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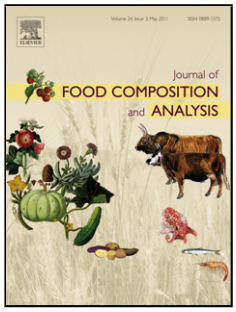

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Development of new apple beverages rich in isothiocyanates by using extracts obtained from ultrasound-treated cauliflower by-products: Evaluation of its physical properties and consumer acceptance

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HIGHLIGHTS

- New apple juices (AJ) enriched with isothiocyanates (ITC) were obtained
- Cauliflower by-products (CBP) were used as source of ITC
- CBP extracts exhibited the highest ITC yield after sonication (20% amplitude, 5 min)
- CBP extracts were included in apple juices at different percentages (0-40%)
- CBP extract inclusion in AJ is appropriate at 10% from a sensorial point of view

ABSTRACT

The objective of this study was to develop a new apple juice beverage enriched with isothiocyanates (ITC) - rich extracts obtained from cauliflower by-products. Ultrasound-assisted extraction (UAE) at different amplitudes (20 – 100 %) and extraction times (0 – 10 min) at a frequency of 24 kHz was employed to obtain ITC-rich extracts. It was found that both amplitude and treatment time had a significant ($p<0.05$) effect on the recovery of ITC from cauliflower leaves and stems, obtaining the highest yields of ITC from leaves (≈3000 μM) and stems (≈7000 μM) after UAE (80% amplitude, 3 min) and UAE (20%, 3 min), respectively. Moreover, the highest recovery of total phenolic compounds (TPC) (≈105 mg gallic acid equivalent (GAE)/L) μM) was found after UAE (100% amplitude, 3 min) of TPC from stems. ITC-rich extracts obtained from caulifower by-products at the optimum UAE conditions were added into apple juice (10-40%), thus increasing the ITC content of the juice and observing the highest values in the new beverage when the highest percentage (40%) was added. Significant differences in smell and taste were found in the apple juices containing 20% and 40% cauliflower extracts compared to control (0% UAE cauliflower waste extracts added) samples. However, the results showed that the beverage

with 10% extract addition preserved well the sensorial properties with regard to control sample and no total color differences (TCD<3) were observed for any new sample compared to control. Therefore, the addition of extracts obtained after UAE of cauliflower wastes can be a useful tool to obtain new beverages rich in ITC although further research dealing with the bioaccessibility and bioavailability of these compounds in the new beverages should be conducted.

KEYWORDS: Cauliflower by-products; apple juice, ultrasound-assisted extraction; isothiocyanates; polyphenols, antioxidant; new food products; food composition; food analysis

1. Introduction

A high consumption of Brassica vegetables has been shown to be correlated with a decrease of cancers such as lung, stomach, colon and rectal cancers (Holst and Williamson, 2004). These beneficial effects have been attributed to their high content in bioactive compounds such as polyphenols and glucosinolates. However, the biological activity of glucosinolates is not simple, as they are hydrolyzed by the endogenous enzyme myrosinase into isothiocyanates, which are also responsible of most of the anticarcinogenic properties of the Brassica vegetables (Zhang, 2004, Veeranki et al., 2015). Unfortunately, isothiocyanates can have undesirable sensory characteristics and tend to be disliked (Cox et al., 2012; Drewnowski and Gomez-Carneros, 2000; Reed et al., 2006). The solution is to extract these compounds from natural vegetable sources as

well as from their derived wastes and by-products and use as health functional ingredients in food products (Barba et al., 2015a; Deng et al., 2014; Galanakis, 2013; Parniakov et al., 2015).

Cauliflower (*Brassica oleracea* var. Botrytis) is one of the main Brassicaceae crops and a great amount of cauliflower wastes and by-products are generated at various stages of production chain (Llorach et al., 2003). About 36% of waste is generated from total weight of cauliflower in terms of leaves and stems (Ospina Machado and Villamizar C, 2004). Cauliflower has total glucosinolates content lower than other common Brassica vegetables (Tiwari and Cummins, 2013). However, although its total glucosinolates content is lower, cauliflower has the highest waste index (ratio of non-edible to edible portion after harvesting), which generates huge quantities of organic solid waste that creates an undesirable odour (Oberoi et al., 2007). Therefore, these wastes can constitute an important source of high-added value compounds which can be used as food additives for the development of new food products and/or nutraceuticals.

For the recovery of valuable compounds from plant food materials, wastes and by-products, several conventional extraction methods based on maceration and heat extraction at high temperatures (>60 ºC) alone and/or combined with different solvents, which can be toxic (i.e., hexane, acetone, methanol, etc.) have been used. Moreover, the use of high temperatures may promote nutritional losses (Barba et al., 2014; Parniakov et al., 2014).

At this stage of development, there is a need to develop new extraction methods that can reduce the extraction time, process temperature and solvent consumption and contribute to higher extraction efficiency and lower energy consumption as compared to conventional extraction techniques (Deng et al., 2014; Galanakis, 2012; Koubaa et al., 2015).

For instance, both food researchers and food industry have shown an increased interest in the use of ultrasound-assisted extraction (UAE) as it may be a good alternative to conventional extraction methods to reocover bioactive compounds (Chemat et al., 2017; Pingret et al., 2013; Rombaut et al., 2014), especially phenolic compounds and isothiocyanates, from different plant food materials, wastes and by-products (Barba et al., 2015b; Deng et al., 2014; Rosello-Soto et al., 2015; Wang et al., 2011). This technique enhances extraction owing to cavitation phenomena, caused by creation of ultrasound pressure waves in the extraction solvents, thus facilitating cell disruption and solvent penetration. UAE can be used alone and/or combined with conventional extraction method as it requires low capital cost (Chemat et al., 2012; Tabasso et al., 2015). Moreover, this methodology can reduce the temperature and solvent amount, as well as the energy consumption, and the wastes generated when other processes are used (Chemat et al., 2017; Li et al., 2013). Moreover, UAE can be combined with green solvents, thus improving high-added value compounds extraction yields (Barba et al., 2014; Grassino et al., 2016; Rosello-Soto et al., 2014; Šic-Žlabur et al., 2015).

Previous studies have assessed and added cauliflower wastes and by-products to foodstuffs such as beef sausage to enhance its nutritional value (Abul-Fadl, 2012) or even in cereal based ready-to-eat expanded snacks as a novel source of dietary antioxidants, fibre and proteins (Stojceska et al., 2008). Regarding beverages, cauliflower wastes and by-products have been only used to enrich a commercial chicken soup with its polyphenol extracts (Llorach et al., 2005). For instance, it will be interesting to work on the enrichment of liquid foods to enhance their nutritional quality using phytochemical extracts rich in bioactive compounds such as polyphenols and isothiocyanates.

The aims of the present work were 1) to prepare a powder rich in phenolic compounds and isothiocyanates from cauliflower wastes; 2) to study the effects of UAE on the

recovery of phenolic compounds and isothiocyanates from cauliflower wastes powders and 2) to develop a beverage with the obtained extracts, rich in isothiocyanates (with potential health benefits). The physical properties (i.e. colour and/or viscosity); as well as the consumer acceptability of the new beverage were also studied.

2. Materials and methods

2.1. Chemicals and reagents

Distilled water, methanol, acetonitrile, potassium phosphate buffer, sulforaphane standard, 1,2-benzendithiol, acetate buffer, 2,4,6-tripyridyl-s-triazine, ferric chloride, ferrous sulphate, 2,2-diphenyl-1-picrylhydrazyl (DPPH), butylated hydroxyl anisole (BHA), Folin-Ciocalteu reagent, $N_{a2}CO_3$ and gallic acid were purchased from Sigma Aldrich (Dorset, UK).

2.2. Samples

2.2.1. Production of cauliflower by-product powder

Cauliflowers were purchased at a local market in Manchester, UK. These were separated into two parts, edible and non-edible. The non-edible part; stem and leaves constituted the cauliflower by-products. Prior to blanching, the stem and leaves were washed with water and immediately drained with paper towel to absorb the excess of water. The blanching procedure followed the conditions predetermined by several studies (Stojceska et al., 2008; Tanongkankit et al., 2010; Volden et al., 2008; Wennberg et al., 2006). Blanching was performed in a hot-water bath at 90±2 °C for 1 min for both cauliflower stem and leaves. Blanched stem and leaves were immediately cooled in cold water, and chopped into small pieces. The prepared cauliflower by-products were dried within 10

min after preparation (Figure 1). The samples were dried at 50-55 °C overnight using a Havest Maid harvest dryer (Hydraflow Industrial Ltd, Upper Hutt, New Zealand) to a final moisture content of approximately 10%. Following drying, the samples were milled using a 0.5 mm mesh screen ZM100 Mill (Retch, Dusseldorf, Germany) to develop a powder. They were then packed and sealed in polyethylene bags and kept at room temperature until needed for analysis or extraction. This process was conducted to enhance the myrosinase activity due to cell damage which occurs during blanching and drying accompanied by the hydrolysis for the formation isothiocyanates (Holst and Williamson, 2004).

2.2.2. Apple juice beverage containing isothiocyanates

Four beverages with cauliflower stem by-products extracts (having the highest isothiocyanates content) levels of 0% (control), 10%, 20% and 40% in apple juice, were prepared. The cauliflower extracts (stored at 4 ^{o}C) were added to the apple juice on the day of the analysis. Apple juice used was the Sainsbury's pure apple juice made from concentrate with a total sugar of 9.2 g/100 mL (packed in 1L box pack).

2.3. Ultrasound-assisted extraction

Fifty grams of cauliflower powder (leaves or stems) were weighed into a beaker and 100 mL of different solvents (distilled water, 70% methanol or 80% acetonitrile) were added. Each beaker was sonicated using the UP400S Ultrasonic Processor (Hielscher, Teltow, Germany) at a working frequency 24 kHz, and power 400W at 20% or 100% amplitudes for 5 min. The experiment was performed in duplicate for each solvent and amplitude. The mixture was then centrifuged (Rotanta 460R Centrifuge, Hettich lab Technology, Tuttlingen, Germany) at 1500 g for 15 min. The supernatant solution was collected and placed into another beaker. The pellet was centrifuged for a second time at 1500 g for 15

min with 100 mL of solvent. The second supernatant was also collected and combined with the previous one. The combined extracts were filtered through a Whatman® paper filter under vacuum conditions. For each sample, isothiocyanates, total phenols and antioxidant activity were determined.

2.4. Optimisation of solid-liquid extraction of isothiocyanates and phenolic compounds from cauliflower wastes powder

A response surface methodology (RSM) using a five-level-two-factor Central Composite Rotatable Design (CCRD) was used. Twenty-three extractions were conducted to generate data for modelling the effects of the independent variables (amplitude, *Amp*, (20, 40, 60, 80 and 100%) and treatment time, *Time*, (0, 3, 5, 7 and 10 min)) on isothiocyanates and total phenolic compounds recoveries. Each experiment was performed in triplicates. Experiments were randomized to minimize the systematic bias in the observed responses due to extraneous factors and for higher precision. All statistical analyses were performed using the software SPSS 19.0 for Windows (SPSS Inc., Chicago, IL, USA)

2.5. Analyses of extracts and beverages

2.5.1. Isothiocyanates

The determination of isothiocyanates was carried out following the protocol of Zhang et al. (1992). Nine hundred microlitres of 100 mM potassium phosphate buffer (pH 8-8.5), 100 µL of standard isothiocyanate (sulforaphane) or the appropriately diluted sample and one mL of methanol containing 80 mM 1,2-benzendithiol were added to a 2.0-mL centrifuge tube. The mixture was then incubated for 90 min at 60 °C. Samples were then cooled at room temperature and absorbance was measured at a wavelength of 365 nm in a spectrophotometer (Genesis 10 UV Spectrophotometer, Cole-Palmer, Illinois, USA). Sulforaphane (Sigma-Aldrich, USA) was used for the calibration curve.

2.5.2. Total phenolic content (TPC)

The content of total phenolic compounds (TPC) was determined by the Folin–Ciocalteu method, which is based on colorimetric oxidation/reduction reaction of phenols (Singleton et al., 1999) with some modifications. 0.5 ml of sample and 2.5 ml of Folin-Ciocalteu reagent (diluted 1:10 with water) were mixed. The tubes were kept in darkness for 5 min and 2 mL of Na_2CO_3 was added. The mixture was incubated for 5 min at 50 °C, and then cooled to room temperature. The absorbance was measured at a wavelength of 760 nm in a spectrophotometer (Genesis 10 UV Spectrophotometer, Cole-Palmer, Illinois, USA). Gallic acid was used for the calibration.

2.5.3. Antioxidant activity

2.5.3.1. 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay

DPPH free radical method was determined according to the method previously reported by Brand-Williams et al. (1995) based on application of DPPH decolorization assay (Sigma-Aldrich, Steinheim, Germany) with some modifications. 0.1 mL of appropriate dilution of the sample extract or BHA was added to a tube containing 3.9 mL of DPPH (Sigma–Aldrich, Lyon, France) coloured radical. The samples were left in the dark for 30 minutes and their absorbance was measured at 517 nm.

2.5.3.2. Ferric Reducing Antioxidant Power (FRAP) assay

The Ferric Reducing Antioxidant Power (FRAP) assay was carried out in accordance to method described by Benzie and Strain (1996) with some modifications. In this assay, reductants (antioxidants) in the sample reduce Fe3+/tripyridyltriazine complex, present in stoichiometric excess, to the blue coloured ferrous form, with an increase in absorbance

at 593 nm. 100 µl of the sample extracts, standard (1 mM ferrous sulphate) or distilled water (blank) were measured in triplicate into test tubes. 300 µl distilled water and 3 ml of FRAP reagent were added to each tube and vortexed for 10 seconds. Cuvettes were filled with the test solutions and their absorbance was measured on the spectrophotometer (Cole-Palmer, Illinois, USA) at 593 nm. The cuvettes were placed in a water bath at 37 °C for 4 min and the absorbance measured again.

2.6. Sensory analysis

A test panel was conducted on the apple juice beverages containing isothiocyanates (section 2.2). Twenty panelists were asked to evaluate the appearance, smell, flavour, texture, aftertaste and overall acceptability of each sample; using a 9-point hedonic scale (1 = unacceptable, 5 = neither acceptable or unacceptable, 9 = acceptable).

2.7. Colour measurement

The colour measurement was performed using a Hunter Ultrascan Pro colorimeter (Hunter Associated Laboratory Inc., Reston, Virginia, USA). Prior to measurements the equipment was calibrated using black, green and white tiles supplied by the manufacturer. Apple juice containing 0% of cauliflower stem by-products extracts (control) was used as standard for comparison with cauliflower stem by-products extract containing samples. Samples were analyzed for the Hunter L*, a*, and b* color values, from which the colour differences ΔL*, Δa* and Δb* were derived. The total colour difference (TCD), Chroma and hue angle of the products were calculated using the following equations (Eq. 1-3).

$$TCD = \sqrt{(L - Lo)^2 + (a - ao)^2 + (b - bo)^2} \quad \text{(Eq. 1)}$$

$$Chroma = \sqrt{(a)^2 + (b)^2} \quad \text{(Eq. 2)}$$

$$Hue = \tan^{-1}\left[\frac{b}{a}\right] \text{ (Eq. 3)}$$

2.8. Statistical analysis

Statistical analysis was performed using software SPSS 19.0 for Windows (SPSS Inc., Chicago, IL, USA). Statistical significance was set up at $p<0.05$. The experimental data obtained by following the CCRD procedures were analyzed by the response surface regression procedure using the following second-order polynomial equation:

$$Y = \beta_0 + \beta_1 \times Amp + \beta_2 \times Time + \beta_3 \times Amp^2 + \beta_4 \times Time^2 + \beta_5 \times Time \times Amp \text{ (Eq. 4)}$$

where Y is the response (isothiocyanates or total phenols values yield); Amp (amplitude) and Time (treatment time) are the uncoded independent variables, β_0 represents the intercept, β_1, β_2, are linear coefficients β_3 β_4, are quadratic coefficients and β_5 is constant coefficient, respectively. The software MINITAB Release 14 (Six Sigma Academy International) was used for regression analysis and analysis of variance (ANOVA).

3. Results and discussion

3.1. Optimization of the extraction process

Figure 2 shows the effect of ultrasound-assisted extraction (UAE) (20% and 100% amplitudes, for 5 min) combined with the most commonly used solvents (distilled water, methanol 70% and acetonitrile 80%) on the recovery of isothiocyanates from stem and leaves extracts.

Many solvents have been studied for the extraction of glucosinolates and isothiocyanates. Vallejo et al. (2002) and Dominguez-Perles et al. (2010) used methanol 70% as solvent for the recovery of isothiocyanates from Brassica vegetables while other

authors used hot methanol 100% (Oerlemans et al., 2006), acidified methanol (Volden et al., 2008) and acetonitrile (Zhang et al., 1992) for the extraction of isothiocyanates. Boiled water was also used as solvent for the extraction of this phytochemical and its breakdown products (Kushad et al., 1999; Sun et al., 2011; Tanongkankit et al., 2012).

As can be seen in Figure 2, distilled water combined with UAE was the best choice for the extraction of isothiocyanates from cauliflower wastes powder. Moreover, the extraction with distilled water and sonication at 20% amplitude for 5 min exhibited the highest isothiocyanates content in both stem and leaves powders, whereas for the other solvents and distilled water at 100% amplitude for 5 min, the extraction of isothiocyanates was rather low. For this reason, distilled water was selected as the most appropriate solvent to establish the experimental design for this study.

3.2. Solid-liquid extraction of isothiocyanates and the total phenols contents of cauliflower wastes powder

Table 1 summarizes the results of the analysis of variance (ANOVA) from the quadratic polynomial model. The quadratic polynomial model was significant for ITC values from both the stem and leaves ($p<0.05$) as well as from TPC values from stem ($p<0.05$) (Table 1). This revealed that they were sufficient to represent the relationship between amplitude and time. However, it was not significant for TPC values from leaves. The value of determination coefficient R^2 was at 81.5% and 95.86% for ITC values from leaves and stems respectively (Table 1). This implied that there was an excellent correlation between the independent variables, amplitude and time from stem and leaves.

The effect of amplitude and treatment time on ITC and total phenol recoveries are also shown, in form of contour plots, in Figure 3. From both Table 1 and Figure 3, amplitude and treatment time showed a significant effect on the recovery of ITC from leaves and stem ($p<0.05$), being an important factor to take into account in the

optimization process. The ITC yield in stems and leaves were higher when the amplitude was between 20 and 28% and the time was between 3 and 3.8 min. However, the ITC yield of both plant materials decreased when the ultrasound amplitude and treatment time were increased (Figure 3). These results were in close agreement to those found by Wang et al. (2011) who found a significant recovery of sinigrin (70.67%) from Indian mustard seed (60 mesh) when UAE (20 kHz and 400 W) was applied, in comparison to conventional solvent extraction by water and ethanol. These authors observed that UAE promoted break-up of the pectinous material found in the vegetal tissue and destruction of plant cells, thus facilitating ITC (sinigrin) extraction.

In addition, in the present study, it was found that amplitude levels higher than 40 and treatment times between 6.6 and 10 min reduced the ITC recovery to zero (Figure 3c). On the other hand, the total phenol yield from leaves was higher when amplitude level was between 20 and 55 and the treatment time was less than 3.4 min (Figure 3b). However, the behaviour of total phenol; was different when stems were used as target material.

Total phenol content was higher when amplitude levels were between 20 and 100 and treatment times were between 3.5 and 10 min (Figure 3d). Moreover, it was also observed that treatment time had a significant effect on the recovery of total phenol from stems while the impact of amplitude on total phenol yield was not significant. The contour plots also showed that the effects of UAE on total phenol recovery differ according to the plant material (stem or leaves) used (Figure 3). However, ITC yield was practically similar in both stem and leaves (Figures 3a and 3c).

Figure 4 which illustrates the effect of ultrasound processing on ITC content of extracts obtained from stem and leaves, confirmed these findings especially for stems. It was obtained that ITC recovery after UAE (20% amplitude and 3 min) was significantly

higher (7000 µM) compared to the other UAE processing conditions. However, ITC yield from stem was higher at 80% amplitude for 3 min (Figure 4). The highest TPC obtained from stem was found when the highest amplitude level (100%) was used for 3 min (Figure 5), whereas TPC obtained from leaves was higher at the control point (0% amplitude; 0 min). If the control conditions are excluded, the maximum total phenol recovery was found when US was applied at the lower amplitude level (20%) for 3 min. This can be attributed to enhanced extraction at low power due to improved diffusion of solvent into the matrix whereas higher treatment time can degrade phenolic compounds.

3.3. Nutritional, quality and sensorial study of apple juices containing different levels of cauliflower by-products extracts

3.3.1. Antioxidant activity of cauliflower by-products extracts in apple juice

Antioxidant activity of the apple juices alone and/or with the extracts obtained from cauliflower wastes extracts added was determined by using DPPH and FRAP methods. Extracts obtained from Ultrasound-treated cauliflower wastes were added into apple juices at different concentrations (from 10% to 40%) and the TPC and antioxidant capacities (FRAP and DPPH values) of the obtained novel functional beverages were compared to a conventional apple juice (without cauliflower wastes extracts added).

Concerning the TPC, the control juice and the apple juice with 10% of cauliflower extract added were closely similar in levels (64 mg GAE/L). However, TPC of the beverage with 40% of cauliflower extracts added was lower. These results suggested that the addition of cauliflower extracts did not increase or even decreased the TPC and the antioxidant capacity of apple juice.

The antioxidant capacity of the apple juice containing 10% cauliflower extract and the control sample showed a higher free radical scavenging ability than the samples

containing 20% and 40% cauliflower extract. In addition, the control and the sample containing 10% cauliflower extract showed a higher total antioxidant capacity as demonstrated by the ability to reduce TPTZ-Fe(III) complex to TPTZ-Fe(II). However, a decrease in the total antioxidant capacity was observed for the apple juices containing 20% and 40% cauliflower extract. Similarly to the results found in our work, Cabello-Hurtado et al. (2012) discovered that the antioxidant activity of glucosinolates extracts measured by DPPH and ABTS assays was also weak.

However, contrary to the results obtained in the present study, Llorach et al. (2005) found that cauliflower by-products extract added in soup exhibit an increase of 3.5 times antioxidant activity compared to soup containing no cauliflower by-product. Indeed, the antioxidant activity of apple juice samples should have increase as cauliflower by-products is known to have a great antioxidant activity (Llorach et al., 2003). It should be noted that the antioxidant capacity of a sample differs according to the food matrix and the individual compounds (Barba et al., 2013). In some previous works, it was shown the different antioxidant capacity of the phenolic compounds depending on their structure and the antioxidant method used for the determination of the total antioxidant capacity (Villaño et al., 2005; 2007). Moreover, the reduction in TPC and associated antioxidant activity might be due to dilution factor i.e. lowering phenolic concentration by including extract with low phenolic concentration.

3.3.2. Colour of apple juice containing cauliflower extracts

Inclusion of any extracts can change not only the nutritional properties of a food product but also its physical properties. In apple juice and all juices, it is essentially the colour and viscosity that are important physical properties to assess the quality of this type of products. Colour appears to play a major role on the quality of food as it is one of the

primary attribute that consumer perceived (Francis, 1995; Goncalves et al., 2007) and is often used for food acceptance and/or acceptability range.

Table 2 shows the colour values for each novel functional apple juice. The control and samples were all quite light, greenish and yellowish according to the ΔL, Δa and Δb mean values. The values varied from one to another but stayed close to the colour of the control. Indeed, the values described perfectly the initial colour of apple juice.

Chroma is an indicator of how dull or vivid a food product is (from 0 to 60). Referring to chroma values for all samples, they presented a dull colour (Table 2). Hue angle defines the colour. The hue angle values of each sample were quite similar (Table 2). The hue angle values indicate that the colour tended towards a dark, green and yellow colour which referred exactly to the finding values of ΔL, Δa and Δb.

The total colour difference (TCD) values enable the validation and/or comparison of the colour of different food samples. When TCD is higher than 3, there is a significant pronounced difference between food samples (Pathare et al., 2013, Adekunte et al, 2010). Apple juice containing cauliflower extracts became darker, greener and yellower than control samples. For each sample, TCD values were lower than 3. However, there was significant differences in TCD between control samples and apple juices containing 40% level of cauliflower extracts. Hence, the inclusion of cauliflower extracts had levels of impact on apple juice colour. Similar findings were observed in other studies where cauliflower extracts were used; Abul-Fadl (2012) used white cauliflower by-products powder to include into beef sausage to enhance its nutritional quality. The cauliflower by-products containing samples exhibited a darkening in colour. Stojceska et al. (2008) found out that the addition of cauliflower extracts to ready-to-eat snack significantly affected the colour of the products.

3.3. Sensory analysis

The sensory analysis results of the effect of the inclusion of cauliflower by-products extract in apple juice are displayed in Figure 6.

3.3.1. Appearance and acceptability of apple juice containing cauliflower extract

Figure 6 show that the appearance of the samples seemed to have similarities with the control. Participants found no significant difference (P>0.05) between the appearance of the control samples and the samples containing different levels of cauliflower extracts. The perceived smell by participants for the control samples differs compared to those of the samples containing cauliflower extracts (Figure 6). However, samples containing 20% and 40% cauliflower extracts showed a significant difference (p<0.05) in smell compared to the control samples. Cauliflower as *Brassica* vegetable is known to release a strong pungent smell due to the hydrolysis of glucosinolates. For this reason, higher percentage of cauliflower extracts inclusion, 20% and 40% induced a strongest distinction in the smell in apple juice compared to the control sample and 10% inclusion sample.

In Figure 6, the 10% and 20% inclusion of cauliflower extract presented a quite similar score. Nevertheless, the 40% inclusion sample presented the lowest score. The flavour of the 40% inclusion sample was characterized as *"too strong", "bland"* and *"dreadful"* by some participants. Indeed, a high amount of extracts might mask the true and meaningful taste of the apple juice. Although, in the figure a difference is noticed, there was significant difference (p<0.05) between the apple juice containing cauliflower extracts and the control samples.

The 10% and 20% inclusion samples presented quite similar scores while the 40% inclusion sample had the lowest score. This meant that the inclusion of cauliflower extracts could change the texture, the viscosity of the apple juice. However, the sensory

evaluation showed that there was no significant difference (p<0.05) between the samples with cauliflower extracts and the control.

As mentioned previously glucosinolates breakdown products released a known pungent and undesirable odour from *Brassica* vegetables such as cauliflower. Therefore, the inclusion of cauliflower extract will probably induce a noticeable aftertaste at high level. The lowest scores for aftertaste were found in the 40% cauliflower extract inclusion. At this high percentage, the unpleasant cauliflower taste was strongly present and was perceived by a participant as *"unpleasant vegetable taste"*. There was however, no statistically significant difference (p<0.05) between the cauliflower extract containing samples and the control samples.

3.3.2. Overall acceptance

The overall acceptance seems to differ between the samples and the control (Figure 6). The 20% inclusion sample presented the lowest score following the 40% and 10% cauliflower extract containing samples.. Control samples showed high acceptability in terms of smell, falvour, texture and after taste. However, panelist judged the control sample and apple juice containing cauliflower extract containing samples to be similar in appearance.

4. Conclusions

From the results obtained, it can be concluded that the optimal conditions for the recovery of isothiocyanates and total phenolic compounds from cauliflower wastes powder were UAE at 20% amplitude for 5 min. Although, the sensory analysis indicated no significant differences (p>0.05) in aftertaste of samples containing cauliflower extracts and control samples, 20% inclusion sample appeared to have the lowest score as there was a

noticeable aftertaste and one of the panelists judged the aftertaste as *"unpleasant vegetable taste"*. It can also be concluded that the development of a beverage using cauliflower by-products extracts is appropriate at the 10% inclusion level. Although at this level, total phenol content and antioxidant activity are similar to that of the control sample, 10% cauliflower extract can enhance the nutritional value of the beverage by enhancing isothiocyanates contents which are known to have chemoprotective effect on health (Zhang, 2004, Veeranki et al., 2015). Further research should be carried out in order to improve the development of this beverage and/or to explore various possibilities with the use of this extract as the anticarcinogenic properties of glucosinolates and its breakdown products such as isothiocyanates are still yet to be explored.

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Figure captions

Figure 1. Set-up experimental design. UAE: Ultrasound-assisted extraction. TPC: Total phenolic compounds. ITC: Isothiocyanates. TAC: Total antioxidant capacity.

Figure 2. Effect of ultrasound processing at 20% and 100% amplitudes on isothiocyanates (ITC) content of extracts with different solvents for 5 min (W=distilled water; M=methanol 70%; A=acetonitrile 80%) from stem (S) and leaves (L).

Figure 3. Effect of ultrasonic amplitude level and time on isothiocyanates (ITC) (leaves (a); stem (c)) and total polyphenols (TP) (leaves (b); stem (d)) values.

Figure 4. Effect of ultrasound processing on isothiocyanates (ITC) content of extracts obtained from stem and leaves.

Figure 5. Effect of ultrasound processing on total phenol content (TPC) (mg gallic acid equivalent (GAE)/L) of extracts obtained from stem and leaves.

Figure 6. Effect of cauliflower extract inclusion on sensory properties of apple juice.

fig.1

CAULIFLOWER WASTES

STEMS

LEAVES

+BLANCHING
+DRYING

1

POWDER DEVELOPMENT

2

Analytical determinations - TPC, ITC, TAC

3

RECOVERY OF TPC, ITC, TAC

+UAE
(Amplitude, time)
+SOLVENT

4

FORMULATION NOVEL FUNCTIONAL APPLE JUICE WITH EXTRACTED COMPOUNDS FROM CAULIFLOWER WASTES

5

✓CONSUMER ACCEPTABILITY

✓NUTRITIONAL AND PHYSICOCHEMICAL CHARACTERIZATION

fig.2

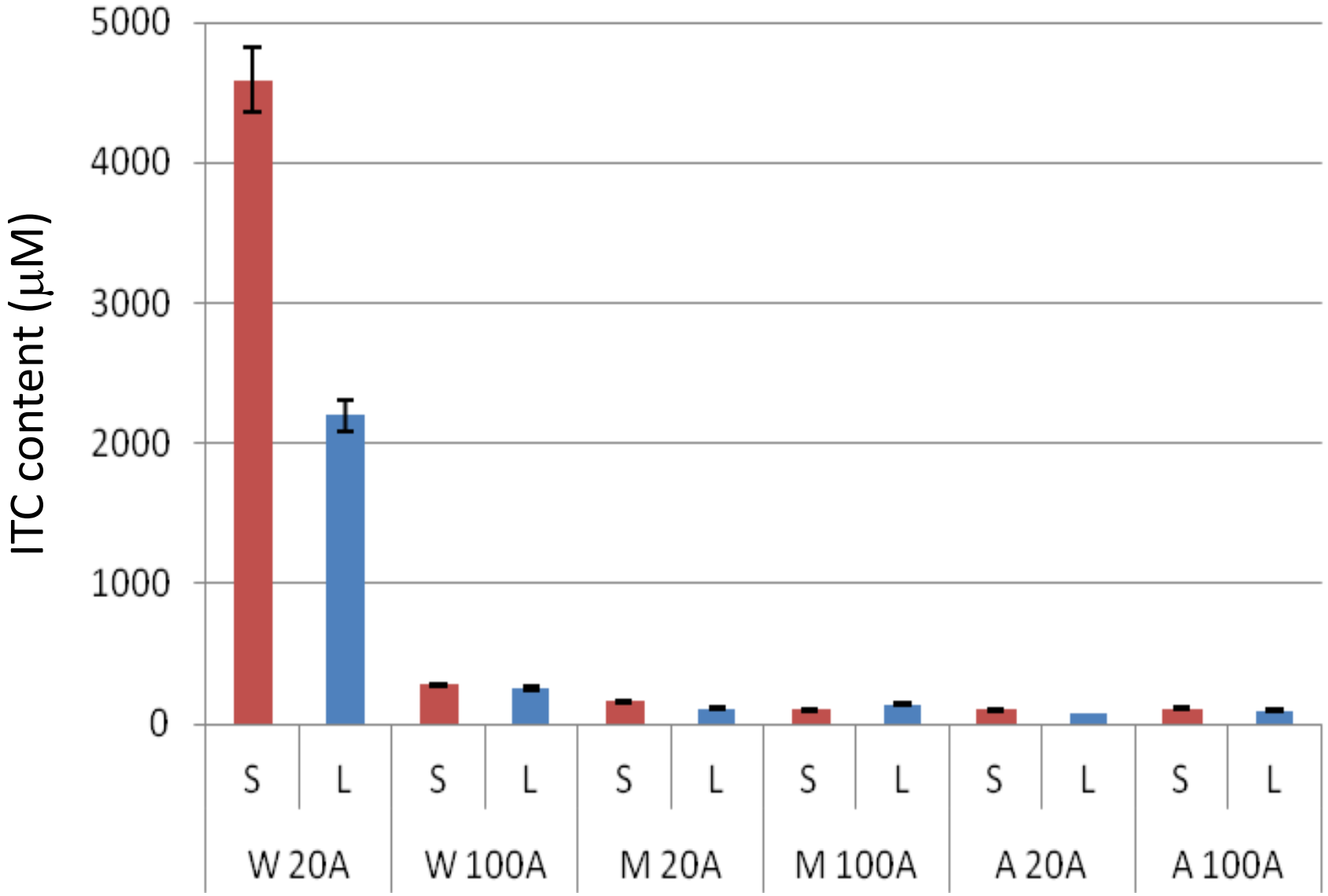

ITC content (μM)
5000
4000
3000
2000
1000
0
S
L
W 20A
W 100A
M 20A
M 100A
A 20A
A 100A

fig.3

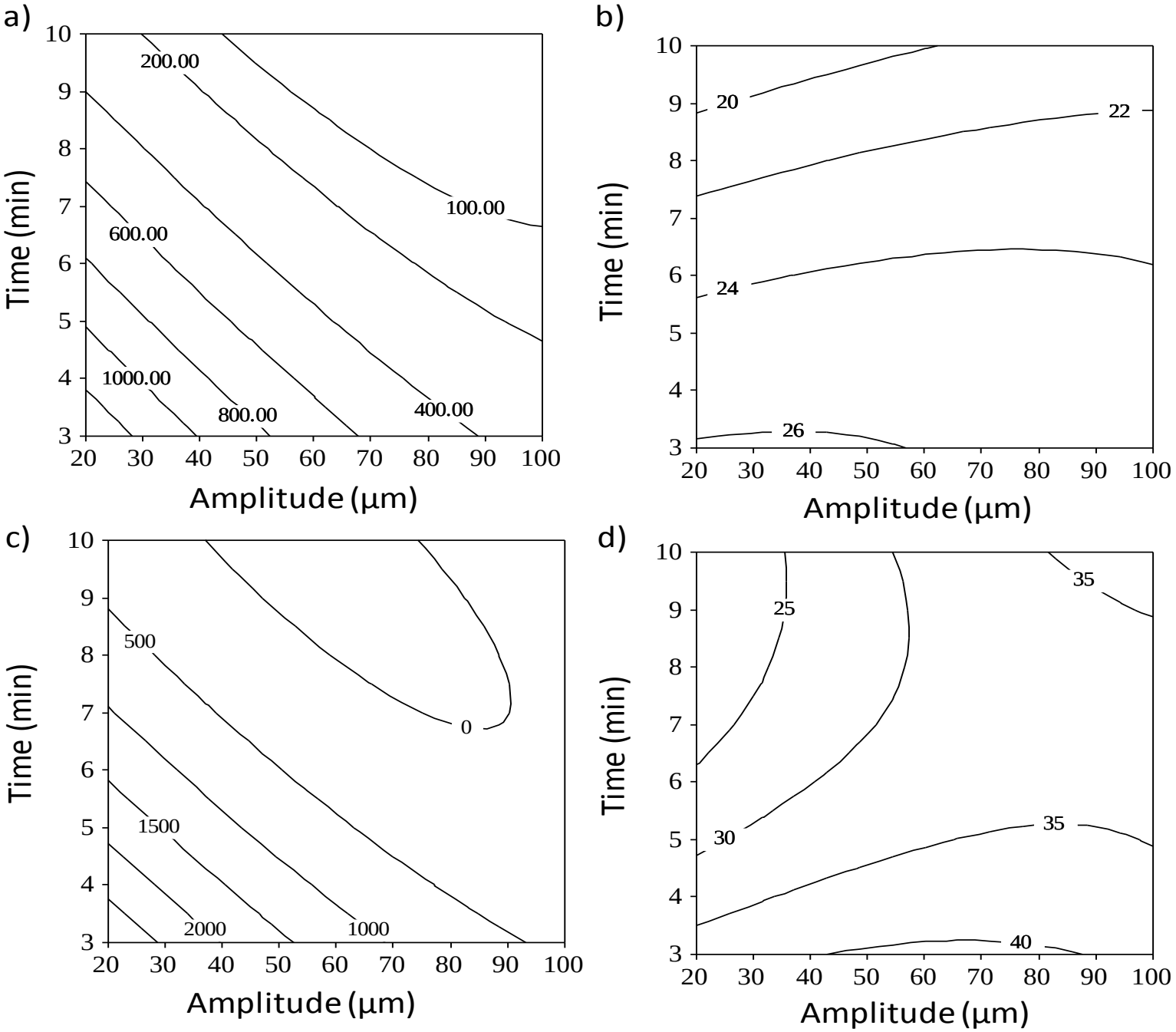

a)
Time (min)
Amplitude (µm)
200.00
100.00
600.00
1000.00
800.00
400.00
b)
Time (min)
Amplitude (µm)
20
22
24
26
c)
Time (min)
Amplitude (µm)
500
0
1500
2000
1000
d)
Time (min)
Amplitude (µm)
25
35
30
35
40

fig.4

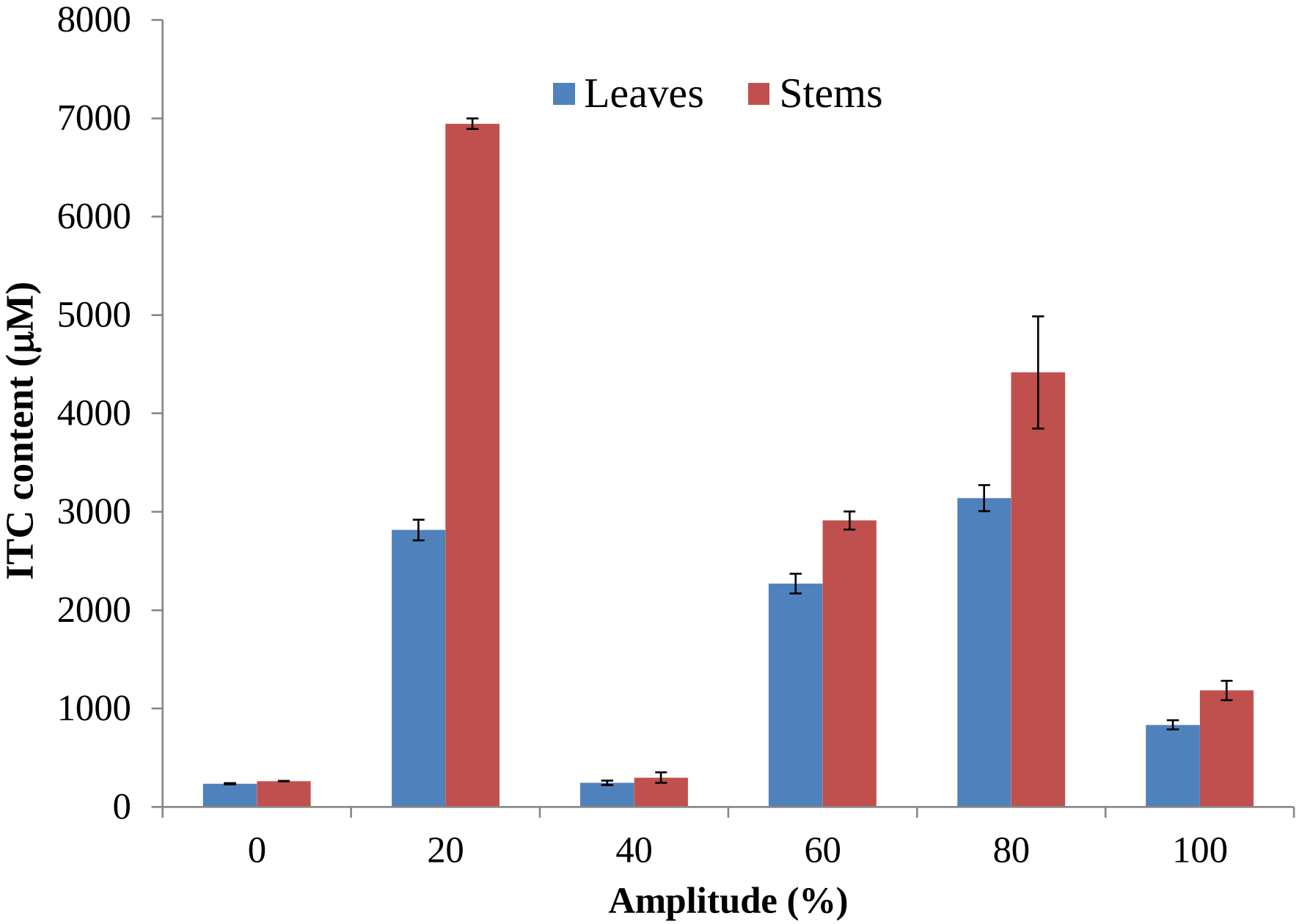

8000
7000
6000
5000
4000
3000
2000
1000
0
Leaves
Stems
ITC content (μM)
0
20
40
60
80
100
Amplitude (%)

fig.5

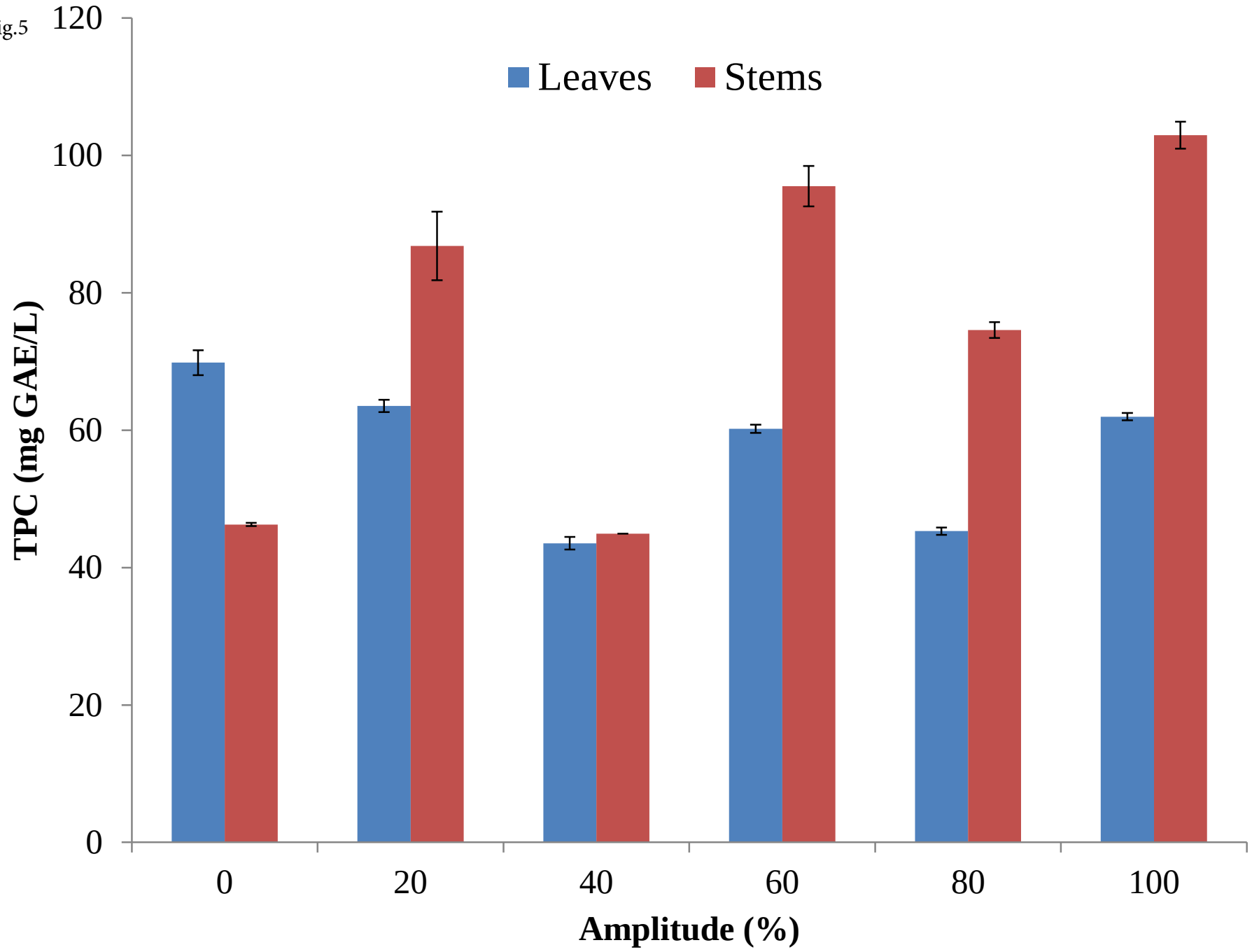
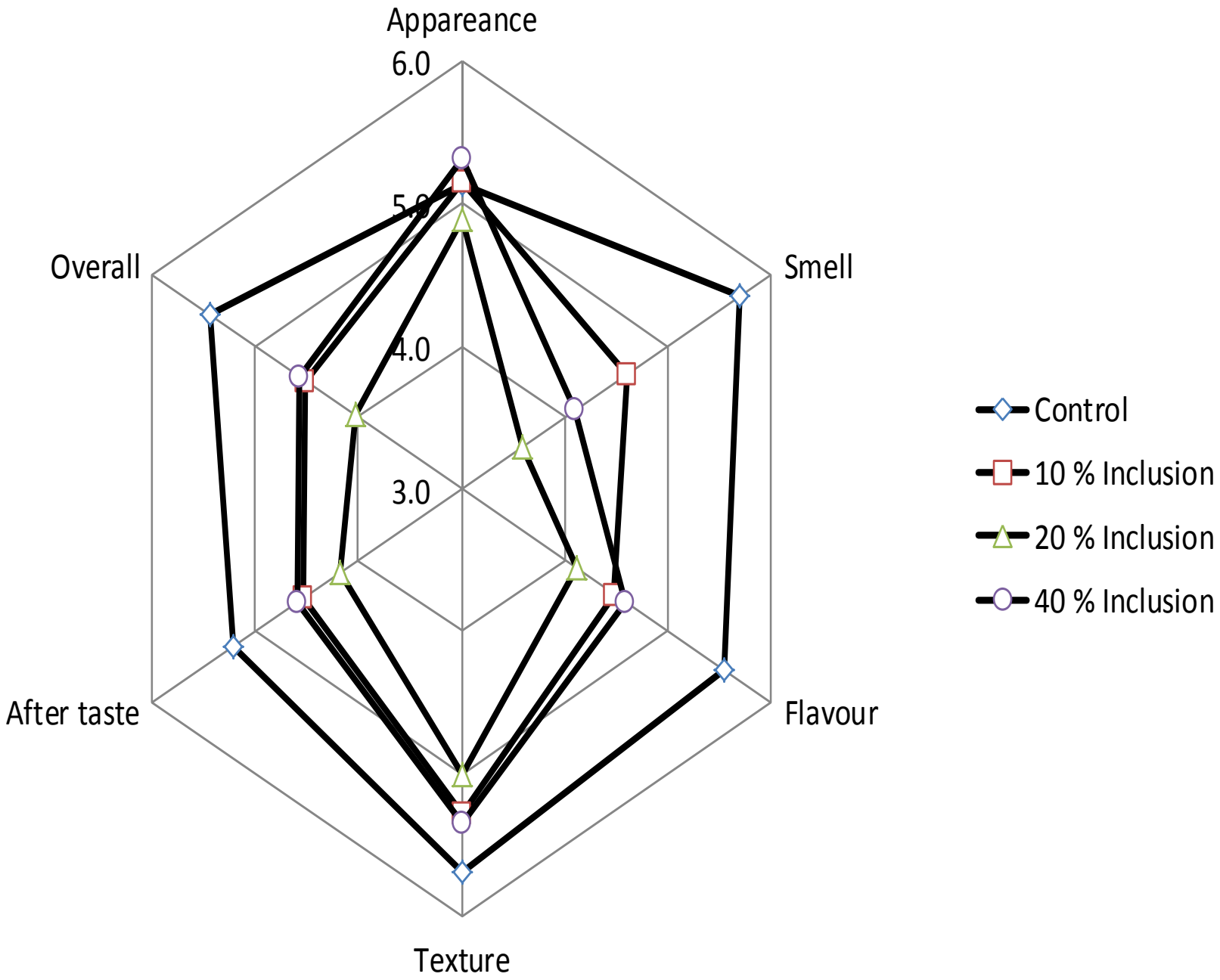

Leaves
Stems
120
100
80
60
40
20
0
TPC (mg GAE/L)
0
20
40
60
80
100
Amplitude (%)

fig.6


Appearance
6.0
5.0
4.0
3.0
Smell
Flavour
Texture
After taste
Overall
Control
10 % Inclusion
20 % Inclusion
40 % Inclusion

Tables

Table 1. Results of analysis of variance (ANOVA) for the fitted quadratic polynomial for the optimization of isothiocyanates (ITC) and total phenolic (TP) compounds values on both stem and leaves

Term	ITC (leaves)	P value	TP (leaves)	P value	ITC (stem)	P value	TP (stem)	P value
Intercept								
β_0	274.03	0.01	23.8844	0.001	210.5	0.03	31.842	<0.001
Linears								
β_1	-315.9	<0.001	0.3856	0.477	-583.7	<0.001	4.603	0.002
β_2	-333.21	<0.001	-3.0149	0.001	-668.6	<0.001	-4.797	0.003
Quadratics								
β_3	147.22	0.136	-0.4414	0.635	429.4	<0.001	-2.751	0.228
β_4	88.59	0.380	-0.9441	0.336	370.4	<0.001	4.179	0.086
Interaction								
β_5	201.42	0.014	0.9672	0.193	675.8	<0.001	3.877	0.037
R^2	81.15%		63.03%		95.86%		64.26%	

P value < 0.05 was significant.

Table 2. Colour values of the four novel functional apple juice samples enriched with extracts from cauliflower by-products.

Sample	Control 0% inclusion	10 % inclusion	20% inclusion	40% inclusion
DL	35.02±0.05	34.94±0.07	33.59±0.49	33.68±0.03
Da	-1.45±0.06	-1.45±0.01	-1.28±0.06	-1.23±0.02
Db	1.71±0.08	1.72±0.12	1.48±0.12	1.99±0.06
Chroma*	2.25±0.10	2.25±0.10	1.96±0.13	2.34±0.06
Hue**	49.69±0.75	49.89±1.64	49.09±1.14	58.39±0.35
TCD	2.24±0.10	2.31±0.07	2.37±0.01	1.96±0.02

*Chroma = $\sqrt{(a)^2 + (b)^2}$. **Hue = $\tan^{-1}\left[\frac{b}{a}\right]$