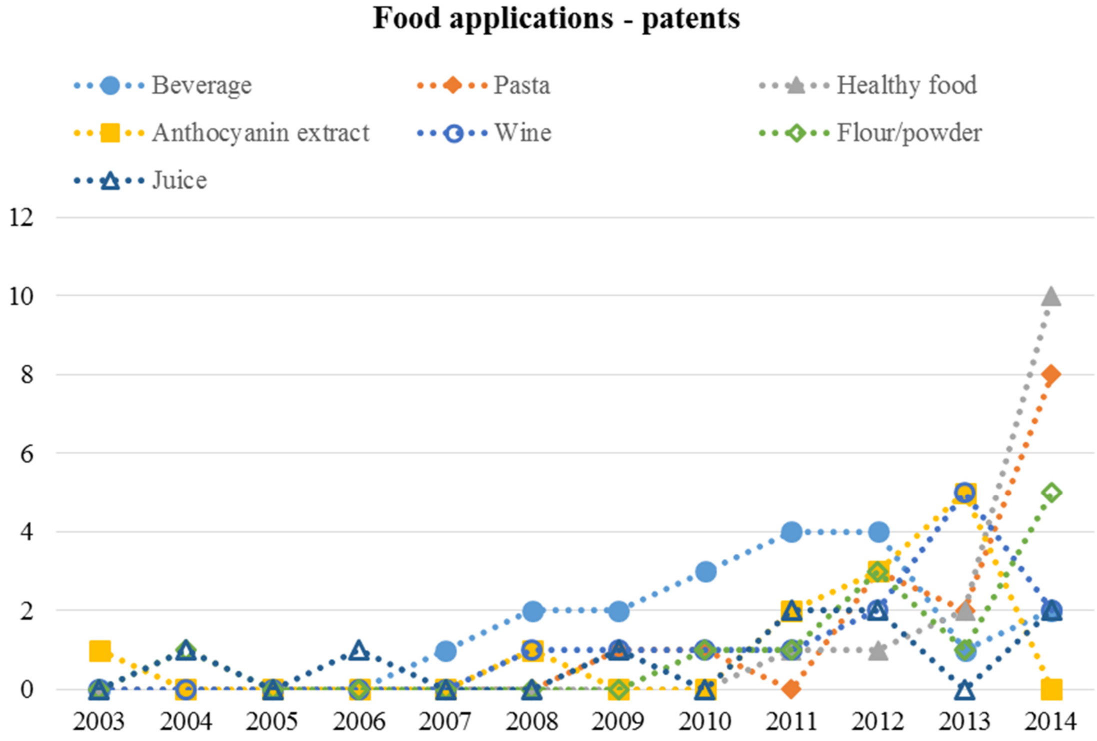

Figure 2.16. Time series map of SP patents by food applications for the period 2003-2014. Only food applications up to a total of three articles were represented.

Research on new food development of beverage or extract for SP wastes as leaf and peel has the potential to be protected by a patent due to the reduced number of food applications.

2.3.9. Text mining

HCA and co-occurrence analysis were performed on statistics obtained by text mining pre-treatment of abstracts of selected articles and patents for the period 2003 to 2014. The words selected were nouns and adjectives with a minimum term and document frequencies of 23 for articles and 34 for patents Dendograms obtained by HCA revealed five word groups for both articles and patents and two major themes were considered based on words frequency. For articles abstracts, “sweet” and “potato” presented the closest similarity in Group 1 with higher term frequencies (1^{st} main theme) (Figure 2.17A). “Acid” was the word more related to “sweet potato” within the group. This may be associated to the different phenolic compounds that did not enter in the analysis due to the criteria applied to term and document frequencies. For the same reason, although "phenolic" ranked high in adjectives frequency (5th) the word did not enter in the representation. "Total content" was next to "sweet potato" and "acid". The other words in Group 1 group were "antioxidant activity" that were closely related to "result" and "effect". The second group of words with higher frequencies (2nd main theme) was formed by "anthocyanin", "purple", level", "concentration" and "treatment" (Group 2). Patents dendogram presented a group of words (Group 1) with much higher frequencies than the others (Figure 2.17B). "Purple sweet potato", "anthocyanins" and "raw material" were the central subject of patents (1st main theme). The 2nd main theme was connected to patent food applications namely "food", "powder", "weight" and "effect".

Co-occurrence networks patterns high-frequency words occurring together and identify content communities. Words are depicted as nodes with a size proportional to their frequency in the documents. Nodes are linked by edges when they occur together and their thickness is proportional to the strength of co-occurrence between them. For articles abstracts, three content communities were detected (Figure 2.18A). The community that included the words "sweet" and "potato" was located at the centre of the network (number 1). The words "total", "acid", "phenolic" and "content" were located closely in this group and presented high co-occurrence. The words "activity" and "effect" belonged to the "sweet potato" group but "antioxidant" and "results" formed a small community on their own (number 2) with several links to core group. "Purple" and "anthocyanin" made a community (number 3) that also was linked by several edges to words of the main group. "DPPH" was a satellite node only linked to the word "activity". The co-occurrence network for patents abstracts presented a different configuration than the one built for articles (Figure 2.18B). Four communities were identified. "Purple", "sweet" and "potato" belonged to the larger community (number 1) and were closely located and strongly linked. The words "raw", "material", "effect", "weight" and "powder" also belonged to this community. All the other three communities in the network were linked to the "purple sweet potato" community but not among each other.

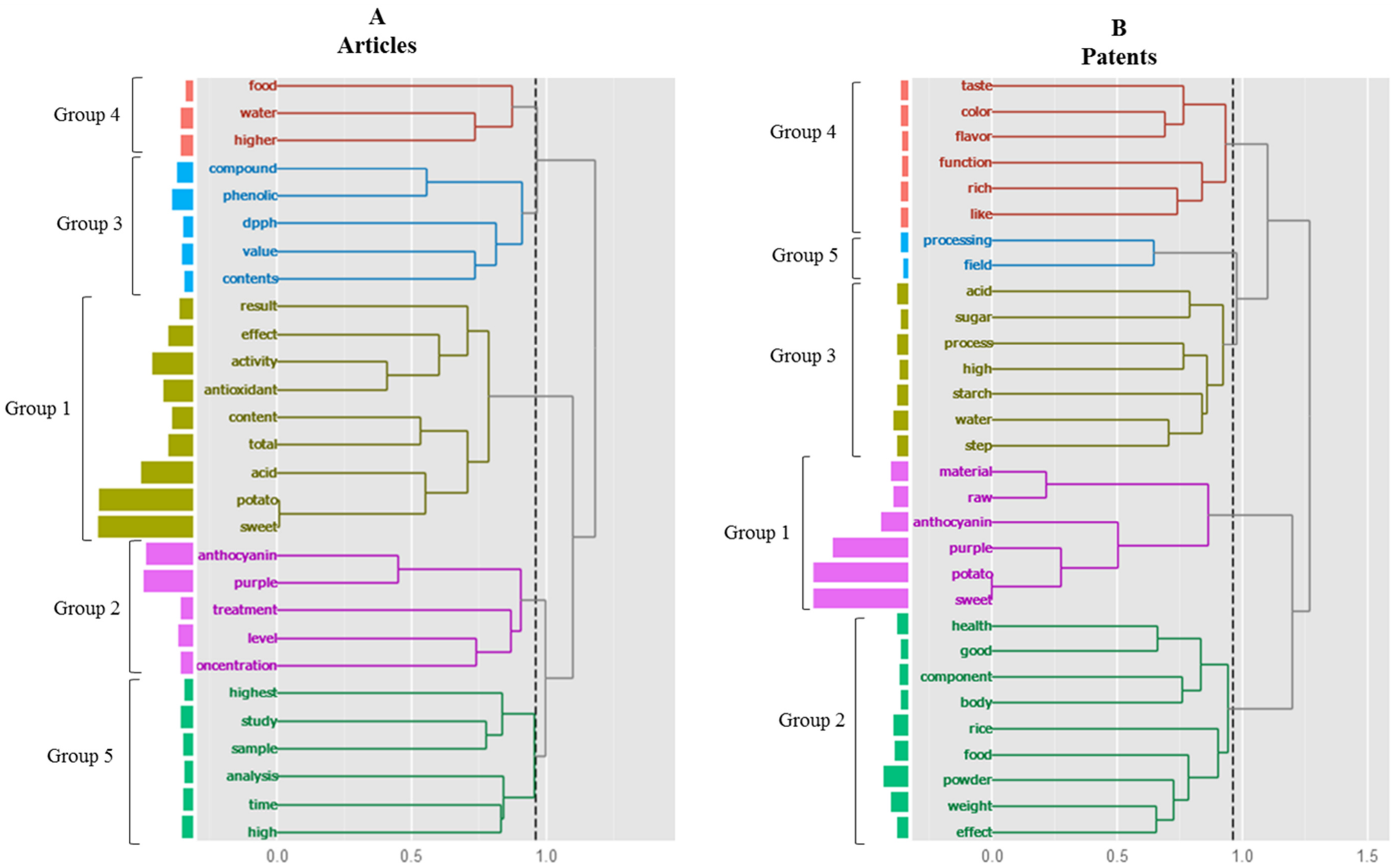

Figure 2.17. Dendograms from abstracts text mining for A) articles and B) patents with indication of word frequencies.

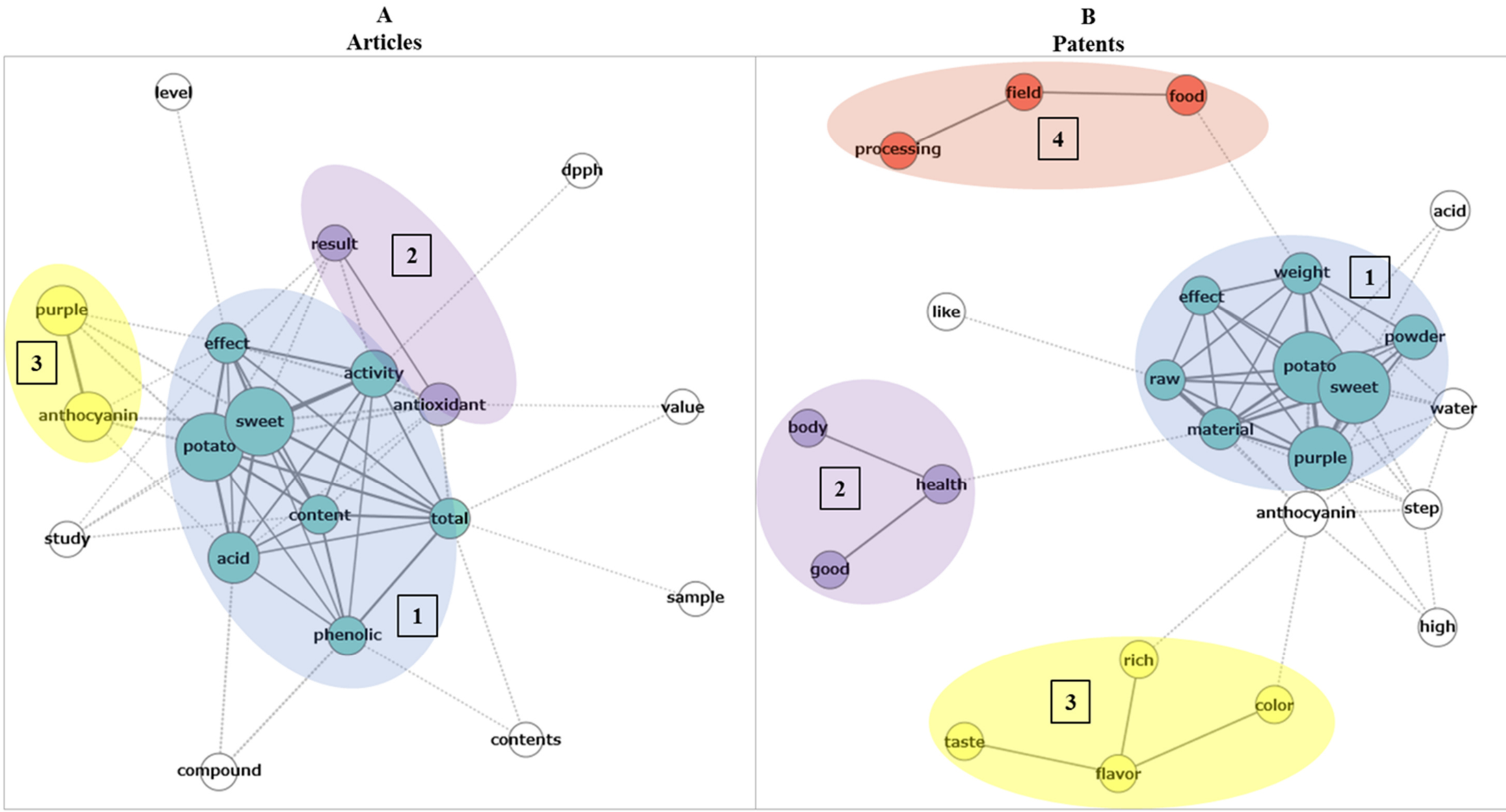

Figure 2.18. Co-occurrence network from abstracts text mining for A) articles and B) patents.

2.4. Conclusions

From the main food applications found in articles and patents for the period 2003-2014, the development of a flour/powder or a beverage could benefit from the knowledge already built for the tuber. As the strategy of combining different SP plant parts presented a low intensity in the period of time studied, it could be undertaken in future research. Rather than using result means comparison tools as verified for the tuber, the use of design of experiments and multivariate statistical tools during new food development could narrow efficiently the knowledge gap found for peels and leaves. Design responses should include DPPH assay combined with other *in vitro* antioxidant assays and the determination of phenolic compounds by spectrophotometric methods as well the content of individual anthocyanins and hydroxycinnamic acids. These results could shape further research on health benefits, expected to be related to the reduction of oxidative stress and improvement of brain and liver functions. Although these health benefits could be inferred from the antioxidant activity and phenolic composition of peels and leaves by comparison to the tuber, validation through clinical and preclinical (animal) studies will be necessary. In addition, text mining applied only to abstract texts revealed the main research features of articles and claims of patents on antioxidant activity of phenolic compounds of sweet potato. Dendograms and co-occurrence networks form text mining analysis may be useful to compare future research lines patterns and trends.

2.5. References

Ahmed, M., Akter, M. S. and Eun, J.-B. (2010a). Impact of alpha-amylase and maltodextrin on physicochemical, functional and antioxidant capacity of spray-dried purple sweet potato flour. *J Sci Food Agric.* **90**: 494-502.

Ahmed, M., Akter, M. S. and Eun, J.-B. (2010b). Peeling, drying temperatures, and sulphite-treatment affect physicochemical properties and nutritional quality of sweet potato flour. *Food Chem.* **121**: 112-118.

Ahmed, M., Akter, M. S. and Eun, J.-B. (2011a). Optimisation of drying conditions for the extraction of beta-carotene, phenolic and ascorbic acid content from yellow-fleshed sweet potato using response surface methodology. *Int J Food Sci Technol.* **46**: 1356-1362.

Ahmed, M., Akter, M. S. T. S. and Eun, J.-B. (2011b). Optimization conditions for anthocyanin and phenolic content extraction form purple sweet potato using response surface methodology. *Int J Food Sci Nutr.* **62**: 91-96.

Ahmed, M., Akter, M. S., Chin, K.-B. and Eun, J.-B. (2009). Effect of maltodextrin concentration and drying temperature on quality properties of purple sweet potato flour. *Food Sci Biotechnol.* **18**: 1487-1494.

Ahmed, M., Akter, M. S., Lee, J.-C. and Eun, J.-B. (2010). Encapsulation by spray drying of bioactive components, physicochemical and morphological properties from purple sweet potato. *LWT-Food Sci Technol*. **43**: 1307-1312.

Ahmed, M., Sorifa, A. M. and Eun, J. B. (2010). Effect of pretreatments and drying temperatures on sweet potato flour. *Int J Food Sci Technol*. **45**: 726-732.

Alam, M. N., Bristi, N. J. and Rafiquzzaman, M. (2013). Review on *in vivo* and *in vitro* methods evaluation of antioxidant activity. *Saudi Pharm J.* **21**: 143-152.

Anastácio, A. and Carvalho, I. S. (2013a). Phenolics extraction from sweet potato peels: Key factors screening through a Plackett-Burman design. *Ind Crop Prod.* **43**: 99-105.

Anastácio, A. and Carvalho, I. S. (2013b). Spotlight on PGI sweet potato from Europe: study of plant part, time and solvent effects on antioxidant activity. *J Food Biochem*. **37**: 628-637.

Baek, L. H. and Jung, G. S. (2004). Production of mixture of *Cordyceps sinensis* powder and sweet potato anthocyanin and mixture prepared thereby. Patent Nr. KR20040064759.

Barnes, S. L. and Sanders, S. A. (2012). Advances in Functional Use of Sweet Potato,[*Ipomoea batatas* (L.) Lam]. *Recent Pat Food Nutr Agric*. **4**:148-154.

Boo, H. O., Chon, S. U., Kim, S. M. and Pyo, B. S. (2005). Antioxidant activities of colored sweet potato cultivars by plant parts. *Food Sci Biotechnol*. **14**: 177-180.

Bovell-Benjamin, A. C. (2007). Sweet potato: a review of its past, present, and future role in human nutrition. *Adv Food Nutr Res*. **52**:1-59.

Carvalho, I. S., Cavaco, T., Carvalho, L. M. and Duque, P. (2010). Effect of photoperiod on flavonoid pathway activity in sweet potato (*Ipomoea batatas* (L.) Lam.) leaves. *Food Chem*. **118**: 384-390.

Cevallos-Casals, B. A. and Cisneros-Zevallos, L. (2004). Stability of anthocyanin-based aqueous extracts of Andean purple corn and red-fleshed sweet potato compared to synthetic and natural colorants. *Food Chem*. **86**: 69-77.

Cevallos-Casals, B. and Cisneros-Zevallos, L. (2001). Bioactive and functional properties of purple sweetpotato (*Ipomoea batatas* (L.) Lam). In: I International Conference on Sweetpotato. Food and Health for the Future 583. pp. 195-203.

Chan, K. W., Khong, N. M. H., Iqbal, S., Umar, I. M. and Ismail, M. (2012). Antioxidant property enhancement of sweet potato flour under simulated gastrointestinal pH. *Int J Mol Sci.* **13**: 8987-8997.

Chang, W.-H., Chen, C.-M., Hu, S.-P., Kan, N.-W., Chiu, C.-C. and Liu, J.-F. (2007). Effect of purple sweet potato leaf consumption on the modulation of the antioxidative status in basketball players during training. *Asia Pac J Clin Nutr*. **16**: 455-461.

Chang, W.-H., Hu, S.-P., Huang, Y.-F., Yeh, T.-S. and Liu, J.-F. (2010). Effect of purple sweet potato leaves consumption on exercise-induced oxidative stress and IL-6 and HSP72 levels. *J Appl Physio*. **109**: 1710-1715.

Chen, C.-M., Lin, Y.-L., Chen, C. Y. O., Hsu, C.-Y., Shieh, M.-J. and Liu, J.-F. (2008). Consumption of purple sweet potato leaves decreases lipid peroxidation and DNA damage in humans. *Asia Pac J Clin Nutr*. **17**: 408-414.

Chen, R. (2014). Purple sweet potato-konjak-containing health rice-shaped food and preparation method thereof. Patent Nr. CN104041752.

Chen, X., Han, Y. and Wang, C. (2014). Sugar-free functional soft sweet rich in purple sweet potato anthocyanin and preparing method thereof. Patent Nr. CN104000000.

Chen, Y. and Wang, C. (2013a). Purple sweet potato and Chinese yam gruel. Patent Nr. CN102972762.

Chen, Y. and Wang, C. (2013b). Processing method of purple sweet potato and Chinese yam gruel. Patent Nr. CN20121514712.

Chen, Y., Yu, X., Xu, Y. and Yu, Y. (2014). Technology for production of purple potato beverage by use of wave band enzyme method. Patent Nr. CN103653138.

Cheng, J. and Cheng, G. (2014). Selenium/germanium traditional Chinese medicine-enriched sweet potato rice noodles capable of prolonging life. Patent Nr. CN103892284.

Chengyu, L. (2009). Anthocyanin oral liquid and beverage and production method thereof. Patent Nr. CN101874619.

Cho, J., Kang, J. S., Long, P. H., Jing, J., Back, Y. and Chung, K. S. (2003). Antioxidant and memory enhancing effects of purple sweet potato anthocyanin and Cordyceps mushroom extract. *Arc Pharmacal Res*. **26**: 821-825.

Cho, K. M., Kim, H. Y., Seo, W. T., Choi, J. S., Cho, E. J., Sin, S. M. and Byun, H. J. (2013). Manufacturing method for granules of *Momordica charantia* having enhanced antioxidant activity and alpha-glucosidase inhibitory activity. Patent Nr. KR20130066960.

Choi, D. S., Park, J. S., Bae, J. O. and Lee, K. J. (2014). Method for manufacturing sweet potato doenjang using colored sweet potato. Patent Nr. KR20140054490.

Choi, M.-S., Park, J.-H., Min, J. Y., Lim, B.-K., Lee, B. H., Jung, G. W Lee, J.-Y.; Karigar, C. S. and Yang, J.-K. (2008). Efficient release of ferulic acid from sweet potato (*Ipomoea batatas*) stems by chemical hydrolysis. *Biotechnol Bioproc*. E. 13: 319-324.

Chon, S. U. and Boo, H. O. (2005). Difference in allelopathic potential as influenced by root periderm colour of sweet potato (*Ipomoea batatas*). *J Agron Crop Sci*. **191**: 75-80.

Chuanbin, W. and Xianxun, S. (2011). Functional beer and brewing method thereof. Patent Nr. CN102250701.

Chun, H. K., Joo, J. Y., Kim, J. H., Kang, S. K. and Chung, Y. C. (2007). Composition for prevention and treatment of anti-gout comprising purple pigments isolated from purple sweet-potato. Patent Nr. KR100759468.

Chun, J.-E., Baik, M.-Y. and Kim, B.-Y. (2014). Manufacture and quality evaluation of purple sweet potato Makgeolli vinegar using a 2-stage fermentation. *Food Sci Biotechnol*. **23**: 1145-1149.

Cui, L., Liu, C.-Q., Li, D.-J. and Song, J.-f. (2011). Effect of processing on taste quality and health-relevant functionality of sweet potato tips. *Agric Sci China* **10**: 456-462.

Dechao, H., Dejuan, H., Peng, S., Xiaowen, X., Hongying, Z. and Jingqiang, Z. (2010). Sweet potato and soybean composite fermented beverage and preparation technology thereof. Patent Nr. CN101828734.

Defa, L. (2009). Purple sweet potato full-nutrition drink and method for preparing same. Patent Nr. CN102085003.

Dehua, M., Yan, L. and Hongwei, X. (2012). Method for extracting anthocyanins from purple sweet potato. Patent Nr. CN102604424.

Dincer, C., Karaoglan, M., Erden, F., Tetik, N., Topuz, A. and Ozdemir, F. (2011). Effects of baking and boiling on the nutritional and antioxidant properties of sweet potato *Ipomoea batatas* (L.) Lam. cultivars. *Plant Food Hum Nutr*. **66**: 341-347.

Ding, H., Gao, Y., Lei, H., Luo, L., Chao, H. and Ruan, R. (2010). *In vitro* antioxidant effects of flavonoids of sweet potato vines. *Int J Food Prop*. **13**: 360-368.

Dini, I., Tenore, G. C. and Dini, A. (2006). New polyphenol derivative in *Ipomoea batatas* tubers and its antioxidant activity. *J Agric Food Chem*. **54**: 8733-8737.

Donado-Pestana, C. M., Salgado, J. M., Rios, A. d. O., dos Santos, P. R. and Jablonski, A. (2012). Stability of carotenoids, total phenolics and *in vitro* antioxidant capacity in the thermal processing of orange-fleshed sweet potato (*Ipomoea batatas* Lam.) cultivars grown in Brazil. *Plant Food Hum Nutr*. **67**: 262-270.

Dong, L. (2014). Purple sweet potato glutinous rice flour and preparation method thereof. Patent Nr. CN103750114.

Fan, G., Han, Y., Gu, Z. and Chen, D. (2008a). Optimizing conditions for anthocyanins extraction from purple sweet potato using response surface methodology (RSM). *LWT-Food Sci Technol*. **41**: 155-160.

Fan, G., Han, Y., Gu, Z. and Gu, F. (2008b). Composition and colour stability of anthocyanins extracted from fermented purple sweet potato culture. *LWT-Food Sci Technol*. **41**: 1412-1416.

Fanzhong, M. (2011). Health-care leisure food with purple sweet potatoes as major ingredients. Patent Nr. CN102100345.

FAOSTAT. (2014). http://faostat.fao.org/ (visited 13 November 2014).

Gan, L.-J., Yang, D., Shin, J.-A., Kim, S.-J., Hong, S.-T., Lee, J. H.; Sung, C.-K. and Lee, K.-T. (2012). Oxidative comparison of emulsion systems from fish oil-based structured lipid versus physically blended lipid with purple-fleshed sweet potato (*Ipomoea batatas* L.) extracts. *J Agric Food Chem*. **60**: 467-475.

Ge, C., Xie, Z. and Yao, M. (2013). Minitype purple sweet potato food and processing food thereof. Patent Nr. CN102948704.

Gong, Z. (2013). Aloe-containing steamed bread and making method thereof. Patent Nr. CN103431282.

Gongjian, F., Zhenxin, G., Yongbin, H., Zhigang, C., Zhendong, Y. and Chunyan, J. (2008). Purple sweet potato wine and producing technique thereof. Patent Nr. CN101215506.

Guan, S. (2014). Purple sweet potato, blueberry, anthocyanin-enriched flour and preparation method thereof. Patent Nr. CN103689356.

Guangyou, Z. (2008). Method for processing sweet potato beverage. Patent Nr. CN101243895

Gundala, S. R., Yang, C., Lakshminarayana, N., Asif, G., Gupta, M. V., Shamsi, S. and Aneja, R. (2013). Polar biophenolics in sweet potato greens extract synergize to inhibit prostate cancer cell proliferation and *in vivo* tumor growth. *Carcinogenesis* **34**: 2039-2049.

Guo, C. (2014). Instant matcha rice powder suitable for the elderly to eat. Patent Nr. CN104041735.

Guo, T. (2014). Purple sweet potato-flavor stomach-strengthening steamed bread and preparation method thereof. Patent Nr. CN103494074.

Gurgel, C., Farias, S., Farias, L. and Moreira, R. (2011). Sensory analysis of sweet potato ice cream. *Rev Bras Prod Agroind*. **13**: 21-26.

Han, H., Li, X., Li, Z., Xu, H. and Dang, Y. (2014). Health-care lemon and purple sweet potato sugar and preparation method thereof. Patent Nr. CN103892026.

Han, Y.-T., Chen, X.-H., Xie, J., Zhan, S.-M., Wang, C.-B. and Wang, L.-X. (2011). Purple sweet potato pigments scavenge ROS, reduce p53 and modulate Bcl-2/Bax to inhibit irradiation-induced apoptosis in murine thymocytes. *Cell Physiol Biochem*. **28**: 865-872.

Harada, K., Kano, M., Takayanagi, T., Yamakawa, O. and Ishikawa, F. (2004). Absorption of acylated anthocyanins in rats and humans after ingesting an extract of *Ipomoea batatas* purple sweet potato tuber. *Biosci Biotechnol Biochem*. **68**: 1500-1507.

Harrison, H. F., Peterson, J. K., Snook, M. E., Bohac, J. R. and Jackson, D. M. (2003). Quantity and potential biological activity of caffeic acid in sweet potato *Ipomoea batatas* (L.) Lam. storage root periderm. *J Agric Food Chem*. **51**: 2943-2948.

Hidaka, Y. and Obayashi, A. (2009). Process for producing reddish purple-colored alcoholic drink by using purple sweet potato. Patent Nr. JP2009027997.

Hiramoto, T., Saiki, K., Hirose, E. and Fukaya, O. (2005). Antioxidative composition. Patent Nr. WO2005012470.

Hong, L. (2011). Black sweet potato juice beverage and preparation method thereof. Patent Nr. CN102224956.

Hong, S. and Seonryu, S. H. (2013). Method for manufacturing rice makkolli using purple sweet potato. Patent Nr. KR101317381.

Hongmei, Z., Meng, Z. and Jianguo, X. (2008). Method for producing purple sweet potato fruit vinegar beverage and product thereof. Patent Nr. CN101238908.

Hou, F., Han, Y., Quan, L. and Li, Z. (2014). Purple sweet potato noodles. Patent Nr. CN104000100.

Huang, D. J., Lin, C. D., Chen, H. J. and Lin, Y. H. (2004). Antioxidant and antiproliferative activities of sweet potato (*Ipomoea batatas* L. Lam 'Tainong 57) constituents. *Bot Bul Acad Sinica* **45**: 179-186.

Huang, M.-H., Chu, H.-L., Juang, L.-J. and Wang, B.-S. (2010). Inhibitory effects of sweet potato leaves on nitric oxide production and protein nitration. *Food Chem*. **121**: 480-486.

Huang, X., Tu, Z., Xiao, H., Li, Z., Zhang, Q., Wang, H., Hu, Y. and. Zhang, L. (2013). Dynamic high pressure microfluidization-assisted extraction and antioxidant activities of sweet potato (*Ipomoea batatas* L.) leaves flavonoid. *Food Bioprod Process*. **91**: 1-6.

Huang, Y. C., Chang, Y. H. and Shao, Y. Y. (2006). Effects of genotype and treatment on the antioxidant activity of sweet potato in Taiwan. *Food Chem*. **98**: 529-538.

Hui, W., Shuqi, F., Guiguo, Y., Wei, W. and Shijie, C. (2012). Method for extracting anthocynins from purple sweet potatoes in continuous countercurrent ultrasonic manner. Patent Nr. CN102618067.

Huntrods, D. (2013). Sweet potato profile. Agricultural Marketing Resource Center. http://www. agmrc. org/commodities__products/vegetables/sweet_potato_profile/ (accessed November 7, 2014).

Hwang, Y. P., Choi, J. H., Choi, J. M., Chung, Y. C. and Jeong, H. G. (2011a). Protective mechanisms of anthocyanins from purple sweet potato against tert-butyl hydroperoxide-induced hepatotoxicity. *Food Chem Toxicol*. **49**: 2081-2089.

Hwang, Y. P., Choi, J. H., Han, E. H., Kim, H. G., Wee, J.-H., Jung, K. O., Jung, K. H; Kwon, K.; Jeong, T. C.; Chung, Y. C. and Jeong, H. G. (2011b). Purple sweet potato anthocyanins attenuate hepatic lipid accumulation through activating adenosine monophosphate-activated protein kinase in human HepG2 cells and obese mice. *Nutr Res*. **31**: 896-906.

Hwang, Y. P., Choi, J. H., Yun, H. J., Han, E. H., Kim, H. G., Kim, J. Y., Park, B. H., Khanla, T., Choi, J. M., Chung, Y. C. and Jeong, H. G. (2011c). Anthocyanins from purple sweet potato attenuate dimethylnitrosamine-induced liver injury in rats by inducing Nrf2-mediated antioxidant enzymes and reducing COX-2 and iNOS expression. *Food Chem Toxicol*. **49**: 93-99.

In, G. S., Jung, Y. M., Kim, B. R., Kim, H. C. and Lee, J. S. (2004). Production of pickled seafoods juice containing germanium and selenium. Patent Nr. KR20040070130.

Ishiguro, K., Yahara, S. and Yoshimoto, M. (2007). Changes in polyphenolic content and radical-scavenging activity of sweetpotato (*Ipomoea batatas* L.) during storage at optimal and low temperatures. *J Agric Food Chem*. **55**: 10773-10778.

Islam, M. S., Yoshimoto, M., Ishiguro, K., Okuno, S. and Yamakawa, O. (2003). Effect of artificial shading and temperature on radical scavenging activity and polyphenolic composition in sweetpotato (*Ipomoea batatas* L.) leaves. *J Am Soc for Hortic Sci*. **128**: 182-187.

Islam, S. (2006). Sweetpotato (*Ipomoea batatas* L.) leaf: its potential effect on human health and nutrition. *J Food Sci*. **71**: R13-R21.

Iwata, S. (2006). Brewed herb vinegar. Patent Nr. JP2006042616.

Jeng, T. L., Ho, P. T., Shih, Y. J., Lai, C. C. and Sung, J. M. (2012). Chemicals contents in non-alcoholic and alcoholic beverages produced from purple-fleshed and orange-fleshed sweet potato varieties. *Food Sci Technol Res*. **18**: 639-644.

Jeong, G. I. (2012). Sweetpotato gruel and method for making thereof. Patent Nr. KR20120078992.

Ji, J. (2013). Pawpaw and purple sweet potato soup and preparation technology thereof. Patent Nr. CN102871074.

Ji, L., Gang, H., Xianggui, C., Guangwei, R., He, L., Bo, L., Li, Y.; Hong, W. and Jiang, X. (2010). Method for preparing mashed purple sweet potato solid beverage. Patent Nr. CN101889710.

Jian, W., Yong, T., Taiming, Q., Yi, W., Daigang, L. and Changqi, W. (2011). Super-fine purple coarse food grain nutrition milk and processing technology of same. Patent Nr. CN102119729.

Jiang, H. (2014). Production method of non-condensed raw purple sweet potato juice. Patent Nr. CN103948102.

Jianyi, L. (2012). Waxberry bean vermicelli. Patent Nr. CN102326748.

Jiao, Y., Yang, Z., Jiang, Y. and Zhai, W. (2012). Study on chemical constituents and antioxidant activity of anthocyanins from purple sweet potato (*Ipomoea batatas* L.). *Int J Food Eng*. **8**: Article 14.

Jing, P., Lan, Y., Zhao, S., Ma, X., Qian, B. and Zhan, X. (2014). Method for rapidly extracting antioxidative purple sweet potato pigment. Patent Nr. CN104098925.

Jinglue, S. (2011). Purple sweet potato beer and production process thereof. Patent Nr. CN102250700.

Jingyu, S., Haibin, T., Cheng, B. and Huaxian, Y. (2012a). Preparation method for pure natural green high-anthocyanin purple sweet potato beverage. Patent Nr. CN102687881.

Jingyu, S., Huaxian, Y., Cheng, B. and Haibin, T. (2012b). Process for extracting anthocyanin from purple sweet potato with double-enzyme method. Patent Nr. CN102337043.

Jinsong, Y., Haide, Z., Haisheng, T. and Hairui, Z. (2012). Method for preparing sweet potato yellow wine containing anthocyanidin. Patent Nr. CN102344872.

Jinsong, Z. and Bing, D. (2009). Fast-food purple sweet potato vermicelli. Patent Nr. CN101558857.

Jiping, S. and Lin, S. (2009). Preparation process of pure natural sweet potato beverage in original color and taste. Patent Nr. CN101601485.

Jo, H. K., Cho, K. M., Seo, W. T., Lee, J. Y., Lee, D. C. and Kim, D. H. (2013). Nuruk prepared with *Rhizopus oryzae* ccs01 strain and purple sweet potato and makgeolli having enhanced anti-oxidative activity prepared by using the nuruk. KR20130098011.

Joglekar, A. M. and May, A. T. (1987). Product excellence through design of experiments. *Cereal Food World* **32**: 857–868.

Johnson, M. and Pace, R. D. (2010). Sweet potato leaves: properties and synergistic interactions that promote health and prevent disease. *Nutr Rev*. **68**: 604-615.

Ju, J.-H., Yoon, H.-S., Park, H.-J., Shin, H.-K., Park, K.-Y., Yang, J.-O., Sohn, M.-S. and Do, M.-S. (2011). Anti-obesity and antioxidative effects of purple sweet potato extract in 3T3-L1 adipocytes *in vitro*. *J Med Food*. **14**: 1097-1106.

Juanying, X. (2012). Black flour. Patent Nr. CN102405947.

Jung, J.-K., Lee, S.-U., Kozukue, N., Levin, C. E. and Friedman, M. (2011). Distribution of phenolic compounds and antioxidative activities in parts of sweet potato (*Ipomoea batata* L.) plants and in home processed roots. *J Food Comp Anal*. 24: 29-37.

Jung, Y. C., Hwang, Y. J., Ha, I. S., Choi, J. M., Jo, S. J., Seo, C. S., Jeong, H.G. and Hwang, Y. P. (2011a). Manufacturing method for purple sweet potato makgealli. Patent Nr. KR20110057810.

Jung, Y. C., Kang, S. K., Chun, S. S., Jeon, E. W., Hwang, Y. J., Woo, J. B., Kang, D. M.;Jin, S. W.; Woo, J. D.; Kim, H. R. and Song, W. Y. (2007). Manufacturing method of beverage composition comprising purple-fleshed sweet potato. Patent Nr. KR100760263.

Jung, Y. C., Kim, S. K., Hwang, Y. J., Ha, I. S., Choi, J. M., Jo, S. J., Jeong, H. G. and Hwang, Y. P. (2011b). Manufacturing method for purple sweet potato grain drink. Patent Nr. KR101141840.

Jyothi, A. N., Moorthy, S. N. and Eswariamma, C. S. (2005). Anthocyanins in sweet potato leaves-varietal screening, growth phase studies and stability in a model system. *Int J Food Prop*. **8**: 221-232.

Kamata, T. (2006). Processed food and drink of purple sweet potato, and method for producing the same. Patent Nr. JP4422049 (B2).

Kano, M., Takayanagi, T., Harada, K., Makino, K. and Ishikawa, F. (2005). Antioxidative activity of anthocyanins from purple sweet potato, *Ipomoea batatas* cultivar Ayamurasaki. *Biosci Biotechnol Biochem*. **69**: 979-988.

Karna, P., Gundala, S. R., Gupta, M. V., Shamsi, S. A., Pace, R. D., Yates, C., Narayan, S. and Aneja, R. (2011). Polyphenol-rich sweet potato greens extract inhibits proliferation and induces apoptosis in prostate cancer cells *in vitro* and *in vivo*. *Carcinogenesis* **32**: 1872-1880.

Kawano, K., Morimura, S., Mori, E., Matsushita, H., Ohta, H. and Kida, K. (2010). Isolation and identification by cytoprotection assay of antioxidative compound contained in vinegar produced from sweet potato-shochu post-distillation slurry. *Food Sci Technol Res*. **16**: 327-332.

Kim, H. W., Kim, J. B., Cho, S. M., Chung, M. N., Lee, Y. M., Chu, S. M., Che, J. H., Kim, S. N., Kim, S. Y., Cho, Y. S., Kim, J. H., Park, H. J. and Lee, D. J. (2012). Anthocyanin changes in the Korean purple-fleshed sweet potato, Shinzami, as affected by steaming and baking. *Food Chem*. **130**: 966-972.

Kim, H. Y. and Mo, E. K. (2014). Antioxidant capacity of japchae, Korean stir-fried sweet potato noodles with vegetables. *Food Sci Biotechnol*. **23**: 361-364.

Kim, J. B., Kim, S. Y., Chung, M. N., Kim, H. R., Kim, H. W., Lee, Y. M., Kim, S. Y., Cho, Y. S., Kim, S. N. and Cho, S. M. (2014). Anti inflammantory composition comprising anthocyanin. Patent Nr. KR20140065554.

Kim, Y. B., Park, D. S., Jeon, J. H., Shin, S. H., Kim, J. J. and Kim, C. H. (2010). Inhibition of alcoholic hangover and gastric ulcer by sweet potato fermentation fractions. Patent Nr. KR20100062308.

Koncic, M. Z., Petlevski, R. and Kalodera, Z. (2013). Antioxidant activity of *Ipomoea batatas* L. Lam. leaf grown in continental Croatia and its effect on glutathione level in glucose-induced oxidative stress. *Int J Food Prop*. **16**: 964-973.

Konczak, I., Okuno, S. and Yoshimoto, M. (2004). Caffeoylquinic acids generated *in vitro* in a high-anthocyanin-accumulating sweet potato cell line. *J Biomed Biotechnol*. **5**: 287-292.

Konczak-Islam, I., Okuno, S., Yoshimoto, M. and Yamakawa, O. (2003a). Composition of phenolics and anthocyanins in a sweet potato cell suspension culture. *Biochem Eng J*. **14**: 155-161.

Konczak-Islam, I., Yoshimoto, M., Hou, D. X., Terahara, N. and Yamakawa, O. (2003b). Potential chemopreventive properties of anthocyanin-rich aqueous extracts from *in vitro* produced tissue of sweetpotato (*Ipomoea batatas* L.). *J Agric Food Chem*. **51**: 5916-5922.

Krishnan, J. G., Padmaja, G., Moorthy, S. N., Suja, G. and Sajeev, M. S. (2010). Effect of pre-soaking treatments on the nutritional profile and browning index of sweet potato and yam flours. *Innov Food Sci Emerg Technol*. **11**: 387-393.

Kurata, R., Adachi, M., Yamakawa, O. and Yoshimoto, M. (2007). Growth suppression of human cancer cells by polyphenolics from sweetpotato (*Ipomoea batatas* L.) leaves. *J Agric Food Chem*. **55**: 185-190.

Kwon, C. M. (2014). Health supplement using *Dendropanax morbifera* lev and method for producing same. Patent Nr. WO2014185653.

Kwon, T. H., Kim, J. W., Lee, B. Y., Mun, E. G., Yu, K. Y., Kim, J., Doo, H. S., Jeong, S. I., Yang, C. M. and Yoon, S. K. (2012). Method for producing Dangmyen with enhanced antioxidative activity adding purple sweet potato and Dangmyen produced by the same method. Patent Nr. KR20120056379.

Lee, B. U. (2013). Colors and improved functional pizza dough and pizza, and its manufacturing method and manufacturing method using the same. Patent Nr. KR20130106041.

Lee, B. Y., Kim, J. W., Jeong, S. I., Lee, Y. E. and Chung, C. H. (2014). Method for producing cookie using makgeolli suljigemi. Patent Nr. KR20140085711.

Li, Y. (2013). Manufacturing method for sweet vegetarian chicken with purple sweet potato starch interlayer. Patent Nr. CN103250807.

Li, B. (2014a). Health food for preventing female climacteric syndrome. Patent Nr. CN103689571.

Li, B. (2014b). Health-keeping traditional Chinese medicine fine dried noodles. Patent Nr. CN103652613.

Li, C. and Zhang, L. (2013). *In vivo* anti-fatigue activity of total flavonoids from sweetpotato *Ipomoea batatas* (L.) Lam. leaf in mice. *Indian J Biochem Biophy*. **50**: 326-329.

Li, C., Dajing, L., Chunquan, L., Jiangfeng, S., Chunju, L. and Haihong, W. (2009). Kraut preparation method and product thereof using sweet potato stem and leaf as raw material. Patent Nr. CN101507485.

Li, C., Wu, Y. and Wang, Y. (2014). Preparation method of flavored nutritional purple sweet potato paste. Patent Nr. CN103932110.

Li, F., Li, Q., Gao, D. and Peng, Y. (2009). The optimal extraction parameters and anti-diabetic activity of flavonoids from *Ipomoea batatas* leaf. *Afr J Tradit Complement Altern Med*. **6**: 195-202.

Li, J. Y., Dong, G. P., Li, M. L., Liu, Z. H. and Lu, Y. (2012). Efficient counter-current chromatographic isolation and structural identification of phenolic compounds from sweet potato leaves. *J Liq Chrom Rel Technol*. **35**: 1517-1527.

Li, J., Li, J., Pang, X. and Li, W. (2012). Sea cucumber anthocyanin flour and preparation method of vermicelli. Patent Nr. CN102813158.

Li, J., Li, X.-d., Zhang, Y., Zheng, Z.-d., Qu, Z.-y., Liu, M., Zhu, S., Liu S. Wang, M. and Qu, L. (2013). Identification and thermal stability of purple-fleshed sweet potato anthocyanins in aqueous solutions with various pH values and fruit juices. *Food Chem*. **136**: 1429-1434.

Li, J., Song, H., Dong, N. and Zhao, G. (2014). Degradation kinetics of anthocyanins from purple sweet potato (*Ipomoea batatas* L.) as affected by ascorbic acid. *Food Sci Biotechnol*. **23**: 89-96.

Li, T. and Li, S. (2013). Preparation method of purple sweet potato wine. Patent Nr. CN103146535.

Li, Z., Zhu, Y., Wang, D., Xie, J., Huang, C., Wang, Z., You, L., Shen, X., Tu, Q. and Li, P. (2013). Preparation method of purple sweet potato fermented product Patent Nr. CN103004988.

Liao, W. C., Lai, Y.-C., Yuan, M.-C., Hsu, Y.-L. and Chan, C.-F. (2011). Antioxidative activity of water extract of sweet potato leaves in Taiwan. *Food Chem*. **127**: 1224-1228.

Lichao, Z., Xin, L., Lihua, H. and Yongqiang, Y. (2009). Method for producing water chestnut sheet jelly. Patent Nr. CN101569387.

Lien, C. Y., Lee, A. Y. F., Chan, C. F., Lai, Y. C., Huang, C. L. and Liao, W. C. (2010). Extraction parameter studies for anthocyanin extraction from purple sweet potato variety TNG73, *Ipomoea batatas*, L. *Appl Eng Agric*. **26**: 441-446.

Lien, C.-Y., Chan, C.-F., Lai, Y.-C., Huang, C.-L. and Liao, W. C. (2012). Ultrasound-Assisted Anthocyanin Extraction of Purple Sweet Potato Variety TNG73, *Ipomoea batatas*, L. *Separ Sci Technol*. **47**: 1241-1247.

Lim, S., Xu, J., Kim, J., Chen, T.-Y., Su, X., Standard, J., Carey, E., Griffin, J., Herndon, B., Katz, B.; Tomich, J. and Wang, W. (2013). Role of anthocyanin-enriched purple-fleshed sweet potato p40 in colorectal cancer prevention. *Mol Nutr Food Res*. **57**: 1908-1917.

Liming, Z., Haiyan, Z., Qingmei, W., Aixian, L., Fuyun, H., Beitao, X. and Shunxu, D. (2011). Sweet potato for processing preserved sweet potato and cultivation method and application thereof. Patent Nr. CN101946613.

Lin, K.-H., Chao, P.-Y., Yang, C.-M., Cheng, W.-C., Lo, H.-F. and Chang, T.-R. (2006). The effects of flooding and drought stresses on the antioxidant constituents in sweet potato leaves. *Bot Stud*. **47**: 417-426.

Lin, S. and Jiping, S. (2009). Preparation process of pure natural sweet red wine made from red potato. Patent Nr. CN101602995.

Liu, C., Chen, J., Li, T., Liang, R., Liu, W. and Luo, S. (2014). *Choerospondias axillaris* peel nutritional jelly drops enriched with polyphenol and preparation method thereof. Patent Nr. CN103960450.

Liu, H. (2013). Method for extracting purple sweet potato anthocyanin. Patent Nr. CN102993154.

Liu, L. (2013). Extraction method of anthocyanin from purple sweet potato. Patent Nr. CN102977067.

Lixin, W., Di, T., Chi, W., Zhihang, Y., Sha, Z., Yan, T., Jingjing, W. and Fenghui, L. (2011). Sweet-potato beverage and manufacturing method thereof. Patent Nr. CN101972017.

Lu, J. (2014a). Anti-cancer vegetable chip and preparation method thereof. Patent Nr. CN103932097.

Lu, J. (2014b). Purple sweet potato chips capable of reducing blood press and preparation method thereof. Patent Nr. CN103932099.

Lu, K. (2014). Seafood flavor thick broad-bean sauce and making method thereof. Patent Nr. CN104106790.

Lu, L.-Z., Zhou, Y.-Z., Zhang, Y.-Q., Ma, Y.-L., Zhou, L.-X., Li, L., Zhou, Z.-Z. and He, T.-Z. (2010). Anthocyanin extracts from purple sweet potato by means of microwave baking and acidified electrolysed water and their antioxidation *in vitro*. *Int J Food Sci Technol*. **45**: 1378-1385.

Luo, H., Wang, X., Wang, F., Wang, C., Gou, R. and Xiao, H. (2014). Purple sweet potato wine and preparation method thereof. Patent Nr. CN103789141.

Ma, J. (2013). Enzyme dietary therapy liquid for removing toxins in human body, and preparation method thereof. Patent Nr. CN103005403.

Maloney, K. P., Truong, V. D. and Allen, J. C. (2012). Chemical optimization of protein extraction from sweet potato (*Ipomoea batatas*) peel. *J Food Sci*. **77**: E307-E312.

Min, J.-Y., Kang, S.-M., Park, D.-J., Kim, Y.-D., Jung, H.-N., Yang, J.-K., Seo, W.-T., Kim, S.-W., Karigar, C. S. and Choi, M.-S. (2006). Enzymatic release of ferulic acid from *Ipomoea batatas* L. (sweet potato) stem. *Biotechnol Bioprocess Eng*. **11**: 372-376.

Mingna, W., Xiaojing, Y., Hailong, H. and Haibin, Z. (2011). Antioxidative yoghurt and preparation method thereof. Patent Nr. CN102125090.

Minyao, L., Yuxia, H., Liang, L., Yuxia, L. and Xiaojin, L. (2011). Method for preparing whole purple sweet potato powder by using microwave vacuum drying. Patent Nr. CN102106521.

Mohanraj, R. and Sivasankar, S. (2014). Sweet Potato (*Ipomoea batatas* [L.] Lam)-a valuable medicinal food: A review. *J Med Food*. **17**: 733-741.

Montilla, E. C., Hillebrand, S., Butschbach, D., Baldermann, S., Watanabe, N. and Winterhalter, P. (2010). Preparative isolation of anthocyanins from japanese purple sweet potato (*Ipomoea batatas* L.) varieties by high-speed countercurrent chromatography. *J Agric Food Chem*. **58**: 9899-9904.

Mou, D., Li, Y., Sun, P. and Zhou, F. (2014). Apple and purple sweet potato clear juice type anthocyanin beverage and preparation method thereof. Patent Nr. CN103976425.

Mu, T., Liu, X., Sun, H., Zhang, M. and Chen, J. (2013a). Method for extracting anthocyanin. Patent Nr. CN103145681.

Mu, T., Xi, L., Sun, H., Zhang, M. and Chen, J. (2013b). Sweet potato stem leaf polyphenol and preparation method thereof. Patent Nr. CN103393882.

Na, K., Lee, K. Y. and Kim, K. T. (2013). Estern prickly pear starch gel including estern prickly pear and the method for making the estern prickly pear starch gel. Patent Nr. KR20130072485.

Nagai, M., Tani, M., Kishimoto, Y., Iizuka, M., Saita, E., Toyozaki, M., Kamiya, T., Ikeguchi, M. and Kondo, K. (2011). Sweet potato (*Ipomoea batatas* L.) leaves suppressed oxidation of low density lipoprotein (LDL) *in vitro* and in human subjects. *J Clin Biochem Nutr*. **48**: 203-208.

Ning, J., Dajing, L., Zhiqiang, L., Chunju, L., Chunquan, L., Jiangfeng, S., Haihong, W., Ying., Z. and Danyu, Z. (2010). Method for preparing antioxidative active extractive of sweet potato leaves. Patent Nr. CN101773593.

Niwa, A., Tajiri, T. and Higashino, H. (2011). *Ipomoea batatas* and *Agarics blazei* ameliorate diabetic disorders with therapeutic antioxidant potential in streptozotocin-induced diabetic rats. *J Clin Biochem Nutr*. **48**: 194-202.

Not_acessible. (2007). Fermented alcoholic drink with purple sweet potato and oriental herb, and process of itself. Patent Nr. KR100700747.

Not_acessible. (2011). Preparation method for rice snack including purple sweet potato. Patent Nr. KR20120130807.

Oba, R. and Noujiyo, H. (2008). Processed food product having yacon as main raw material, and method for producing the same. Patent Nr. JP2008141966.

Oba, R. and Saegusa, T. (2003). Method for producing fermented liquor containing anthocyanin. Patent Nr. JP4251388.

Oba, R. and Sasaki, Y. (2006). Method for producing anthocyanin-containing low calorie lactic acid bacteria beverage. Patent Nr. JP3973108.

Oiwa, K. and Oiwa, M. (2007). Unrefined sake vinegar of purple sweet potato, and method for producing the same. Patent Nr. JP2007274984.

Ojeda, G. A., Sgroppo, S. C. and Zaritzky, N. E. (2014). Application of edible coatings in minimally processed sweet potatoes (*Ipomoea batatas* L.) to prevent enzymatic browning. *Int J Food Sci Technol*. **49**: 876-883.

Oki, T., Osame, M., Masuda, M., Kobayashi, M., Furuta, S., Nishiba, Y., Kumagai, T., Sato, T. and Suda, I. (2003). Simple and rapid spectrophotometric method for selecting purple-fleshed sweet potato cultivars with a high radical-scavenging activity. *Breeding Sci*. **53**: 101-107.

Oladejo, A. O. (2011). An evaluation of some peeling methods for sweet potato (*Ipomea batatas*). Master's Thesis. University of Agriculture-Abeokuta, Nigeria.

Padda, M. S. and Picha, D. H. (2007). Methodology optimization for quantification of total phenolics and individual phenolic acids in sweetpotato (*Ipomoea batatas* L.) roots. *J Food Sci*. **72**: C412-C416.

Panda, S. H. and Ray, R. C. (2007). Lactic acid fermentation of beta-carotene rich sweet potato (*Ipomoea batatas* L.) into lacto-juice. *Plant Food Hum Nutr*. **62**: 65-70.

Panda, S. H., Naskar, S. K., Sivakumar, P. S. and Ray, R. C. (2009a). Lactic acid fermentation of anthocyanin-rich sweet potato (*Ipomoea batatas* L.) into lacto-juice. *Int J Food Sci Technol.* **44**: 288-296.

Panda, S. H., Panda, S., Sivakumar, P. S. and Ray, R. C. (2009b). Anthocyanin-rich sweet potato lacto-pickle: production, nutritional and proximate composition. *Int J Food Sci Technol*. **44**: 445-455.

Panda, S. K., Swain, M. R., Singh, S. and Ray, R. C. (2013). Proximate compositions of a herbal purple sweet potato (*Ipomoea batatas* L.) wine. *J Food Process Preservation* **37**: 596-604.

Panda, V. and Sonkamble, M. (2012). Phytochemical constituents and pharmacological activities of *Ipomoea batatas* L. (Lam) - a review. *Int J Res Phytochem Pharm*. **2**: 25-34.

Park, K.-H., Kim, J.-R., Lee, J.-S., Lee, H. and Cho, K.-H. (2010). Ethanol and water extract of purple sweet potato exhibits anti-atherosclerotic activity and inhibits protein glycation. *J Med Food* **13**: 91-98.

Peng, C. A. (2013). Purple sweet potato bread processing method. Patent Nr. CN103039561.

Peng, Z., Li, J., Guan, Y. and Zhao, G. (2013). Effect of carriers on physicochemical properties, antioxidant activities and biological components of spray-dried purple sweet potato flours. *LWT-Food Sci Technol*. **51**: 348-355.

Peschel, W., Sánchez-Rabaneda, F., Diekmann, W., Plescher, A., Gartzía, I., Jiménez, D., Lamuela-Raventos, R., Buxaderas, S. and Codina, C. (2006). An industrial approach in the search of natural antioxidants from vegetable and fruit wastes. *Food Chem*. **97**: 137-150.

Philpott, M., Ferguson, L. R., Gould, K. S. and Harris, P. J. (2009). Anthocyanidin-containing compounds occur in the periderm cell walls of the storage roots of sweet potato (*Ipomoea batatas*). *J Plant Physiol*. **166**: 1112-1117.

Pi, Z. and Li, L. (2014). Extraction method of purple sweet potato anthocyanin. Patent Nr. CN103665072.

Pochapski, M. T., Fosquiera, E. C., Esmerino, L. A., dos Santos, E. B., Farago, P. V., Santos, F. A. and Groppo, F. C. (2011). Phytochemical screening, antioxidant, and antimicrobial activities of the crude leaves extract from *Ipomoea batatas* (L.) Lam. *Pharmacogn Mag*. **7**: 165-170.

Qiang, Z. (2012). Sweet potato leaf beer and preparation process thereof. Patent Nr. CN102453645.

Qin, Y. (2014). Honey freeze-dried powder and preparation method thereof. Patent Nr. CN104082651.

Qiong, Z. (2014). Formula of purple sweet potato beverage. Patent Nr. CN103636793.

Qiu, F., Luo, J., Yao, S., Ma, L. and Kong, L. (2009). Preparative isolation and purification of anthocyanins from purple sweet potato by high-speed counter-current chromatography. *J Sep Sci*. **32**: 2146-2151.

Qiuyun, H. (2012). Method for producing whole sweet potato powder. Patent Nr. CN102511754.

Quanan, D. and Yubo, L. (2012). Anthocyanidin rice wine and method for preparing same. Patent Nr. CN102757878.

Quanan, D., Zhaojiang, L., Yubo, L. and Xiaoling, L. (2011). Method for rapidly extracting anthocyanin from purple sweet potato through crushing method. Patent Nr. CN102161652.

Rabah, I. O., Hou, D. X., Komine, S. I. and Fujii, M. (2004). Potential chemopreventive properties of extract from baked sweet potato (*Ipomoea batatas* Lam. cv. Koganesengan). *J Agric Food Chem*. **52**: 7152-7157.

Rabah, I. O., Hou, D. X., Komine, S., Shono, M. and Fujii, M. (2005). Increase in antioxidant and cytotoxicity through apoptosis-induction on HL-60 of sweet potato (*Ipomoea batatas* Lam. cv. Koganesengan) by sub-critical water treatment. *Food Sci Technol Res*. **11**: 122-126.

Rao, X., Chen, L. and Wang, L. (2014). Production method for purple sweet potato peanuts. Patent Nr. CN103828997.

Ray, R. C., Panda, S. K., Swain, M. R. and Sivakumar, P. S. (2012). Proximate composition and sensory evaluation of anthocyanin-rich purple sweet potato (*Ipomoea batatas* L.) wine. *Int J Food Sci Technol*. **47**: 452-458.

Redovnikovic, I. R., Bogovic, M., Belko, D., Delonga, K., Fabek, S., Novak, B. and Toth, N. (2012). Influence of potassium fertilisation on the levels of phenolic compounds in sweet potato (*Ipomoea batatas* L.) leaves. *J Hortic Sci Biotech*. **87**: 47-51.

Roy, S., Banerjee, A., Tarafdar, J. and Mitra, S. (2012). Tuber quality assessment of orange-fleshed sweet potato (*Ipomoea batatas*) cultivars and their genetic relatedness as revealed by SDS-PAGE of tuber proteins. *Indian J Agric Sci*. **82**: 482-488.

Rumbaoa, R. G. O., Cornago, D. F. and Geronimo, I. M. (2009). Phenolic content and antioxidant capacity of Philippine sweet potato (*Ipomoea batatas*) varieties. *Food Chem*. 113: 1133-1138.

Ryu, S. H. and Lee, Y. N. (2013). Manufacturing method of premix with purple sweet potato and cut noodles, wheat flakes and cold noodles using the same. Patent Nr. KR101324519.

Saeki, A., Oda, K. and Isobe, T. (2006). Method for producing purple sweet potato (*Ipomoea batatas*) vinegar. Patent Nr. JP2006197826.

Saigusa, N., Kawashima, N. and Ohba, R. (2007). Maintaining the anthocyanin content and improvement of the aroma of an alcoholic fermented beverage produced from raw purple-fleshed sweet potato. *Food Sci Technol Res*. **13**: 23-27.

Saigusa, N., Terahara, N. and Ohba, R. (2005). Evaluation of DPPH-radical-scavenging activity and antimutagenicity and analysis of anthocyanins in an alcoholic fermented beverage produced from cooked or raw purple-fleshed sweet potato (*Ipomoea batatas* cv. Ayamurasaki) roots. *Food Sci Technol Res*. **11**: 390-394.

San, Q., Lu, J., Zheng, Y., Li, J., Zhou, Z., Hu, B., Zhang, Z., Fan, S., Mao, Z., Wang, Y.-J. and Ma, D. (2009). Purple sweet potato color ameliorates cognition deficits and attenuates oxidative damage and inflammation in aging mouse brain induced by D-Galactose. *J Biomed Biotechnol*. Article ID 564737.

Sasaki, K., Han, J., Shimozono, H., Villareal, M. O. and Isoda, H. (2013). Caffeoylquinic acid-rich purple sweet potato extract, with or without anthocyanin, imparts neuroprotection and contributes to the improvement of spatial learning and memory of SAMP8 mouse. *J Agric Food Chem.* **61**: 5037-5045.

Sasaki, K., Oki, T., Kobayashi, T., Kai, Y. and Okuno, S. (2014). Single-laboratory validation for the determination of caffeic acid and seven caffeoylquinic acids in sweet potato leaves. *Biosci Biotechnol Biochem*. **78**: 2073-2080.

Sasaki, Y. and Ohba, R. (2004). Antioxidant activity and optimal manufacturing conditions of purple sweet potato lactic acid bacteria drink. *Food Sci Technol Res*. **10**: 447-452.

Seo, W. S., Taek, K. Y., Cha, J. Y. and Lee, Y. S. (2010). Fermented alcoholic drink with purple sweet potato and producing method of thereof. Patent Nr. KR101067682.

Shan, S., Zhu, K.-X., Peng, W. and Zhou, H.-M. (2013). Physicochemical properties and salted noodle-making quality of purple sweet potato flour and wheat flour blends. *J Food Process Preservation* **37**: 709-716.

Shao, Y. (2014). Anthocyanin sausage and preparation method thereof. Patent Nr. CN103653026.

Shao, Y.-Y. and Huang, Y.-C. (2008). Effects of steaming and kneading with presteaming treatments on the physicochemical properties of various genotypes of sweet potato (*Ipomoea batatas* L.). *J Food Process Eng*. **31**: 739-753.

Shi, F. (2013). Preparation method of purple sweet potato health product composition. Patent Nr. CN103190613.

Shuhua, Z., Jie, Z., Rongxin, G., Yuchen, L., Lili, Z., Changbao, C. and Peiying, S. (2012). Purple sweet potato anthocyanin capsule. Patent Nr. CN102302471.

Shuyong, Z. (2011). Method for extracting purple sweet potato anthocyanin. Patent Nr. CN102020868.

Soison, B., Jangchud, K., Jangchud, A., Harnsilawat, T., Piyachomkwan, K., Charunuch, C. and Prinyawiwatkul, W. (2014). Physico-functional and antioxidant properties of purple-flesh sweet potato flours as affected by extrusion and drum-drying treatments. *Int J Food Sci Technol.* **49**: 2067-2075.

Song, B. J., Sapper, T. N., Burtch, C. E., Brimmer, K., Goldschmidt, M. and Ferruzzi, M. G. (2013). Photo- and thermodegradation of anthocyanins from grape and purple sweet potato in model beverage systems. *J Agric Food Chem*. **61**: 1364-1372.

Song, J., Li, D., Liu, C. and Zhang, Y. (2011). Optimized microwave-assisted extraction of total phenolics (TP) from *Ipomoea batatas* leaves and its antioxidant activity. *Innov Food Sci Emerg Technol*. **12**: 282-287.

Stegmann, J. and Grohmann, G. (2003). Hypothesis generation guided by co-word clustering. *Scientometrics* **56**: 111-135.

Suda, I., Ishikawa, F., Hatakeyama, M., Miyawaki, M., Kudo, T., Hirano, K., Ito, A., Yamakawa, O. and Horiuchi, S. (2008). Intake of purple sweet potato beverage affects on serum hepatic biomarker levels of healthy adult men with borderline hepatitis. *Eur J Clin Nutr*. **62**: 60-67.

Sugawara, A., Ko, K., Fukazawa, H., Yoshimoto, M., Takagaki, K. and Mori, S. (2005). Method for producing processed product of leaf and stem of sweet potato. Patent Nr. JP2005278596.

Sun, F. (2014). Purple sweet potato health care noodle and preparation method thereof. Patent Nr. CN103976251.

Sun, H., Mu, T., Liu, X., Zhang, M. and Chen, J. (2014a). Purple sweet potato (*Ipomoea batatas* L.) anthocyanins: Preventive effect on acute and subacute alcoholic liver damage and dealcoholic effect. *J Agric Food Chem*. **62**: 2364-2373.

Sun, H., Mu, T., Xi, L. and Song, Z. (2014b). Effects of domestic cooking methods on polyphenols and antioxidant activity of sweet potato leaves. *J Agric Food Chem*. **62**: 8982-8989.

Sun, H., Mu, T., Xi, L., Zhang, M. and Chen, J. (2014c). Sweet potato (*Ipomoea batatas* L.) leaves as nutritional and functional foods. *Food Chem*. **156**: 380-389.

Sun, Y., Yang, B. and Wu, L. (2013). Health-care food capable of delaying female climacteric syndrome and preparation method thereof. Patent Nr. CN103330201.

Suzuki, S., Kitani, S. and Yasutani, I. (2006). Sweet potato stem extract and use thereof. Patent Nr. WO2006014028.

Taira, J., Taira, K., Ohmine, W. and Nagata, J. (2013). Mineral determination and anti-LDL oxidation activity of sweet potato (*Ipomoea batatas* L.) leaves. *J Food Comp Anal*. **29**: 117-125.

Takagaki, K. and Tsubata, M. (2009). Antioxidant food. Patent Nr. JP2009011163.

Takenaka, M., Nanayama, K., Isobe, S. and Murata, M. (2006). Changes in caffeic acid derivatives in sweet potato (*Ipomoea batatas* L.) during cooking and processing. *Biosci Biotechnol Biochem*. **70**: 172-177.

Tang, X., Tang, J. and Tang, H. (2005). Insoluble diet fibre preparation method and its product. Patent Nr. CN1620921.

Tao, Y., Cheng, H., Zhang, Y. and Zhang, D. (2014). Sweet potato powder and preparation method thereof. Patent Nr. CN103535660.

Teow, C. C., Truong, V.-D., McFeeters, R. F., Thompson, R. L., Pecota, K. V. and Yencho, G. C. (2007). Antioxidant activities, phenolic and beta-carotene contents of sweet potato genotypes with varying flesh colours. *Food Chem*. **103**: 829-838.

Tian, Q. G., Konczak, I. and Schwartz, S. J. (2005). Probing anthocyanin profiles in purple sweet potato cell line (*Ipomoea batatas* L. cv. Ayamurasaki) by high-performance liquid chromatography and electrospray ionization tandem mass spectrometry. *J Agric Food Chem*. 53: 6503-6509.

Tianyin, L. (2011). Sweet potato beverage and preparation method thereof. Patent Nr. CN101953489.

Tokusoglu, O. and Yildirim, Z. (2012). Effects of cooking methods on the anthocyanin levels and antioxidant activity of a local Turkish sweetpotato *Ipomoea batatas* (L.) lam cultivar hatay kirmizi: boiling, steaming and frying effects. *Turk J Field Crops* **17**: 87-90.

Tong, W., Lin, Q. and Tong, H. (2014). Rye glutinous rice flour and preparation method thereof. Patent Nr. CN103815238.

Truong, V. D., Hu, Z., Thompson, R. L., Yencho, G. C. and Pecota, K. V. (2012). Pressurized liquid extraction and quantification of anthocyanins in purple-fleshed sweet potato genotypes. *J Food Comp Anal*. **26**: 96-103.

Tsukada, S., Ikeda, K., Yoshimoto, M., Kurata, R., Fujii, M. and Ko, N. (2004). Agent for suppressing cancer cell growth and method for producing the same. Patent Nr. JP2004352681.

Wang, F., Tan, X., Zhang, Y., Shi, Y. and Tan, Y. (2013). Method for producing low-methanol purple sweet potato liquor. Patent Nr. CN103243003.

Wang, K. (2014). Spleen-invigorating appetizing healthy rice and preparation method thereof. Patent Nr. CN104106779.

Wang, Q. (2014a). Selenium-rich granulated toffee. Patent Nr. CN103609808.

Wang, Q. (2014b). Sweet-orange flavour nutritious and healthcare toffee. Patent Nr. CN103609813.

Wang, S., Xiao, J., Zhang, Y., Jiang, W. and Sun, L. (2013). Extraction and purification method for anthocyanin in purple sweet potato. Patent Nr. CN103193839.

Wang, S.-M., Yu, D.-J. and Song, K. B. (2011). Quality characteristics of purple sweet potato (*Ipomoea batatas*) slices dehydrated by the addition of maltodextrin. *Hortic Environ Biotechnol*. **52**: 435-441.

Wang, Y. (2014). Simple and convenient method for rapidly extracting anthocyanin from purple sweet potatoes. Patent Nr. CN103601712.

Wang, Y., Liu, F., Cao, X., Chen, F., Hu, X. and Liao, X. (2012). Comparison of high hydrostatic pressure and high temperature short time processing on quality of purple sweet potato nectar. *Innov Food Sci Emerg Technol*. 16: 326-334.

Wang, Z. W. (2006). Capsule of purple sweet potato, its production and application. Patent Nr. CN1733053.

Wei, H. (2014). Composite purple sweet potato health care rice and preparation method thereof. Patent Nr. CN103689339.

Wireko-Manu, F., Ellis, W. and Oduro, I. (2010). Production of a non-alcoholic beverage from sweet potato (*Ipomoea batatas* L.). *Afr J Food Sci*. **4**: 180-183.

Wong, W.-C., Wu, J., Chen, J. J. and Quirk, B. (2013). Method for producing purple sweet potato juice and dried powder. Patent Nr. US2013309355.

Wu, T.-Y., Tsai, C.-C., Hwang, Y.-T. and Chiu, T.-H. (2012). Effect of antioxidant activity and functional properties of chingshey purple sweet potato fermented milk by *Lactobacillus acidophilus*, *L. delbrueckii* subsp *lactis,* and *L. gasseri* strains. *J Food Sci.* 77: M2-M8.

Wu, X. (2013). Preparation method of vegetable crisp of combination purple sweet potato and purple cabbage. Patent Nr. CN103461911.

Xiangdong, Y. (2012). Anthocyanidin beverage formula and its production technology. Patent Nr. CN102389135.

Xianxiang, L. (2008). Method for separating and extracting purple sweet potato pigment. Patent Nr. CN101235215.

Xiaoling, L., Zesheng, Z., Ping, S., Shuqing, M. and Hui, F. (2009). Preparation method for cation exchange resin secondarily purified anthocyanins pigment from purple sweet potato. Patent Nr. CN101343298.

Xiaosong, L. (2010). Rice/flour starch food containing proanthocyanidin and anthocyanin. Patent Nr. CN101869237.

Xiaoyan, W. (2012). Health food using purple sweet potato and sea-buckthorn as main raw materials and preparation method thereof. Patent Nr. CN102488196.

Xiguang, Q., Hui, Z., Li, W. and Haifeng, Q. (2012). Hawthorn and purple sweet potato cloudy juice drink and preparation method thereof. Patent Nr. CN102599584.

Xingcang, J. (2011). Processing method of purple sweet potato bean curd. Patent Nr. CN102187905.

Xu, D. (2014). Anthocyanidin-rich noodle and making method thereof. Patent Nr. CN103989060.

Xu, G. and Xu, H. (2014a). Purple sweet potato anthocyanin childhood nutrition noodles. Patent Nr. CN103494078.

Xu, G. and Xu, H. (2014b). Purple sweet potato anthocyanin fine dried noodles. Patent Nr. CN103494077.

Xu, W., Liu, L., Hu, B., Sun, Y., Ye, H., Ma, D. and Zeng, X. (2010). TPC in the leaves of 116 sweet potato (*Ipomoea batatas* L.) varieties and Pushu 53 leaf extracts. *J Food Comp Anal*. **23**: 599-604.

Xuesong, H. (2012). Purple sweet potato rice dumpling and preparation method thereof. Patent Nr. CN102657310.

Yan, Z., LI, C., Huang, W., Wang, X. and Wang, F. (2012). Production method of blackberry and purple sweet potato compound juice and product of production method. Patent Nr. CN102835707.

Yang, R. (2014a). Blood pressure-reduction and swelling-reduction flour and preparation method thereof. Patent Nr. CN103689333.

Yang, R. (2014b). Chicken liver-containing blood-enriching health flour and preparation method thereof. Patent Nr.CN103689331.

Yanxiang, G. (2011). Processing technology of clear purple sweet potato concentrated juice. Patent Nr. CN102119773.

Yao, M., Zhang, N. and Jin, X. (2014). New purple sweet potato anthocyanin production method by extraction of purple sweet potato. Patent Nr. CN103980244.

Yasumoto, T., Naoki, H., Tokeshi, K., Tsunami, K. and Takumi, Y. (2006). Method for acquiring sweet potato functional essence. Patent Nr. JP2005348660.

Ye, J., Meng, X., Yan, C. and Wang, C. (2010). Effect of purple sweet potato anthocyanins on beta-amyloid-mediated PC-12 cells death by inhibition of oxidative stress. *Neurochem Res*. **35**: 357-365.

Ye, X. J., Morimura, S., Han, L. S., Shigematsu, T. and Kida, K. (2004). *In vitro* evaluation of physiological activity of vinegar produced from barley-, sweet potato-, and rice-shochu post-distillation slurry. *Biosci Biotechnol Biochem*. **68**: 551-556.

Ye, X. and Li, K. (2004). Extracting process for purple sweet potato antocynidin and mucin. Patent Nr. CN1554710.

Yin, Q., Chen, M. and Liu, Y. (2003). Process for extracting anthocyanin of purple sweet potato by utilizing citric acid solution. Patent Nr. CN1460694.

Yongbin, H., Xiaoyuan, S., Yanping, F., Peilin, Z., Deping, F. and Peiqi, C. (2012a). Purple sweet potato-blackberry composite pulp beverage and its preparation method. Patent Nr. CN102356903.

Yongbin, H., Yanping, F., Wenxiang, J. and Deping, F. (2012b). Puffed glutinous rice purple sweet potato crisp chip and production method thereof. Patent Nr. CN102726689.

Yoon, B. and Park, Y. (2004). A text-mining-based patent network: Analytical tool for high-technology trend. *J High Technol Manage Res*. **15**: 37-50.

Yuan, K., Jia, S., Lin, Y., Zhu, L. and Yan, H. (2013). Natural food additive. Patent Nr. CN103462037.

Yuanzheng, S., Genna, B., Liuyong, Z., Hua, L and Guowen, M. (2011). Antioxidant milk product and preparation method thereof. Patent Nr. CN101990951.

Yubao, X. (2010). Green tea vermicelli and production process thereof. Patent Nr. CN101744067.

Yubo, L. (2010). Anthocyanin rice wine and making method thereof. Patent Nr. CN101906371.

Yunli, Y. (2008). Method for extracting purple sweet potato anthocyanin pigments by employing invoice process. Patent Nr. CN101255453.

Zeng, S., Chen, J., Liu, W., Zhang, L., Zhang, Y., Zheng, B. and Chen, L. (2014). Purple sweet potato spring roll wraps and making process thereof. Patent Nr. CN103719196.

Zhang, J., Tang, G., Zhang, M. and Zhang, Y. (2014). Purple sweet potato and coarse-cereal compound nutrient health rice with high protein high protein content and rich selenium, zinc and calcium. Patent Nr. CN103689342.

Zhang, L. W. (2007). Process of producing purple preserved sweet potato with Jishu-18 sweet potato. Patent Nr. CN101019630.

Zhang, X., Wu, Z., Weng, P., Yang, Y. and Chen, J. (2014). Preparation method for antioxidative soybean oil containing purple sweet potato anthocyanins. Patent Nr.CN104082432.

Zhang, Z. (2013). Fermentation wine and preparation method thereof, and liqueur and preparation method thereof. Patent Nr. CN103320252.

Zhang, Z.-F., Fan, S.-H., Zheng, Y.-L., Lu, J., Wu, D.-M., Shan, Q. and Hu, B. (2009). Purple sweet potato color attenuates oxidative stress and inflammatory response induced by D-galactose in mouse liver. *Food Chem Toxicol*. **47**: 496-501.

Zhang, Z.-F., Lu, J., Zheng, Y.-L., Wu, D.-M., Hu, B., Shan, Q., Cheng, W., Li, M.-Q. and Sun, Y.-Y. (2013). Purple sweet potato color attenuates hepatic insulin resistance via blocking oxidative stress and endoplasmic reticulum stress in high-fat-diet-treated mice. *J Nutr Biochem*. **24**: 1008-1018.

Zhao, G. (2013). Blueberry chew product and preparation method thereof. Patent Nr. CN103431265.

Zhao, J.-G., Yan, Q.-Q., Lu, L.-Z. and Zhang, Y.-Q. (2013). *In vivo* antioxidant, hypoglycemic, and anti-tumor activities of anthocyanin extracts from purple sweet potato. *Nutr Res Prac*. **7**: 359-365.

Zhao, J.-G., Yan, Q.-Q., Xue, R.-Y., Zhang, J. and Zhang, Y.-Q. (2014). Isolation and identification of colourless caffeoyl compounds in purple sweet potato by HPLC-DAD–ESI/MS and their antioxidant activities. *Food Chem*. **161**: 22-26.

Zhao, R., Li, Q., Long, L., Li, J., Yang, R. and Gao, D. (2007). Antidiabetic activity of flavone from *Ipomoea batatas* leaf in non-insulin dependent diabetic rats. *Int J Food Sci Technol*. **42**: 80-85.

Zhao, X., Xiangyan, C., Chen, F., Chen, J., Wang, X. and Deng, P. (2013). Ultra-fine fruit-vegetable nutrient mungbean noodle and preparation method thereof. Patent Nr. CN103053909.

Zhao, Z., Jin, Y., Zeng, H. and Tu, C. (2014). Novel granular tea and preparation technology thereof. Patent Nr. CN104115976.

Zhenchang, W., Yuejin, H., Hongjiang, S., Xiaolong, M., Quanneng, Z., Hong, Y. and Shixuan, S. (2012). Preparation method of purple sweet potato powder. Patent Nr. CN102697005.

Zheng, Y., Chunyang, L., Ning, J. and Naifu, W. (2009). Production method of raw juice of purple sweet potato and product thereof. Patent Nr. CN101507518.

Zhenxin, G., Shuyu, Z., Yongqi, Y. and Chunyan, X. (2012a). Purple sweet potato vinegar and brewing process thereof. Patent Nr. CN102690750.

Zhenxin, G., Yongqi, Y and Shuyu, Z. (2012b). Purple sweet potato vinegar healthcare beverage. Patent Nr. CN102697122.

Zhichao, Z. (2011). Solid instant food of purple sweet potato and soymilk and preparation method thereof. Patent Nr. CN102224847.

Zhou, C. (2014). Freeze-dried instant bean vermicelli and production method thereof. Patent Nr. CN104012837.

Zhou, Y., Li, Q. and Dai, W. (2014). Purple sweet potato wine and preparation method thereof. Patent Nr. CN104017689.

Zhu, F., Cai, Y.-Z., Yang, X., Ke, J. and Corke, H. (2010). Anthocyanins, hydroxycinnamic acid derivatives, and antioxidant activity in roots of different Chinese purple-fleshed sweetpotato genotypes. *J Agric Food Chem*. **58**:7588-7596.

Zou, G. (2014). Extraction method of purple sweet potato essence, purple sweet potato drink and processing method thereof. Patent Nr. CN104041770.

2.6. Supplementary Tables

Table S2.1. Classification of the 122 selected articles for the period 2003-2014 by sweet potato variety (purple or not purple) and plant part. See S1 for article references.

Variety	Sweet potato plant part								
	Tuber	**Leaf**	**Stem**	**Leaf & stem**	**Vine**	**Peel**	**Cell line**	**Waste**	**Other**
Purple	a1, a2, a5, a6, a17, a25-a27, a29, a30, a35-a38, a41-a43, a46, a49, a58, a61-a63, a65, a67, , a70, a75-a77, a80, a83, a86-a94, a96, a99, a102-a105, a107, a109-a111, a113-a115, a118, a119, a122 (**56**)	a14, a15, a16, a45, a47 a68 (**6**)					a100 (**1**)	a48 (**1**)	
Not purple	a3, a4, a7, a12, a13, a21, a23, a24, a39, a54, a69, a71, a72, a73, a74, a81, a82, a85, a98, a101, a116 (**21**)	a9, a11, a28, a33, a34, a50, a55, a56, a57, a59, a60, a64, a79, a84, a95, a97, a106, a112, a117, a120, a121 (**21**)	a18, a66 (**2**)		a20, a22, a40 (**3**)	a8, a31, a78 (**3**)	a51-a53 (**3**)	a108 (**1**)	a44[a] a10[b] a19[b] a32[c] (**4**)

[a] Flower, leaf, stem, petiole and root
[b] Leaf, stem and root
[c] Leaf, vein, root

Table S2.2. Classification of the 170 selected patents in the period 2003-2014 by sweet potato variety (purple or not purple) and plant part. See S2 for patent references.

Variety	**Sweet potato plant part**							
	Tuber	**Leaf**	**Stem**	**Vine**	**Peel**	**Leaf & stem**	**Waste**	**Other**
Purple	p1-p5, p7, p9-p14, p16, p19-p21, p23, p25-p27, p29- p44, p46- p48, p50, p52-p54, p56-p58, p62, p63, p65, p66, p68, p71-p78, p80, p86-p100, p102-p107, p109-p118, p120-p122, p125-p132, p139, p141-p145, p147-p149, p151-p159, p162-p167, p169-p170 (**131**)				p6 (**1**)		p51, p67 (**2**)	
Not purple	p8, p15, p18, p22, p24, p28, p55, p60, p64, p70, p84, p108, p123, p124, p133, p134, p137, p139, p140, p146, p150, p160, p161, p168 (**24**)	p18, p61, p69, p82 (**4**)	p81 (**1**)	p83 (**1**)		p45, p79, p59, p101 (**4**)	p85 (**1**)	p17[a] (**1**)

[a] Not mentioned

Table S2.3. Indication of the types of studies of the 122 selected articles by sweet potato plant part for the period 2003-2014. More than one type of study could be attributed for an article.

Type of study	Sweet potato plant part						
	Tuber	**Leaf**	**Stem**	**Vine**	**Peel**	**Cell line**	**Waste**
Bioavailability	a13 (**1**)						
Clinical	a30 (**1**)	a14, a15, a16, a47, a68 (**5**)					
Cultivar/ genotype/ variety	a19, a24, a35, a39, a41, a67, a70, a85, a86, a99, a102, a121 (**12**)	a19, a40, a44, a45, a60, a64, a68, a84, a97, a106 (**10**)	a19, a44 (**2**)				
Formulation	a2, a77, a103 (**3**)						
New food	a12, a26, a27, a41, a72, a73, a74, a75, a83, a87, a88, a90, a92, a94, a104, a105, a113, a114, a115 (**19**)						a48, a108 (**2**)
Optimization	a4, a6, a25, a61, a71, a102 (**6**)	a57, a95 (**2**)					
Phenolic compounds	a23, a42, a43, a58, a76, a80, a122 (**7**)	a33, a50, a79, a117 (**4**)		a22 (**1**)	a31, a78 (**2**)	a53, a100 (**2**)	
Plant part	a10, a19, a32, a44 (**4**)	a9, a10, a19, a32, a44 (**5**)	a9, a10, a19, a32, a44 (**5**)				
Preclinical (animal)	a17, a29, a30, a36, a37, a38, a63, a69, a82, a89, a91, a96, a109, a110, a111, a119 (**16**)	a28, a47, a55, a56, a57, a107, a112 (**7**)					
Processing method	a1, a3, a21, a24, a35, a44, a49, a65, a81, a93, a98, a101, a118 (**13**)	a44, a120 (**2**)	a44 (**1**)	a20 (**1**)	a8 (**1**)		
Processing variables	a3, a5, a7, a10, a54, a62 (**6**)	a9, a10, a34, a59 (**4**)	a9, a10, a18, a66 (**4**)		a8 (**1**)		

(*cont.*)

Type of study	Sweet potato plant part						
	Tuber	**Leaf**	**Stem**	**Vine**	**Peel**	**Cell line**	**Waste**
Production/ cultivation		a11, a40, a45, a64, a84 (**5**)				a51, a52 (**2**)	
Storage	a39, a115 (**2**)						

Table S2.4. Indication of the type of antioxidant assays (*in vitro* and *in vivo*) used in the 122 selected articles by sweet potato plant part for the period 2003-2014.

Assay		Sweet potato plant part						
		Tuber	Leaf	Stem	Vine	Peel	Cell line	Waste
In vitro	DPPH	a2, a5, a10, a13, a17, a21, a23, a24, a32, a35, a39, a41, a42, a43, a46, a65,a70, a75, a76, a77, a81, a82, a83, a86, a88, a90, a99, a101, a104, a105, a113, a114, a115, a116, a118, a122 (**36**)	a9, a10, a32, a34, a40, a50, a60, a64, a68, a95, a106 (**11**)	a10, a32 (**2**)	a20, a22 (**2**)		a53 (**1**)	
	H_2O_2		a60 (**1**)					
	NO		a33 (**1**)					
	ABTS	a13, a24, a41, a99, a114, a118 (**6**)	a33, a106 (**2**)					
	FRAP	a13, a23, a41, a43, a63, a76, a77, a104, a122 (**9**)	a9, a15, a106 (**3**)		a20 (**1**)	a8 (**1**)		
	SOD	a42, a105 (**2**)	a34, a60, a64 (**3**)		a22 (**1**)			
	HO•		a34 (**1**)		a22 (**1**)			
	ORAC	a99, a105 (**2**)	a84, a120 (**2**)					
	RP	a32, a35, a42, a65, a86, a122 (**6**)	a9, a32, a33, a50, a60, a64 (**6**)	a32 (**1**)				
	TAA		a9, a79 (**2**)					
	FTC	a32, a42, a44, a86, a114 (**5**)	a32, a44, a79 (**4**)	a32, a44 (**2**)				
	TBARS	a10, a27, a44, a77, a91, a114 (**6**)	a10, a15, a33, a44, a68 (**5**)	a10, a44 (**2**)	a22 (**1**)			

(*cont.*)

Assay		Sweet potato plant part						
		Tuber	**Leaf**	**Stem**	**Vine**	**Peel**	**Cell line**	**Waste**
	CBI	a13 (**1**)	a50 (**1**)					
	ICA	a13, a43, a35, a86 (**4**)	a33, a60 (**2**)					
In vivo	FRAP	a63 (**1**)	a15 (**1**)					
	GSH	a14, a36, a38, a102, a110 (**5**)	a16, a50 (**2**)					
	GSH Px	a109, a111 (**3**)						
	GSt	a36, a38, a119 (**2**)						
	SOD	a105, a109, a110, a111, a91, a119 (**6**)	a112, a50 (**2**)					
	CAT	a109, a91 (**2**)						
	GGT	a96 (**1**)						
	TBARS	a17, a36, a37,a38, a65, a77, a90, a91, a107, a109 (**10**)	a15, a16 (**2**)					
	LDL	a17, a46, a76, a96 (**4**)	a14, a16, a112, a68 (**4**)					
	Other	a29, a32, a36, a37, a38, a69, a76, a90, a91, a105, a107, a109, a110, a111, a119 (**15**)	a14, a15, a16, a32, a33, a47, a50, a55, a60, a97, a112, a121 (**12**)	a32 (**1**)	a20, a32 (**2**)			a48, a108 (**2**)

ABTS: 2-2'-azino-bis (3 ethylbenzothiazoline-6-sulfonic acid)
CAT: Catalase
CBI: β-carotene bleaching inhibition
DPPH: 2,2-diphenyl-1-Picrylhydrazyl
FRAP: Ferric reducing-antioxidant power
FTC: Ferric thiocyanate
GGT: γ-Glutamyl transpeptidase
GSH Px: Glutathione peroxidase
GSH: Reduced glutathione
GSt: Glutathione-S-transferase
H_2O_2: Hydrogen peroxide
HO•: Hydroxyl radical
ICA: Iron (II) chelating activity
LDL: Low-density lipoprotein
NO: Nitric oxide
ORAC: Oxygen radical absorbance capacity
RP: Reducing power
SOD: Superoxide
SOD: Superoxide dismutase
TAA: Total antioxidant activity
TBA: Thiobarbituric acid-reactive substances

Table S2.5. Indication of the phenolic composition of sweet potato by plant part found in the 122 selected articles for the period 2003-2014.

Compounds	Sweet potato plant part						
	Tuber	**Leaf**	**Stem**	**Vine**	**Peel**	**Cell line**	**Waste**
TPC	a1, a2, a3, a4, a5, a6, a7, a13, a21, a24, a32, a35, a39, a40, a54, a63, a65, a71, a74, a75, a77, a81, a82, a83, a85, a86, a87, a89, a99, 101, a103, a104, a105, a111, a113, a114, a118 (**37**)	a9, a11, a14, a15, a16, a28, a32, a33, a47, a50, a60, a64, a68, a79, a95, a97, a106, a120, a121 (**19**)	a18, a32 (**2**)		a8 (**1**)		a108 (**1**)
TMA	a1, a2, a5, a6, a25, a26, a27, a35, a40, a42, a58, a61, a62, a65, a72, a73, a74, a77, a89, a94, a99, a101, a102, a103, a104, a105, a118, a119 (**29**)	a45, a47 (**2**)				a53 (**1**)	
TFC	a5, a13, a32, a35, a40, a65, a77, a83, a111 (**9**)	a9, a32, a33, a34, a50, a56, a57, a60, a64 (**9**)	a32 (**1**)				
Hydroxycinnamic acids	a19, a23, a39, a44, a71, a89, a98, a116, a122 (**9**)	a10, a11, a19, a28, a33, a40, a44, a59 a84, a106, a117, a120 (**11**)	a18, a19, a44,a66 (**4**)	a20 (**1**)	a31 (**1**)	a51, a52 (**2**)	a48 (**1**)
Hydroxybenzoic acids		a11 (**1**)	a18, a66 (**2**)				
Flavonoids		a11 (**2**)					
Flavonols		a11, a28, a33, a59, a84 (**5**)		a20 (**1**)			
Anthocyanins	a26, a42 a27, a30, a49, a58, a63, a67, a70, a80, a87, a88, a94, a102, a115, a119, a122 (**17**)	a11 (**1**)			a78 (**1**)	a52, a53, a100 (**3**)	

TPC: total phenolic compounds, TMA: total monomeric anthocyanins; TFC: total flavonoid content
TPC, TMA and TFC were determined by spectrophotometric methods

Table S2.6. Indication of statistical analysis used in the 122 selected articles on sweet potato by plant part for the period 2003-2014.

Statistical analysis	Sweet potato plant part						
	Tuber	**Leaf**	**Stem**	**Vine**	**Peel**	**Cell line**	**Waste**
Student t-test	a43, a76, a82, a83, a89 (**5**)	a11, a15, a16, a17, a47, a50 (**6**)					
ANOVA	a1, a2, a3, a4, a13, a24, a35, a36, a37, a38, a39, a40, a42, a46, a63, a69, a72, a77, a85, a91, a93, a95, a113, a116 (**24**)	a9, a14, a15, a16, a33, a56, a60, a64, a68, a84, a117 (**11**)	a66 (**1**)		a8, a31 (**2**)		
Duncan test	a1, a2, a3, a4, a5, a6, a7, a21, a35, a77, a81, a85, a86, a92, a99, a102, a103, a104, a105, a115, a118 (**21**)	a33, a34, a60 (**3**)	a18, a66 (**2**)				
Dunnet test		a15, a97 (**2**)					
Tukey's test	a13, a24, a36, a37, a38, a39, a46, a63, a91, a109, a110 (**11**)	a56, a84 (**2**)		a20 (**1**)			
Fisher's LSD	a13, a32, a41, a42, a72, a73, a116 (**7**)	a32, a64, a68 (**3**)	a32 (**1**)		a31 (**1**)		
Scheffé test	a69 (**1**)						
Factorial design	a24; a73, a118 (**3**)	a9 (**1**)					
Screening design					a8 (**1**)		
Optimization	a4, a5, a25, a61, a102 (**5**)	a95 (**1**)					
Correlation	a24, a39, a40, a72, a73, a74, a86, a99, a102, a116, a118 (**11**)	a60, a106, a120, a121 (**4**)					

(*cont.*)

Statistical analysis	Sweet potato plant part						
	Tuber	**Leaf**	**Stem**	**Vine**	**Peel**	**Cell line**	**Waste**
Hierarquical Cluster Analysis					a8 (**1**)		
Principal Components Analysis	a72, a73, a74, a75, a83, a85 (6)	a9 (1)					
Discriminant analysis	a72 (**1**)						
Regression analysis	a70 (**1**)	a117 (**1**)			a8 (**1**)		
Not mentioned	a10, a12, a119, a122	a10	a10				

Table S2.7. Indication of the health benefits from the 29 clinical and preclinical articles on sweet potato by plant part for the period 2003-2014.

Type of study	Health benefits related to antioxidant activity	Sweet potato plant part	
		Tuber	**Leaf**
Clinical	Anticancer activity		a47
	Decrease oxidative stress		a15, a16
	Decrease pro-inflammatory cytokine secretion		a15
	Enhance antioxidant defence		a16
	Modulate antioxidative status		a14, a15
	Reduce DNA oxidation		a14
	Reduce lipid oxidation		a14
	Selectively absorbed after ingestion	a30	
	Suppression of low-density lipoprotein oxidation.		a68
Preclinical (animal)	Ameliorate diabetic disorders	a69	
	Ameliorates cognition deficits	a91	
	Ameliorates Inflammation in brain	a91	
	Anticancer benefits		a28,
	Antidiabetic activity		a57, a112
	Anti-fatigue activity		a56
	Apoptosis in prostate cancer		a47
	Attenuates dimethylnitrosamine-induced liver injury	a38	
	Attenuates hepatic insulin resistance via blocking oxidative stress	a110	
	Attenuates inflammatory response in liver	a109	
	Attenuates Oxidative Damage in brain	a91	
	Attenuates oxidative stress induced by D-galactose in mouse liver	a109	
	Cancer-preventing effects	a82	
	Cytoprotective activity against γ-radiation	a29	
	Decreased the serum levels of hepatic biomarkers	a96	
	Growth Suppression of Human Cancer Cells		a55
	Imparts neuroprotection	a89	
	Improvement of Memory	a89	
	Improvement of Spatial Learning	a89	
	Inhibition of tumor growth	a111	
	Memory enhancing effects	a17	
	Prevention of Colorectal cancer	a63	
	Preventive effect on acute and subacute alcoholic liver damage	a119	
	Protection against *t*-BHP-induced hepatotoxicity	a36	
	Protection on induced hepatic lipid accumulation	a37	
	Selectively absorbed after ingestion	a30	
	Treatment of Alzheimer's disease		a107

t-BHP: tert-butyl hydroperoxide

Table S2.8. Indication of the food applications of sweet potato by plant part found in the 122 selected articles for the period 2003-2014.

Food applications	**Sweet potato plant part**						
	Tuber	**Leaf**	**Stem**	**Vine**	**Peel**	**Cell line**	**Waste**
Alcoholic drink	a87, a88 (**2**)						
Anthocyanin extract	a17, a26, a30, a36, a37, a38, a46, a67, a107, a109, a110, a111, a115, a119, a122 (**15**)						
Baked	a21, a44, a49 (**3**)	a120 (**1**)					
Beverage	a30, a41, a46, a94, a96 (**5**)						
Boiled	a20, a21, a44, a98, a101 (**5**)	a120 (**1**)		a20 (**1**)			
Dried slices	a103 (**1**)						
Extract	a11, a23, a28, a29, a82, a89, a100, a102, a122 (**9**)	a55, a56, a117 (**3**)				a51, a52, a53 (**3**)	
Fermented			a20 (**1**)				
Flour/powder	a1, a2, a3, a4, a5, a6, a7, a10, a13, a19, a24, a25, a27, a32, a35, a36, a39, a40, a42, a43, a47, a54, a58, a61, a62, a63, a64, a69, a70, a77, a85, a86, a93, a99, a118 (**35**)	a9, a10, a19, a32, a33, a34, a45, a50, a57, a59, a60, a68, a79, a84, a95, a97, a106, a112 (**18**)	a10, a18, a19, a32, a66 (**5**)	a22 (**1**)	a8, a31 (**2**)		
Fresh	a21, a71, a76, a78, a80 (**5**)	a120, a121 (**2**)					
Fresh cut	a116 (**1**)						
Fried	a44, a101 (**2**)	a120 (**1**)					
Kneaded flour	a35 (**1**)						

(*cont.*)

Food applications	Sweet potato plant part						
	Tuber	**Leaf**	**Stem**	**Vine**	**Peel**	**Cell line**	**Waste**
Lactic acid beverage	a72, a74, a90 (**3**)						
Microwaved	a44, a65 (**2**)	a120 (**1**)					
Milk	a105 (**1**)						
Nectar	a104 (**1**)						
Pasta[a]	a92, a114 (**2**)						
Pickle	a73 (**1**)						
Pigment	a12, a91 (**2**)						
Roasted	a24 (**1**)						
Steamed	a35, a44, a49, a101 (**4**)	a120 (**1**)	a20 (**1**)				
Stir fried	a44 (**1**)	a14, a15, a16 (**3**)					
Vinegar	a113 (1)						a48, a108 (**2**)
Wine	a75, a83 (**2**)						

[a] including noodles

Table S2.9. Indication of the number of food applications on sweet potato by plant part found in the selected 170 patents for the period 2003-2014.

Food applications	Sweet potato plant part						
	Tuber	**Leaf**	**Stem**	**Vine**	**Peel**	**Leaf & stem**	**Waste**
Alcoholic drink	p16, p62, p75 (**3**)						p67 (**1**)
Anthocyanin extract	p10, p21, p31, p41, p52, p53, p58, p71, p78, p87, p103, p110 (**12**)						
Beer	p29 (**1**)	p69 (**1**)			p6 (**1**)		
Beverage	p8, p84, p15, p4, p9, p20, p26, p30, p37, p38, p48 p54, 91, p105, p118, p34, p122, p163, p170 (**19**)	p82 (**1**)					
Bread	p13, p68, p128 (**3**)						
Candy/toffee	p121, p139, p153, p154 (**4**)						
Capsule	p77, p88 (**2**)						
Chewing gum	p113 (**1**)						
Chip	p140 (**1**)						
Composition	p7, p17, p76, p133 (**4**)						
Cookie	p135 (**1**)						
Encapsulation material	p166 (**1**)						
Enriched oil	p166 (**1**)						
Essence	p170 (**1**)	p101 (**1**)					
Extract	p41, p102, p106, p145, p155, p162 (**6**)	p61 (**1**)	p81 (**1**)			p79, p59 (**2**)	p85 (**1**)

(*cont.*)

Food applications	Sweet potato plant part						
	Tuber	**Leaf**	**Stem**	**Vine**	**Peel**	**Leaf & stem**	**Waste**
Fibre				p83 (**1**)			
Flour/powder	p1, p36, p57, p70, p89, p94, p115, p126, p150, p160. p161 (**11**)						
Functional food	p66, p80, p141 (**3**)						
Gel	p60 (**1**)						
Healthy food	p12, p44, p95, p98, p119, p120, p125, p127, p142, p147, p151, p152, p156, p164, p165 (**15**)						
Jelly	p49 (**1**)						
Juice	p22, 918, p40, p96, p99, p100, p116, p131, p144 (**9**)						
Lactic acid beverage	p65 (**1**)						
Liquor	p64 (**1**)						
Milk	p27, p107 (**2**)						
Pasta[a]	p108, p2,8, p42, p73, p33, p46, p114 p123, p130, p137, p149, p157, p158, p159, p168 (**15**)						
Paste	p138 (**1**)						
Pickle		p45 (**1**)					
Pigment	p93, p92, p132 (**3**)						
Pizza dough	p43 (**1**)						
Porridge	p2, p3, p24, (**1**)						
Preserve	p50, p111 (**1**)						*(cont.)*

Food applications	Sweet potato plant part						
	Tuber	**Leaf**	**Stem**	**Vine**	**Peel**	**Leaf & stem**	**Waste**
Sauce	p124 (**1**)						
Sausage	p148 (**1**)						
Snack	p11, p63, p90, p104, (**4**)						
Soup	p25 (**1**)						
Sugar	p129 (**1**)						
Supplement	p5, p55, p134, p136 (**4**)						
Tea	p167 (**1**)						
Tofu	p97 (**1**)						
Vinegar	p23, p74, p117 (**3**)						
Wine	p14, p19, p32, p35, p39, p47, p72, p86, p109, p112, p143, p169 (**12**)						p51 (**1**)
Yogurt	p56 (**1**)						

[a] including noodles

Chapter 3

Spotlight on PGI sweet potato from Europe: Study of plant part, time and solvent effects on antioxidant activity

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Spotlight on PGI sweet potato from Europe: Study of plant part, time and solvent effects on antioxidant activity

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Abstract

Sweet potato (SP) has long been used as food and in traditional medicine. For the first time, total antioxidant activity (TAA), reducing power (RP), ferric-reducing antioxidant power (FRAP) and 1,1-diphenyl-2-picryl-hydrazyl (DPPH) antioxidant activities from European sweet potato vines holding a Protected Geographic Indication (PGI) were studied through a factorial design. Plant part was the main influential factor followed by solvent acidification. Principal component analysis revealed total phenolic content, total flavonoid content, FRAP and RP assays more related to sample differences (stems or leaves) and DPPH to extraction conditions, namely solvent acidification. The in vitro antioxidant activities of leaves were 1.25 g AAE/100 g dw for TAA and 50.8 g GAE/100 g dw for RP. DPPH scavenging activity was higher than FRAP (i.e., 58.6 and 29.3 and mM Trolox/g dw, respectively). Stems results were 1.3 to 9.2 times lower than leaves. These outcomes confirmed SP leaves a potential resource of antioxidants in the human diet that may add supplementary value to PGI agriculture products.

Practical application

Vines, especially leaves, from PGI sweet potatoes could be advantageously used as a natural source of antioxidants, with potential to be used as a food in the diet. Focus on sweet potato of European origin may contribute to increase interest on its cultivation and consumption. In addition, insights on the potential added value present in Protected Geographic Indication (PGI) agriculture products may enhance protected demonization turnover for producers. Industries that pursuit food with functional applications may consider sweet potato vines in their new product development processes, aiming for health benefits.

3.1. Introduction

Consumer demand for foods that contain health-promoting components beyond traditional nutrients has stove interest in functional foods (Carvalho et al. 2006, 2010). Sweet potato (SP) (*Ipomoea batatas* L. Lam.) can be considered per se a functional food due to its appreciable amounts of biological active compounds, such as polyphenols. It is a traditional food in many countries where not only tubers but also vines, raw or cooked, are also commonly consumed (Lako et al. 2007; Huang et al. 2009; Isabelle et al. 2010). Polyphenols present in SP leaves were related to antioxidative/radical scavenging activity physiological functions (Islam et al. 2002) that could prevent or moderate oxidative stress-related diseases. They showed the highest polyphenol content (5.35 g gallic acid equivalent/100 g dw) among 12 vegetables, near twice the content of purslane (*Portulaca oleraceae*) and three times of green onions (*Allium fistulosum*) (Huang et al. 2009). Phenolic compounds associated with antioxidant activity of SP leaf extracts are flavonoids (Chu et al. 2000; Islam et al. 2002) and phenolic acids (Islam 2006; Truong et al. 2007). High in vitro antioxidant activity of SP leaves correlates with these compounds, with several potential food applications (Islam 2006). Extraction conditions are a key parameter in the determination of antioxidant activity. Besides solid–solvent ratio and temperature, time of contact and pH can affect the efficiency extraction of antioxidants, and it is relevant to study their main effects and interactions. SP origin in published studies is mostly from Asia, which in 2009 cultivated 82 % of total world production, Africa and America. To our knowledge, no studies were published on the antioxidant activity of SP leaves from Europe. Although this region produced only 0.06 % of world crop, mainly at the Iberia Peninsula, it was the second best-performing region in culture yield (FAOSTAT 2009). In addition, vines from SP with Protected Geographical Indication (PGI) have not been studies yet. The European Union PGI status is used to denote agricultural products and foods closely linked to a geographical area, where they are produced and/or processed and/or prepared (European Commission Agriculture and Rural Development 2010). As SP from Portugal has this denomination, the vines used in this study have two novelty features, Europe origin and PGI status. The objectives of the present study were to study antioxidant activity of vines of SP with a European PGI denomination and analyze the effects of vine part, time and solvent. Correlation between total phenolic content and antioxidant activities was also assed. These results may contribute to raise interest in SP vines produced in Europe and its potential for use as a functional food or ingredient. In addition, it may signal extra value present in PGI agriculture products for turnover improvement.

3.2. Materials and methods

3.2.1. Reagents

Aluminum chloride (Merck, Darmstadt, Germany), ammonium molybdate (Panreac, Barcelona, Spain), ascorbic acid (Sigma, St. Louis, MO), 2,2-diphenyl-2-picryl-hydrazyl (DPPH) (Calbiochem, Nottingham, U.K.), ferric chloride (Merck), Folin–Ciocalteau reagent (Merck), gallic acid (Fluka, St. Louis, MO), glacial acetic acid (BDH-Prolabo, West Chester, PA), hydrochloric acid (Riedel, Seelze, Germany), methanol (BDH-Prolabo), potassium ferricyanide (Merck), quercetin (Alfa Aesar, Ward Hill, MA), sodium acetate (Merck), sodium phosphate (Merck), sodium carbonate (Merck), sulfuric acid (Merck), trichloroacetic acid (BDH-Prolabo), 2,4,6-tripyridyl-2-trizine (TPTZ) (Sigma), Trolox (Sigma Aldrich, Poole, U.K.), dipotassium hydrogen phosphate (Merck) and potassium dihydrogen phosphate (Merck).

3.2.2. Equipment

All determinations are spectrophotometric, and absorbance readings were made in an Ultrospec 1100 pro-UV/visible spectrophotometer (GE Healthcare Life Sciences, Buckinghamshire, U.K.).

3.2.3. Plant Material

Plant material was collected from SP plants (Lira variety) from Aljezur (Portugal) that holds a PGI status (European Union 2008). SP vines used as propagation material were collected at an early morning of July and kept in an isothermal container during transport to the laboratory within 1.5 h. Vines had green-purple stems and stalks, and green glabrous with entire shape leaves. Each vine was cut into three equal parts; and leaves, stalks and stems were cut after forming three groups: the upper (younger), middle and bottom sections of the vine. The plant material used in this study was from the bottom section, where stem length was between 15 and 38 cm, average of 25 cm, with two to six leaves, average of four. Leaves and stems were stored in open trays at 10C and relative humidity of 95 % (S600 PLH phitoclim, Aralab, Rio de Mouro, Portugal) for 48 h. Whole leaves and stems were cut in portions of 1.5 cm approximately, dried in monolayer regime at 60C in a Binder BD 53 incubator (Binder, Tuttilgen, Germany) for 24 h and 48 h, respectively. After cooling in an exsiccator, samples were grounded to a fine powder using a mortar and pestle, and stored in sealed glass tubes at 4C until analysis. Moisture loss during drying was of 76.49 ± 3.09 and 80.07 ± 0.04 % for leaves and stems, respectively.

3.2.4. Extraction of Antioxidants

Plant extracts were prepared by the addition of 1 mL of 80 % aqueous methanol to 0.05 g of dried plant material; tubes were sealed, involved in aluminum foil, vortexed and kept in an orbital shaker (Edmund Buhler Gmg H-Ks 15, Hechingen, Germany) at a 45° angle for 24 h at 37C. The same conditions of extraction were used in the second and third extractions, after tubes were centrifuged (Hettich Universal 320, Tuttlingen, Germany) at 3,000 ¥ g for 10 min. Solvent was decanted, and 0.75 mL of fresh solvent was added to plant material. The volume of solvent used per extraction experiment was collected in glass tubes, filtered and diluted to a final volume of 5 mL of extract, with solid: solvent ratio of 1:100. Extracts were hold at 4 ºC until analysis.

3.2.5. Total Phenolic Content and Total Flavonoid Content

Total phenolic content (TPC) and total flavonoid content (TFC) were determined according to methods described by Huang et al. (2006), with slight modifications. A calibration curve of gallic acid in the range of 12.5–100 mg/mL (r^2 = 0.995) was constructed, and the TPC was expressed as g gallic acid equivalent (GAE) in 100 g of dried material. TFC was quantified from a standard calibration curve that was constructed in the range of 7–55 g/mL of quercetin (r^2 = 0.9991), and results were expressed in g of quercetin equivalent (QE) by 100 g of dry weight.

2.3.6. Antioxidant Activity

Four different methods were used to evaluate antioxidant activity. Total antioxidant activity (TAA) was assayed by phosphomolybdenum method according to Prieto et al. (1999), with slight modifications. A linear calibration curve for acid ascorbic in the range of 28–201 mg/mL (r^2 = 0.9992) was constructed, and results were expressed as g of ascorbic acid equivalent (AAE) in 100 g of dry material. Reducing power (RP) determination was made by ferricyanide method described by Huang et al. (2010), with minor modifications. A calibration curve for acid gallic was made at the range of 200 and 540 mg/mL of gallic acid, with a regression coefficient of 0.9997, and results were expressed in g GAE per 100 g of dried material.
The method described by Benzie and Strain (1996) was used the determination of ferric-reducing antioxidant power (FRAP), with some modifications. An aliquot of 200 mL was added to 3 mL of FRAP reagent (buffer acetate 300 mM, pH 3.6; 40 mM of HCl; 10 mM of TPTZ and 20 mM ferric chloride). After the mixture stood at 20C for 4 min in the dark, the absorbance at 593 nm was measured against a blank. A calibration curve for Trolox was made in the range of 50 and 175 mM, with a regression coefficient of 0.9991 (data not shown), and results were expressed in mM Trolox equivalent (TE) per gram of dry weight.
Free radical DPPH scavenging capacity was determined by the method of Brand-Williams et al. (1995), where 2 mL of 0.15 mM DPPH was added to 100 mL of extract (at adequate dilutions) or Trolox. Tubes were sealed, vortexed and incubated in the dark at room temperature during 30 min. Absorbance was measured at 517 nm. A calibration curve for Trolox was made in the range of 100 and 800 mM, with a regression coefficient of 0.996, and results were expressed in mM TE per gram of dry weight.

3.2.7. Statistical Analysis

Design Expert ver. 6.0.0 software (Stat-Ease 2000) was used to plan a complete factorial 2 x 3 x 2 experimental design, with three replicates. Plant part (factor A) was tested at two levels (stems or leaves), and solvent (factor C) varied as acidified or non-acidified 80 % aqueous methanol. Time (factor B) was tested at three levels: 24, 48 or 72 h. The magnitude and significance of main (A, B and C), two interactions (AB, AC and BC) and the three interaction (ABC) effects were accessed by regression analysis of variance (ANOVA). Magnitude of effects was accessed by the percentage of contribution of each model term relative to the sum of squares, and significant terms were confirmed by respective values. Refined models were obtained after removal non-significant terms, exclusion of influential results and power transformation to comply with ANOVA assumptions. R^2 and adjusted R^2 were also used to verify model adequacy. A principal components analysis (PCA) was performed using SPSS software, version 17.0 (SPSS, Inc., Chicago, IL), after Kaiser–Meyer–Olkin (KMO) and Bartlett's tests, in order to analyze correlation between dependent variables and confirm patterns in data.

3.3. Results and discussion

Extraction of plant polyphenols is a key step for polyphenol and antioxidant activity determinations; and important factors, like plant part (factor A), time (factor B) and solvent (factor C), need to be assessed in order to evaluate its impact on antioxidant activity. Plant part was elected as a factor as different color of stems (green-purple) and leaves (leaves) were indicative of the presence of different compounds and possibly different antioxidant activities. Time and solvent may have conflicting actions on the solubilization and degradation of polyphenols (Robards 2003) and were included in the design to study factor interaction. Time associated with boiling or stirring extraction methods for SP. leaves varied from 5 min (Huang et al. 2010) to 24 h (Carvalho et al. 2010) (Table 3.1). To study the effect of longer times, extraction was allowed for 24, 48 and 72 h. Extraction with acidic solvents may release compounds initially part of polymers or bounded to others. It may also contribute to the solubilization and diffusion of phenolic compounds due to cell wall disintegration. In addition, the use of water in combination with organic solvents ensures the extraction of polyphenols due to the creation of a moderately polar medium. Enhanced recovering of polyphenols from plant material, including flavonols with low polymerization degree, had been reported (Robards 2003). From the different acidification/solvent combinations reported for SP vines, it was chosen to test acidified or non-acidified 80 % methanol.

3.3.1. Full and Reduced Factorial Models

According to Table 3.2, main effect plant part (A) was dominant for TPC, TFC, RP and FRAP results, with a percentage of contribution of total variability higher than 75 %, and highly significant ($P < 0.001$). For DPPH, solvent (factor C) as main effect explained 71 % of result variability. Full-model ANOVA analysis exposed interaction between plant part and solvent (AC) to be significant for all assays although more important for TAA. Interaction plant part–time (AB) was only significant for RP. For TFC, interactions time– solvent (AC) to be significant for all assays although more important for TAA. Interaction plant part–time (AB) was only significant for RP. For TFC, interactions time–solvent (BC) and plant part–time–solvent (ABC) were still significant ($P < 0.05$), but with small contribution in explaining variability. In order to comply with ANOVA assumptions, TFC, TAA and DPPH results were modified by square root or inverse square root power transformation. TFC refined model confirmed only plant part and solvent as significant effects. Adequacy of final models was also confirmed by high R^2 values with similar adjusted R^2 results.

3.3.2. Effect of Plant Part, Time and Solvent on TPC and TFC

For TPC and TFC, plant part presented a highly significant positive effect on TPC and TFC estimated means (Figure 3.1). Solvent had effect on both assays, but in lesser magnitude than plant part. Non-acidified solvent increased TPC for stems, but for leaves, it had an opposite effect. Solvent effect on TFC results was lower than those observed for TPC. Therefore, acidification of 80 % methanol enhanced extraction of polyphenolic compounds from leaves, but not flavonoid content. Opposite behavior was observed for stems.

Table 3.1. Indication of extraction conditions published and tested in this study for sweet potato vines.

Origin	Plant part[a]	Storage	Cooked	Drying	Method	Acidified	Time (h)	Tempera-ture[b] (ºC)	Solvent	%Solvent	Reference
China	V	no	no/yes	n.m.[c]	sonnication	no	0.5	n.m.	ethanol	80	Cui et al. 2011
China	L	no	no	no	boilling	no	0.67	80	ethanol	70	Xu et al. 2010
Taiwan	L	no	no	freeze	boilling	no	0.08	(100)	water	100	Huang et al. 2010
Taiwan	V	no	no	no	boilling	no	0.33	100	water	100	Liao et al. 2011
Singapure	L	no	no	freeze	sonnication	yes	0.25	37-39	acetone	70	Isabelle et al. 2010
Japan	S, L	no	no	freeze	n.m.	no	n.m.	hot	methanol	100	Ishida et al. 2000
Japan	S,L	no	no	freeze	boilling	no	0.25	(68)	methanol	80	Islam et al. 2002
Japan	L	no	no	freeze	boilling	no	0.25	(78.88)	ethanol	80	Yoshimoto et al. 2002
Japan	L	no	no	freeze	stirring	no	1	ambient	methanol	70	Nagai et al. 2011
Fiji	L	no	yes	freeze	stirring	yes	0.5	(77)	acetonitrile	100	Lako et al. 2007
Korea	L	no	no	no	maceration	no	n.m.	n.m.	ethanol	80	Jung et al. 2011
USA	L	no	no	freeze	sonnication	yes	0.17	n.m.	methanol	80	Huang et al. 2009
Portugal	L	no	no	hot air	stirring	no	24	n.m.	methanol	100	Carvalho et al. 2010
Portugal	S (-1) L (+1)	48 h 10 ºC 95 %RH	no	hot air	stirring	yes (-1) no(+1)	24 (-1) 48 (0) 72 (+1)	37	methanol	80	This study

[a]V: vine, L:leaves, S:stems
[b]values in brackets were estimated
[c]n.m.: not mentioned

Table 3.2. Effects list from ANOVA for full factorial model and significant main factors and interactions for each dependent variable.

	TPC	**TFC**	**TAA**	**RP**	**FRAP**	**DPPH**
Full model						
Source of variance	%SS †	%SS	%SS	%SS	%SS	%SS
Plant part (A)	92.80***	97.50***	59.10***	75.67***	85.94***	25.71***
Time (B)	0.46ns	0.18ns	0.81ns	7.87***	0.71ns	0.28ns
Solvent (C)	0.71ns	0.46***	26.32***	0.48ns	0.06ns	71.12***
AB	0.28ns	0.10ns	0.29ns	4.56***	0.42ns	0.01ns
AC	1.33*	0.30***	8.28***	4.70***	3.05**	1.29***
BC	0.22ns	0.32*	0.16ns	0.63ns	0.77ns	0.12ns
ABC	0.01ns	0.25*	0.08ns	1.15ns	0.01ns	0.03ns
Refined model						
Power transforms	no	square root	(square root)$^{-1}$	no	no	square root
Significant main effects	A***	A*** C**	A*** C***	A*** B***	A***	A*** C***
Significant interactions	AC***		AC*	AB*** AC***	AC*	AC*
Model (p)	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
R^2	0.941	0.993	0.940	0.928	0.890	0.989
Adjusted R^2	0.938	0.983	0.935	0.913	0.883	0.988

ns: P>0.05, * P<0.05, ** P<0.01, ***P<0.001
† percentage of sum of squares
A: plant part; B: time, C: solvent.
TPC, total phenolic content; TFC, total flavonoid content; TAA, total antioxidant activity; RP, reducing power; FRAP, ferric-reducing antioxidant power; DPPH, 1,1-diphenyl-2-picryl-hydrazyl.

3.3.3. Effect of Plant Part, Time and Solvent on TAA and RP

Solvent and time have different effects on the magnitude and significance of TAA and RP antioxidant measurements. As TAA was transformed by inverse square root function, graphical interpretation should be done accordingly. Therefore, solvent affected more leaves than stems results, and higher antioxidant activity was observed for acidified solvent in comparison with non-acidified solvent (Figure 3.2).

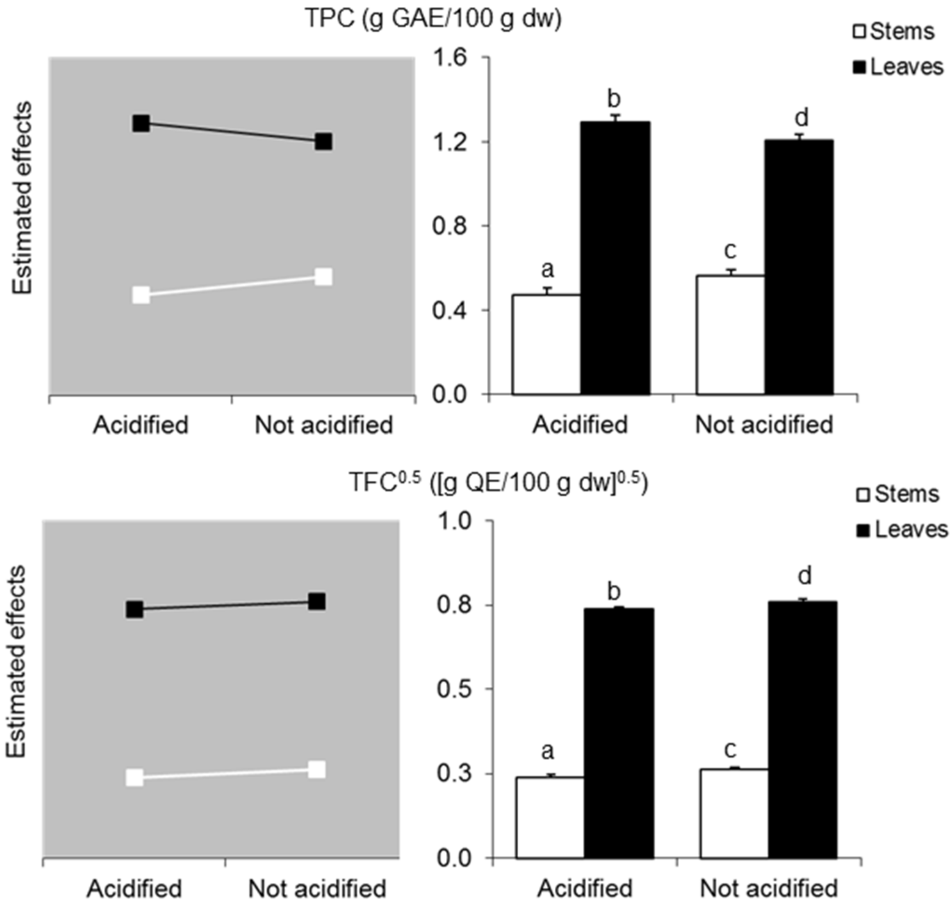

Figure 3.1. Interaction and bar graphs of TPC and $(TFC)^{0.5}$ estimated means for plant part with solvent. Different letters indicate significant different values at $p < 0.05$.

For RP, interaction of plant part with solvent had the same direction but different magnitude than TPC. Thus, other compounds than those quantified by the Folin–Ciocalteau method contribute to the reducing power of stems. Time influenced only leaves results and had a positive effect. As time did not affect influenced only leaves results and had a positive effect. As time did not affect neither TPC nor TFC, this enhanced reduced power was possibly not associated with polyphenols or flavonoids influenced only leaves results and had a positive effect. As time did not affect neither TPC nor TFC, this enhanced reduced power was possibly not associated with polyphenols or flavonoids.

3.3.4. Effect of Plant Part, Time and Solvent on FRAP and DPPH

FRAP plant part–solvent (AC) interaction (Figure 3.3) presented a behavior similar to TPC but not to RP, as would be expected because RP and FRAP assays are based on similar reaction mechanisms (Berker et al. 2009). Solvent had more effect than plant part on DPPH, for both stems and leaves. Lower results obtained for acidified solvent may be related to slower reaction time leading to less radical scavenging activity. The same differences were observed for acidified and non-acidified 50 % methanol (Pérez-Jiménez and Saura-Calixto 2006).

3.3.5. Variables Correlations and Pattern in Data

For SP leaves, high correlations between TPC/TAA, TPC/RP (Gupta and Prakash 2008) and TPC/DPPH (Truong et al. 2007) were reported. To evaluate how these correlations were affected in this study, PCA was performed. PCA was appropriate as all two-pair variables. Pearson correlation coefficients were significant at a 0.05 level, KMO statistic was higher than 0.5 and Bartlett's test of sphericity was significant. Two components that explained 97 % of data variability were extracted. According to the loading plot (Figure 3.4A), all variables were strong positively correlated to the first component (PC1). TFC, TPC, RP and FRAP were more correlated as they were more close to the PC1 axis than TAA or DPPH. DPPH was positively highly correlated with PC2 and TAA negatively correlated at the same component. Data pattern from score plot (Figure 3.4B) confirmed plant part as the major influential factor, as PC1 separated stems and leaves results. PC2, which split data in acidified and non-acidified solvent, relates more to DPPH.

3.3.6. Antioxidant Activity of SP Leaves and Stems

Caution should be taken when comparing antioxidant activity data, as difference in method, solvent and analytical conditions may lead to very diverse results. For comparison purposes, results obtained with 24 h time and non-acidified solvent will be used (Table 3.3, in bold). TPC for European SP leaves and stems were 1.22 and 0.60 g GAE/100 g dw, respectively.

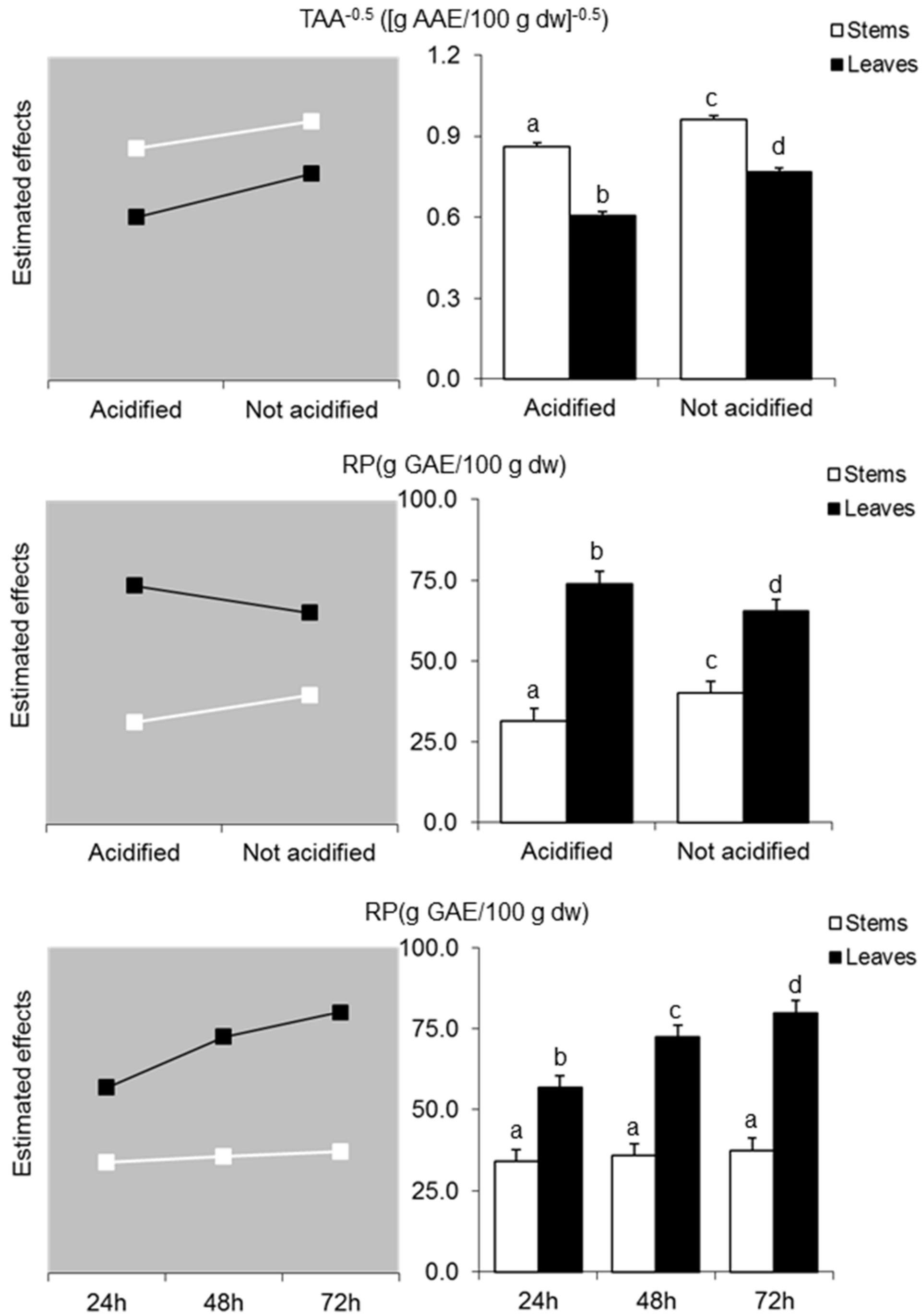

Figure 3.2. Interaction and bar graphs of $(TAA)^{-0.5}$ and RP estimated means for plant part with solvent and with time. Different letters indicate significant different values at $p < 0.05$.

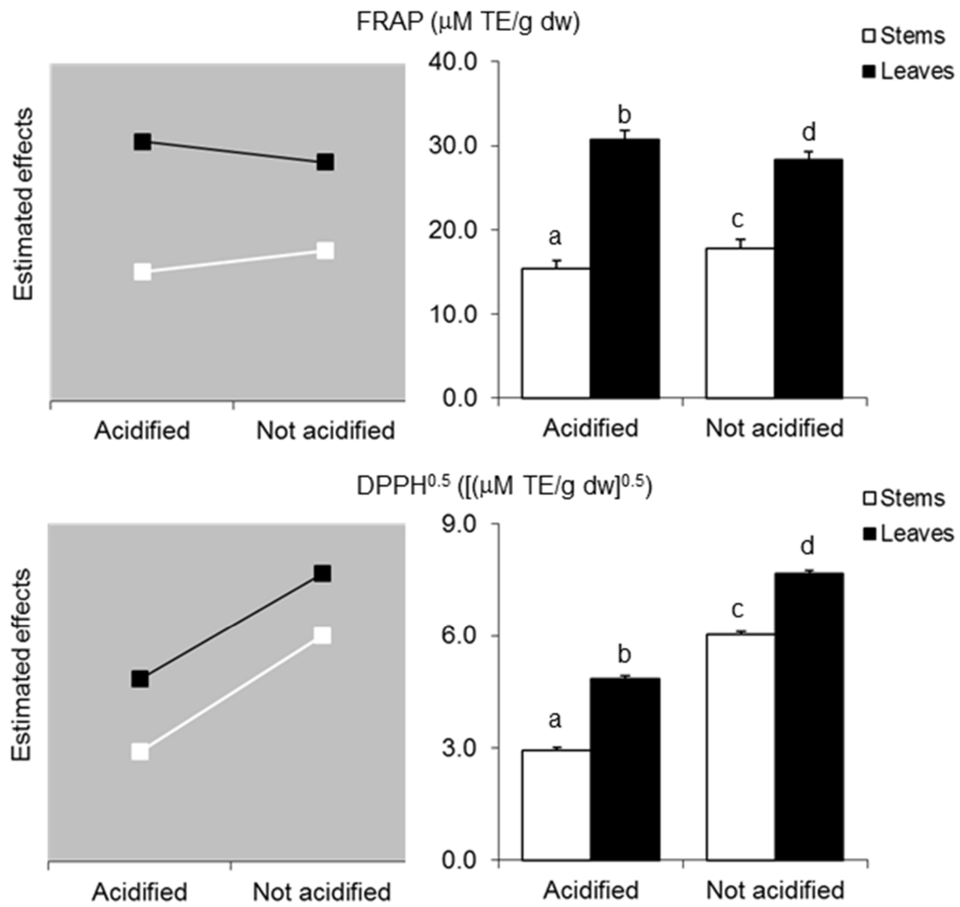

Figure 3.3. Interaction and bar graphs of FRAP and $(DPPH)^{0.5}$ estimated means for plant part with solvent. Different letters indicate significant different values at $p < 0.05$.

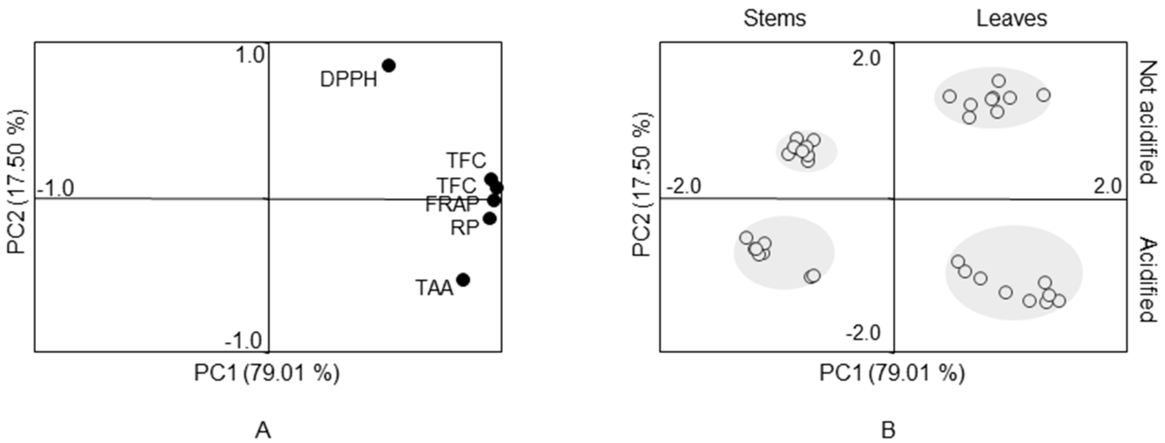

Figure 3.4. Loading plot for variables (A) and score plot for treatments (B) obtained from principal components analysis.

These values are lower than those published, which ranged from 0.85 and 5.35 g GAE/100 g dw from Fiji (Lako et al. 2007) and the U.S.A. (Huang et al. 2009), respectively. Besides the influence of genotype and edaphic–climatic conditions, this study included a fresh storage (Table 3.1) prior to analysis that may have contributed to these lower results. TFC for SP leaves (0.59 g QE/100 g dw and 0.064 g QE/100 dw) (Table 3.3) were also lower than those reported for leaves from China, 1.85 mg/100 g. (Chu et al. 2000). Still, European SP leaves had higher TPC than broccoli or spring onion (Isabelle et al. 2010), while SP stems were superior to lettuce or cucumber (Lako et al. 2007). As SP leaves and stems had FRAP results lower than DPPH (Table 3.3), samples had radical scavenging activity superior to metal-ion-reducing power. SP leaves from this study had higher FRAP results than peas, similar to carrots, but lower than broccoli (Ou et al. 2002). FRAP determined in this study for SP stems is higher than onions and peas. DPPH results were very different than those reported for SP leaves, with activities between 199 and 227 mM TE/g dw (Truong et al. 2007). However, even with a storage step prior to analysis, SP leaves and stems had higher DPPH activity than onions (Gorinstein et al. 2009). These results confirm that leaves and stems from European SP could be considered as a potential supplementary resource of antioxidants in human diet and have additional value added for PGI agricultural products.

3.4. Conclusion

Plant part (stems or leaves) presented the largest main significant effect ($P < 0.001$) for all assays, while for DPPH activity was the most influential factor. Time of extraction had the only significant effect on RP assay, as main effect and interaction with plant part. Although factors selected present different effects on assays, variables were highly correlated in a principal component that explained 78 % of data variability. European SP vines, especially leaves, can be considered as a natural source of antioxidants, with potential to be used as a food in the diet or as ingredient for functional applications aiming for health benefits. It explains how extra values may be present in PGI agriculture products with possible turnover of positive contribution. In this study, leaves and stems were from the bottom section of the SP vines that were stored for 48 h (10 ºC and 95 % RH) prior to analysis.

Table 3.3. Phenolic, flavonoid content and antioxidant activities of SP stems and leaves.

Plant Part	Time (h)	Solvent	TPC*	TFC†	TAA‡	RP*	FRAP§	DPPH§
Leaves	24	Acidified	1.20 ± 0.21	0.52 ± 0.04	2.65 ± 0.41	63.0 ± 6.9	29.7 ± 3.5	23.2 ± 1.3
	24	**Not acidified**	**1.22 ± 0.07**	**0.59 ± 0.05**	**1.06 ± 0.36**	**50.8 ± 2.2**	**29.3 ± 2.8**	**7.9 ± 2.0**
	48	Acidified	1.26 ± 0.08	0.54 ± 0.03	2.83 ± 0.10	73.8 ± 3.5	30.1 ± 4.1	23.8 ± 1.9
	48	Not acidified	1.18 ± 0.02	0.66 ± 0.06	1.45 ± 0.27	71.0 ± 0.8	27.0 ± 1.3	8.4 ± 0.5
	72	Acidified	1.32 ± 0.09	0.56 ± 0.04	1.64 ± 0.09	89.2 ± 3.9	32.0 ± 0.3	58.8 ± 0.4
	72	Not acidified	1.30 ± 0.11	0.56 ± 0.03	1.04 ± 0.01	70.8 ± 7.2	29.3 ± 2.9	36.7 ± 1.5
Stems	24	Acidified	0.42 ± 0.07	0.050 ± 0.011	2.70 ± 0.18	28.8 ± 9.2	13.5 ± 3.0	23.7 ± 3.2
	24	**Not acidified**	**0.60 ± 0.02**	**0.064 ± 0.006**	**1.35 ± 0.22**	**39.6 ± 2.8**	**18.0 ± 1.3**	**9.1 ± 0.7**
	48	Acidified	0.47 ± 0.12	0.064 ± 0.004	1.63 ± 0.09	34.8 ± 5.1	16.2 ± 3.1	58.6 ± 4.5
	48	Not acidified	0.57 ± 0.02	0.068 ± 0.011	1.08 ± 0.06	37.0 ± 0.9	17.6 ± 0.4	34.0 ± 1.6
	72	Acidified	0.45 ± 0.10	0.066 ± 0.012	1.81 ± 0.15	35.3 ± 9.1	16.0 ± 3.4	62.4 ± 6.9
	72	Not acidified	0.60 ± 0.02	0.067 ± 0.002	1.13 ± 0.01	39.6 ± 2.0	18.3 ± 0.7	38.3 ± 1.8
Leaves/Stems	24	Acidified	2.9	10.4	1.0	2.2	2.2	1.0
	24	**Not acidified**	**2.0**	**9.2**	**0.8**	**1.3**	**1.6**	**0.9**
	48	Acidified	2.7	8.3	1.7	2.1	1.9	0.4
	48	Not acidified	2.1	9.7	1.3	1.9	1.5	0.2
	72	Acidified	3.0	8.6	0.9	2.5	2.0	0.9
	72	Not acidified	2.2	8.3	0.9	1.8	1.6	1.0

* g GAE/100 g dw.
† g QE/100 g dw.
‡ g AAE/100 g dw.
§ mM TE/g dw.
Results ate mean _ SD for *n* = 3.
TPC, total phenolic content; TFC, total flavonoid content; TAA, total antioxidant activity; RP, reducing power; FRAP, ferric-reducing antioxidant power; DPPH, 1,1-diphenyl-2-picryl-hydrazyl.

How plant age and postharvest storage affected the antioxidant value SP vines and the identification of compounds responsible for the biological properties of SP leaves and stems will be addressed in future works.

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3.5. References

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. Anal. Biochem. 239, 70–76.

BERKER, K., GÜÇLÜ, K., TOR,I˙., DEMIRATA, B. and APAK, R. 2009. Total antioxidant capacity assay using optimized ferricyanide/prussian blue method. Food Anal. Method 3, 154–168.

BRAND-WILLIAMS,W., CUVELIER, M.E. and BERSET, C. 1995. Use of a free radical method to evaluate antioxidant activity. LWT-Food Sci. Technol. 28, 25–30.

CARVALHO, I., MIRANDA, I. and PEREIRA, H. 2006. Evaluation of oil composition of some crops suitable for human nutrition. Ind. Crop. Prod. 24, 75–78.

CARVALHO, I.S., CAVACO, T., CARVALHO, L.M. and DUQUE, P. 2010. Effect of photoperiod on flavonoid pathway activity in sweet potato (*Ipomoea batatas* (L.) Lam.) leaves. Food Chem. 118, 384–390.

CHU, Y., CHANG, C. and HSU, H. 2000. Flavonoid content of several vegetables and their antioxidant activity. J. Sci. Food Agric. 80, 561–566.

CUI, L., LIU, C., LI, D. and SONG, J. 2011. Effect of processing on taste quality and health-relevant functionality of sweet potato tips. Agric Sci. China 10, 456–462.

EUROPEAN COMMISSION AGRICULTURE AND RURAL DEVELOPMENT. 2010. PDO and PGI Agricultural Products: a 14.2 billion euro turnover for over 800 products, Newsletter, IP/09/1593.

EUROPEAN UNION. 2008. Publication of an application pursuant to Article 6(2) of Council Regulation (EC) No. 510/2006 Batata Doce de Aljezur EC No: PT-PGI-0005-0517-03.01.2005. Off. J. Eur. Union C324, 31–33.

FAOSTAT. 2009. FAO statistical database. http://faostat.fao.org/ (accessed October, 2011).

GORINSTEIN, S., PARK, Y., HEO, B., NAMIESNIK, J., LEONTOWICZ, H., LEONTOWICZ, M., HAM, K., CHO, J. and KANG, S. 2009. A comparative study of phenolic compounds and antioxidant and antiproliferative activities in frequently consumed raw vegetables. Eur. Food Res. Technol. 228, 903–911.

GUPTA, S. and PRAKASH, J. 2008. Influence of antioxidant components on antioxidant activity of dehydrated green leafy vegetables. Food Sci. Technol. Res. 14, 104–109.

HUANG, M., CHU, H., JUANG, L. and WANG, B. 2010. Inhibitory effects of sweet potato leaves on nitric oxide production and protein nitration. Food Chem. 121, 480–486.

HUANG, Y., CHANG, Y. and SHAO, Y. 2006. Effects of genotype and treatment on the antioxidant activity of sweet potato in Taiwan. Food Chem. 98, 529–538.

HUANG, Z.,WANG, B., EAVES, D.H., SHIKANY, J.M. and PACE, R.D. 2009. Total phenolics and antioxidant capacity of indigenous vegetables in the southeast United States: Alabama Collaboration for Cardiovascular Equality Project. Int. J. Food Sci. Nutr. 60, 100–108.

ISABELLE, M., LEE, B.L., LIM, M.T., KOH,W., HUANG, D. and ONG, C.N. 2010. Antioxidant activity and profiles of common vegetables in Singapore. Food Chem. 120, 993–1003.

ISHIDA, H., SUZUNO, H., SUGIYAMA, N., INNAMI, S., TADOKORO, T. and MAEKAWA, A. 2000. Nutritive evaluation on chemical components of leaves, stalks and stems of sweet potatoes (*Ipomoea batatas* poir). Food Chem. 68, 359–367.

ISLAM, M.S., YOSHIMOTO, M., YAHARA, S., OKUNO, S., ISHIGURO, K. and YAMAKAWA, O. 2002. Identification and characterization of foliar polyphenolic composition in sweetpotato (*Ipomoea batatas* L.) genotypes. J. Agric. Food. Chem. 50, 3718–3722.

ISLAM, S. 2006. Sweetpotato (*Ipomoea batatas* L.) leaf: Its potential effect on human health and nutrition. J. Food Sci. 71, R13–R121.

JUNG, J.K., LEE, S.U., KOZUKUE, N., LEVIN, C.E. and FRIEDMAN, M. 2011. Distribution of phenolic compounds and antioxidative activities in parts of sweet potato (*Ipomoea batata* L.) plants and in home processed roots. J. Food. Compos. Anal. 24, 29–37.

LAKO, J., TRENERRY, V.,WAHLQVIST, M., WATTANAPENPAIBOON, N., SOTHEESWARAN, S. and PREMIER, R. 2007. Phytochemical flavonols, carotenoids and the antioxidant properties of a wide selection of Fijian fruit, vegetables and other readily available foods. Food Chem. 101, 1727–1741.

LIAO,W.C., LAI, Y.C., YUAN, M.C., HSU, Y.L. and CHAN, C.F. 2011. Antioxidative activity of water extract of sweet potato leaves in Taiwan. Food Chem. 127, 1224–1228.

NAGAI, M., TANI, M., KISHIMOTO, Y., IIZUKA, M., SAITA, E., TOYOZAKI, M., KAMIYA, T., IKEGUCHI, M. and KONDO, K. 2011. Sweet potato (*Ipomoea batatas* L.) leaves suppressed oxidation of low density lipoprotein (LDL) in vitro and in human subjects. J. Clin. Biochem. Nutr. 48, 203–208.

OU, B., HUANG, D., HAMPSCH-WOODILL, M., FLANAGAN, J.A. and DEEMER, E.K. 2002. Analysis of antioxidant activities of common vegetables employing oxygen radical absorbance capacity (ORAC) and ferric reducing antioxidant power (FRAP) assays: A comparative study. J. Agric. Food. Chem. 50, 3122–3128.

PÉREZ-JIMÉNEZ, J. and SAURA-CALIXTO, F. 2006. Effect of solvent and certain food constituents on different antioxidant capacity assays. Food Res. Int. 39, 791–800.

PRIETO, P., PINEDA, M. and AGUILAR, M. 1999. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: Specific application to the determination of vitamin E. Anal. Biochem. 269, 337–341.

ROBARDS, K. 2003. Strategies for the determination of bioactive phenols in plants, fruit and vegetables. J. Chromatogr. A 1000, 657–691.

TRUONG, V., MCFEETERS, R.F., THOMPSON, R.T., DEAN, L.L. and SHOFRAN, B. 2007. Phenolic acid content and composition in leaves and roots of common commercial sweetpotato (*Ipomea batatas* L.) cultivars in the United States. J. Food Sci. 72, C343–C349.

XU,W., LIU, L., HU, B., SUN, Y., YE, H., MA, D. and ZENG, X. 2010. TPC in the leaves of 116 sweet potato (*Ipomoea batatas* L.) varieties and Pushu 53 leaf extracts. J. Food Compos. Anal. 23, 599–604.

Chapter 4

Phenolics extraction from sweet potato peels: Key factors screening through a Plackett–Burman design

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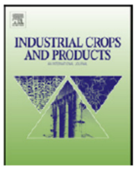

Phenolics extraction from sweet potato peels: Key factors screening through a Plackett–Burman design

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Abstract

Sweet potato peels usually considered as waste in food service operations may be upgraded into value added functional foods with high phenolic content and antioxidant activity. The effect of nine extraction variables was identified as relevant in previous phenolics extraction studies, because solvent solid ratio, time, pH, peeling cut depth, particle size, temperature, solvent, sample amount and agitation were combined in a 12 run Plackett–Burman design to screen for key factors. The extraction of phenolic compounds was only significantly ($p < 0.05$) affected by solvent: solid ratio (positively) and peel cut depth (negatively) with similar effect magnitude. These factors effects presented similar direction, size and significance on extracts antioxidant activity measured by FRAP. Regression models on FRAP transformed by squared root function revealed the two factor interaction peel cut depth and sample amount as the third most influencing factor. Beyond the usage of sweet potato peels as a new constituent for human food, it should also be tested for ingredient optimized in the production of functional foods.

Keywords: Sweet potato; *Ipomoea batatas;* Peels; Phenolic compounds; Plackett–Burman design.

4.1. Introduction

Sweet potato (*Ipomoea batatas* L.) ranked as 3rd in the root and tubers crops world production in 2010 (FAO, 2010). It was identified as an excellent novel source of natural health promoting for the functional food market (Bovell-Benjamin, 2007). Sweet potato roots and leaves contain biologically active compounds with significant medicinal value as antioxidant properties that might beneficiate human health (Huang et al., 2006). This crop was already claimed in patented functional compositions related to suppression of intestinal cancer (Iwasaki, 2003) and anti-aging food (McDaniel, 2011). Although usually treated as waste, sweet potato peels (SPP) had also received attention for food and feed application as they possessed high levels of phenolics (Zhu et al., 2010) and can reach almost three times more antioxidant activity than the other plant tissues (Cevallos-Casals and Cisneros-Zevallos, 2002). It was recently demonstrated in animal models that SPP possessed a potent wound healing activity possibility associated to an underlying antioxidant mechanism (Panda et al., 2011). In addition, SPP extracts might be beneficial against Alzheimer's disease as it reduced the level of lipid peroxidation and increased catalase activities in biochemical studies using the brain tissue of mice (Kim et al., 2011). These findings reinforce the drive for recycling agro industry wastes as peels into valuable adding products (Peschel et al., 2006), namely at the post-harvest and peeling steps of the supply chain. Losses during storage and transportation reached 6.3 % of 2007 sweet potato world production. Regarding peeling, industrial operations reduced by 2 % and 14 % the root fresh mass with steam and lye-peeling methods, respectively (Van Hal, 2000). Values higher than 18 % waste reported for abrasion peeling of potatoes (Singh and Shukla, 1995) are expected for sweet varieties, as roots are usually more irregular. In food service sector, preparation losses mainly due to peels from tubers and vegetables ranged from 3 to 8 % of the food delivered (Engstrom and Carlsson-Kanyama, 2004). The development process aiming peels valorisation needs inputs on the extraction of compounds with potential health-promoting and/or disease-preventing properties. Plackett–Burman designs (PBDs) have been used to study the influence of a large number of factors on phenolic compounds extraction with a small number of experimental trials (Dopico-Garcia et al., 2007; Luque-Rodríguez et al., 2007; Priego-Capote et al., 2004). While focused in identifying main effects, PBD may provide information on two factors interactions and increase research efficiency (Phoa et al., 2009). The objective of this study was to screen by PBD the factors that alone or combined influence the extraction of phenolic compounds with antioxidant activity from SPP obtained as waste in food service operations.

4.2. Materials and methods

4.2.1. Reagents

Ferric chloride (Merck); Folin Ciocalteau reagent (Merck); gallic acid (Fluka); glacial acetic acid (BDH-Prolabo); hydrochloric acid (Riedel); sodium acetate (Merk); Sodium carbonate (Merk); trichloroacetic acid (BDH-Prolabo); 2,4,6-tripyridyl-2- triazine (TPTZ) (Sigma); Trolox (Sigma Aldrich).

4.2.2. Plant material

Sweet potatoes were purchased at the market and transported to the lab on the same day. SPs were washed under tap water and air dried during the night. Roots were twice hand peeled in one direction with a cut depth circa 1.5 mm, corresponding to 8.4 ± 2.1 and 7.8 ± 2.0 % of fresh root for the first and second layer, respectively. Peels were dried at 60 ◦C during 48 h, milled and classified into two different sizes, all less than 200 µm (70 mesh) and all less than 600 µm (30 mesh).

4.2.3. Extraction process

Twelve treatments were made in triplicate employing the experimental plan as depicted in Table 4.1. Dried SPP varied in the layer depth of the hand cut (factor D) and particle size (factor E). Extracts were prepared at the programmed solvent: solid ratio (factor A) by the addition of proper solvent (factor G), acidified or not (factor C) taking the solid sample scale (factor H) in account. Tubes or Erlenmeyer's were sealed, involved in aluminum foil, and kept in an orbital shaker (Edmund Buhler Gmg H-Ks 15, Germany) at 45◦ angle with agitation (factor J), time (factor B) and temperature (factor F) according to the experimental plan.

Table 4.1. Information on published design of experiments used in extraction optimization studies of phenolics from fruit and vegetable peels.

Order	Run	A: Solvent: solid	B: Time (min)	C: pH	D: Cut depth (mm)	E: Particle size (μm)	F :Temperature (ºC)	G: Solvent (water)	H: Sample (g)	J: Agitation (rpm)	K: Dummy 1	L: Dummy 2	TPC (mg GAE/g dw)	FRAP (μM TE/g dw)
1	4	50 (+)	180 (+)	7.0 (+)	3.0 (+)	<600 (+)	40 (+)	tap (+)	1 (+)	200 (+)	(+)	(+)	4.19 ± 0.07	1.67 ± 0.20
2	1	10 (-)	180 (+)	2.3 (-)	3.0 (+)	<600 (+)	40 (+)	distilled (-)	0.1 (-)	50 (-1)	(+)	(-)	1.02 ± 0.20	1.40 ± 0.43
3	2	10 (-)	60 (-)	7.0 (+)	1.5 (-)	<600 (+)	40 (+)	tap (+)	0.1 (-)	50 (-)	(-)	(+)	2.77 ± 0.23	1.11 ± 0.08
4	9	50 (+)	60 (-)	2.3 (-)	3.0 (+)	<200 (-)	40 (+)	tap (+)	1 (+)	50 (-)	(-)	(-)	2.53 ± 0.39	1.57 ± 0.30
5	5	10 (-)	180 (+)	2.3 (-)	1.5 (-)	<600 (+)	27 (-)	tap (+)	1 (+)	200 (+)	(-)	(-)	3.97 ± 0.12	2.68 ± 0.82
6	10	10 (-)	60 (-)	7.0 (+)	1.5 (-)	<200 (-)	40 (+)	distilled (-)	1 (+)	200 (+)	(+)	(-)	3.14 ± 0.27	3.72 ± 0.83
7	12	10 (-)	60 (-)	2.3 (-)	3.0 (+)	<200 (-)	27 (-)	tap (+)	0.1 (-)	200 (+)	(+)	(+)	1.77 ± 0.06	1.31 ± 0.11
8	3	50 (+)	60 (-)	2.3 (-)	1.5 (-)	<600 (+)	27 (-)	distilled (-)	1 (+)	50 (-)	(+)	(+)	5.87 ± 0.18	8.11 ± 0.50
9	11	50 (+)	180 (+)	2.3 (-)	1.5 (-)	<200 (-)	40 (+)	distilled (-)	0.1 (-)	200 (+)	(-)	(+)	3.70 ± 0.22	4.83 ± 0.43
10	7	50 (+)	180 (+)	7.0 (+)	1.5 (-)	<200 (-)	27 (-)	tap (+)	0.1 (-)	50 (-1)	(+)	(-)	6.21 ± 0.30	4.37 ± 0.52
11	8	10 (-)	180 (+)	7.0 (+)	3.0 (+)	<200 (-)	27 (-)	distilled (-)	1 (+)	50 (-1)	(-)	(+)	1.62 ± 0.07	0.71 ± 0.08
12	6	50 (+)	60 (-)	7.0 (+)	3.0 (+)	< 600 (+)	27 (-)	distilled (-)	0.1 (-)	200 (+)	(-)	(-)	3.26 ± 0.52	2.46 ± 0.86

After centrifugation (Hettich Universal 320, DJB Lovecare, United Kingdom) at 3000 × g for 10 min, extract was filtered and diluted to proper final volume. Extracts were hold at −18 ◦C until analysis.

4.2.4. Evaluation of extracts

Total phenolic content (TPC) was determined according to methods described by Huang et al. (2006) with slight modifications. A calibration curve of gallic acid in the range 12.5–100 µg/mL (R^2 = 0.995) was constructed, and the TPC was expressed as g gallic acid equivalent (GAE) in 100 g of dried material. Ferric-reducing antioxidant power (FRAP) was according to the method described by Benzie and Strain (1999) was used, with some modifications. An aliquot of 200 l was added to 3 mL of FRAP reagent (buffer acetate 300 mM, pH 3.6; 40 mM of HCl; 10 mM of TPTZ and 20 mM ferric chloride). After the mixture stood at 20 ◦C for 4 min in the dark, the absorbance at 593 nm was measured against a blank. A calibration curve for Trolox was made at the range of 50 and 175 µM with a regression coefficient of 0.9991 (data not shown) and results were expressed in µM Trolox equivalent (TE) per gram of dry weight. All absorbance readings were made in a T70+ UV/Vis Spectrometer (PG Instruments Ltd., United Kingdom).

4.2.5. Statistical analysis

A 12 run non-geometric PBD with nine factors and two dummy variables were varied at two levels in a combined pattern of Hamada and Wu matrices (Table 1). Average of responses TPC and FRAP were obtained from treatment replicates (three). Data analysis was performed by first determining main effects size and significance followed by regression analysis for the assessment of two factor interactions.

4.2.5.1. Main effects

Significant main effects were assessed by half-probability plots, Lenth's method and Analysis of Variance (ANOVA). In halfprobability plots, the absolute values of the nine main effect estimates were plotted against half-normal scores. Scale was defined by partitioning the right half of the normal distribution in nine parts of equal area. In the presence of a small number of active main effects, inactive factors scores should follow a straight line and the active ones should look like outliers (Heyden et al., 2001). Lenth's method determined a pseudo standard error that was plotted in a standardized Pareto chart obtained by dividing the absolute value of the estimated effects by the standard error (Zhao et al., 2011). Evidence of a significant effect was given when respective bar extents Lenth's distance. In addition, ANOVA at a 95 % significant level and Hierarquical Cluster Analysis (HCA) using between groups linkage method with Euclidean distance were performed to aid interpretation (Tyssedal and Niemi, 2011).

4.2.5.2. Two factors interaction

When the number of significant main effects was not too large, regression analysis was used to investigate two-factor interactions (2FI's) based on Phoa et al. (2009) research. First, all main factors identified by previous approaches and all possible two factors interactions were submitted to a forward method selection during regression analysis and variables with a confidence level higher than 95 % were considered as significant. Second, all main effects are added to the model again and a new variable selection was performed. New two factors interactions on the model were analysed again for significance. Process was repeated until goodness of fit indices, as squared coefficient of determination (R^2), adjusted squared coefficient of determination (R^2_{Ajd}) and regression standard deviation measured by mean squared error (MSE) stopped changing. When it was necessary to distinguish between competing models, residual analysis was done. Backward and stepwise methods were also used for comparison purposes. Design Expert 6.0.11 (Stat-Ease Corporation, Minneapolis, USA) was used for all statistical analyses except cluster analysis performed by PASW Statistic 18.0 software (SPSS, Chicago, USA).

Table 4.2. Effect of different factors on TPC extracts obtained from comparative, screening or optimization studies on food or food wastes. Main effects were classified as significant (Y) or not significant (N), with indication of a positive (+) of negative (-) effect direction and/or tested factor range when applicable.

Material	Extraction method[a]	A: Solvent: solid	B: Time (min)	C: pH	D: Layer depth	E: Particle size (μm)	F :Temperature (°C)	G: Solvent	H: Sample (g)	J: Agitation	Reference
Comparative studies:											
Pomegranate peels	PW		Y(-) 5-30			Y(-) 212-1400[b]	Y(-) 40-90				Çam and Hisil (2010)
Green tea extract	C	Y(-) 33-100	Y(+) 3-30				Y(+) 22-100			Y(+) no - yes	Molan et al. (2009)
Sweet potato (raw)	C				Y(-) skin-pith						Padda and Picha (2008)
Screening (Plackett Burman):											
White grapes	C/UA						Y(-) 40-50	Y			Dopico-García et al. (2007)
Red grape skins	SH		Y(+) 20-40	Y(-)			Y(+) 60-90	Y	N 1-3		Luque-Rodríguez et al. (2007)
Olive oil industry waste	UA						N 20-30				Priego-Capote et al. (2004)
Optimisation:											
Mashua tubers	C		Y(+) 5-120	Y(-) 1.4-5				Y			Chirinos et al. (2007)
Jabuticaba skins	HP						Y(+) 40-80				Santos and Meireles (2011)
Grape peel	SF						Y(-) 37-46				Gaafor et al. (2011)
Palm heart fruit pulp	UA	Y(+) 10-100	Y(+) 15-45								Borges et al. (2011)
Betel nut seed	UA	Y(+) 23-57	Y(+) 23-57				Y(+) 33-67				Han et al. (2011)
White distilled grape pomace	V						Y/N 40-50	Y			Guerrero et al. (2008)

[a] Extraction methods: C: conventional solid liquid; HP: high pressure carbon dioxide assisted extraction; PW: pressurized water extraction; SF: supercritical fluid extraction; SH: superheated liquid extraction; UA: solid liquid ultrasound assisted; V: laboratory-scale vertical extraction.
[b] 212 and 1400 corresponds to 62–212 and 560–1400 fractions, respectively.

4.3. Results and discussion

4.3.1. Factors selection

The selection of factors to be included in the screening design was based on variables tested in comparative, screening and optimization studies on polyphenols extraction with different plant materials and methods (Table 4.2). Temperature, time, solvent and solvent: solid ratio were included in most of the studies. For temperature, all situations were observed: positive effect (Molan et al., 2009; Luque-Rodríguez et al., 2007; Santos and Meireles, 2011; Han et al., 2011), negative (Çam and Hisil, 2010; Dopico-Garcia et al., 2007; Ghafoor et al., 2010), positive and negative depending on solvent used (Guerrero et al., 2008) and no effect (Priego-Capote et al., 2004). Time presented always a significant positive effect on TPC with one exception (C¸ am and Hisil, 2010). The effect of solvent: solid ratio also varied, positive (Borges et al., 2011; Han et al., 2011) or negative (Molan et al., 2009). When studied, the increase of pH affected TPC negatively. Factors as peeling cut depth (Padda and Picha, 2008), particle size (Çam and Hisil, 2010), amount of sample (Luque-Rodríguez et al., 2007) and agitation (Molan et al., 2009) were studied in much lesser extent, both alone or combined with other factors. Therefore, all nine factors presented in Table 2 were included in a PBD to screen respective effect on aqueous extraction of TPC from dried SPP. FRAP assay was also included to compare extraction efficiency with antioxidant activity.

4.3.2. Factors levels

Factors levels were defined in line with food service operational constraints and conventional solid liquid extractions (Table 1). Solvent solid ratio varied from 10 to 50, with the objective to assess influence at the approximate middle range for studies with opposite conclusions (Molan et al., 2009; Borges et al., 2011). Time of contact had 2-h span and ranged from 60 to 180 min to assess longer time effects than those used in previous studies (Çam and Hisil, 2010; Molan et al., 2009; Luque-Rodríguez et al., 2007; Chirinos et al., 2007; Borges et al., 2011; Han et al., 2011). To gather information on the effect of pH higher than 5, neutral pH was tested in addition to the acidic conditions reported as favourable to phenolics extraction (Luque-Rodríguez et al., 2007; Chirinos et al., 2007). Root cut depth was related to layer thickness obtained by hand peeling, included in the study as previous studies reported that Phenolic compounds in sweet potato were mainly present at the outer 5 mm of tissue of the root (Van Hal, 2000). The effect of using different cuts in food service operations was accessed by analysis peels obtained from 1.5 or 3.0 mm cut depths, phenolic compounds were mainly present at the outer 5 mm of tissue of the sweet potato root (Van Hal, 2000). Particle size was varied considering the availability of one only sieve mesh, 0.2 or 0.6 mm. Temperature levels tested (27 or 40 ◦C) were both in the mild range in the attempt to reduce both phenolics degradation and energy costs. For the solvent factor, tap water available in food service operations was tested in opposition to distilled one normally used in extraction studies. With the purpose to rationalize further optimization studies, the effect of scale on the bioactives extraction was ascertained by sample weight at 0.1 or 1 g. Finally, agitation was varied between reduced and high rotations for quantification of factor effect.

4.3.3. Total phenolic content and antioxidant activity

TPC of SPP varied between 1.02 ± 0.20 and 6.21 ± 0.30 mg GAE/g dw (Table 1) which was encouraging for conventional solid liquid extraction. This range was higher than results reported for mashua tubers (0.2 mg GAE/g dw) by conventional extraction (Chirinos et al., 2007) and for fruit skins (3.5 mg GAE/g dw) by more friendly phenolic extraction methods (Santos and Meireles, 2011). However, SPP presented lower TPC than other vegetables wastes, as cucumber (∼18 mg GAE/g dw) or artichoke (∼11 mg GAE/g dw) (Peschel et al., 2006). A wider variation for FRAP results was observed in comparison to TPC with a range of 0.71–8.11 µM TE/g dw. Pearson correlation between TPC and FRAP was 0.77 ($p < 0.01$) that was in line with correlations reported by Molan et al. (2009).

4.3.3.1. Main effects

Half-normal probability plot for TPC (Figure 4.1A) revealed solvent: solid ratio (factor A) and cut depth (factor D) as possible active effects as they deviate from a straight line through formed by the remaining non-significant factors normally distributed around zero. Lenth's method confirmed these two factors as active (Figure 4.1B) with a threshold significance of 0.01, indicated by ANOVA results (Table 3).

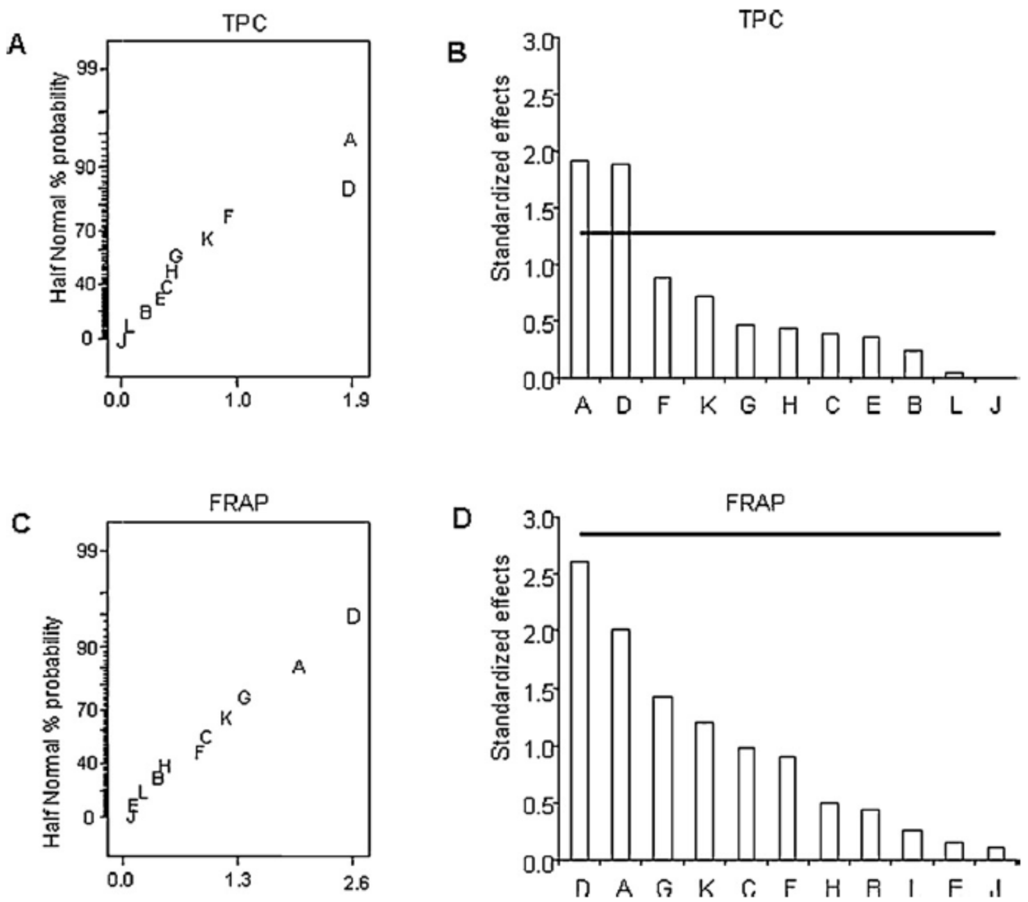

Figure 4.1. Graphical analyses of Plackett–Burman designs for all factor TPC and FRAP main effects. Letters in half normal probability plots (A and C) represents factors effect size scaled in absolute equally-spaced quantities. In Pareto charts (B and D) bars represent standardized effect size in decreasing order and line is the Lenth's distance. For factors identification (A–J) see legend of Table 4.1.

Table 4.3. ANOVA results of TPC and FRAP from Plackett-Burman design.

	Source	**DF**[a]	**MSS**[b]	**F value**	**P value**[c]
TPC Average	Model	2	10.76	15.7	***
	A-Solvent:solid	1	10.94	15.98	**
	D-Cut depth	1	10.57	15.43	**
	Residual	9	0.68		
	Cor Total	11			
FRAP Average	Model	2	16.36	8.53	**
	A-Solvent:solid	1	12.17	6.34	*
	D-Cut depth	1	20.56	10.72	**
	Residual	9	1.92		
	Cor Total	11			

[a] degrees of freedom
[b] mean sum of square
[c] significance *p<0.05; ** p<0.01; ***p<0.001

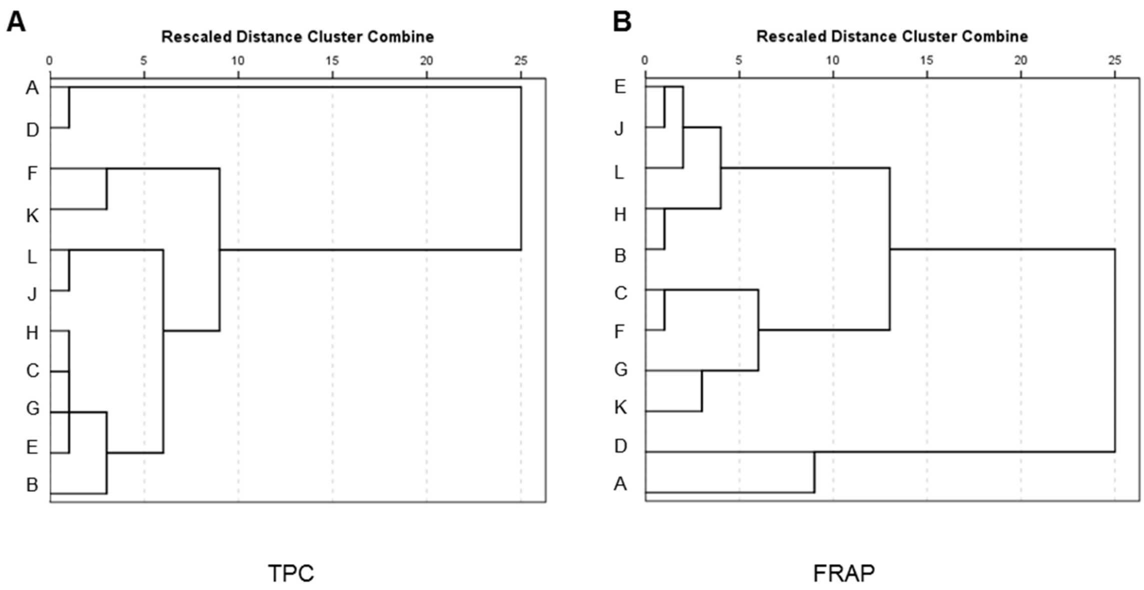

Figure 4.2. Hierarchical dendrogram from HCA for TPC (A) and FRAP (B) main factors effect. For factors identification (A–J) see legend of Table 4.1.

For FRAP, no factor was selected by both graphical methods (Figure 4.1C and D) but ANOVA identified the same key factors for TPC although with less significance. HCA confirmed solvent: solid and cut depth and significant main effects for TPC by way of them formed a group with lower distance (more similarities) as depicted in Figure 4.2A. As three factors groups could be identified (Tyssedal and Niemi, 2011) possible two factor interactions were investigated in regression analysis. For FRAP, solvent: solid ratio and peel cut depth formed the group with less similarities (Figure 4.2B) underlying differences from TPC.

4.3.3.2. Factors interactions

No significant interactions were identified for TPC by forward (˛in = 0.05) or stepwise (α_{in} = α_{out} = 0.05) selection during regression analysis (Figure 4.3A). In decreasing absolute size effect, backward method (˛out = 0.05) revealed significant interactions of solvent: solid ratio with pH > particle size > solvent > time > agitation > temperature. However, model presented lower R^2_{Ajd} and higher MSE than forward and stepwise procedures. Therefore, the calculus approach used in this study did not identify factors interactions beside two main effects for

TPC. Regarding magnitude, phenolic compounds extraction was enhanced by higher solvent solid ratios for emerging technologies (Borges et al., 2011; Han et al., 2011) but not by conventional method (Molan et al., 2009). Cut depth negative effect was also observed previously by Padda and Picha (2008). As standardized residuals departed from normality (data not shown), FRAP data transformation by log function was required.

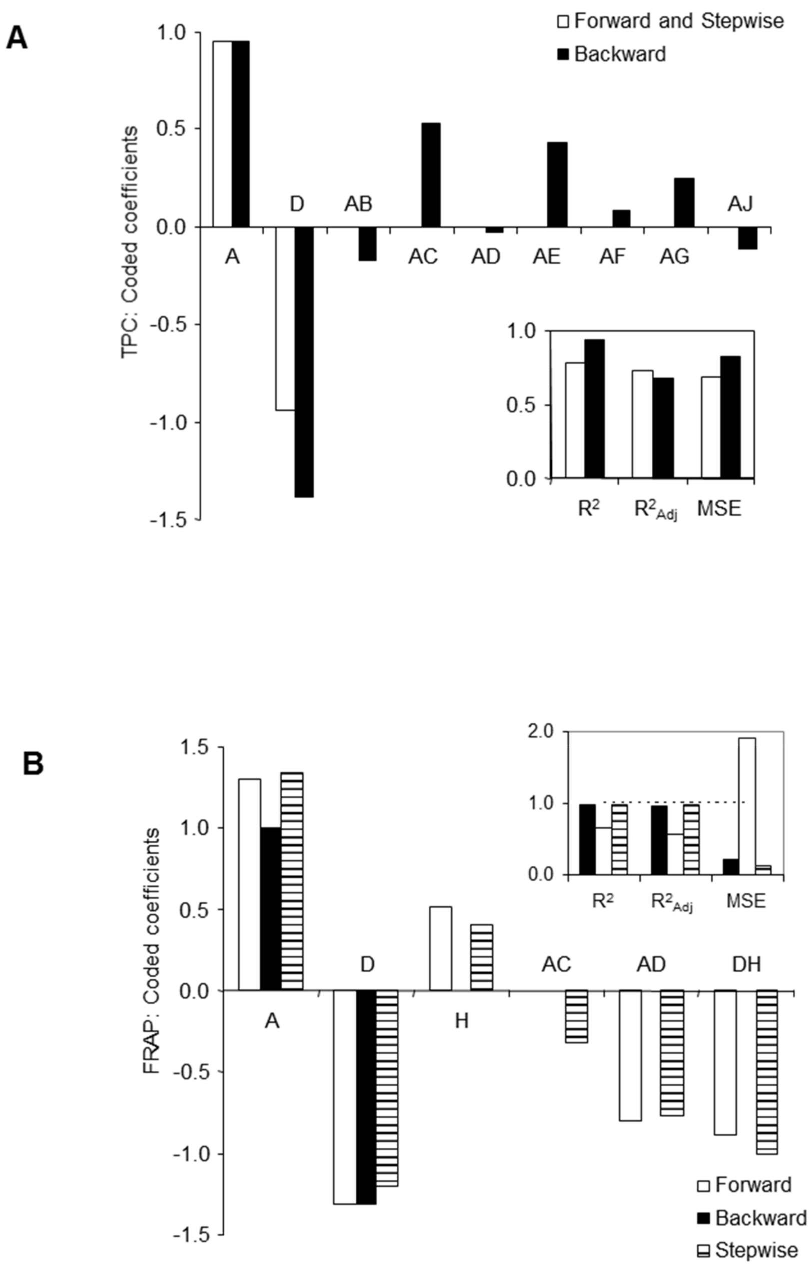

Figure 4.3. Coded regression coefficients for TPC (A) and FRAP (B) final regressions models obtained by forward (α_{in} = 0.05), backward (α_{out} = 0.05) and stepwise (α_{in} = ˛out = 0.05) selection procedures, with indication of R^2, R^2_{Ajd} and mean square error (MSE). For factors identification (A–J) see legend of Table 4.1.

The best regression model for FRAP data transformed by squared root function was obtained by the stepwise procedure with the highest R^2 and R^2_{Ajd} and lowest MSE (Figure 4.3B). Significant main effects ($p < 0.05$) were solvent: solid ratio (factor A), cut depth (factor D) and sample amount (factor H). Interactions DH and AD were third and fourth in absolute effect size, both with negative direction. Cut depth was the most negative influential factor both as main effect and in interaction. Solvent pH combined with solvent solid ration had negative effect. In resume, the factors that influenced more both TPC and FRAP were solvent solid ratio and root cut depth. Future optimization studies should vary solvent solid ratio at the highest tested level (50) and cut depth at the lower. The benefits on including the second layer of peeled sweet potato need assessment regarding its detrimental effect on phenolics concentration and antioxidant activity. Whilst no influential factor interactions were identified for TPC, higher antioxidant activities were obtained at both lower pH and sample amount. These factors setting will contribute to the enhance efficacy of future optimization studies.

4.4. Conclusions

The extraction of phenolic compounds from sweet potato peels was only significantly ($p < 0.05$) affected by solvent: solid ratio (positively) and peel cut depth (negatively) with similar effect size. These variables had a similar effect on the size and importance of antioxidant activity as measured by FRAP. Regression models revealed significant main effects and two factors interactions for FRAP but not for TPC. After transformation of FRAP by the squared root function, sample size was selected by stepwise procedure as main effect and in interaction with solvent: solid ratio. In addition, a small positive influence of pH in interaction with solvent: solid ratio on antioxidant activity was observed. These results encourage the utilization of SPP obtained from food services operations as valuable sources for biological active compounds with antioxidant activity.

4.5. References

Benzie, I., Strain, J., 1999. Ferric reducing/antioxidant power assay: direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. Methods in Enzymology 299, 15–27.

Borges, G.S.C., Vieira, F.G.K., Copetti, C., Gonzaga, L.V., Fett, R., 2011. Optimization of the extraction of flavanols and anthocyanins from the fruit pulp of *Euterpe edulis* using the response surface methodology. Food Research International 44, 708–715.

Bovell-Benjamin, A.C., 2007. Sweet potato: a review of its past, present, and future role in human nutrition. Advances in Food and Nutrition Research 52, 1–59.

Çam, M., Hisil, Y., 2010. Pressurized water extraction of polyphenols from pomegranate peels. Food Chemistry 123, 878–885.

Cevallos-Casals, B.A., Cisneros-Zevallos, L.A., 2002. Bioactive and functional properties of purple sweetpotato (*Ipomoea batatas* (L.) Lam). Acta Horticulturae 583, 195–203.

Chirinos, R., Rogez, H., Campos, D., Pedreschi, R., Larondelle, Y., 2007. Optimization of extraction conditions of antioxidant phenolic compounds from mashua (*Tropaeolum tuberosum* Ruíz & Pavón) tubers. Separation and Purification Technology 55, 217–225.

Dopico-Garcia, M.S., Valentao, P., Guerra, L., Andrade, P.B., Seabra, R.M., 2007. Experimental design for extraction and quantification of phenolic compounds and organic acids in white Vinho Verde grapes. Analytica Chimica Acta 583, 15–22.

Engstrom, R., Carlsson-Kanyama, A., 2004. Food losses in food service institutions –examples from Sweden. Food Policy 29, 203–213.

FAO, 2010. FAOSTAT. Available from: http://faostat.fao.org/default.aspx (accessed March 2012).

Ghafoor, K., Park, J., Choi, Y.-H., 2010. Optimization of supercritical fluid extraction of bioactive compounds from grape (*Vitis labrusca* B.) peel by using response surface methodology. Innovative Food Science and Emerging Technologies 11, 485–490.

Guerrero, M.S., Sineiro Torres, J., Nunez, M.J., 2008. Extraction of polyphenols from white distilled grape pomace. Optimization and modeling. Bioresource Technology 99, 1311–1318.

Han, L., Zhang, H., Luo, S., Luo, K., 2011. Optimization of ultrasound-assisted extraction of total phenol from betel (*Areca catechu* L.) nut seed and evaluation of antioxidant activity in vitro. African Journal of Biotechnology 10, 9289–9296.

Heyden, Y.V., Nijhuis, A., Smeyers-Verbeke, J., Vandeginste, B.G.M., Massart, D.L., 2001. Guidance for robustness/ruggedness test in method validation. Journal of Pharmaceutical and Biomedical Analysis 24, 723–753.

Huang, Y., Chang, Y., Shao, Y., 2006. Effects of genotype and treatment on the antioxidant activity of sweet potato in Taiwan. Food Chemistry 98, 529–538.

Iwasaki, T., 2003. Nutritious supplemental composition for suppression against onset of large intestinal cancer and manufacturing method thereof. U.S. Patent 2003 0157127 A1.

Kim, J.K., Choi, S.J., Cho, H.Y., Kim, Y.J., Lim, S.-T., Kim, C.-J., Kim, E.K., Kim, H.K., Peterson, S., Shin, D.-H., 2011. Ipomoea batatas attenuates amyloid β peptideinduced neurotoxicity in ICR mice. Journal of Medicinal Food 14, 304–309.

Luque-Rodríguez, J.M., Luque, M.D., Perez, P., 2007. Dynamic superheated liquid extraction of anthocyanins and other phenolics from red grape skins of winemaking residues. Bioresource Technology 98, 2707–2713.

McDaniel, D.H., 2011. Methods and compositions for identifying, producing and using plant-derived products for modulating cell function and aging. U.S. Patent 2011/0159121 A1.

Molan, A.L., De, S., Meagher, L., 2009. Antioxidant activity and polyphenol content of green tea flavan-3-ols and oligomeric proanthocyanidins. International Journal of Food Sciences and Nutrition 60, 497–506.

Padda, M.S., Picha, D.H., 2008. Phenolic composition and antioxidant capacity of different heat-processed forms of sweetpotato cv. 'Beauregard'. International Journal of Food Science and Technology 43, 1404–1409.

Panda, V., Sonkamble, M., Patil, S., 2011. Wound healing activity of *Ipomoea batatas* tubers (sweet potato). Functional Foods in Health and Disease 10, 403–415.

Peschel, W., Sanchez-Rabaneda, F., Diekmann, W., Plescher, A., Gartzia, I., Jimenez, D., Lamuela-Raventos, R., Buxaderas, S., Codina, C., 2006. An industrial approach in the search of natural antioxidants from vegetable and fruit wastes. Food Chemistry 97, 137–150.

Phoa, F.K.H., Wong, W.K., Xu, H., 2009. The need of considering the interactions in the analysis of screening designs. Journal of Chemometrics 23, 545–553.

Priego-Capote, F., Ruiz-Jiménez, J., Luque de Castro, M.D., 2004. Fast separation and determination of phenolic compounds by capillary electrophoresisdiode array detection. Application to the characterisation of alperujo after ultrasound-assisted extraction. Journal of Chromatography A 1045,239–246.

Santos, D.T., Meireles, M.A.A., 2011. Optimization of bioactive compounds extraction from jabuticaba (*Myrciaria cauliflora*) skins assisted by high pressure CO_2. Innovative Food Science and Emerging Technologies 1, 398–406.

Singh, K.K., Shukla, B.D., 1995. Abrasive peeling of potatoes. Journal of Food Engineering 26, 431–442.

Tyssedal, J., Niemi, R., 2011. Graphical aids for the analysis of two-level non-regular designs. Norges Teknisk-Naturvitenskapelige Universitet Statistica 3, 1–21.

Van Hal, M., 2000. Quality of sweet potato flour during processing and storage. Food Reviews International 16, 1–37.

Zhao, B.-S., Fu, Y.-J., Wang, W., Zu, Y.-G., Gu, C.-B., Luo, M., Efferth, T., 2011. Enhanced extraction of isoflavonoids from *Radix Astragali* by incubation pretreatment combined with negative pressure cavitation and its antioxidant activity. Innovative Food Science and Emerging Technologies 12, 577–585.

Zhu, F., Cai, Y.Z., Yang, X.S., Ke, J.X., Corke, H., 2010. Anthocyanins, hydroxycinnamic acid derivatives, and antioxidant activity in roots of different Chinese purplefleshed sweetpotato genotypes. Journal of Agricultural and Food Chemistry 58, 7588–7596.

Chapter 5

Phenolics extraction from sweet potato peels: Modelling and optimization by Response Surface Modelling and Artificial Neural Network

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Phenolics extraction from sweet potato peels: Modelling and optimization by Response Surface Modelling and Artificial Neural Networks

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Abstract

Sweet potato peels (SPP) are a major waste generated during root processing and currently have little commercial value. The aqueous extraction of phenolics with free radical scavenging activity from SPP was modelled and optimized by Response Surface Methodology (RSM) and Artificial Neural Network (ANN). Solvent: solid ratio (30-60 mL/g), time (30-90 min) and temperature (25-75 ºC) were varied according to a Central Composite Design. Temperature, and solvent: solid ratio, alone and in interaction, presented a positive effect in TPC and in ABTS and DPPH methods ($p<0.05$). Time had a negative influence in ABTS method and TPC. Optimized extraction conditions obtained by both RSM and ANN were 60 mL/g, 30 min and 75 ºC. The obtained responses in the optimized conditions were as follow: 11.87 ± 0.69 mg GAE/g DM for TPC, 12.91 ± 0.42 mg TE/g DM for ABTS and 46.35 ± 3.08 mg TE/g DM for DPPH. SPP presented similar behaviour in optimum conditions and phenolic content obtained from peels of potato, tea fruit and bambangan. Predictive models and the optimized extraction conditions from this investigation may offer an opportunity for food processors to generate products with high potential health benefits.

Keywords Sweet potato peels • Phenolic content • Radical scavenging activity • Extraction • Response surface methodology • Artificial neural network.

5.1. Introduction

Sweet potato (*Ipomoea batatas* L.) is a most used agro product in the world, especially in Asia and Africa where it is used in traditional diets. This root has also been profusely included in the composition of innovative applications such as ice-cream (Gurgel et al. 2011) or non-carbonated drink (Wireko-Manu et al. 2010). Further examples of developed functional sweet potato products are chips, flakes, yogurts and juices (Barnes and Sanders, 2012). Agro by-products represent an important source of phytochemicals such as phenolic compounds, possessing a wide range of functional activities. Peels are accounted as one of the major wastes generated during the processing of sweet potato with currently little commercial value (Maloney et al. 2012) and few research has been performed to upgrade it. Sweet potato peels (SPP) contain a high phenolic content with free radical scavenging activities (Zhu et al. 2010) which were related to health benefits such as wound healing (Panda et al. 2011). Studies on SPP valorisation have already covered applications such as production of bioethanol (Oyeleke et al. 2012), pharmaceuticals (Manpreet et al. 2005) and biosurfactants (Saharan et al. 2011). Recycling of agro wastes into high valuable products with potential health benefits could be achieved through extraction. As processing conditions are recognised to influence the removal of phenolics which are located particularly in the peel (Peschel et al. 2006), process modelling and optimization may encourage food operators to upgrade agro-waste. The optimization of the extraction of bioactive compounds from SPP was previously investigated by Response Surface Methodology (RSM) by Maloney and coworkers (2012). RSM is a reputed statistical technique proposed to improve processes by the means of its modelling capabilities to locate optimized points for various processes (Ilzarbe et al. 2008). Artificial neural network (ANN) has been applied in parallel with RSM method in prediction and modelling of extraction of phenolics with improvement in data fitting (Cheok et al. 2012; Wen et al. 2012). Phenolics extraction optimization studies were performed on fruit and vegetable peels such as apple (Junjian et al. 2013), grape (Casazza et al. 2012; Ghafoor et al. 2010), jabuticaba (Santos and Meireles 2011), pomegranate (Amyrgialaki et al. 2014; Tabaraki et al. 2012), orange (Dahmoune et al. 2014), tangerine (Londoño-Londoño et al. 2010), bambangan (Prasad et al. 2011), banana (González-Montelongo et al. 2010), mangosteen (Cheok et al. 2012), rambutan (Prakash Maran et al. 2013), tea fruit (Xu et al. 2012) as well as and potato (Amado et al. 2014; Singh et al. 2011; Wijngaard et al. 2012). For the six aforementioned peel materials, the experimental design was based on a Central Composite Design (CCD). The objective of this work was to model the effect of solvent: solid ratio, time and temperature on the extraction of phenolic compounds with radical scavenging activity from SPP through a CCD by RSM and ANN, and define the best conditions to obtain infusions with high phenolic content and biological activity.

5.2. Materials and methods

5.2.1. Reagents

2,2-Diphenyl-2-picrylhydrazyl (DPPH), Folin–Ciocalteau reagent, 2-2'-azino-bis (3 ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), gallic acid, sodium persulfate and Trolox were purchased from Sigma-Aldrich Co. Ltd. (Poole, U.K.). Sodium carbonate was purchased from VWR (West Chester, PA). Absolute ethanol was bought from Merck (Nottingham, U.K.). All reagents were of analytical grade.

5.2.2. Plant material

Sweet potatoes were purchased at local markets and transported to the lab at the same day. Roots were washed under tap water and air-dried during the night. Peeling was done manually with a cut depth of circa 1.5 mm, and peels were then dried at 60 ºC for 48 h. The dried material was then milled and sieved until all particles were smaller than 600 µm (30 mesh).

5.2.3. Extraction process

Twenty extractions runs were performed with respect to the CCD experimental plan presented in Table 5.1. 25 mL of distilled water at the proper temperature were transferred into a 250 mL screw cap flask with an adequate quantity of SPP powder regarding the experiment. The mixtures were stirred at constant rate of 200 rpm on a hot plate stirrer (HS0707V2, Favorit, Malaysia) at the respective run temperatures measured by digital food thermocouple (Model HI 98501, Hanna Instruments, Bedfordshire, UK). Extracts were filtered through a filter paper with a vacuum pump aspirator (DOA-P604-BN, Cast Manufacturing Inc., USA) and volume filled up to 25 mL. Extracts were hold at -18 ºC by the day of analysis.

Table 5.1. Coded and actual values of independent variables used for the central composite design and total phenolics content and radical scavenging activity of sweet potato peel extraction trials.

Std	Independent variables				Results		
Or	Coded[a]	X_1 (mL/g)	X_2 (min)	X_3 (ºC)	TPC (mg GAE/g DM)	ABTS assay (mg TE/g DM)	DPPH assay (mg TE/g DM)
1	−−−	30	30	25	5.77 ± 0.04	5.71 ± 0.42	20.61 ± 1.28
2	−−+	30	30	75	7.89 ± 0.09	8.18± 0.34	19.29 ± 0.43
3	−+−	30	90	25	7.01 ± 0.19	6.18 ± 0.22	21.97 ± 0.75
4	−++	30	90	75	6.09 ± 0.10	7.17± 0.27	23.05 ± 0.35
5	+−−	60	30	25	5.72 ± 0.21	4.97 ± 0.49	31.05 ± 0.59
6	+−+	60	30	75	11.35 ± 0.16	12.85 ± 0.14	45.91 ± 0.52
7	++−	60	90	25	6.51 ± 0.11	3.91 ± 0.30	37.89 ± 0.70
8	+++	60	90	75	11.11 ± 0.22	10.90± 0.08	45.32 ± 2.13
9	a00	20	60	50	5.64 ± 0.02	5.15± 0.06	13.76 ± 0.33
10	A00	70	60	50	9.36 ± 0.39	7.77 ± 0.36	49.79 ± 0.69
11	0a0	45	10	50	7.08 ± 0.19	8.25 ± 0.21	30.88 ± 0.98
12	0A0	45	110	50	7.24 ± 0.17	6.94 ± 0.05	31.49 ± 0.17
13	00a	45	60	8	6.47 ± 0.03	4.15 ± 0.24	31.25 ± 1.57
14	00A	45	60	92	11.86 ± 0.29	11.94± 0.10	33.38 ± 2.95
15	0	45	60	50	8.78 ± 0.25	8.06± 0.12	34.68 ± 0.33
16	0	45	60	50	8.92 ± 0.75	7.87 ± 0.50	31.09 ± 0.77
17	0	45	60	50	8.17 ± 0.17	7.97± 0.16	33.18 ± 0.07
18	0	45	60	50	8.09 ± 0.20	7.66 ± 0.47	33.18 ± 1.55
19	0	45	60	50	7.71 ± 0.24	8.00 ± 0.61	32.73 ± 1.00
20	0	45	60	50	8.65 ± 0.11	8.39 ± 0.29	33.61 ± 1.25

[a] - , level -1; + , level+1; a, -1.68; A,+1.68; 0, central point.
Std Or, standard order; X_1, solvent: solid; X_2, time; X_3 ,temperature; TPC, total phenolic content; ABTS, 2-2'-azino-bis (3 ethylbenzothiazoline-6-sulfonic acid); DPPH, 2,2-diphenyl-1-picrylhydrazyl; GAE, gallic acid equivalent; TE, Trolox equivalent; DM, dried material.
Results were expressed as the mean ± SD of three determinations.

5.2.4. Evaluation of extracts

Total phenolic content (TPC), ABTS radical scavenging activity and DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity were determined regarding the method described previously by Prieto et al. (1999), Re et al. (1999) and Blois (1958), respectively, with minor modifications (Nunes et al. 2012). In addition, DPPH assay values were determined using absolute ethanol as a solvent. TPC was expressed in mg gallic acid equivalent (GAE) per gram of dried material (DM) while ABTS and DPPH values were reported as mg of Trolox equivalent (TE)/g DM. Determinations were carried out in triplicate and data were reported as means ± SD. All absorbance readings were made by a T70+ UV/Vis Spectrometer (PG Instruments Ltd, United Kingdom).

5.2.5. Statistical Analysis

A Central Composite Design (CCD) for the aqueous solid–liquid extraction of SPP was used with three independent variables, solvent: solid ratio (X_1), time (X_2) and temperature (X_3) on three responses, TPC, ABTS and DPPH assays (Table 5.1). The experimental plan contained 20 runs, with 8 factorial points, 6 axial points and 6 central points. In order to have equal precision of estimation in all directions, design was made rotatable by an axial distance of 1.68, which corresponded to the 4-th root of the number of factorial points. Therefore, variables were coded at five levels (-1.68,-1, 0, 1, 1.68). Pearson correlations were conducted to examine the relationship between the antioxidant assays. A three-dimensional scatter-plot was built to compare the conditions of phenolic compounds extraction among studies performed on fruit and vegetable peels. The software used for the establishment of the experimental design, analysis of the data and creation of the plots was JMP® Pro 10.0.2 (www.jmp.com) provided at no cost by SAS (www.sas.com). Figures 5.2 and 5.5 were constructed by Microsoft® Excel 2013. For RSM analysis, data were approximated to a second-order polynomial equation by the least-square regression method with the selection of the significant coefficients by the backward method (α_{out}=0.05) with no rules (model hierarchy was not mandatory). The coefficient of determination (R^2), adjusted R^2 (R^2_{adj}) and root mean square error (RMSE) were used to access the models performance. Fitted models were used to build surface plots and extraction conditions optimization was performed by maximizing the desirability function. For the analysis by ANN, a simple single layer structure with three inputs, X_1, X_2 and X_3, one hidden layer with seven nodes and TPC, ABTS and DPPH assays as outputs was exploited to model the experimental data obtained from CCD design (Figure 5.1). The data were divided into two subsets, training and validation and network topology was trained by k-fold cross validation method (k=6). One hidden layer was considered previously adequate by trial and error for adjusting extraction data (Cheok et al. 2012). The hyperbolic-tangent activation function was used as transfer function in the hidden layer to the output layer. The number of neurons in the hidden layer was adjusted iteratively to maximize performance fitting determined by R^2 and RMSE. The rate of change in each predicted output was determined varying a given input while keeping all other input variables constant. Optimization was performed by maximizing the desirability function.

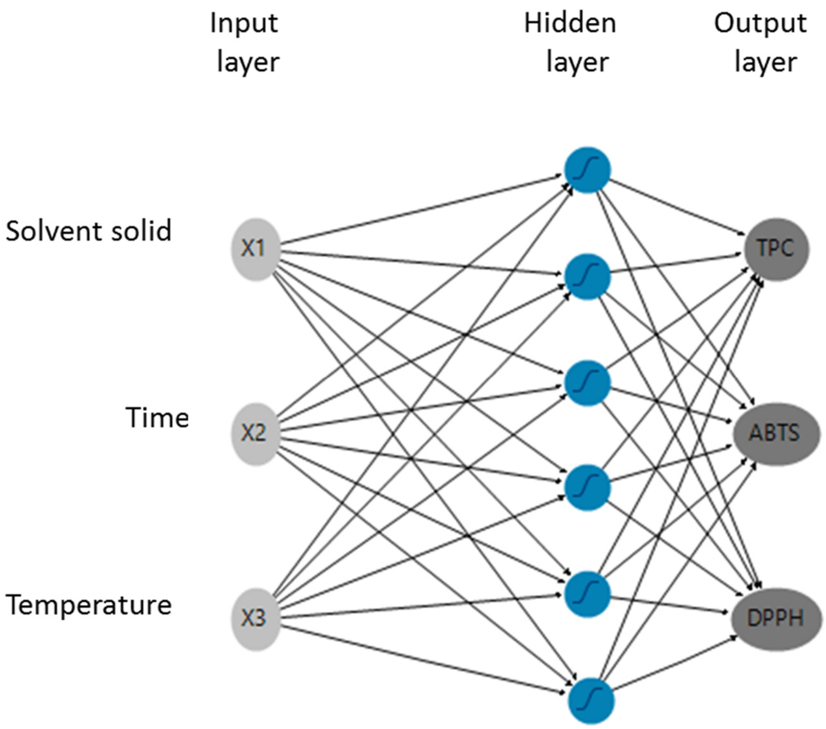

Figure 5.1. Diagram of the Artificial Neural Network architecture. X_1, solvent: solid; X_2, time; X_3, temperature; TPC, total phenolic content; ABTS, 2-2'-azino-bis (3 ethylbenzothiazoline-6-sulfonic acid); DPPH, 2,2-diphenyl-1-picrylhydrazyl.

5.3. Results and discussion

5.3.1. Design and variables selection

Data from optimization studies on the extraction of phenolics (measured by the Folin-Ciocalteau assay) and free radical scavenging activity from fruit and vegetable peels were recorded and presented in Table 2. Studies on peels valorisation through the extraction of phenolic compounds had been much more focused on fruits than on vegetables. However, only two studies included the measurement of ABTS free radical inhibition and/or DPPH free radical scavenging activities expressed in TE (Wijngaard et al. 2012). Research on optimization were performed by conventional solid-liquid extraction as well as innovative processes have been investigate such as high pressure carbon dioxide extraction (Santos and Meireles 2011), supercritical fluid extraction (Casazza et al. 2012) and pressurized liquid extraction (Wijngaard et al. 2012). According to Table 5.2, most of the experimental plans were based on CCD with three independent factors. Solvent: solid ratio was included as an independent variable in four optimization studies and it was considered a fixed factor in the majority of them. On the other hand, time and temperature were considered as independent factors in many investigations done on peels, although not always combined. Regarding the extraction of phenolic compounds from SPP, solvent: solid ratio and depth cut were identified as critical factors in a previous screening study (Anastácio and Carvalho 2013). The most favourable level identified for depth cut, 1.5 mm, was used as a fixed condition in this work. Time and temperature when varied between 60-180 min and 27-40 ºC did not influenced the extraction of phenolics from SPP (Anastácio and Carvalho, 2013). They were included in this optimization study as independent variables with a wider range of variation. Thus, the design selection for the aqueous extraction process was a three-factor CCD with solvent: solid ratio (X_1), time (X_2) and temperature (X_3) as independent variables and TPC, ABTS and DPPH assays as responses. This design and independent will provide useful information on the body of knowledge regarding extraction of phenolic compounds with antiradical activity from fruit and vegetable peels.

Table 5.2. Information on published design of experiments used in extraction optimization studies of phenolics from fruit and vegetables peels.

Nr	Peel material	Extraction method [a]	Solvent	Design (n/r)[b]	Solvent: solid (mL/g)	Time (min)	Tempe-rature (°C)	Other variables	TPC	ABTS assay	DPPH assay	(Reference)
	Fruits											
1	Apple	US[c]	Aqueous ethanol	BBD (3/17)	57	20-40	30-50	Enzyme amount	7.0	N	N	(Junjian et al.,2013)
2	Bambangan	C	Aqueous ethanol	CCD (3/20)	20-50	30	30-65	Ethanol concentration	14.6	N	N	(Prasad et al. 2011)
3	Banana	C	Methano l	CCD (3/16)	6.7	21-141	25-55	Number of extractions	22	49	31	(González-Montelongo et al. 2010)
4	Grape	SF	Carbon dioxide	F[d] (3/16)		30	37-46	Pressure and modifier	0.44	N	N	(Ghafoor et al. 2010)
5	Grape	C	Ethano l	F (3/11)	10-30	540-1740	25	---	3.22	N	N	(Casazza et al. 2012)
6	Jabuticaba	HP	Carbon dioxide	F (2/10)		45	40-80	Pressure and solid -liquid/ CO_2	13	N	N	(Santos and Meireles 2011)
7	Mangosteen	C	Aqueous methanol	CCD (3/20)	5.4-200	22-382	25	Methanol (%)	140.6	N	N	(Cheok et al. 2012)
8	Orange	US	Aqueous acetone	CCD	250	5-15	27	Ultrasound power and acetone concentration	13.57	N	N	(Dahmoune et al. 2014)
9	Pomegranate	US	Aqueous ethanol	CCD (3/16)	50	10-30	30-60	Ethanol concentration	91.98	N	N	(Tabaraki et al. 2012)
10	Pomegranate	C	Aqueous ethanol	CCD (3/16)	60	60-300	---	Ethanol concentration and pH	325	N	N	(Amyrgialaki et al. 2014)

(cont.)

Nr	Peel material	Extraction method [a]	Solvent	Design (n/r)[b]	Solvent: solid (mL/g)	Time (min)	Tempe-rature (ºC)	Other variables	TPC	ABTS assay	DPPH assay	(Reference)
11	Rambutan	US	Water	CCD (4/30)	10-20	10-30	30-50	Ultrasound power	5.52	N	N	(Prakash Maran et al. 2013)
12	Tangerine	US	Water	F (2/4)	10	30-90	40	Peels humidity	19.6	N	N	(Londoño-Londoño et al. 2010)
13	Tea fruit	C	Aqueous ethanol	CCD (3/20)	40	10-40	30-70	Ethanol concentration	47.5	N	N	(Xu et al. 2012)
	Vegetables											
14	Potato	MW	Aqueous methanol	F (3/20)	10	5-15	---	Power and % solvent	3.94	N	N	(Singh et al. 2011)
15	Potato	C	Water	BBD (3/17)	20000	40-60	40-80		3.94	N	3.52	(Wijngaard et al. 2012)
16	Potato	PL	Carbon dioxide	CCD (2/13)			65-135	Ethanol concentration	3.68	N	3.39	(Wijngaard et al. 2012)
17	Potato	C	Aqueous ethanol	CCD (3/20)	2000	5–150	25-90	Ethanol concentration	10.3	N	Y[e]	(Amado et al. 2014)

[a] C: conventional; HP: high pressure carbon dioxide assisted; MW: microwave assisted; PL: pressurized liquid; SF: supercritical fluid; US: ultrasound assisted,
[b] BBD: Box–Behnken design; CCD: Central Composite Design; F: factorial design; (n: number of factors/r: number of runs)
[c] cellulase enzymolysis extraction
[d] orthogonal array design L_{16}:4^5
[e] not expressed in TE units
X_1, solvent: solid; X_2, time; X_3, temperature; TPC: total phenolic content by Folin-Ciocalteau method (mg GAE/g dw); ABTS and DPPH assays (mg TE/g dw); N not included in the study).

5.3.2. Independent variables levels

According to Table 5.2, the widest range for solvent: solid ratio (X_1) was tested for a conventional extraction of phenols from mangosteen peels with a minimum value of 5.4 and maximum value of 200 mL/g (Cheok et al. 2012). The time of extraction (X_2) was studied at very different levels whereas the conventional extraction for grape peels had the largest range, from 540 to 1740 min (Casazza et al. 2012). The lowest level studied was five minutes for phenolics extraction from potato peels (Singh et al. 2011). Regarding temperature (X_3) of conventional extractions, the lowest and highest temperatures studied were 25 ºC (González-Montelongo et al. 2010) and 90 ºC (Amado et al. 2014), respectively. Thus, minimum and maximum levels for the independent variables in the design were 30 to 60 mL/g for solvent: solid ratio, 30 to 90 min for time and 25 to 75 ºC for temperature. These settings were adjusted near to the median of values reported for fruit and vegetable peels studies (Table 5.2).

5.3.3. Total phenolic content and free radical scavenging activity

TPC results of SPP varied between 5.64 and 11.86 mg GAE/g DM (Table 5.1). This range was wider than reported value in the previous factor screening design (Anastácio and Carvalho 2013) whereas an improvement of phenolic extraction was achieved. ABTS results of SPP presented a similar range of variation to TPC (3.91-12.85 mg TE/g DM), so these two variables are strongly associated with a Pearson's correlation coefficient (R) of 0.957 ($p<0.001$). DPPH assay results had values between 13.76 and 49.79 mg TE/g DM, and were higher than ABTS results. ABTS free radicals could be dissolved in aqueous media whereas DPPH free radicals can only be dissolved in organic media (Wojdylo at al. 2007). The differences observed for the two assays might be justified by higher antiradical activities of SPP lipophilic compounds in comparison to hydrophilic compounds. Still, DPPH and ABTS assays results values were moderately correlated ($R= 0.521$; $p<0.05$). A lower correlation of TPC with DPPH method ($R=0.666$; $p<0.01$) than with ABTS was observed. The higher ability of SPP's extracts to scavenge DPPH free radicals in comparison to inhibit ABTS free radicals may be related to compounds that are not determined by the Folin-Ciocalteau assay. Regarding other studies, TPC results were in line with those observed by Zhu et al. (2010) for the SPP methanolic infusions ranged from 8 to 20 mg GAE/g DM. However, TPC values was lower than other vegetable wastes such as broccoli (~30 mg GAE/g DM) or chicory (~13 mg GAE/g DM)

(Peschel et al. 2006). Regarding relationships between variables, a moderate correlation coefficient between TPC and ABTS was obtained for banana peels (R > 0.55) (González-Montelongo et al. 2010) while DPPH and ABTS assays results were more related (R > 0.70) than what was observed in this work.

5.3.4. Extraction modelling by RSM

RSM analysis fitted experimental values were onto second order polynomial equations. According to ANOVA results for (Table 5.3), TPC, ABTS and DPPH assays fitted models were significant at a confidence level of 0.001, and p-values of the lack of fit were all higher than 0.05 confirmed suitability of the selected design. Prediction equations were presented in Supplementary data. The coded regression coefficients sign and magnitude gives a direct measure of the contribution of the various independent variables in each response (Figure 5.2). For TPC response, temperature of extraction (X_3) presented the highest positive effect followed by the interaction term between solvent: solid and temperature (X_1X_3).

Table 5.3. Results of ANOVA for TPC, ABTS and DPPH methods with significant variables obtained by backward stepwise multiple regression method (p<0.05).

Source	TPC			ABTS assay			DPPH assay		
	DF	MSS	F value[a]	DF	MSS	F value[a]	DF	MSS	F value[a]
Model	6	10.53	48.42***	7	65.60	42.80***	3	487.60	96.29***
X_1	1	14.74	67.78***	1	6.95	32.48***	1	1351.25	266.84***
X_2									
X_3	1	30.76	141.47***	1	35.19	164.46***	1	48.11	9.50**
X_1X_2									
X_1X_3	1	10.19	46.88***	1	12.08	59.83***	1	63.45	12.53**
X_2X_3	1	2.07	9.52**	1	1.86	8.70*			
X_1^2	1	1.69	7.78*	1	2.21	10.34**			
X_2^2	1	3.71	17.10**	1	3.48	16.26**			
X_3^2				1	3.10	14.50**			
Residual	13	0.22		12	0.21				
Lack of Fit	8	0.21	0.97 ns	7	0.20	0.91 ns	5	7.39	1.84 ns
Pure Error	5	0.22		5	0.22		11	4.01	

[a] Significance indicated by the p-value: ns p>0.05; * p<0.05; ** p<0.01; *** p<0.001

TPC, total phenolic content; ABTS, ABTS radical scavenging assay; DPPH, 2,2-diphenyl-1-picrylhydrazyl; DF, degrees of freedom; MSS, mean sum of squares; X_1, solvent: solid; X_2, time; X_3, temperature.

In addition, the interaction term time-temperature (X_2X_3) and quadratic terms for time (X_2^2) and solvent: solid (X_1^2) presented a negative influence in the extraction of phenols. Compared to TPC response, ABTS fitted model include time (X_2) as main factor with a negative effect. Main factors and interactions terms with positive effect on ABTS were similar to TPC, with a decreasing order of X_3>X_1X_3>X_1. In general, terms with negative impact presented lower magnitudes than positive terms. A different fitted model was observed for DPPH assay, where all significant terms had a positive effect in the order of X_1>X_1X_3 >X_3. In general, temperature (X_3), interaction solvent: solid-temperature (X_1X_3) and solvent: solid (X_1) terms presented a positive influence on TPC, ABTS and DPPH assays responses.

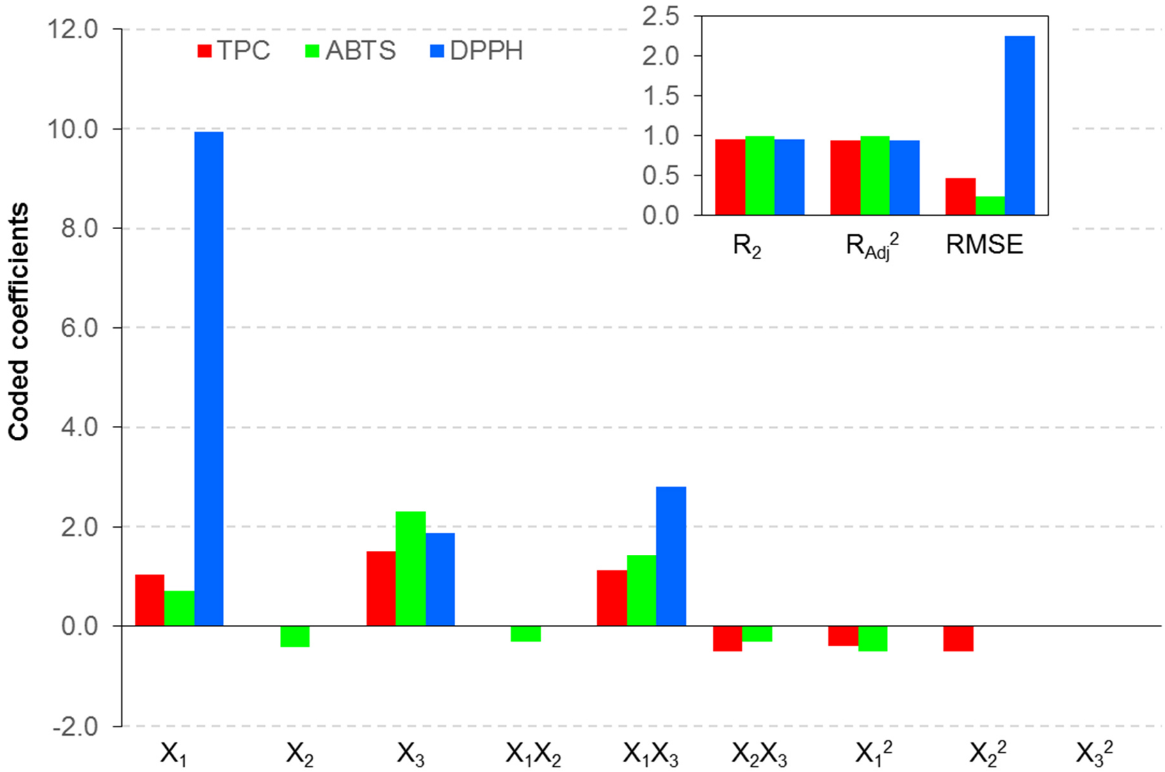

Figure 5.2. Significant coded coefficients for TPC, ABTS and DPPH assays models obtained by CCD design for sweet potato peel extracts. Model performance indicators were represented in the upper right graph. X_1, solvent: solid; X_2, time; X_3 ,temperature; TPC, total phenolic content; ABTS, 2-2'-azino-bis (3 ethylbenzothiazoline-6-sulfonic acid); DPPH, 2,2-diphenyl-1-picrylhydrazyl; R^2, coefficient of determination; R^2_{Adj}, adjusted coefficient of determination; RMSE, root mean squared error.

The significant negative terms observed TPC and ABTS models had much less magnitude than the positive ones. Time (X_2) was the least influential variable for SPP extracts and as the main effect it was only significant for ABTS. The fitted models explained 96 % of the variability observed for TPC and 99 % for ABTS whereas it was slightly lower (94 %) for DPPH assay, as indicated by the coefficients of determination (R^2) (Figure 5.2). Adjusted coefficients of determination (R^2_{Adj}) were similar in value with R^2 for all three models. Root mean squared error (RMSE) was much higher for DPPH method than the others responses which demonstrates the existence of a higher variance of the fitted model. To exemplify the combined effects of variables, surface plots of solvent: solid and temperature were constructed for TPC, ABTs and DPPH assays responses (Figure 5.3). Positive interactions between solvent: solid and temperature could be confirmed. By analysing the slopes for each variable, it is anticipated that the upper level of solvent: solid and temperature will be optimal for the extraction of phenols.

5.3.5. Extraction modelling by ANN

The ANN equation models were presented in Supplementary data. R^2 values for ANN models were 0.931 for TPC and 0.936 for both ABTS and DPPH assays. Regarding RMSE, TPC, ABTS and DPPH models presented values of 0.501, 0.458 and 1.77, respectively. Only DPPH assay model presented a higher R^2 and lower RMSE than RSM models. As ANN is not able to provide insights of the models as directly as RSM approach, the rate of change of a response with change in the given input variable was computed with the remaining fixes at their mean value (Figure 5.4). The greater the influence of the variable the higher the slope and range of change in the response. It could be observed that temperature (X_3) presented the highest influence on TPC followed by solvent: solid ratio (X_1) with a positive effect. Due to its low slope, time (X_2) had minimum impact on TPC response. For ABTS results, temperature (X_3) also presented the higher effect and positive slope. The influence of solvent solid (X_1) was similar to time (X_2) but opposite direction, solvent: solid ratio had a positive effect while time presented a negative influence. For DPPH assay values, solid: solvent was the main influential factor. Time and temperature presented both a positive slope that was lower than solvent: solid slope.

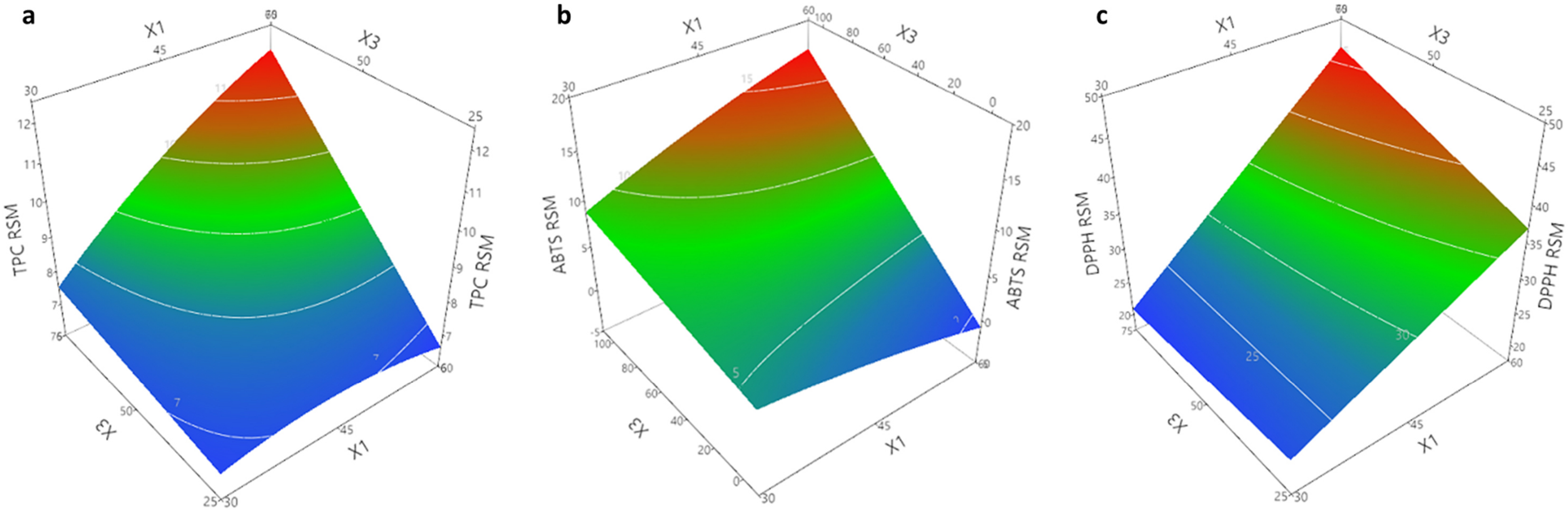

Figure 5.3. Response surface for the effects of solvent: solid ratio (X_1, mL/g) and temperature (X_3, ºC) on (a) TPC (mg GAE/g DM), (b) ABTS assay (mg TE/g DM) and (c) DPPH assay (mg TE/g DM). Time was fixed at 60 min. TPC, total phenolic content; ABTS, 2-2'-azino-bis (3 ethylbenzothiazoline-6-sulfonic acid); DPPH, 2,2-diphenyl-1-picrylhydrazyl; RSM, Response Surface Methodology; GAE, gallic acid equivalent; TE, Trolox equivalent; DM, dried material.

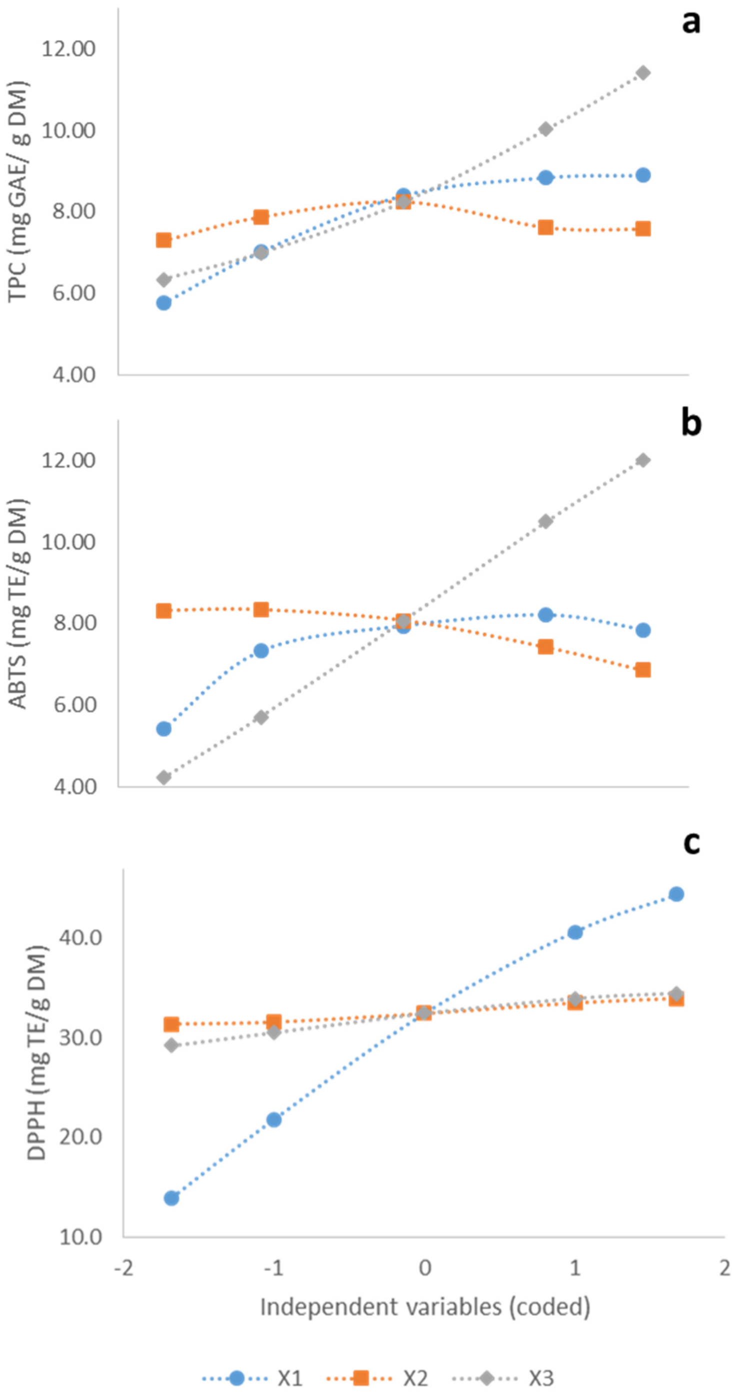

Figure 5.4. Analysis of the impact of coded variables X_1, X_3 and X_3 on ANN models for TPC, ABTS and DPPH assays. X_1, solvent: solid; X_2, time; X_3, temperature; TPC, total phenolic content; ABTS, 2-2'-azino-bis (3 ethylbenzothiazoline-6-sulfonic acid); DPPH, 2,2-diphenyl-1-picrylhydrazyl.

In summary, ANN models revealed that temperature and solvent: solid ratio have always a positive effect on responses. Time influence was always smaller than the other factors. Temperature presented a strong positive effect on both TPC and ABTS responses but less pronounced in DPPH assay results.

5.3.6. Extraction optimization

The operational extraction conditions that maximized simultaneously TPC, ABTS and DPPH assays by RSM were a solvent: solid ratio of 60 mL/g, a time of 30 minutes and a temperature of 75 ºC. These optimized settings had a desirability of 0.94. For solvent: solid ratio and temperature, the optimal point was located at the maximum of the variables range. This was consequence of a pronounced effect of these two variables individually and in interaction. An optimized temperature value at the upper limit of the experimental design was also obtained for juticaba (Santos and Meireles 2011), pomegranate (Tabaraki et al. 2012), rambutan (Prakash Maran et al. 2013) and potato peels (Wijngaard et al. 2012). At optimal extraction conditions, the predicted TPC, ABTS and DPPH assays values were 11.87 ± 0.69 mg GAE/g DM, 12.91 ± 0.42 mg TE/g DM and 46.35 ± 2.71 mg TE/g DM, respectively. When each response was maximized individually, the same optimal point was obtained for ABTS and DPPH methods. However, the optimal setting for time changed to 48.1 when TPC response was optimized individually. Predicted responses when maximizing TPC were 11.98 ± 0.61 mg GAE/g DM for TPC, 12.29 ± 0.33 mg TE/g DM for ABTS and 46.35 ± 2.71 mg TE/g DM for DPPH assay. These results were not statistically different ($p<0.05$) from the optimization for all responses. The optimal extraction conditions obtained by ANN approach were identical to RSM. ANN predicted responses were 11.44 mg GAE/g DM for TPC, 12.84 mg TE/g DM for ABTS and 47.1 mg TE/g DM for DPPH method. This optimal presented a desirability value of 0.91. When optimized individually, solvent: solid and temperature factors solution did not change from 60 mL /g and 75 ºC, respectively. However, the optimal extraction time was 42.3, 22.7 and 86.1 minutes when the desirability function was maximized only for TPC, ABTS and DPHH, respectively. Predicted responses were in line with the overall optimum.

Considering that the conventional extraction conditions were different, than those used in in study, SPP water extracts presented a higher optimal TPC value than potato peels but much lower than fruits peels (Table 5.2). SPP optimal ABTS value was than banana peels but optimal

DPPH assay was higher than potato and banana peels. When optimal solvent: solid ratio, time and temperature were represented in a scatter 3D plot with TPC as weights for bubble size (Figure 5.5), SPP was also placed next to potato, forming a group with tea fruit and bambangan peels. Thus, the aqueous extraction of phenols from SPP had a high valorisation potential within agro peels.

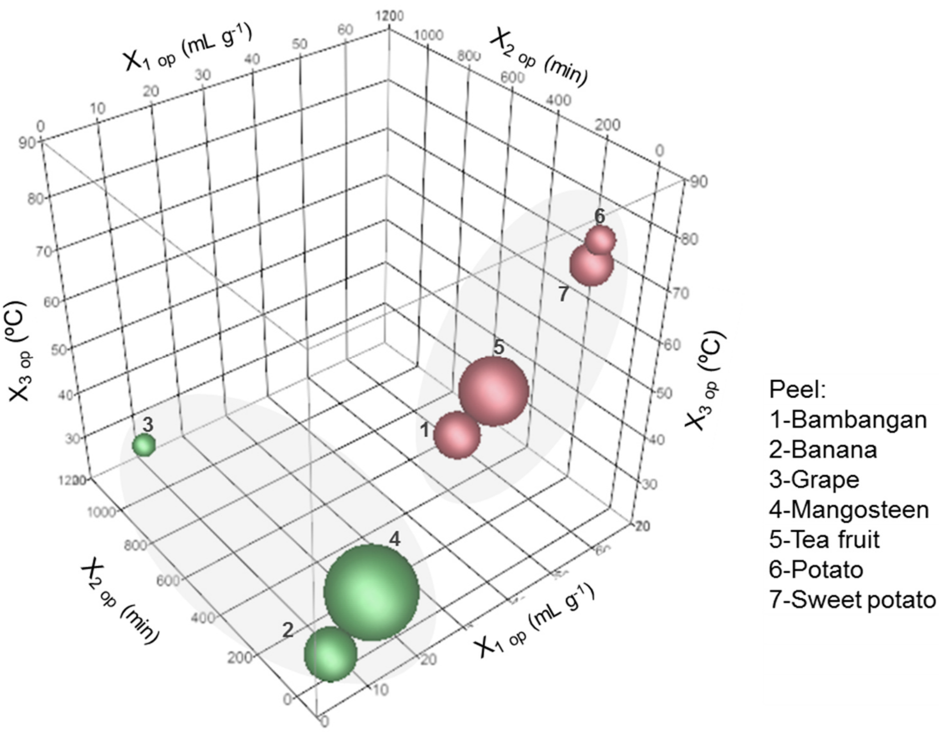

Figure 5.5. Three-dimensional scatter-plot of the optimized conditions for the extraction of phenolics from sweet potato peel and from other fruit and vegetable peels. Similar peels in optimized solvent: solid ratio ($X_{1\ op}$), time ($X_{2\ op}$) and temperature ($X_{3\ op}$) with total phenolic content as bubble size weights were under the same elliptic shadow.

5.4. Conclusions

Extraction modelling and optimization of phenolic compounds measured by Folin-Ciocalteau assay with ABTS and DPPH antiradical activities from SPP were performed by RSM and ANN based on a CCD experimental plan. Independent variables temperature and solvent solid have had a positive impact as main factors and combined on all responses. Time presented a negative influence as main effect for ABTS method and interaction with temperature for TPC and ABTS method. RSM and ANN models presented the same optimal extraction conditions by maximization of the desirability function. The optimal settings were a solvent: solid ratio of 60 mL/g, time of 30 min and temperature of 75 ºC with experimental values of 11.87 ± 0.69 mg GAE/g DM, 12.91 ± 0.42 mg TE/g DM and 46.35 ± 2.71 mg TE/g DM for TPC, ABTS and DPPH assays, respectively. SPP optimized conditions for the aqueous extraction of phenolic compounds with antiradical activity may represent an opportunity for food processors to transform this by-product from a liability to an asset. Future research will focus on the development of food applications with potential health benefits based on SPP aqueous extracts.

Conflicts of interest

The Authors declares that there is no conflict of interest.

5.5. References

Amado IR, Franco D, Sánchez M, Zapata C, Vázquez JA (2014) Optimization of antioxidant extraction from *Solanum tuberosum* potato peel waste by surface response methodology. Food Chem 165: 290-299.

Amyrgialaki E, Makris DP, Mauromoustak A, Kefalas, P (2014) Optimisation of the extraction of pomegranate (*Punica granatum*) husk phenolics using water/ethanol solvent systems and response surface methodology Ind Crop Prod 59: 216-222.

Anastácio A, Carvalho, IS (2013) Phenolics extraction from sweet potato peels: Key factors screening through a Plackett–Burman design Ind. Crop Prod 43:99-105.

Barnes, SL, Sanders, SA (2012) Advances in Functional use of Sweetpotato, [*Ipomoea batatas* (L) Lam]. Recent Pat Food Nutr Agric 4:1-7.

Blois MS (1958) Antioxidant determinations by the use of a stable free radical. Nature 181:1199–1200.

Casazza AA, Aliakbarian B, De Faveri D, Fiori L Perego P (2012) Antioxidants from winemaking wastes: A study on extraction parameters using response surface methodology. J Food Biochem 36:28-37.

Cheok CY, Chin NL, Yusof YA, Talib RA, Law CL (2012) Optimization of total phenolic content extracted from *Garcinia mangostana* Linn hull using response surface methodology versus artificial neural network. Ind Crop Prod 40:247-253.

Dahmoune F, Moussi K, Remini H, Belbahi A, Aoun O, Spigno G, Madani K (2014) Optimization of ultrasound-assisted extraction of phenolic compounds from *Citrus sinensis* L peels using response surface methodology. Chem Eng 37:889-894.

Ghafoor K, Park J, Choi Y-H (2010) Optimization of supercritical fluid extraction of bioactive compounds from grape (*Vitis labrusca* B) peel by using response surface methodology. Innov Food Sci Emerg Technol 11:485-490.

González-Montelongo R, Lobo MG, González M (2010) The effect of extraction temperature, time and number of steps on the antioxidant capacity of methanolic banana peel extracts. Sep Purif Technol 71:347-355.

Gurgel CSS, Farias SMdOC, Faria, LRG, Moreira RT (2011) Sensory analysis of sweet potato ice cream. Rev Bras Prod Agroindus 13:21 - 26

Ilzarbe L, Álvarez MJ, Viles E, Tanco M (2008) Practical applications of design of experiments in the field of engineering: a bibliographical review. Quality Reliab Eng Int 24:417-428.

Junjian R, Mingtao F, Yahui L, Guowei L, Zhengyang Z, Jun L (2013) Optimisation of ultrasonic-assisted extraction of polyphenols from apple peel employing cellulase enzymolysis. Int J Food Sci Technol 48:910-917.

Londoño-Londoño J, Lima VRd, Lara O, Gil A, Pasa, TBC, Arango GJ, Pineda JRR (2010) Clean recovery of antioxidant flavonoids from citrus peel: Optimizing an aqueous ultrasound-assisted extraction method. Food Chem 119:81-87

Maloney KP, Truong VD, Allen JC (2012) Chemical optimization of protein extraction from sweet potato (*Ipomoea batatas*) peel. J Food Sci 77:E307-312.

Manpreet, S, Sawraj, S, Sachin, D, Pankaj, S, & Banerjee, U C (2005) Influence of process parameters on the production of metabolites in solid-state fermentation. Malays J Microbiol 1:1-9

Nunes, R, Anastácio, A, Carvalho, IS (2012) Antioxidant and free radical scavenging activities of different plant parts from two *Erica* species. J Food Quality 35:307-314.

Oyeleke SB, Dauda BEN, Oyewole OA, Okoliegbe IN, Ojebode T (2012) Production of bioethanol from cassava and sweet potato peels. Adv Environ Biol 6:241-245.

Panda V, Sonkamble M, Patil S (2011) Wound healing activity of *Ipomoea batatas* tubers (sweet potato). Funct Food Health Dis 10:403-415.

Peschel W, Sánchez-Rabaneda F, Diekmann W, Plescher A, Gartzía I, Jiménez D, Lamuela-Raventós R, Buxaderas S, Codina C (2006) An industrial approach in the search of natural antioxidants from vegetable and fruit wastes. Food Chem 97:137-150.

Prakash Maran J, Manikandan S, Vigna Nivetha C, Dinesh R (2013) Ultrasound assisted extraction of bioactive compounds from *Nephelium lappaceum* L fruit peel using central composite face centred response surface design. Arabian J Chem .

Prasad KN, Hassan FA, Yang B, Kong KW, Ramanan RN, Azlan A, Ismail A (2011) Response surface optimisation for the extraction of phenolic compounds and antioxidant capacities of underutilised *Mangifera pajang* Kosterm peels. *Food Chem 128:*1121-1127.

Prieto P, Pineda M, Aguilar M (1999) Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of Vitamin E1. Anal Biochem 269:337–341.

Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C (1999) Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Biol Med 26:1231 - 1237.

Saharan B, Sahu R, Sharma D (2011) A review on biosurfactants: fermentation, current developments and perspectives. Gen Eng Biotechnol J, 2011:1-14.

Santos DT, Meireles MAA (2011) Optimization of bioactive compounds extraction from jabuticaba (*Myrciaria cauliflora*) skins assisted by high pressure CO2. Innov Food Sci Emerging Technol 12:398-406.

Singh A, Sabally K, Kubow S, Donnelly DJ, Gariepy Y, Orsat V, Raghavan GS (2011) Microwave-assisted extraction of phenolic antioxidants from potato peels. Molecules 16: 2218-2232.

Tabaraki R, Heidarizadi E, Benvidi A (2012) Optimization of ultrasonic-assisted extraction of pomegranate (*Punica granatum* L) peel antioxidants by response surface methodology. Sep Purif Technol 98:16-23.

Wen L, Yang B, Cui C, You L, Zhao M (2012) Ultrasound-assisted extraction of phenolics from longan (*Dimocarpus longan Lour*) fruit seed with artificial neural network and their antioxidant activity. Food Anal Method 5:1244-1251.

Wijngaard HH, Ballay M, Brunton N (2012) The optimisation of extraction of antioxidants from potato peel by pressurised liquids. Food Chem 133:1123-1130.

Wireko-Manu FD, Ellis WO, Oduro I (2010) Production of a non-alcoholic beverage from sweet potato (*Ipomoea batatas* L). Afr J Food Sci 4:180 - 183.

Wojdylo A, Oszmianski J, Czemerys R (2007) Antioxidant activity and phenolic compounds in 32 selected herbs. *Food Chem 105:*940-949.

Xu P, Bao J, Gao J, Zhou T, Wang Y (2012) Optimization of extraction of phenolic antioxidants from tea (*Camellia sinensis* L) fruit peel biomass using response surface methodology. BioResources 7:2431-2443.

Zhu F, Cai YZ, Yang X, Ke J, Corke H (2010) Anthocyanins, hydroxycinnamic acid derivatives, and antioxidant activity in roots of different chinese purple-fleshed sweetpotato genotypes. J Agric Food Chem *58:*7588-7596.

5.6. Supplementary data

RSM Predictive equations

X_1: solvent: solid (30-60 mL/g)

X_2: time (30-90 min)

X_3: temperature (25-75 ºC)

TPC=

$$
\begin{aligned}
&8.57911098092693\\
&+ 1.03876552171705 * \left(\frac{(X1 - 45)}{15}\right)\\
&+ 1.50070162440203 * \left(\frac{(X3 - 50)}{25}\right)\\
&+ \left(\frac{(X1 - 45)}{15}\right) * \left(\left(\frac{(X3 - 50)}{25}\right) * 1.12875\right)\\
&+ \left(\frac{(X2 - 60)}{30}\right) * \left(\left(\frac{(X3 - 50)}{25}\right) * -0.50875\right)\\
&+ \left(\frac{(X1 - 45)}{15}\right) * \left(\left(\frac{(X1 - 45)}{15}\right) * -0.3851748800979\right)\\
&+ \left(\frac{(X2 - 60)}{30}\right) * \left(\left(\frac{(X2 - 60)}{30}\right) * -0.5053830328996\right)
\end{aligned}
$$

ABTS assay=

$$
\begin{aligned}
&7.94499053006147\\
&+ 0.71731725600671 * \left(\frac{(X1 - 45)}{15}\right)\\
&+ -0.4212645535247 * \left(\frac{(X2 - 60)}{30}\right)\\
&+ 2.30149385615806 * \left(\frac{(X3 - 50)}{25}\right)\\
&+ \left(\frac{(X1 - 45)}{15}\right) * \left(\left(\frac{(X2 - 60)}{30}\right) * -0.30875\right)\\
&+ \left(\frac{(X1 - 45)}{15}\right) * \left(\left(\frac{(X3 - 50)}{25}\right) * 1.42625\right)\\
&+ \left(\frac{(X2 - 60)}{30}\right) * \left(\left(\frac{(X3 - 50)}{25}\right) * -0.29625\right)\\
&+ \left(\frac{(X1 - 45)}{15}\right) * \left(\left(\frac{(X1 - 45)}{15}\right) * -0.5037624679552\right)
\end{aligned}
$$

DPPH assay=

$$
\begin{aligned}
&31.7055\\
&+ 9.94701951133673 * \left(\frac{(X1 - 45)}{15}\right)\\
&+ 1.87687576221541 * \left(\frac{(X3 - 50)}{25}\right)\\
&+ \left(\frac{(X1 - 45)}{15}\right) * \left(\left(\frac{(X3 - 50)}{25}\right) * 2.81625\right)
\end{aligned}
$$

ANN Predictive equations

X_1: solvent: solid (30-60 mL/g)

X_2: time (30-90 min)

X_3: temperature (25-75 ºC)

TanH: hyperbolic tangent

TPC =

3.0298

$-2.1187 * TanH (0.5 * (-4.4344 + 0.056085 * X_1 + 0.022434 * X_2 + 0.0063245 * X_3))$

$-6.0797 * TanH (0.5 * (-2.6474 + 0.063560 * X_1 - 0.019351 * X_2 + 0.025436 * X_3))$

$- 4.3914 * TanH (0.5 * (-1.7177 + 0.018516 * X_1 + 0.0094428 *X_2 + 0.0064907 * X_3))$

$+ 5.5140 * TanH (0.5 * (-0.62970 + 0.056814 * X_1 + 0.0026407 * X_2 - 0.020855* X_3))$

$- 8.0205 * TanH (0.5 * (-1.1013 + 0.044306 * X_1 - 0.026027 *X_2 - 0.018798 * X_3))$

$- 15.5538 * TanH (0.5 * (2.5852 - 0.043737 * X_1 + 0.010170 * X_2 - 0.019675 * X_3))$

ABTS assay=

3.5384

$- 11.5766 * TanH (0.5 * (-4.4344 + 0.056085 * X_1 + 0.022434 * X_2 + 0.0063245 * X_3))$

$- 10.3526 * TanH (0.5 * (-2.6474 + 0.063560 * X_1 - 0.019351 * X_2 + 0.025436 * X_3))$

$+ 13.2513 * TanH (0.5 * (-1.7177 + 0.018516 * X_1 + 0.0094428 * X_2 + 0.0064907 * X_3))$

$+ 5.7319 * TanH (0.5 * (-0.62970 + 0.056814 * X_1 + 0.0026407 * X_2 - 0.020855 * X_3))$

$- 6.8284 * TanH (0.5 * (-1.1013 + 0.044306 * X_1 - 0.026027 *X_2 - 0.018798 * X_3))$

$- 22.3724 * TanH (0.5 * (2.5852 - 0.043737 * X_1 + 0.010170 * X_2 - 0.019675 * X_3))$

DPPH assay=

22.5131

$- 2.6371 * TanH(0.5 * (-4.4344 + 0.056085 * X_1 + 0.022434 * X_2 + 0.0063245 * X_3))$

$- 19.3363 * TanH(0.5 * (-2.6474 + 0.063560 * X_1 - 0.019351 * X_2 + 0.025436 * X_3))$

$+ 0.71650 * TanH(0.5 * (-1.7177) + 0.018516 * X_1 + 0.0094428 * X_2 + 0.0064907 * X_3))$

$+ 21.6820 * TanH (0.5 * (-0.62970 + 0.056814 * X_1 + 0.0026407 * X_2 - 0.020855 * X_3))$

$- 11.8997 * TanH (0.5 * (-1.1013 + 0.044306 * X_1 - 0.026027 * X_2 - 0.018798 * X_3))$

$- 46.3678 * TanH (0.5 * (2.5852 + -0.043737 * X_1 + 0.010170 * X_2 - 0.019675 * X_3))$

Chapter 6

Development of a beverage benchtop prototype based on sweet potato peels: optimization of antioxidant activity by a mixture design

The content of this Chapter was submitted as Anastácio, A. and Carvalho, I.S. Development of a beverage benchtop prototype based on sweet potato peels: optimization of antioxidant activity by a mixture design. *Food Science and Nutrition*.

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Development of a beverage benchtop prototype based on sweet potato peels: optimization of antioxidant activity by a mixture design

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Abstract

A beverage benchtop prototype related to oxidative stress protection was developed based on sweet potato peels phenolics. Formula components were sweet potato peel (*Ipomoeas batatas*) aqueous extract (SPPE), sweet potato leaves water extract (SPLE) and honey solution (HonS). According to linear squares regression (LSR) models, SPLE presented higher positive effect on total phenolic content (TPC), FRAP and DPPH assays than the other components. All antagonist interactions were not significant. The optimum formula obtained by artificial neural networks (ANN) analysis was 50.0 % of SPPE, 21.5 % of SPLE and 28.5 % of HonS. Predicted responses of TPC, FRAP, DPPH assays and soluble solids were 309 mg GAE/L, 476 mg TE/L, 1098 mg TE/L and 12.3 ºBrix, respectively. Optimization with LSR models was similar to ANN. Beverage prototype results positioned next to commercial vegetable and fruit beverages thus it has an interesting potential to the market of health and wellness.

Keywords: *Ipomoeas batatas*; Sweet potato peels; sweet potato leaves; honey; beverage; phenolics; antioxidant activity; mixture design; artificial neural network; optimization.

6.1. Introduction

Oxidative stress accelerates aging and contributes to several degenerative diseases due to the presence of excessive free radicals inside cells. These reactive oxygen species can cause cell injury due to structural damage and activation of gene regulatory proteins leading to disease (Adly 2010). As antioxidants, phenolics may protect cell constituents against oxidative damage. There is a growing interest in agro-wastes valorisation into functional products due to the increasing sustainable development demand from society (Spatafora and Tringali 2012). Sweet potato (*Ipomoea batatas*) peels are one of the major wastes generated during root processing (Maloney et al. 2012). Owing to their high phenolic content and antioxidant activity, sweet potato peels were considered to be a valuable source for biological active compounds for functional foods (Anastácio and Carvalho 2013a). Sweet potato had demonstrated a protective effect on oxidative-stress-related neurodegenerative diseases such as atherosclerosis (Miyazaki et al. 2008) and Alzheimer (Ye et al. 2010). Since peels can reach almost three times more antioxidant activity than any other sweet potato plant tissues (Cevallos-Casals and Cisneros-Zevallos 2001), they may also have health benefits related to oxidative stress prevention. To leverage the conversion of sweet potato peels from a liability to an asset, studies on its application on innovative foods are needed. The use of by-products as a source of active ingredients to produce functional beverages was already considered a promising approach (Corbo et al. 2014). Due to an increased demand for intake of dietary antioxidants, the development of an antioxidant rich beverage should be considered (Nanasombat et al. 2015). The consumption of beverages rich in polyphenols limit the risk of various degenerative diseases associated with oxidative stress due to their role in protecting cell constituents against damage (Pandey and Rizvi 2009). Additionally, the growth of the health and wellness beverage market is set to outperform the wider soft and hot drinks industry until 2016 (Cowland 2013). Sweet potato was the base of a non-alcoholic beverage with properties similar to fruit juices (Weirko-Manu et al. 2010), and claimed in a patented functional juice (Barnes and Sanders 2012) and anti-aging food (McDaniel 2010). The addition of peel extracts of citrus (Kim et al. 2012) and plums (de Beer et al. 2012) to beverages raised their antioxidant activity. Thus, an antioxidant beverage based on sweet potato peels is a promising path for its valorisation. Using also sweet potato leaves as a formula component enhances the recycling concept of the new product. Leaves were identified as potential source of antioxidants (Anastácio and Carvalho 2013b) that could prevent or moderate oxidative stress related diseases (Islam 2006). There are few patented beverages with sweet potato peels or leaves. Sweet potato peels (Chuanbin and Xianxun 2011) and leaves (Qiuyun 2012) were used for the production of beers. Regarding non-alcoholic beverages, only leaves were patented for a juice beverage (Hong 2011). The beverage prototype could have poor taste acceptability due to astringency related to phenolic content (de Beer et al. 2012). Honey was also added to the formulations as a sweetener and also as a functional component because its radical scavenging activity can ameliorate oxidative stress (Erejuwa et al. 2012). Honey was already used in combination with sweet potato tuber in patented functional foods, as curd (Luo 2014), cake (Tong et al. 2014), preserved strip (Lin 2014, Laijin et al. 2009) and candied (Yong 2012). The assessment of synergistic and antagonistic interactions between components is relevant for functional foods (Wang et al. 2011) and can be achieved through a mixture design methodology on the beverage formula. This approach was used in the optimization of a nutraceutical fruit juice (Lawless et al. 2012) and a cardio protection beverage (Gunathilake et al. 2013). Artificial neural network (ANN) has been applied in parallel with multivariate techniques to increase modelling performance. Few applications of ANNs have been published for optimization of mixture designs related to food. The aim of this study was to optimize the antioxidant activity of a beverage benchtop prototype made from sweet potato peel aqueous extract (SPPE), sweet potato leaves aqueous extract (SPLE) and honey solution (HonS) as components, through a mixture design approach with linear squares regression (LSR) and ANN analysis. To evaluate its potential as a functional food, the antioxidant activity of this beverage was compared with both commercial and laboratory drinks.

6.2. Materials and Methods

6.2.1 Chemicals

2,2-Diphenyl-2-picrylhydrazyl (DPPH), Folin–Ciocalteau reagent, gallic acid and Trolox were purchased from Sigma-Aldrich Co. Ltd. (Poole, U.K.). Iron (III) chloride, sodium acetate, sodium carbonate, glacial acetic acid and 2,4,6-tripyridyl-2-triazine (TPTZ), were purchased from VWR (West Chester, PA). Absolute ethanol was purchased from Merck (Nottingham, U.K.). All reagents were of analytical grade.

6.2.2. Preparation of the sweet potato extracts

Sweet potatoes were purchased at the local market, washed under tap water and air dried during the night. Roots were peeled with a cut depth circa 1.5 mm in one direction with a hand peeler. Sweet potato leaves were cut from vines collected from the field at Aljezur (Portugal), washed under tap water and dried with paper towels. Peels and leaves were dried at 60 ºC in a Binder BD 53 incubator (Binder, Tuttilgen, Germany) during 48 h and 24 h, respectively. The dried materials were milled until all particles were smaller than 600 µm (30 mesh). Extractions were performed, in a screw cap flask (100 mL) in a stirring hot plate (HS0707V2, Favorit, Malaysia) stirred at constant speed of 200 rpm at 75 ºC during 45 min. A solid: solvent ratio of 16.7 mg/mL was used for peels and 8.3 mg/mL for leaves. After filtration with a vacuum pump aspirator (DOA-P604-BN, Cast Manufacturing Inc., USA) and centrifugation (Hettich Universal 320, Tuttlingen, Germany) at 3000 g for 10 min, SPPE and SPLE were stored at -18 ºC.

6.2.3. Preparation of the honey solution

Rosemary honey from regional origin was purchase in the local market. Because beverage mixtures were prepared by volume for formulation convenience, a honey solution (HonS) was used with a concentration of 0.535 g/mL. It presented soluble solids (SS) of 44 ºBrix measured by a pocket digital refractometer (Pocket Pal-1 ATAGO, Japan).

6.2.4. Beverage evaluation

Total phenolic content (TPC), ferric-reducing antioxidant power (FRAP) and DPPH (2,2-Diphenyl-2-picrylhydrazyl) radical scavenging activity were determined according to the method described by Prieto et al. (1999), Benzie and Strain (1996) and Blois (1958), respectively, with minor adaptations indicated in Nunes et al. (2012). TPC was expressed in mg of gallic acid equivalent (GAE) by 1 L of beverage, while FRAP and DPPH assays in mg of Trolox equivalent (TE) by 1L of beverage. All absorbance readings were made in a T70+ UV/Vis Spectrometer (PG Instruments Ltd, United Kingdom). SS were measured as indicated for honey solution. Determinations were carried out in triplicate and data were reported as mean ± standard deviation.

6.2.5. Experimental design and statistical analysis

The experimental plan comprising a total of 24 runs was obtained using a *I*-optimal randomized mixture design for three components, SPPE, SPLE and HonS (Table 6.1). The proportional levels of components were set at 0.5-1.0 (50-100 %) for SPPE and 0-0.5 (0-50 %) for both SPLE and Hon. Beverage mixtures were blended volumetrically.

Table 6.1. Mixture design and total phenolic content, antioxidant activity and soluble solids of beverage formulations based on sweet potato peel. SPPE: sweet potato peel water extract; SPLE: sweet potato leaves water extract; HonS: honey solution;

Std ord[a]	Run	Components			Responses[b]			
		SPPE	SPLE	HonS	TPC (mg GAE/L)	FRAP (mg TE/L)	DPPH assay (mg TE/L)	SS (ºBrix)
4	1	0.667	0.167	0.166	287 ± 4	454 ± 9	1147 ± 282	7.5 ± 0.2
8	2	0.500	0.000	0.500	258 ± 15	283 ± 5	637 ± 213	21.0 ± 0.2
9	3	0.500	0.150	0.350	287 ± 9	406 ± 9	874 ± 72	15.5 ± 0.2
12	4	0.500	0.500	0.000	325 ± 5	626 ± 30	1634 ± 262	0.7 ± 0.1
12	5	0.500	0.500	0.000	337 ± 5	660 ± 20	2038 ± 246[c]	0.7 ± 0.1
2	6	0.850	0.000	0.150	275 ± 19	432 ± 31	1000 ± 289	6.8 ± 0.2
9	7	0.500	0.150	0.350	297 ± 13	415 ± 13	982 ± 21	15.1 ± 0.2
8	8	0.500	0.000	0.500	250 ± 6	307 ± 13	587 ± 80	21.0 ± 0.1
5	9	0.645	0.000	0.355	258 ± 3	347 ± 16	739 ± 135	15.2 ± 0.2
4	10	0.667	0.167	0.166	296 ± 18	513 ± 11	1325 ± 36	7.4 ± 0.1
2	11	0.850	0.000	0.150	265 ± 14	435 ± 15	974 ± 120	7.0 ± 0.1
6	12	0.650	0.350	0.000	276 ± 41b	589 ± 42	1699 ± 138	0.7 ± 0.1
6	13	0.650	0.350	0.000	320 ± 16	609 ± 16[c]	1573 ± 64	0.0 ± 0.0
1	14	1.000	0.000	0.000	255 ± 13	499 ± 19	1204 ± 71	0.8 ± 0.1
4	15	0.667	0.167	0.166	308 ± 12	507 ± 22	1269 ± 71	7.5 ± 0.1
11	16	0.500	0.372	0.128	333 ± 8	573 ± 30	1399 ± 109	6.0 ± 0.1
3	17	0.850	0.000	0.150	278 ± 6	460 ± 18	1017 ± 181	6.8 ± 0.1
5	18	0.645	0.000	0.355	261 ± 9	368 ± 16	815 ± 97	12.4 ± 0.1
3	19	0.850	0.150	0.000	269 ± 9	488 ± 98	1449 ± 32	0.8 ± 0.1
3	20	0.850	0.150	0.000	273 ± 13	550 ± 7	1462 ± 66	0.7 ± 0.1
1	21	1.000	0.000	0.000	255 ± 7	518 ± 17	924 ± 503[c]	0.8 ± 0.0
8	22	0.500	0.000	0.500	256 ± 8	364 ± 19	722 ± 520	20.9 ± 0.0
7	23	0.618	0.382	0.000	321 ± 20	712 ± 31[c]	1760 ± 116	0.6 ± 0.1
10	24	0.500	0.350	0.150	327 ± 3	616 ± 28	1447 ± 119	6.7 ± 0.0

[a] standard order
[b] mean ± standard deviation of three determinations
[c] outlier

TPC, FRAP, DPPH assays and SS results were approximated to a Scheffé polynomial model by the LSR method ($P< 0.05$). Analysis of variance (ANOVA) was computed to determine coefficients significance. Diagnostic tools such as normal plot of the residuals distribution, outlier detection and Box–Cox plots were computed to check models adequacy. The coefficient of determination (R^2) and adjusted coefficient of determination (R^2_{Adj}) were used to access models performance. For the analysis by ANN, a simple single layer structure was used with the three components SPPE, SPLE and HonS as inputs, one hidden layer with three nodes and TPC, FRAP, DPPH assays and SS as outputs (Figure 6.1). The data were divided into two subsets, training and validation and network topology was trained by k-fold cross validation method (k=6). Inputs were multiplied by weights and computed by hyperbolic-tangent activation function in the hidden layer. The number of neurons in the hidden layer was adjusted iteratively to maximize performance fitting determined by R^2 and root mean squared error (RMSE). Optimization was performed by maximizing the desirability function. To compare SPPE beverage with other antioxidant beverages, data was represented by Box–Cox plots. JMP® Pro 10.0.2 software (www.jmp.com) provided at no cost by SAS (www.sas.com) was used in the design of experiments, data analysis and creation of the graphics, except Figure 6.2 which was made with Microsoft® Excel 2013.

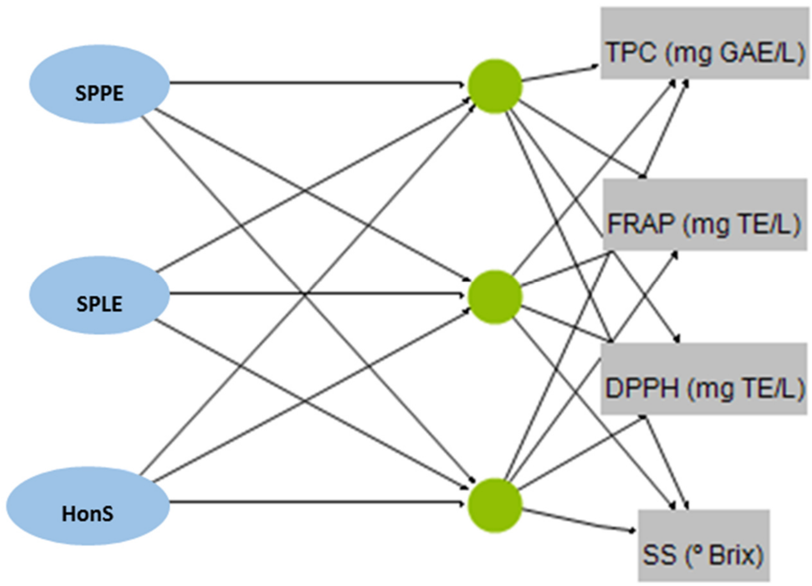

Figure 6.1. Diagram of the Artificial Neural Network architecture.

6.3. Results and discussion

6.3.1. Beverage concept and components selection

The concept associated to the SPPE functional beverage was to provide a product based on sweet potato wastes, rich in antioxidants and sweetened with HonS. Valorisation of SPPE was the driving force of this study and the inclusion of SPLE reinforces product appeal regarding recycling and sustainability. A common limiting factor in waste valorisation is its practical suitability (Peschel et al. 2006). The direct use of SPPE as they are obtained by aqueous extraction in the liquid form addresses this issue. A sweetener was added to the beverage formula to mask the probable astringency related to its high phenolic content. HonS was chosen because it also contributes to antioxidant activity.

6.3.2. Component levels selection

Restrictions on mixture components were established according to beverage concept. Thus, as the functional beverage was intended to be based on sweet potato peels, a minimum of 50 % was established for this component. Its upper level was set to maximum (100 %) to gain a better knowledge of its potential. The other two components, SPLE and HonS were then varied in the remaining design space, between 0 and 50 %.

6.3.3. Mixture design selection

The study objective was to optimize a beverage formulation based on predictive models. The selection of the mixture design selection had to incorporate the restriction applied to SPPE (minimum of 50 %). Optimal designs efficiency is based on providing a high reliability of information with a reduced number of runs. *I-optimal* designs are adequate for the objective of this study because the average variance is minimized over restricted design region (Yeh et al. 2010). The number of run replicates was chosen so the Scheffé polynomial model could be used to fit experimental data. It was observed that the antioxidant activity of a combination of foods may not be equal to the weighted sum of the antioxidant activity derived from the individual components (Wang et al. 2011). With this model, cross terms were included to access synergistic or antagonistic effects beside the linear main-effect terms.

6.3.4. Design responses selection

Although *in vitro* antioxidant assays lack specificity, they are useful to access the potential of a new product at the initial stages of development. TPC based on Folin-Ciocalteau reagent, DPPH assay and FRAP were chosen as design responses mainly because they are profusely used in food products such as beverages. FRAP was considered a reasonable screen assay for the ability to maintain redox status in cells or tissues but for DPPH radical, no similarity to the highly reactive radicals involved in lipid peroxidation was found (Prior et al. 2005). These responses allowed the characterization of the antioxidant function present in beverage formulations by different reaction mechanisms. The FRAP mechanism is totally electron transfer while DPPH radical may be neutralized either by electron transfer or by hydrogen atom transfer (radical quenching) (Prior et al. 2005). Soluble solids were also included as a response as an indicator of formulations sweetness/perceived astringency.

6.3.5 Beverage formula modelling

To proceed modelling by LSR method, pseudo-components defined as X_{SPPE}=((SPPE-0.5)/0.5), X_{SPLE}=(SPLE/0.5) and X_{HonS}=(HonS/0.5) were used. After running diagnostic plots (data not shown), no data transformation was required and outliers detected in each response were not considered for modelling. According to ANOVA results (Table 6.2), all models were significant ($P< 0.001$) and presented non-significant lack of fit. The significance, sign and magnitude of the coefficients of the model depicted in Figure 6.2 can assess the impact of beverage components in each response. The coefficient estimate of the linear terms X_{SPPE}, X_{SPLE} and X_{HonS} represented the fitted response at the three vertices of the triangle while the coefficients of the cross terms $X_{SPPE}*X_{SPLE}$, $X_{SPPE}*X_{HonS}$, $X_{SPLE}*X_{HonS}$ and $X_{SPPE}*X_{SPLE}*X_{HonS}$ were indicative of the curvature across each edge of the factor space. Synergistic or antagonistic effects of component pairs could be evaluated by positive or negative coefficients of the cross terms. For the TPC model, all linear pseudo-components were significant ($P< 0.001$) and had a positive contribution. X_{SPLE} term presented 1.2 times more effect than X_{SPPE} or X_{HonS}.

Table 6.2. Analysis of variance (ANOVA) and results of TPC, FRAP, DPPH assay and SS. TPC: total phenolic content; FRAP: ferric-reducing antioxidant power; DPPH: 1,1-Diphenyl-2-picrylhydrazyl radical scavenging activity; SS: soluble solids; LSM: least squared regression; ANN: artificial neural network.

	TPC			**FRAP**			**DPPH assay**			**SS**		
ANOVA												
Source	DF[a]	MSS[b]	F value[c]	DF	MSS	F value	DF	MSS	F value	DF	MSS	F value
Model	6	2922.48	49.08***	6	35860.70	42.44***	6	436866.00	105.08***	6	195.01	518.87***
Lack of Fit	8	70.53	1.45 ns	7	1048.16	1.57 ns	8	3475.00	0.70 ns	8	0.27	0.56 ns
Pure Error	8	48.56		8	667.27		7	4937.21		9	0.47	
C. Total	22			21			21			23		
LSM												
R^2 [d]		0.948			0.944			0.977			0.995	
R^2_{Adj} [e]		0.922			0.922			0.967			0.993	
RMSE		7.29			31.99			61.27			0.703	
ANN												
R^2		0.995			0.996			0.972			0.998	
RMSE		1.87			6.60			57.39			0.320	

[a] degrees of freedom
[b] mean sum of squares
[c] P value: ns $P > 0.05$; *** $P < 0.001$
[d] coefficient of determination
[e] adjusted coefficient of determination

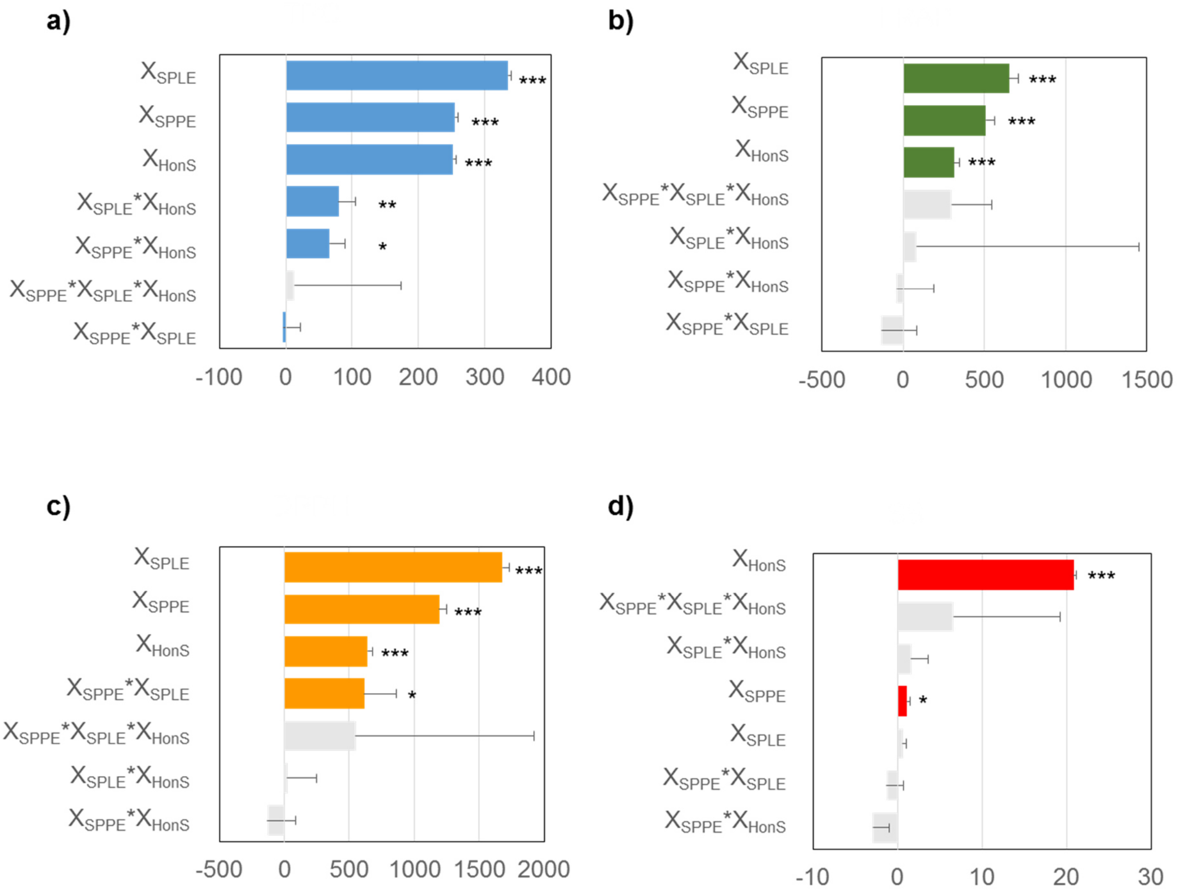

Figure 6.2 Effect of beverage pseudo-components X_{SPPE}, X_{SPLE} and X_{HonS} on TPC (a), FRAP (b), DPPH assay (c) and SS (d) obtained by least squares regression method. $^{*}P < 0.05$; $^{**}P < 0.01$and $^{***}P < 0.001$.

The significant cross effect terms were the positive interactions $X_{SPLE}*X_{HonS}$ and $X_{SPPE}*X_{HonS}$. These synergetic effects were over four times lower than the magnitude of the X_{SPLE} individual term. Therefore, the overall pseudo components effects order for TPC was $X_{SPLE} > X_{SPPE} \sim X_{HonS} > X_{SPLE}*X_{HonS} > X_{SPPE}*X_{HonS}$. Regarding antioxidant activity, linear terms were significant ($P< 0.001$) and had a positive contribution on both FRAP and DPPH assay. A synergetic effect between X_{SPPE} and X_{SPLE} was observed for DPPH assay with a magnitude more than 1.7 lower than the respective individual terms. As this effect was not significant for FRAP, the synergetic interaction between phenolics from sweet potato leaves and peels may be more related to a radical quenching mechanism. Thus, FRAP model presented an positive effect order of $X_{SPLE} > X_{SPPE} > X_{HonS}$ while overall pseudo components effects order for DPPH assay was $X_{SPLE} > X_{SPPE} > X_{HonS} \sim X_{SPPE}*X_{SPLE}$. As expected, pseudo-components effect on SS was quite different than for the other responses. X_{HonS} was the dominant significant term but

X_{SPPE} also presented a positive contribution although much smaller in magnitude. TPC, FRAP, DPPH assays and SS models presented R^2 values higher than 0.94 (Table 6.2). As R^2_{Adj} values were in line with R^2, models fitting quality was considered high. In summary, SPLE was the component with highest influence on beverage phenolic content and antioxidant activity, followed by SPPE and HonS. HonS played the major influence in SS response with SPPE contributing also to the beverage perceived sweetness. SPPE, SPLE and HonS when combined at the levels of the mixture design presented predominantly positive effects regarding the beverage antioxidant activity. The interactions of SPPE with HonS and of SPPE with HonS were significantly synergetic for TPC were significant for. SPPE and SPLE presented also a significantly synergetic effect for DPPH assay. No significant antagonistic effects were observed. Predictive equations were depicted in Table 6.3 and represented in ternary plots in Figure 6.3. The ANN approach provided more complex predictive models than LSR (Table 6.2) as outliers were not removed and components were used as such (not transformed into pseudo-components).

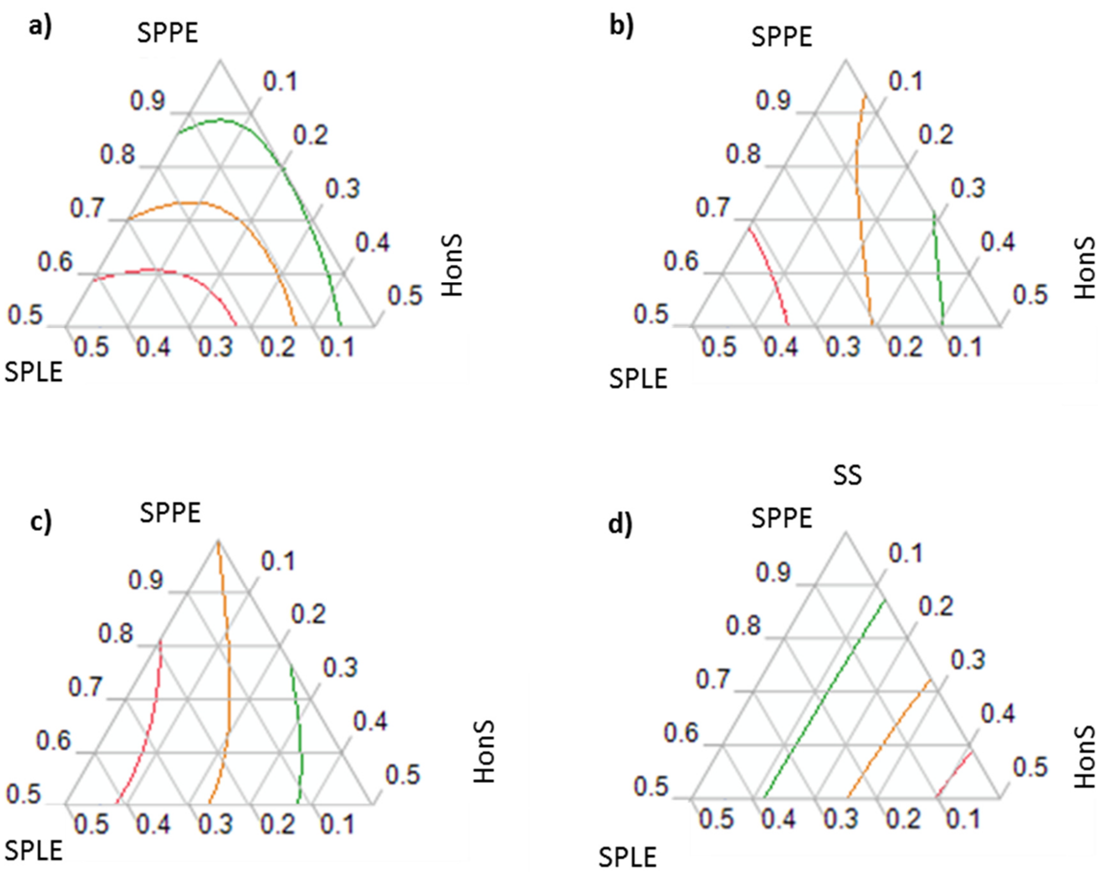

Figure 6.3. SPP beverage ternary plots of models obtained by LSR for a) TPC, b) FRAP, c) DPPH assay and d) SS.

Table 6.3. Predictive equations for TPC, FRAP, DPPH assay and SS by Least Squared Regression and Artificial Neural Network methodologies.

	Least-Squared Regression
	Pseudo components
	X_{SPPE}=((SPPE-0.5)/0.5)
	X_{SPLE}=(SPLE/0.5)
	X_{HonS}=(HonS/0.5)
TPC=	+ 259.72*X_{SPPE}+328.24*X_{SPLE}+252.12* X_{SPLE}
	- 44.184*X_{SPPE}*X_{SPLE}
	+ 53.969*X_{SPPE}*X_{SPLE}
	+ 96.8013* X_{SPLE} *X_{SPLE}
	+ 138.49* X_{SPPE} *X_{SPL}*X_{SPLE}
FRAP=	+ 502.18*X_{SPPE} +643.46 *X_{SPLE}+310.91*X_{SPLE}
	- 121.72*X_{SPPE} *X_{SPLE}
	- 24.209*X_{SPPE}*X_{SPLE}
	+106.49* X_{SPLE}*X_{SPLE}
	+ 275.38* X_{SPPE}*X_{SPLE}*X_{SPLE}
DPPH= assay	+1200.0*X_{SPPE}+1659.5*X_{SPLE}+641.17*X_{SPLE}
	+ 560.05*X_{SPPE} * X_{SPLE}
	-153.08*X_{SPPE}*X_{SPLE}
	+ 70.611* X_{SPLE} *X_{SPLE}
	+ 729.80*X_{SPPE} *X_{SPLE}*X_{SPLE}
SS=	+1.3142*X_{SPPE}+0.5411*X_{SPLE} +20.838*X_{SPLE}
	-1.5822*X_{SPPE}*X_{SPLE}
	-3.4708*X_{SPPE}*X_{SPLE}
	+ 1.7349*X_{SPLE}*X_{SPLE}
	+ 7.3174*X_{SPPE}*X_{SPLE}*X_{SPLE}
	Artificial Neural Network
	Input components
	H1=0.45597+1.14435*SPPE+1.24526*SPLE+0.83444*HonS
	H2=-1.13170-0.60170*SPPE+0.77913*SPLE-5.19503*HonS
	H3=0.038834+2.23013*SPPE-1.47347*SPLE+0.47381*HonS
TPC=	-1099.22
	+1996.22*TanH(0.5***H1**) -208.4685*TanH(0.5***H2**) -138.4977*TanH(0.5***H3**)
FRAP=	-3375.974
	+5840.550*TanH(0.5***H1**) -187.7337*TanH(0.5***H2**)-179.4318*TanH(0.5***H3**)
DPPH= assay	-8351.47686
	+15064.63*TanH(0.5***H1**)+99.1915*TanH(0.5***H2**)-402.2132*TanH(0.5***H3**)
SS=	+240.3730
	-354.1427*TanH(0.5***H1**)-5.35792*TanH(0.5***H2**)-9.59026*TanH(0.5***H3**)

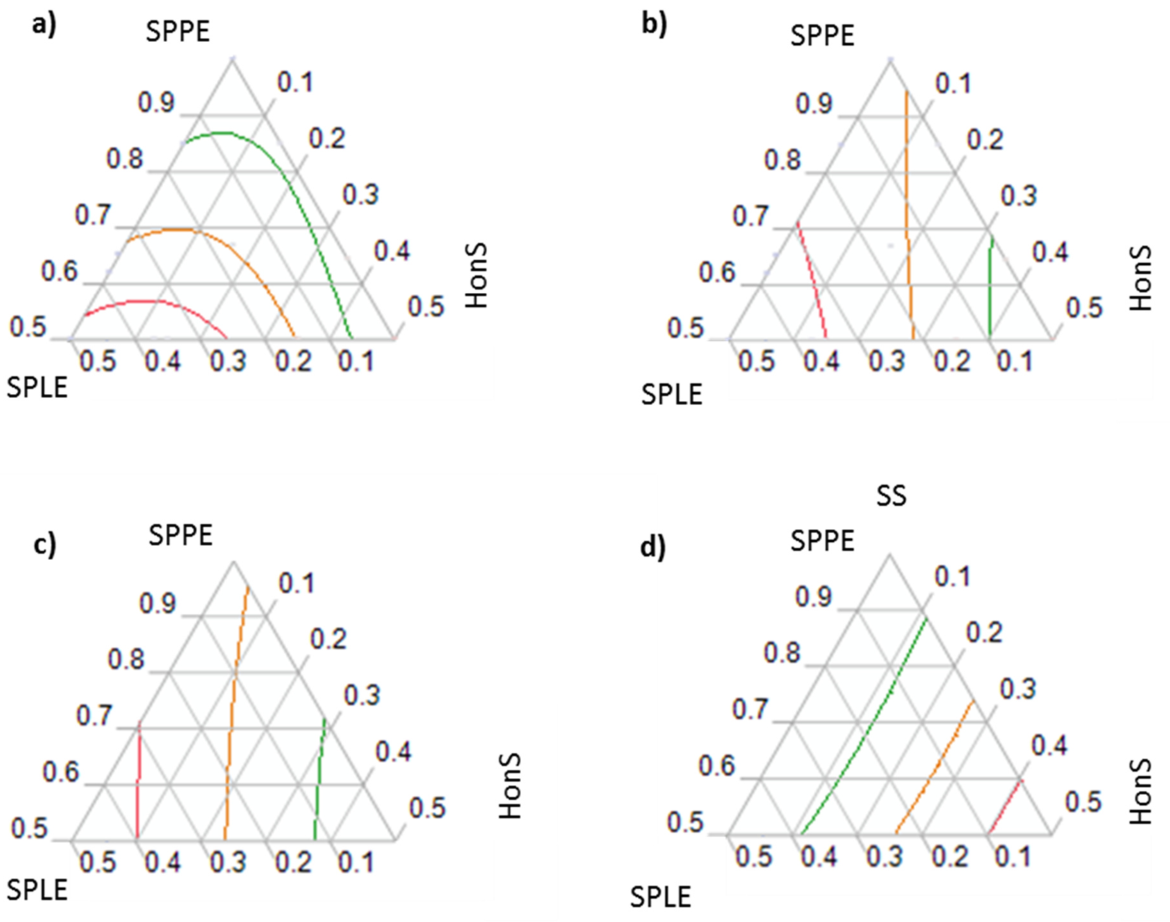

Figure 6.4. SPP beverage ternary plot of model obtained by ANN for a) TPC, b) FRAP, c) DPPH assay and d) SS.

All ANN models presented better fitting performance than LSR, except for DPPH assay and regarding R^2. When represented in a ternary plot, ANN models had similar trends as LSR across the design area for all responses (Figure 6.4).

6.3.6. Beverage formula optimization

The desirability function was used to simultaneously optimize all the responses. Formula optimization was performed by two different criteria. For the first criteria (C1), all the responses were maximized and weighted equally in the overall desirability function while for the second criteria (C2), settings were equal to C1 plus the additional requirement of a minimum of 12.3 ºBrix for SS. This constrain in SS corresponded to the overall SS mean of commercial and laboratory prototype beverages with antioxidant activity. With the optimization criteria C1, the optimal formula obtained from LSR models was 50.0 % of SPPE, 36.2 % of SPLE and 13.8 % of HonS (Figure 6.5) with predicted responses of 323 ± 3 mg GAE/L, 572 ± 30 mg TE/L, 1391 ± 72 mg TE/L and 6.5 ± 0.6 ºBrix for TPC, FRAP, DPPH assays and SS, respectively. The desirability value was 0.588 and optimum was located in an extreme of the design space. Similar results were obtained by ANN models (Figure 6.5). The optimal formulation by ANN models was 50.0 % of SPPE, 38.3 % of SPLE and 11.2 % of HonS and predicted responses were of 331 mg GAE/L for TPC, 582 mg TE/L for FRAP, 1469 mg TE/L for DPPH assay and 5.7 ºBrix for SS. Using C2 optimization criteria, the design space for optimization was reduced so SPPE could vary between 50 and 70 %, SPLE between 0 and 23 % and HonS between 27 and 50%. With this constraint, the optimized beverage formula was 50.0 % SPPE, 22.1 % SPLE and 27.9 % HonS. Predicted values for TPC, FRAP, DPPH assays and SS were (308 ± 10) mg GAE/L, 483 ± 38 mg TE/L, 1106 ± 8 mg TE/L and 12.3 ± 0.8 ºBrix, respectively. By increasing HonS component 1.2 times, the antioxidant activity decreased 17 % for FRAP and 22 % for DPPH assay. The value of the desirability function for the formula obtained by C2 (0.543) was similar to C1 and had higher probable taste acceptability. Using ANN models, optimum was similar to LSR models, with 50.0 % SPPE, 21.5 % SPLE and 28.5 % HonS as optimal formula. Predicted responses were of 309 mg GAE/L for TPC, 476 mg TE/L for FRAP, 1098 mg TE/L for DPPH assay and 12.3 ºBrix for SS. There were no relevant differences between LSR and ANN optimization results, thus future developments should use predictive equations obtained with higher efficiency. As all runs of the mixture design was used in ANN modelling, the chosen optimized beverage prototype formula was 50.0% SPPE, 21.5 % SPLE and 28.5 % HonS.

6.3.7. Comparison with antioxidant beverages

In order to benchmark the SPPE beverage, statistics from data on the phenolic content, antioxidant activity and soluble solids of commercial and laboratory prototype beverages from scientific journals were computed.

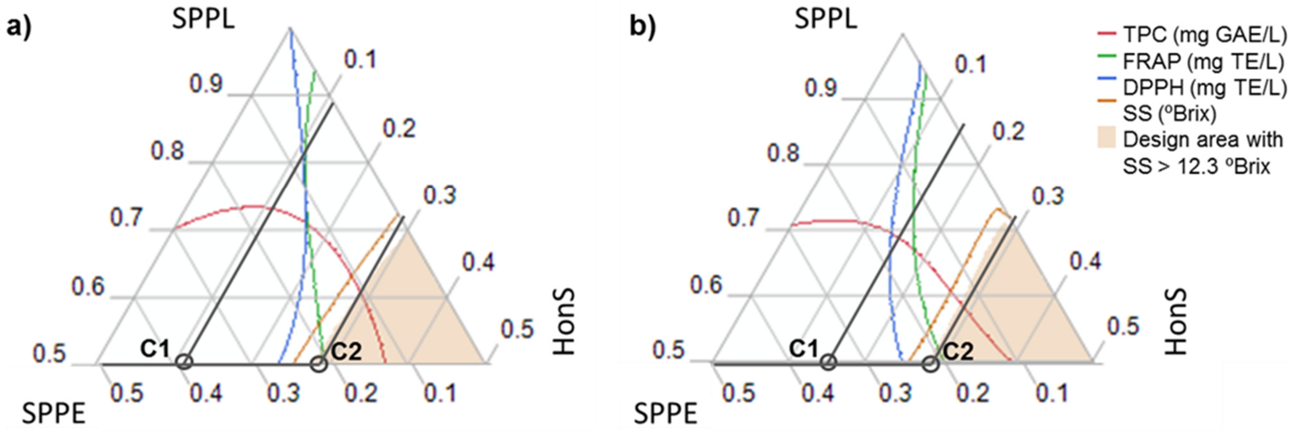

Figure 6.5. Optimal SPP beverage formula for criteria C1 (no restrains) and C2 (SS > 12.3 ºBrix) obtained by a) LSR models and b) ANN models.

The commercial antioxidant beverages considered were fruit juices (González-Molina et al. 2012, Pulido et al. 2003, Seeram et al. 2008, de Beer et al. 2012, Pellegrini et al. 2003), vegetable juices (Wootton-Beard and Ryan 2012), herbal infusions (Ho et al. 2010, Bravo et al. 2007, Pellegrini et al. 2003) and teas (Bravo et al. 2007, Pulido et al. 2003, Ho et al. 2010). Three laboratory beverages were also used for comparison, a plum peel enriched nectar (de Beer et al. 2012), a cardio protection fruit beverage (Gunathilake et al. 2013) and a nutraceutical fruit juice (Lawless et al. 2012). The range of TPC response for SPPE beverage was within fruit juices range of variation but it was lower than the other commercial and laboratory beverages (Figure 6.6). Regarding SPPE beverage antioxidant activity, FRAP range was within herbal infusion and tea, and above vegetable juice variations. However, DPPH assay box-plot showed that SPPE beverage overlapped fruit juice variation and was higher than vegetable juice and plum peel enriched beverage. Finally, SS box plots confirmed HonS component selected levels as adequate. In general, SPPE beverage benchtop prototype was competitive to fruit and vegetable juices regarding antioxidant activity. It presented however less phenolics than the commercial and laboratory beverages used for comparison.

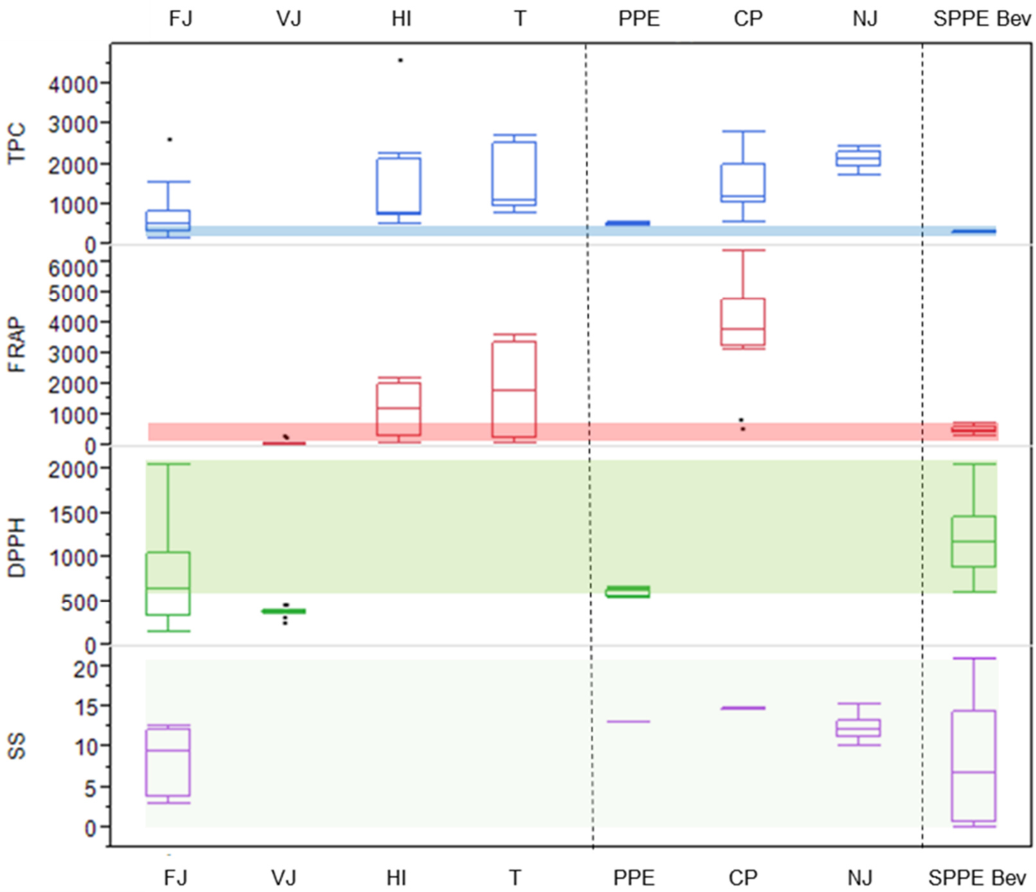

Figure 6.6. Box plots on TPC (mg GAE/L), FRAP (mg TE/L), DPPH assay (mg TE/L) and SS (ºBrix) data of commercial and laboratory drinks and SPP beverage. Shaded areas corresponded to the range of variation for SPP beverage. Beverage categories: FJ; fruit juice; VJ, vegetable juice; HI, herbal infusion; T, tea; PPE, plum peel enriched nectar; CP; cardio protective beverage; NJ, nutraceutical juice; SPP Bev, sweet potato peel beverage.

6.4. Conclusions

The optimized formula obtained by ANN models for the SPPE beverage benchtop prototype was 50.0 % of SPPE, 21.5 % of SPLE and 28.5 % of HonS. TPC, FRAP, DPPH assays and SS predicted responses for the optimal formula were 309 mg GAE/L, 476 mg TE/L, 1098 mg TE/L and 12.3 ºBrix, respectively. LSR models revealed that components presented positive effect on TPC, FRAP, DPPH assays in the order SPLE>SPPE>HonS. Synergetic effects were observed between SPPE and HonS and SPLE and HonS for TPC. The interaction between SPPE and SPLE was synergetic for DPPH method. Antagonist effects in all responses were not significant (P> 0.05). Sweet potato peel beverage was positioned next to commercial vegetable and fruit beverages in terms of antioxidant activity. Further research on beverage composition is relevant before initiating studies on product quality, safety and stability.

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Conflict of interest

The authors declare no conflict of interest.

6.5. References

Adly, A. A. (2010). Oxidative stress and disease: an updated review. *Research Journal of Immunology,* **3,** 129-45.

Anastácio, A. & Carvalho, I. S. (2013a). Phenolics extraction from sweet potato peels: Key factors screening through a Plackett–Burman design. *Industrial Crops and Products,* **43,** 99-105.

Anastácio, A. & Carvalho, I. S. (2013b). Spotlight on PGI sweet potato from Europe: Study of plant part, time and solvent effects on antioxidant activity. Journal of Food Biochemistry, **37**, 628–637.

Barnes, S. & Sanders, S. (2012). Advances in functional use of sweetpotato,[*Ipomoea batatas* (L.) Lam]. *Recent Patents on Food, Nutrition & Agriculture*, **4**, 148-154.

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

Blois, M. S. (1958). Antioxidant determinations by the use of a stable free radical. *Nature,* **181**, 1199–1200.

Bravo, L., Goya, L. & Lecumberri, E. (2007). LC/MS characterization of phenolic constituents of mate (*Ilex paraguariensis*, St. Hil.) and its antioxidant activity compared to commonly consumed beverages. *Food Research International,* **40,** 393-405.

Cevallos-Casals, B. & Cisneros-Zevallos, L. (2001). Bioactive and functional properties of purple sweetpotato (*Ipomoea batatas* (L.) Lam). In: I International Conference on Sweetpotato. Food and Health for the Future 583. pp. 195-203.

Chuanbin, W. & Xianxun, S. (2011). Functional beer and brewing method thereof. Patent Nr. CN102250701 (B); CN102250701 (A), 2011-11-23.

Corbo, M. R., Bevilacqua, A., Petruzzi, L., Casanova, F. P. & Sinigaglia, M. (2014). Functional beverages: the emerging side of functional foods. *Comprehensive Reviews in Food Science and Food Safety,* **13,** 1192-1206.

Cowland, D. (2013). Health and Wellness Beverages Outperform Wider Drinks Industry. Euromonitor International.

De Beer, D., Steyn, N., Joubert, E. & Muller, N. (2012). Enhancing the polyphenol content of a red-fleshed Japanese plum (*Prunus salicina* Lindl.) nectar by incorporating a polyphenol-rich extract from the skins. *Journal of the Science of Food and Agriculture,* **92,** 2741-2750.

Erejuwa, O. O., Sulaiman, S. A. & Ab Wahab, M. S. (2012). Honey-a novel antidiabetic agent. *International Journal of Biological Sciences,* **8,** 913-934.

González-Molina, E., Gironés-Vilaplana, A., Mena, P., Moreno, D. A. & García-Viguera, C. (2012). New Beverages of lemon juice with elderberry and grape concentrates as a source of bioactive compounds. *Journal of Food Science,* **77,** C727-C733.

Gunathilake, K. D. P. P., Rupasinghe, H. P. V. & Pitts, N. L. (2013). Formulation and characterization of a bioactive-enriched fruit beverage designed for cardio-protection. *Food Research International,* **52,** 535-541.

Ho, S.-C., Wu, S.-P., Lin, S.-M. & Tang, Y.-L. (2010). Comparison of anti-glycation capacities of several herbal infusions with that of green tea. *Food Chemistry,* **122,** 768-774.

Hong, L. (2011). Black sweet potato juice beverage and preparation method thereof. Patent Nr. CN102224956 (A), 2011-10-26.

Islam, S. (2006). Sweetpotato (*Ipomoea batatas* L.) leaf: its potential effect on human health and nutrition. *Journal of Food Science,* **71,** R13-R121.

Kim, J. K., Baik, M. Y., Hahm, Y. T. & Kim, B. Y. (2012). Development and optimization of a drink utilizing Citrus (*Citrus unshiu*) peel extract. *Journal of Food Process Engineering,* **35,** 557-571.

Laijin, W., Qiming, G. & Nenlian, Z. (2009). Processing method of honey dried sweet potato. Patent Nr. CN101491322 (B); CN101491322 (A), 2009-07-29.

Lawless, L. J., Threlfall, R. T., Meullenet, J. F. & Howard, L. R. (2012). Consumer-based optimization of blackberry, blueberry and concord juice blends. *Journal of Sensory Studies,* **27,** 439-450.

Lin, M. (2014). Dried walnut and honey health-care pumpkin and sweet potato strips and production method thereof. Patent Nr. CN103518928 (A), 2014-01-22.

Luo, J. (2014). Sweet potato honey bean curd and preparation method thereof. Patent Nr. CN104054838 (A), 2014-09-24.

Maloney, K. P., Truong, V. D. & Allen, J. C. (2012). Chemical optimization of protein extraction from sweet potato (*Ipomoea batatas*) peel. *Journal of Food Science,* **77,** E307-E312.

McDaniel, D. H. (2010). Methods and compositions for identifying, producing and using plant-derived products for modulating cell function and aging. Google Patents.

Miyazaki, K., Makino, K., Iwadate, E., Deguchi, Y. & Ishikawa, F. (2008). Anthocyanins from purple sweet potato *Ipomoea batatas* cultivar Ayamurasaki suppress the development of atherosclerotic lesions and both enhancements of oxidative stress and soluble vascular cell adhesion molecule-1 in apolipoprotein E-deficient mice. *Journal of Agricultural and Food Chemistry,* **56,** 11485-11492.

Nanasombat, S., Thonglong, J. & Jitlakha, J. (2015). Formulation and characterization of novel functional beverages with antioxidant and anti-acetylcholinesterase activities. Functional *Foods in Health and Disease,* **5,** 1-16.

Nunes, R., Anastácio, A. & Carvalho, I. S. (2012). Antioxidant and free radical scavenging activities of different plant parts from two *Erica* species. *Journal of Food Quality,* **35,** 307-314.

Pandey, K. B. & Rizvi, S. I. (2009). Plant polyphenols as dietary antioxidants in human health and disease. *Oxidative Medicine and Cellular Longevity,* **2,** 270-278.

Peschel, W., Sánchez-Rabaneda, F., Diekmann, W., Plescher, A., Gartzía, I., Jiménez, D., Lamuela-Raventos, R., Buxaderas, S. & Codina, C. (2006). An industrial approach in the search of natural antioxidants from vegetable and fruit wastes. *Food Chemistry,* **97,** 137-150.

Prieto, P., Pineda, M. & Aguilar, M. (1999). Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. *Analytical Biochemistry,* **269,** 337-341.

Prior, R. L., Wu, X. & Schaich, K. (2005). Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *Journal of Agricultural and Food Chemistry,* **53,** 4290-4302.

Pulido, R., Hernandez-Garcia, M. & Saura-Calixto, F. (2003). Contribution of beverages to the intake of lipophilic and hydrophilic antioxidants in the Spanish diet. *European journal of Clinical Nutrition,* **57,** 1275-1282.

Qiuyun, H. (2012). Method for producing whole sweet potato powder. Patent Nr. CN102511754 (A), 2012-06-27.

Spatafora, C. & Tringali, C. (2012). Valorization of vegetable waste: identification of bioactive compounds and their chemo-enzymatic optimization. *Open Agriculture Journal,* **6,** 9-169.

Tong, J., Zong, J. & Lu, Z. (2014). Honey-flavor sweet potato fruit and preparation method thereof. Patent Nr. CN103931858 (A), 2014-07-23.

Wang, S., Meckling, K. A., Marcone, M. F., Kakuda, Y. & Tsao, R. (2011). Synergistic, additive, and antagonistic effects of food mixtures on total antioxidant capacities. *Journal of Agricultural and Food Chemistry,* **59,** 960-968.

Weirko-Manu, F., Ellis, W. & Oduro, I. (2010). Production of a non-alcoholic beverage from sweet potato (*Ipomoea batatas* L.). *African Journal of Food Science,* **4,** 180-183.

Wootton-Beard, P. C. & Ryan, L. (2012). Combined use of multiple methodologies for the measurement of total antioxidant capacity in UK commercially available vegetable juices. *Plant Foods for Human Nutrition,* **67,** 142-147.

Ye, J., Meng, X., Yan, C. & Wang, C. (2010). Effect of purple sweet potato anthocyanins on β-amyloid-mediated PC-12 cells death by inhibition of oxidative stress. *Neurochemical Research,* 35**,** 357-365.

Yeh, M.-F., Cece, A. & Presser, M. (2010).Custom Designs Using JMP® Design of Experiments and SAS® PROC OPTEX. SAS Global Forum 2010. Paper 196.

Yong, L. (2012). Honey sweet potato. Patent Nr. CN102511606 (A), 2012-06-27.

Chapter 7

General conclusions

General conclusions

Functional foods demand a continuous innovative approach to develop convenient and healthy foods according to consumer's expectations. The commencing stages involve the conversion of an essential concept often originated from academic research into a prototype. A framework for the valorisation of agro wastes into a food application through the development of a benchtop prototype with added value was applied to sweet potato (SP). A functional beverage with attributes related to oxidative stress protection was developed using sweet potato peels (SPP) and sweet potato leaves (SPL) as components. The developing processes involved the following steps: literature and patent review, preliminary study, key factors screening, extraction optimization and beverage formula optimization. Total phenolic content and antioxidant activity used to evaluate experiments was measured by Folin-Ciocalteau method and *in vitro* screening assays, respectively.

Literature review for the period 2003-2014 showed that SPP presented lower research amount on phenolic compounds with antioxidant activity then SPL. SPP also presented a lower number of patented food products than SPL for the same period of time. *In vitro* antioxidant activity was mainly evaluated by the DPPH assay. The experimental designs used in research were predominantly the comparative type and design of experiments was used in reduced number. Flour/powder was the most studied food application with health benefits in articles while for beverage was the top patented food. The enhancement of antioxidant defence was a health benefit established for SPL by clinical trials while for SPP they were no studies. An antioxidant beverage was the elected food application with health benefits for the valorisation of SPP and SPL. Text mining analysis on articles and patents abstracts could show the state of the art regarding SP food applications by dendograms and co-occurrence networks.

A preliminary study on phenolic content and antioxidant activity of sweet potato vines by a factorial design revealed that plant part (stems or leaves) presented the largest main significant effect ($P < 0.001$) on TPC, TFC, TAA, RP and FRAP while for DPPH assay solvent was the most influential factor. Time of extraction presented a significant effect only on RP assay, as main effect and interaction with plant part. SPL flour reached up to 122 ± 0.07 mg GAE/g dw for TPC, 0.59 ± 0.05 g QE/100 g dw for TFC, 1.06 ± 036 g AAE/100 g dw for TAA, 50.8 ± 2.2 g GAE/100 g dw for RP, 29.3 ± 2.8 mM TE/g dw for FRAP and 7.9 ± 2.0 mM TE/g dw for

DPPH method. Principal components analysis revealed that TFC, TPC, RP and FRAP were highly correlated while TAA was more correlated with DPPH assay.

The extraction of phenolic compounds with antioxidant activity from SPP flour was screened by a Plackett-Burman design to select key factors among nine variables such as solvent: solid ratio, time, pH, peeling cut depth, particle size, temperature, solvent, sample amount and agitation. Solvent: solid ratio and peel cut depth presented similar effect size but opposite direction (positive and negative, respectively) on TPC ($p < 0.05$). These two factors had a similar effect size on FRAP. Regression models revealed significant main effects and two factors interactions for FRAP but not for TPC. After transformation of FRAP by the squared root function, sample size was selected by the stepwise regression procedure as main effect and in interaction with solvent: solid ratio. In addition, a small positive influence of pH in interaction with solvent: solid ratio on antioxidant activity was observed. TPC and FRAP presented a Pearson a correlation (R) of 0.77.

In the development stage regarding extraction optimization, phenolic compounds with ABTS and DPPH radical scavenging activities from SPP flour were modelled and optimized by a CCD experimental plan. In decreasing order, temperature factor, the interaction between solvent: solid and temperature factors, and solvent: solid factor presented positive effects on TPC and ABTS methods. An inverse order was observed for DPPH assay. Optimized extraction conditions obtained by both RSM and ANN were 60 mL/g, 30 min and 75 ºC. The obtained responses at the optimized conditions were as follow: 11.87 ± 0.69 mg GAE/g DM for TPC, 12.91 ± 0.42 mg TE/g DM for ABTS method and 46.35 ± 3.08 mg TE/g DM for DPPH method. SPP presented similar behaviour in both optimal conditions and phenolic content obtained from peels of potato, peels of tea fruit and peels of bambangan (mango).

The optimized formula for the antioxidant beverage benchtop prototype obtained by ANN models was 50.0 % of SPPE, 21.5 % of SPLE and 28.5 % of HonS. Predicted results for the optimal formula on TPC, FRAP, DPPH assay and SS were 309 mg GAE/L, 476 mg TE/L, 1098 mg TE/L and 12.3 ºBrix, respectively. LSR models revealed that components presented positive effect on TPC, FRAP and DPPH assays in the order SPLE>SPPE>HonS. Synergetic effects were observed between SPPE and HonS and SPLE and HonS for TPC. The interaction between SPPE and SPLE was synergetic for DPPH assay. Antagonist effects in all responses were not significant (P> 0.05). The beverage benchtop prototype was positioned next to commercial vegetable and fruit beverages in terms of antioxidant activity. Further research on beverage composition is relevant before initiating studies on product quality, safety and stability.

These findings reduced knowledge gaps on the field of food application with health benefits related to SPL and especially SPP. Predictive models from this study may help researchers in their functional food development processes. Transfer of this knowledge to food processors may help transform SPP and SPL from a liability to an asset through their valorisation into innovative products for the market of health and wellness.

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