EFFECT OF APPLE AND CARROT POMACE EXTRACTS ON PORCINE INTESTINAL EPITHELIAL CELLS IPEC-1

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Abstract

Agro-industrial waste can be a valuable ingredient of animal feed that can reduce feed costs, minimize environmental impact, and improve the sustainability of animal production. However, the use of waste in animal nutrition needs further in vitro and in vivo tests, in order to establish the best inclusion rate for the achievement of best performances. The present paper aims to investigate the effect of individual and combination of apple and carrot pomace extracts on the porcine intestinal epithelial cells line IPEC-1. IPEC-1 cell cultures were treated with apple and carrot extracts in different dilutions for 24h and cell cytotoxicity was measured using MTT. Interaction between fruit pomace extracts were analyzed using the Chou and Talalay method. Exposure of IPEC-1 cells to individual pomace extracts or their mixture induced a dose dependent decrease of cell viability. This decrease was more pronounced for apple extract, that has proven a high cytotoxic effect at the lowest dilutions. The exposure of cells to the combination of both apple and carrot extracts results in an intermediate effect on cell viability than that produced by the exposure to each individual extract. The interaction between the two extracts was mainly antagonist for lower concentrations turning into synergic effect for the concentrated extracts. These toxicity data should be considered in formulation of feed for swine in order to find the best inclusion rate of apple and carrot pomaces.

Key words*: apple pomace, carrot pomace, intestinal epithelial cell, citotoxicity*

INTRODUCTION

Agro-industrial waste presents a significant problem due to its potential environmental and health impacts, including pollution, greenhouse gas emissions, and soil degradation [1]. However, this waste represents a valuable resource for a circular bioeconomy, offering opportunities for recycling, reuse, and the production of valuable products [2]. It also contains abundant bioactive compounds - such as dietary fibers, antioxidants, and other nutrients - that can be extracted and applied across diverse industries [3].

Globally, apple processing generates as much as 12 million tons of waste annually - apple pomace (AP) - consisting of peels, pulp, seeds, cores, stems, and combinations of these parts [4]. AP is rich in polyphenols, which can act as natural antioxidants and exhibit antimicrobial, anticancerogenic, and antidiabetic properties, offering significant prospects for use in the pharmaceutical, cosmetic, and food industries [5].

Carrot pomace, the residue left after carrot processing, is also a valuable source of dietary fiber, carbohydrates, and minerals, and contains variable levels of protein and fat. It is also rich in bioactive substances such as beta-carotene and polyphenols [6].

Agro-industrial waste can be a valuable and sustainable source of animal feed that can reduce feed costs, minimize environmental impact, and improve the sustainability of animal production [7].

However, use of waste in animal nutrition requires, needs further in vitro and

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in vivo tests, in order to establish the best inclusion rate for the achievement of best performances. This depends on various factors including the extract toxicity, bioavailability, absorption of the bioactive compounds at the gut level etc. In this context, the present paper aims to investigate the effect of individual and combination of apple and carrot pomace extracts on the porcine intestinal epithelial cells line IPEC-1.

MATERIAL AND METHODS

Preparation of fruit pomace extracts. Apple (a mix of apple cultivars) and carrot (common orange carrot) dried pomace were individually mixed with methanol 80% with a ratio sample: solvent ratio of 1:7 (w/v) and extracted overnight. The supernatant was recovered by centrifugation, filtered and then concentrated to remove the solvent. The organic residues were dissolved into water.

Assessment of the total polyphenol concentration. Polyphenols were extracted in 80% acetone solution (1:7, w/v) using Folin-Ciocalteu method. The total polyphenol content was calculated based on a calibration curve, using gallic acid as a standard. The results were expressed as milligrams of gallic acid equivalents (mg GAE)/100 g sample.

Cell culture and measurement of cell viability. $2x10^5$ IPEC-1 cells/mL were seeded in 96 well plates and grown in DMEM/F-12 medium supplemented with 5% foetal bovine serum, a mixture of penicillin (100UI/mL) and streptomycin (50µg/mL), 15mM Hepes, 2mM L-glutamine, ITS (10 µg/mL insulin, 5µg/L epidermal growth factor, 5ng/mL sodium selenite and 5µg/mL transferrin) at 370C. After 1 day, IPEC-1 cells were treated with apple and carrot extracts in different dilutions [1/10 (0.1)-1/100 (0.01)] for another day. Cell cytotoxicity was measured using MTT[3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] test assay. Results are expressed as optical density values.

Analysis of the interaction between fruit pomace extracts. Chou and Talalay method was used to investigate the interaction type after the exposure of IPEC-1 cells to different dilutions of individual fruit pomace extracts and their combinations. The interaction was analyzed based on isobologram method described by [8]. The CompuSyn software version 1.0 was used to generate the isobolograms and to calculate the fraction affected (Fa) and the combination index (CI) values for mixtures of apple and carrot extracts.

Statistical analysis. The differences between the groups were analyzed with one-way ANOVA followed by Fisher PSLD test for multiple comparisons using StatView software 6.0. P values lower than 0.05 were considered significant.

RESULTS AND DISCUSSION

Fruit pomaces represent a source of bioactive compounds (dietary fiber, vitamins, polyphenolic components [9] and can be used as ingredients in food or feed [10]. For example, apple pomace was used in piglets diet in order to reduce the negative effects associated with weaning, to reduce the incidence of diarrhea and to increase the animal performances [11, 12].

The addition of apple and carrot dried pomace increase feed conversion ratio and improved fatty acid composition of pork meat [13]. Dried fruit and vegetable pomaces contain biologically active substances in considerable amount.

Our previous studies on apple pomace have shown that the cultivar can influences the concentration of dietary fiber, total polyphenols and epicatechin and different minerals [14]. The use of fried fruit pomace as feed ingredient can enrich meat in bioflavonoids, but can also improve growth parameters and can reduce oxidative stress in swine [15]. For this reason, the fruit and vegetable pomaces, through their content in bioactive compounds can represent valuable feed additives. However, in vitro studies have shown that some bioactive compounds as polyphenols can exhibit cytotoxic effects, particularly at high concentrations [16]. As their cytotoxic effects depends on cell type, polyphenols type and concentration, our study aimed to investigate the effect of individual and combination of apple and carrot pomace extracts on the porcine intestinal epithelial cells line IPEC-1. These data will be taken into account when these wastes will be used as ingredient in swine feed.

In our study, the total polyphenol content measured by Folin-Ciocalteu method was 0.34mg/mL (3.4mg gallic acid equivalents -GAE/g dry weight - DW in apple extract and respectively 0.13 mg/mL (1.3mg GAE/g DW) in carrot extract. Similar content of total polyphenols in apple and carrot pomaces was found by other studies. Li and colaborators have found in apple pomace aqueous-methanol extracts a concentration of 5.56 mg gallic acid equivalents (GAE)/g dry weight (DW) pomace of free polyphenolic compounds, with the major free phenolic compounds identified as chlorogenic acid, quercetin-3-O-galactoside, quercetin-3-O-rhamnoside, and phloridzin [17]. For carrot pomace the total polyphenol content was 85 mg GAE/g DW [18].

Exposure of IPEC-1 cells to individual pomace extracts or their mixture induced a dose dependent decrease of cell viability (Fig.1). This decrease was more pronounced for apple extract, that has proven a high cytotoxic effect at higher concentrations (96.4% and 57.48% reduction in cell viability for dilutions of 0.1 and 0.2 respectively, compared with 22.71% and 14% for the same dilutions for carrot pomace extract. Similarly, Butkeviciute and colaborators observed an important cytotoxic effect of the whole apple and apple peel extracts on HT-29 colon adenocarcinoma and U-87 human glioblastoma lines [19]. Other study shown that cellular proliferation was significantly inhibited in breast cancer cells by 10 mg/mL apple peel extracts [20]. However, the carrot extracts show a selective cytotoxicity depending on the cell type. For example, carrot oil extract decreases viability of 11 human acute myeloid leukemia cell lines, but not of healthy peripheral mononuclear cells [21].

In our study, the exposure of cells to the combination of both apple and carrot extracts results in an intermediate effect on cell viability that that produced by the exposure to each individual extract (Fig.1).

Isobolograms represent graphical methods used to analyze the interaction between drug combinations or plant extracts and allow to establish if the combined effect of these substances is synergistic (greater than the sum of individual effects), additive (equal to the sum of individual effects), or antagonistic (less than the sum of individual effects) [22].

In our paper, isobolograms and combination index method were used in order to assess the types of interactions between the apple and carrot extracts. Table 1 shows the combination index values for the extract combination responsible for an inhibition level (IL) of 25, 50, 75 and 90%. DRI represent the dose reduction index requested for lowering the cell viability with 25, 50, 75 and 90%. According to Chou and Talalay method, the effect is synergistic for CI values lower than 1.1, additive for values between 0.9 - 1.1 and antagonist for CI higher than 1.1. With the exception of extracts combination at fa =0.9 where a synergic effect was obtained (CI=0.62) all the combination indexes are higher than 1.1 indicating an antagonistic effect. DRI values ranging from 0.122 to 2.148 for apple pomace extract and from 4.635 to 6.207 for carrot pomace extract indicate the factor by which the two extracts in combination are reduced by synergy/antagonism. Fig 2A-D shows the interactions between combination index, fraction affected and extracts dilutions. The synergy between the two extracts combination is indicated in the fig 2B by the point positioned below the diagonal lines of the toxins at 0.9 of affected fraction. In Table 2 are presented the dose-effect curve parameters for cell viability. The median effect dose required to produce 50% inhibition of cell viability is 0.036 for apple pomace extract, 0.586 for carrot and 0.229 for the combination apple and carrot.

Apple contain flavonols, especially quercetin, flavanols but also phloretin, a dihydrochalcone and chlorogenic acid, a phenolic acid [23]. Carrot polyphenols are mainly hydroxycinnamic acid derivatives, including chlorogenic acid [24]. Literature data have shown that the antiproliferative effect of apple and carrot extracts are related to their content in polyphenols. In particular, it was shown that phloretin, chlorogenic acid, quercetin and caffeic acid, as polyphenols found in apple and carrot are able to significantly reduce the viability of two human colon cancer cells (HT29 and LoVo) [25].

Table 1. Values of interaction parameters for apple and carrot extracts combination

Cell Viability	CI value*	Extract dilution		DRI	
		Apple	Carrot	Apple	Carrot
fa = 0.25 (IL25)	8.39	0.051	1.955	0.122	4.635
fa = 0.5 (IL50)	3.34	0.036	0.586	0.317	5.109
fa = 0.75 (IL75)	1.38	0.025	0.175	0.826	5.631
fa = 0.9 (IL90)	0.62	0.018	0.052	2.148	6.207

* *CI is combination index values for combinations of fruit pomaces extracts that are responsible for different inhibition levels (25, 50, 75 and 90).*
DRI is Dose reduction index indicating reduction fold for cell viability (25, 50, 75 and 90) for fruit pomaces extracts combinations compared with individual extract.
Fa is fraction affected by the fruit pomace extracts.

Table 2. Dose effect relationship parameters for apple, carrot extracts and their combination on the cell viability

Drug/Combo	Dm	m	r
Apple	0.03646	-3.1727	-0.9780
Carrot	0.58625	-0.9120	-0.6428
Apple + Carrot	0.22948	-0.8438	-0.7781

Dm is the median-effect dose which is required to produce 50% inhibition of the assessed parameters.
m - slope for the relationship between dose and effect

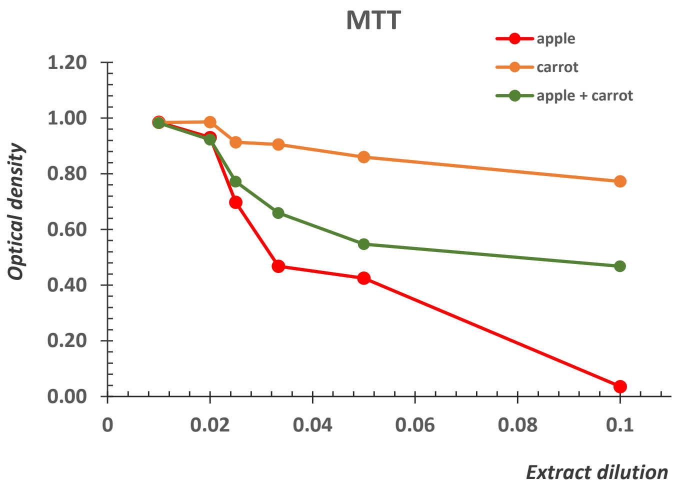

Fig.1. The effect serial dilutions of apple and carrot pomace extracts on IPEC-1 cells viability

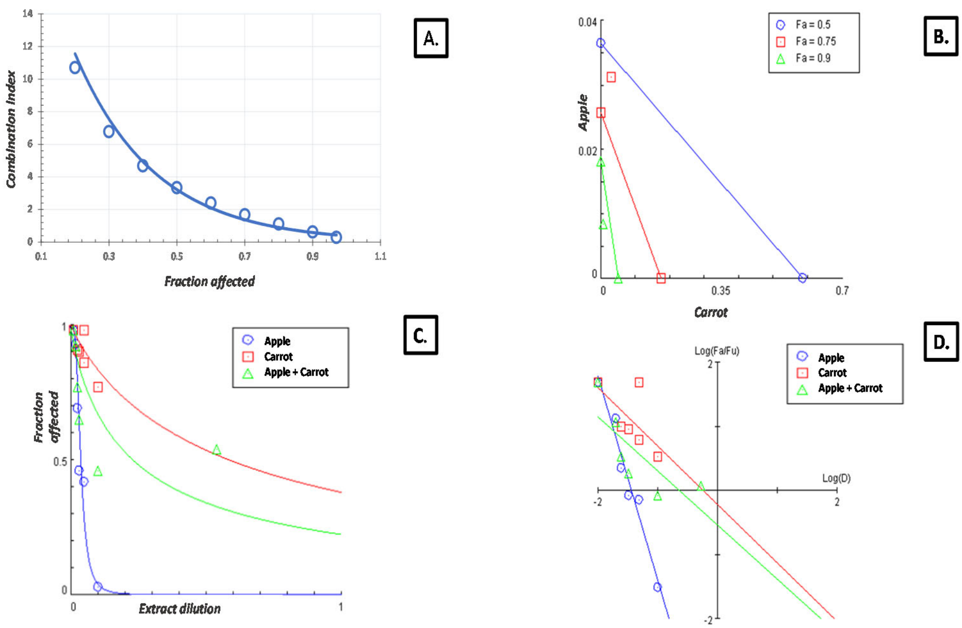

Fig 2A-D. Interactions between combination index, fraction affected and apple and carrot pamaces extracts dilutions.

CONCLUSION

Agro-industrial waste can be a valuable and sustainable ingredients for animal feed due to the high content in bioactive compounds. Exposure of intestinal porcine epithelial cells to individual apple and carrot pomace extracts or to their mixture induced a dose dependent decrease of cell viability; this decrease was more pronounced for apple pomace extract, less for carrot pomace extract and intermediate for apple and pomace combination. The interaction between the two extracts was mainly antagonist for lower concentrations turning into synergic effect for the concentrated extracts. These toxicity data should be considered in formulation of feed for swine in order to find the best inclusion rate of apple and carrot pomaces.

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