CRISTIANA PIANGIOLINO[1] , VINCENZO NOBILE[2], FEDERICA ZANZOTTERA[1] , FRANCESCO PUOCI[3]*
*Corresponding author
1. Principium SA, Via Luganetto 4, 6962 Viganello (TI), Switzerland.
2. Farcoderm srl, Via Mons. Angelini 21, 27028 S. Martino Siccomario (PV), Italy.
3. University of Calabria, Dept. of Pharmacy, Health and Nutritional Sciences , 87036 Rende (CS), Italy.


Francesco Puoci

Potential control in calories intake
and prolonged satiety effect promoted by apple polyphenols

KEYWORDS: apple, polyphenols, antioxidant, glycemic control, calories, weight management

Abstract Growing evidence is rising about the potential of a conscious and responsible nutrition to promote health and reduce cardiovascular risk. Weight management and healthy life-style are the primary approach. The current article provides the scientific rational for the use of dietary supplementation based on standardized apple polyphenols to control of calories intake by reducing sugars and carbohydrates absorption.
In vitro data are reported, showing the superior antioxidant and hypoglycaemic activity of an apple complex named PCQ, with respect to other standard references. A human trial was run to confirm PCQ capacity to reduce glucose concentration, to slow down its absorption rate, to decrease the glycaemic index and promote the satiety effect.

INTRODUCTION

The WHO attested that the worldwide prevalence of obesity nearly doubled between 1980 and 2008. Based on the latest estimates in European Union countries, overweight affects 30-70 percent and obesity affects 10-30 percent of adults (1). In the USA, the National Health and Nutrition Examination Survey (NHANES) has estimated that the percentage of overweight adults is dramatically increased touching 68,5 percent and the obeses are by now 34,9 percent (2). Consequently the promotion of a policy for weight control and calories intake reduction is strongly needed.
In the last decades, there has been a growing appreciation and awareness about the link between fruit and vegetables enriched diet and improved health. In particular, apple and its polyphenols have been reported to inhibit cancer cell proliferation, to decrease lipid oxidation, to lower total and LDL cholesterol levels and also to exert antioxidant and anti-inflammatory activities (3).
The apple polyphenols, including phloridzin, chlorogenic acid and quercetin, are ranked the second highest in antioxidant properties among all commonly consumed fruits and vegetables in the United States (4). In addition, there is a growing evidence that the above mentioned polyphenols have the capacity to modulate the body's glucose intake. Phloridzin (PHLO) is reported to impede glucose absorption in small intestine and kidneys by competing at the site of adhesion with its enteric sugar transporters and facilitated transporters, such as GLUT2. It also promotes renal glycosuria (5), positively influences glucose metabolism at muscular level by decreasing GLUT4 availability and delays hepatic glucose release.
Chlorogenic acid (CHLA) inhibits Glucose-6-phosphatase, the key enzyme to balance neoglucogenesis and glycogenolysis in the liver, thus delaying glucose hepatic release and controlling blood glycaemia (6).
The mechanism of Quercetin (QUE), involving draining properties and fat storage limitation (7) is also relevant in the weight control strategy.
These polyphenols work in synergy to modulate glucose absorption, lower postprandial glycaemic load and regulate insulinaemic levels. This is an effective approach for weight management because it helps reducing calories intake and promotes a prolonged sense of satiety, by avoiding the post-prandial blood sugar peak and delaying the absorption of glucose.

METHODS

Apple extract

SelectSIEVE® Apple PCQ (PCQ) is naturally obtained through soft-processing of food grade apple pomace through a proprietary technology GSMT™, which confers a unique and complete profile of standardized biophenols, including PHLO, QUE and CHLA. The extract is standardized at 90 percent in polyphenols: 15-30 percent Dihydrocalcones (PHLO), 10-25 percent Hydroxycinnamic Acid (CHLA) and 15-25 percent Flavonols (QUE).

Antioxidant Properties

The antioxidant properties of PCQ were investigated in different *in vitro* experimental models and compared to another commercially available apple extract characterized by similar composition (PAE: > 80 percent in polyphenols, 5 percent in PHLO):

- the scavenging activity against the lipophilic radical DPPH (1,1-diphenyl-2-picrylhydrazyl) and the hydrophilic radical ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) were measured through spectrophotometric methods according to the literature methods (8) (9) and expressed as IC50.

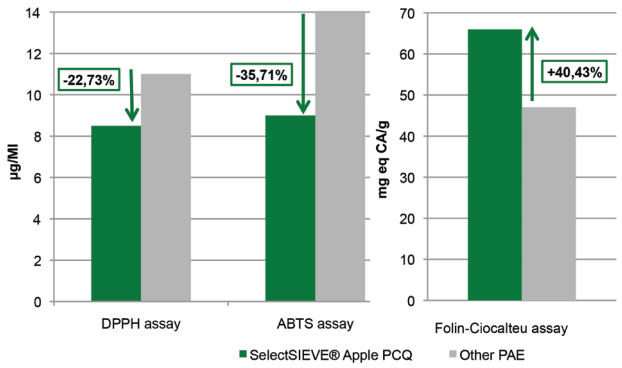

Figure 1. Antioxidant performance of PCQ and PAE reference via DPPH, ABTS and Folin-Ciocalteau assays.

- the inhibition capacity against the peroxyl radicals was also determined according to the literature protocol and compared to other well-known natural extracts and pure synthetic reagents such as PHLO (10), QUE and CHLA (11).
- the total phenol content was assessed by means of Folin-Ciocalteau assay (12) and reported as equivalent to catechins (CA).

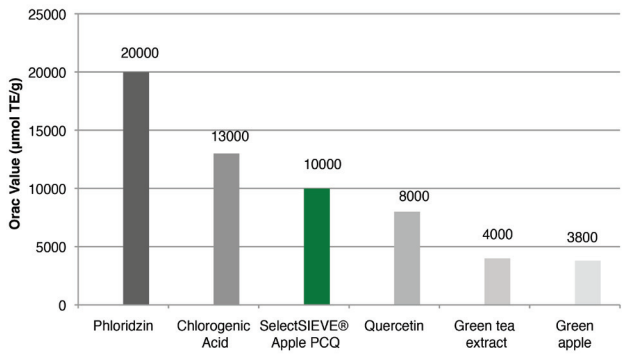

Figure 2. ORAC chart reporting PCQ value in comparison to strongly antioxidant botanicals and pure biophenols.

- the influence on the enzymatic function of SOD (superoxide dismutase), CAT (catalase) and GSH-Px (glutathione peroxidase) was also evaluated. SOD activity was measured as inhibition capacity to reduce Cytochrome C in the presence of a superoxide system (xanthine/xanthine oxidase) (13); CAT performance was evaluated through its hydrogen peroxide decomposition (14); GSH-Px catalytic ability was investigated in the coupled reaction (15):

H_2O_2 + GSH → GS + H_2O (via GSH-Px)
GS + NADPH → GSH NADP (via GSH-R)

Hypoglycaemic Properties

PCQ was shown *in vitro* to impact the activity of the enzymes involved in carbohydrates digestion:

- The determination of α-amylase inhibition degree provided the ingredient ability to inhibit the activity of α-amylase, the enzyme responsible for breaking down long-chain carbohydrates into oligosaccharides at the beginning of digestion. The reference reaction is the starch digestion catalyzed by α-amylase in the presence of known amount of the apple extract and the detection of residual starch by the Lugol's reactive.
- The α-glucosidase inhibition rate expressed the ingredient capacity to inhibit the activity of α-glucosidase, the enzyme acting upon hydrolization of 1,4–α bonds of oligosaccharides and release of glucose. The reference reaction is the sucrose metabolism mediated by α-glucosidase in the presence of known amount of the apple extract, measured according to the Fehling assay.
- The determination of β-glucosidase inhibition degree showed the ingredient activity against the β-glucosidase, the enzyme that catalyzes the hydrolysis of lactose to be absorbed by the intestine. The reference reaction is the Fehling assay based on lactose digestion by β-glucosidase.

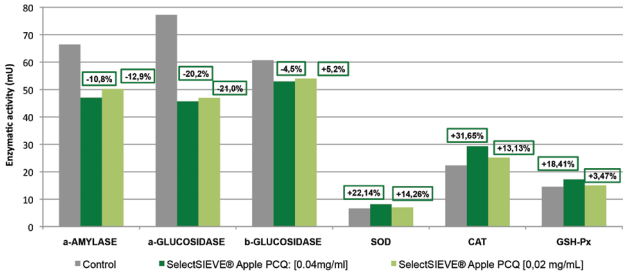

Figure 3. Influence on the enzymatic activity promoted by PCQ with respect to the standard (blanc) at different concentrations.

Oral Glucose Tolerance Test

The effectiveness of PCQ apple extract in decreasing glycaemia levels after glucose load was investigated in humans by carrying out an oral glucose tolerance test (OGTT) on 10 subjects having normal glucose absorption curves.

Subjects were selected by a physician from a panel of healthy male and female volunteers belonging to Caucasian ethnicity, aged between 18 and 50 years old, with basal glycaemia lower than 110 mg/dl (6.11 mmol/L).

The trial was performed in a certified medical analysis laboratory, as follows:

- OGTT#1: each subject ingested 75 g glucose;
- OGTT#2: each subject ingested 75 g glucose + 500 mg PCQ.

A double blind cross-over study design was chosen to improve data comparison and to control study biases.

Patients were instructed to fast 8-12 hours prior to the test.

The basal value of glycaemia was checked preliminary by electrochemical method, than the fasting blood sample was collected by venipuncture. Subjects were administered with 150 ml of a glucose standard solution equivalent to 75 g anhydrous glucose (OGTT#1) or 150 ml of the glucose standard solution + PCQ (OGTT#2) based on the randomization list. Subjects

were invited to consume the glucose load (or the glucose load + PCQ) within 5 minutes. Blood samples were then collected by venipuncture after 30, 60, 90, 120, and 180 minutes from the initial intake. The glucose concentration was measured by means of the Hexokinase/ Glucose-6-Phosphate Dehydrogenase (HK/G6P-DH) enzymatic assay.

SUBSTRATE / TEST	PCQ	Blanc	PAE	PHLO	QUE	CHLA	Green tea	Green apple	Figure
DPPH (IC50: µg/ml)	**8,5**		11,0						1
ABTS (IC50: µg/ml)	**9,0**		14,0						1
ORAC (µmol TE/g)	**10.000**			20.000	8.000	13.000	4.000	3.800	2
Folin-Ciocalteau (mg eq CA/g)	**66,0**		47,0						1
SOD (mg/ml)	**8,14; 7,01***	6,65							3
CAT (mg/ml)	**29,33; 25,20***	22,28							3
GSH-Px (mg/ml)	**17,20; 15,03***	14,52							3
α-amylase (mg/ml)	**47,04; 50,24***	66,42							3
α-glucosidase (mg/ml)	**45,69; 46,99***	77,22							3
β-glucosidase (mg/ml)	**52,96; 53,98***	60,69							3

Table 1. Raw data referring to *in vitro* anti-oxidant and hypoglycaemic activity of apple concentrated extract. * results have been collected at 0,04 mg/ml and 0,02 mg/ml.

RESULTS

The ability of PCQ to fight against different oxidative stress models and its enzymatic activity *in vitro* are summarized in Table 1.
Collected data upon the antioxidant activity of the apple extract, showed its great performance in the inhibition of organic free radicals such as DPPH and ABTS when compared to another commercially available apple extract (PAE) by 23 percent and 36 percent respectively (Figure 1). In terms of catechin equivalents, the apple complex was found to be stronger than the reference PAE by 40 percent (Figure 1). The ORAC value of PCQ corresponded to 10000 µmol TE/g and exceeded some reference compounds and botanical extracts (Figure 2). Such antioxidant performance was also confirmed by the activation of the enzymes SOD, CAT and GSH-Px, which all underwent a consistent improvement as shown in Figure 3.
Following the *in vitro* pre-screening investigation, PCQ was further proved to modulate those enzymes involved in the digestion and absorption of carbohydrates such as the α-amylase/α-glucosidase/β-glucosidase cascade. Raw data are reported in Table 1, while the comparison between different ingredient dosages and the standard enzymatic activity (blanc) is represented in Figure 3. The enzymatic assays were carried out by miming a model for both long- and short-term absorption: starting from a suggested daily dosage of 200 mg, two functional concentrations were calculated corresponding to 100 percent bioavailability (0,04mg/mL) and 50 percent partial absorption (0,02mg/mL). Both antioxidant and hypoglycemic performances of the apple concentrated complex showed to be dosage-dependent: such a trend was still established when the dosage is up to 3 times (600 mg/day), thus leading to almost complete inhibition of α-glucosidase (-92 percent) and strong modulation of α-amilase (-74 percent) with respect to the standard enzyme activity (data not shown, available upon request).
Based on the promising *in vitro* evidences, the influence of the apple complex on the glycemic metabolism was investigated through a clinical trial based on Oral Glucose Tolerant Test. The glucose concentration curves with and without the apple complex PCQ are reported in Figure 4a and clearly show a different trend. The most evident result is the decrease of the glycemix load (peak registered 30 minutes after the intake), for the group administered with glucose + PCQ, corresponding to a reduction of post-prandial peak by 14,0 percent.
Furthermore the rate of glucose increase, represented by the slope of the OGGT curves between 0 and 30 minutes after the intake (tendency lines in Figure 4a), is decreased by 27,2 percent in the study group.
Finally the total concentration of absorbed glucose, equivalent to the sugar assumption in the first 60 minutes from

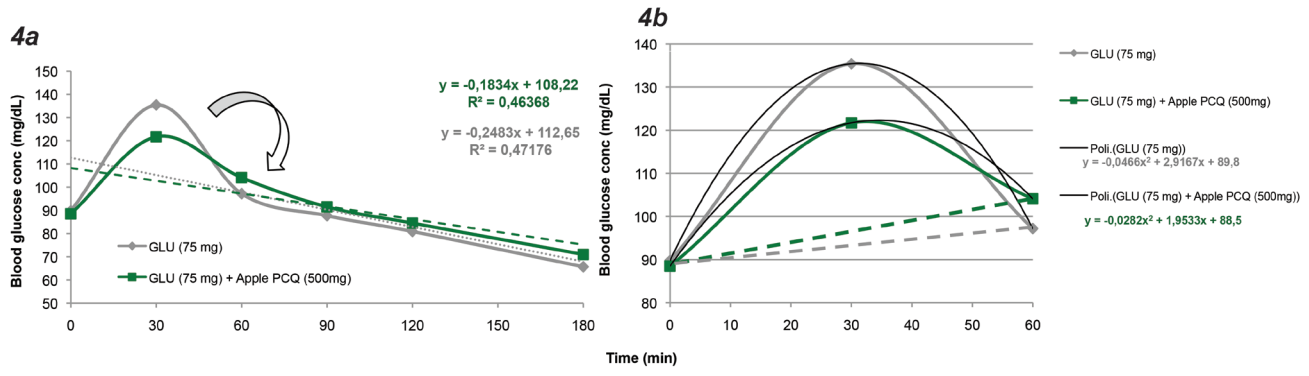

Figure 4. Oral glucose tolerance test in humans: blood glucose concentration curves with tendency lines for study and control groups (a); OGTT curves approximation between 0 and 60 minutes for mathematical estimation of the areas under the curves.

the intake, was decreased by 39,2 percent in the active group (glucose + PCQ) with respect to control. OGTT curves between 0 and 60 minutes were interpolated by a quadratic equation: the comparison of glucose concentrations was measured as the difference between the areas under the approximated curves, calculated by integral equation.

DISCUSSION

The well-known health benefits of apples have been attributed to the presence of polyphenols as dietary antioxidants, whose consumption could prevent the risk for chronic diseases such as cardiovascular and neurodegenerative diseases, cancer, diabetes and metabolic syndrome.
Different studies have shown a relationship between the increased levels of mitochondrial ROS and hyperglycemia, leading to potential vascular complications. Therefore it is important to control both blood glucose level and cellular redox status to manage body weight and obesity complications, including cardiovascular disease, hypertension, stroke (16).
Indeed among the trendy slimming approaches, there is the inhibition of carbohydrates-hydrolyzing enzymes α-amylase, α-glucosidase and β-glucosidase.
This article reviews the state-of-the-art about the biophenol-linked antioxidant and anti-hyperglycemic properties of apple extracts and describes the specific performance of a concentrated complex named PCQ.

Commercially available extracts, pure polyphenol compounds and PCQ were compared through free radicals inhibition assays and enzymatic models. All tests evidenced an extremely powerful scavenging activity for PCQ with respect to pure reference compounds or other substances generally recognized as strong antioxidants. Such performance was further confirmed by the *in vitro* improvement of SOD, CAT and GSH-Px activity. Such enzymes work in synergy to neutralize free radicals and oxidant agents: SOD catalyzes superoxide anion dismutation and production of hydrogen peroxide, which is neutralized in water via CAT or GSH-Px depending on its concentration.
The ability of PCQ to strongly inhibit α-amylase, α-glucosidase and β-glucosidase enzymes suggests the full blockage of the carbohydrates digestion, thus consequently limiting calories intake. This function was then studied through a clinical trial, in order to confirm the effective ability of the apple ingredient to decrease the glucose load in the blood after a single administration.
The experimental results pointed out that:

- the post-prandial glycemic peak recorded at 30 minutes was strongly decreased when compared to control, making PCQ effective in the control of glucose intake;
- the overall glucose concentration and its corresponding rate of absorption in the first 60 minutes were consistently reduced with respect to the control, confirming a lower absorption of carbohydrates, sugars and resultant calories after the meal;
- in between 30 and 60 minutes a trend inversion occurs: both groups kept blood glycaemia levels constant during the

following two hours, although the study group underwent a slighter drop in glycaemia with respect to control, thus reducing the risk of hypoglycemic crisis, favouring a satiety sensation and delaying the need of eating.

CONCLUSION

Reported hypoglycaemic and antioxidant effects of the apple complex PCQ are connected to the synergic coexistence of functional biophenols such as PHLO, QUE and CHLA. Oxidative stress, responsible for different cardiovascular degenerative processes and aging, as well as glycemic and anabolic metabolism can be controlled through the intake of apple biophenols.
All the collected data support the use of a dietary supplement based on apple polyphenols such as PCQ complex for the weight management, thanks to its ability to decrease calories intake and prolong the long-term post-prandial satiety effect.

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