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Distinct Effects of two Almond Cultivars on Agreeability and Gastrointestinal Motility in Healthy Subjects: more than mere Nutraceuticals

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ABSTRACT

Background: Almonds are healthy nutraceuticals, which vary across different cultivars. We compared the composition, agreeability and gastrointestinal effects of two almond cultivars from different areas.
Methods: Californian Carmel (CA*cv*) and local Apulian Filippo Cea (FC*cv*) cultivars were compared for the chemical composition and sensory evaluation according to visual analogue and semiquantitative scales in 60 volunteers. Gallbladder/gastric motility (ultrasonography) and orocecal transit time (H_2-breath test) were studied in another 24 subjects by comparing the effects of a standard liquid test meal with isovolumetric almond test meals (24 g of CA*cv* or FC*cv* almonds).
Results: Proteins prevailed in CA*cv*, while FC*cv* contained more lipids and 10-times more total phenol content than CA*cv*. For agreeability, CA*cv* scored higher than FC*cv* for smell, texture and appearance, although different perceptions existed in lean (scores for smell, taste, texture, appearance higher for CA*cv* than FC*cv*), obese (CA*cv* better than FC*cv* only for appearance) and elderly subjects (CA*cv* better than FC*cv* only for texture). Gallbladder emptying was stronger with FC*cv* than CA*cv*. Antral dilatation after ingestion of both cultivars was greater than the dilatation observed after the test meal. Gastric emptying, however, was similar after FC*cv*, CA*cv* and the test meal. The orocecal transit time in response to both cultivars was shorter than after the test meal.
Conclusions: Differences in composition and effects of FC*cv* and CA*cv* cultivars support their potential use as valuable nutraceutical tools, to be confirmed in further clinical studies.

Key words: breath test – Mediterranean diet – monounsaturated fatty acids – nuts – orocecal transit time – ultrasonography.

Abbreviations: AUC: area under curve; BMI: body mass index; CA*cv*; Carmel cultivar; FC*cv*: Filippo Cea cultivar; MUFA: monounsaturated fatty acids; OCTT: orocecal transit time; VAS: visual analogue scale.

INTRODUCTION

The Mediterranean diet is typically rich in fruits, vegetables, whole grains, fibres, legumes, nuts and seeds, olive oil (as a source of monounsaturated fatty acids [MUFA]) and with a moderate consumption of wine. An appropriate and systematic consumption of the Mediterranean diet is associated with a reduction in global mortality in particular for cardiovascular causes and for cancer, and also might prevent the onset of type 2 diabetes mellitus [1].

Almonds (*Amygdalus communis* L.), a component of the Mediterranean diet, provide nutrients and phytochemicals [2]. Almonds are rich in fats (~50%, mainly MUFA), although the specific content differs depending on harvest and variety [3], and also are an excellent source of vitamin E, manganese, magnesium, copper, phosphorus, fibre, riboflavin, protein, phenols and polyphenols [4]. Moderate and regular consumption of almonds and nuts (~30 g daily) are associated with health-promoting effects [2, 5, 6] and could be recommended, as a nutraceutical tool, in metabolic diseases (i.e. glycaemic control in diabetics [7], hyperuricemia [8], hyperlipidaemia [9, 10]), to reduce the risk factors for coronary heart disease [11], and to improve the intestinal microbiota profile [12, 13]. Thus, the consumption of almonds is gaining interest locally and worldwide. Factors such as the almond genotype [14, 15], the growing region [16, 17], the climatic conditions during the growing season [18], the harvest time [19], the storage conditions might influence the chemical

composition of almonds and their nutraceutical effects. Given the high almond consumption, the Italian production cannot cover the whole domestic demand, and a significant amount of almonds must be imported. In Apulia, two varieties of almonds are the most popular, i.e., the native Filippo Cea *cultivar* (FC*cv*), produced only locally and used mainly for high-quality confectionary, and the imported Californian Carmel *cultivar* (CA*cv*), which is widely available in local supermarkets. The aim of the present study was therefore to compare the properties of the two almonds for chemical and texture analysis and for individual sensory perception. As information on the effects of almonds on gastrointestinal motility is scarce, we also compared the evoked gastrointestinal motility of both *cultivars*, which show distinctive nutraceutical features.

MATERIAL AND METHODS

Chemical and texture analysis of almonds

Chemical and texture analyses were performed in representative samples of both *cultivars*. Protein content (total nitrogen x 5.18), ashes, and moisture content of almonds were determined according to the AACC methods 46-11A, 08-01 and 44-15A, respectively [20]. Fat content was determined with a Soxhlet apparatus, using diethyl ether (Sigma Aldrich, Milan, Italy) for extraction. Total carbohydrates were calculated as difference. The lipid fraction, extracted by the Soxhlet method was subjected to the UV spectrophotometry analyses carried out according to the official methods of European Communities 2568/91 [21]. The fatty acid composition was determined by gas-chromatographic analysis of fatty acid methyl esters according to the AOCS method [22], as previously reported [23]. The total phenol content was measured according to Pasqualone et al. [24]. The texture analysis was performed by a Texture Analyzer (Z1.0 TN, Zwick GmbH & Co. KG, Ulm, Germany) equipped with a 1 kN load cell and the software Text Expert 2. Almonds were placed on their longest side, and penetrated with a 2 mm diameter cylindrical probe. The following parameters were measured: pre-test speed 200 mm min^{-1}, test speed 1 mm s^{-1}, penetration distance 4 mm. The results (mean of 15 samples) were expressed as the maximum force (N) under the force-deformation within 4 mm deformation.

Subjects

A total of 84 healthy volunteers were enrolled; 60 subjects underwent the study on sensory evaluation while 24 subjects underwent the gastrointestinal motility study. All subjects were healthy volunteers and enrolled at a tertiary referral centre (Clinica Medica "A. Murri", Dept. of Biomedical Science and Human Oncology, University of Bari) in the province of Bari (Apulia region, about 4M inhabitants, Southern Italy). All subjects gave their informed consent and, at entry, underwent a full clinical evaluation in order to exclude clinically evident diseases. Exclusion criteria were diagnosis of organic diseases, therapies potentially influencing sensory perception or gastrointestinal motility, and history of peanut, tree nut, and seed allergy. The study was no-profit and approved by the Ethics Review Board of the University Hospital Policlinico in Bari (n. Almond1-1292-17).

Sensory evaluation

Each subject performed organoleptic assessment of both almond *cultivars* in double blind and random fashion. Quantitative visual analogue scales (VAS 0-100 mm on a horizontal line) were used to record the degree of appreciable odour, taste, chewing and visual perception (view) of almonds. Semi-quantitative scales (score 0-3) were used to record specific perceptions: smell (aroma, flavour), taste (sweet, salt, bitter, sour, persistence of food taste in mouth after swallow), mouth tactile sensations (hardness, crunchiness, chewiness, stickiness, oiliness, astringency), and visual aspect (shape, roughness, seed colour, pleasantness) [25]. Before each test the mouth was washed with plain water. Food, drink, smoking and physical activity were forbidden before and during the test. Potential differences in sensory perception were investigated according to body size (i.e. body mass index, BMI<30 Kg/m^2 and BMI≥ 30 Kg/m^2) and age (i.e. age <65 years and age ≥65 years).

Test meals

The standard test meal (Nutridrink®; Nutricia, Milano, Italy) consisted of 200 mL liquid suspension containing 12 g (20%) protein, 11.6 g (19%) fat, and 36.8 g (61%) carbohydrates for a total of 300 kcal, 1260 kJ, 455 mOsm/L. Lactulose (10 g = 15 mL Lattulac®, SOFAR, Trezzano Rosa, Milan, Italy) was added to the test meal in order to simultaneously assess the orocecal transit time (OCTT). The final volume of the meal was therefore 215 mL. The almond test meal consisted of 24 g of FC*cv* or CA*cv* (i.e. 12 almonds) with 175 mL of water and 15 mL lactulose (final volume 215 mL). Thus, the three test meals were different in composition but isovolumetric. Each meal was ingested at room temperature over one min in the presence of the examiner. Each subject underwent the motility tests on 3 different days ingesting a standard test meal, FC*cv* or CA*cv* almond test meal in a random fashion.

Gallbladder and gastric motility

Gallbladder, gastric motility and orocecal transit time were studied simultaneously [26-34]. Time-dependent changes of fasting and postprandial gallbladder volumes (mL) and antral areas (cm^2) were measured from frozen sonograms on a portable scanner (Noblus, Hitachi Medical, Tokyo, Japan) equipped with a 3.5 MHz convex transducer. Experiments started at 8 am after an overnight fast of at least 12h. Gallbladder volume and antral area were measured before the meal at -10, -5 and 0 min and after the meal every 5 min during the first 30 min and every 15 min thereafter up to 120 min. Indices of gallbladder emptying were fasting volume (mL), residual volume (minimum volume measured postprandially, in mL and percent of fasting volume).

Indices of gastric emptying were antral (basal) area (cm^2), maximal postprandial antral area recorded at time 0, i.e., 5 min after meal ingestion, postprandial and minimal postprandial antral areas during the 2h emptying curve. Postprandial areas were also normalized to maximal areas after subtraction of basal areas, i.e. $100 \times (A_t - A_{bas})/(A_{max} - A_{bas})$, where A_t = postprandial area at any given time; A_{bas}=basal area; A_{max}=maximal antral area. For both gallbladder and stomach, further indices included area under the emptying curve (AUC expressed as mL and % x 120 min), and half-emptying time

($T_{1/2}$, min). $T_{1/2}$ was calculated by linear regression analysis from the linear part of the emptying curves and was the time at which 50% decrease of gallbladder volume and antral area were observed.

Orocecal transit time

OCTT was measured by the lactulose H_2-breath test according to standard guidelines [32, 35-38]. During the 10 days before the test, antibiotics, probiotics, or other drugs known to affect gastrointestinal motility or intestinal microbiota were prohibited. A special diet was given the day before the test, to avoid the presence of non-absorbable or slowly fermentable food in the intestinal tract. The diet consisted of meat, fish, eggs and olive oil, and water as drink. Breath samples were taken before the meal and, subsequently, every 10 min up to 180 min after the ingestion of meal, during which a rise of 10 p.p.m. above baseline on two consecutive measurements (i.e. OCTT in min) was observed in all subjects. Time-dependent changes of H_2 in expired breath were studied using a pre-calibrated, portable hydrogen-sensitive electrochemical device (EC60-Gastrolyzer; Bedfont Scientific, Medford, NJ, USA). Results are expressed as H_2 excretion in parts per million (p.p.m.). Accuracy of the detector was ±2 p.p.m.

Statistical analysis

Analyses were performed using the statistical software NCSS10 (NCSS LLC, Kaysville, UT, USA) [39]. Values were expressed as mean ± standard error of the mean (SEM), and differences were evaluated by the paired- or unpaired two-tailed Student's *t*-test, as appropriate. For non-normal distribution, variables were expressed as median and corresponding interquartile range (IQR), and differences were evaluated by the nonparametric Wilcoxon test. Differences between the indices of motility were checked by the analysis of variance (ANOVA) followed by Fisher's least significant difference (LSD) test. A two-sided probability (P) of less than 0.05 was considered statistically significant.

RESULTS

The general characteristics of the enrolled subjects according to the test type are depicted in Table I. Subjects undergoing the sensory evaluation were significantly older and had a greater BMI than the subjects undergoing the motility studies. The difference is due to the original study design: whereas a wider age and BMI range was necessary for the studies on sensory evaluation, a narrower range was necessary for the motility studies. Both advanced age [40] and obesity [26], in fact, can act as potentially confounding factors of gastrointestinal motility.

Table I. Clinical characteristics of enrolled subjects

	Sensory evaluation	Motility studies
Number	60	24
Males: Females	30:30	10:14
Age years (range)	51.4±2.4 (18-80)	28.7±0.9* (23-37)
BMI, Kg/m^2 (range)	27.9±0.7 (19.5-48.8)	22.1±0.5* (19.5-24.7)

BMI: body mass index; data are expressed as mean±SEM; *P<0.01 between groups.

Chemical and structural analysis of almonds

Almond *cultivars* had a different composition (Table II). CA*cv* had significantly (P<0.001) higher content of protein and slightly lower content of lipids. Moisture, carbohydrates and ashes contents were similar. The analysis of fatty acids composition showed that the content (%) of stearic and oleic acid was less (P<0.001) while the content of palmitic (P<0.01) and linoleic (P<0.001) acid was higher in CA*cv* than FC*cv*. Also, total phenol content was about 10 times lower in the CA*cv* than FC*cv* while K_{232}, a marker of autoxidation degradation of the lipid fraction, was significantly higher (P=0.002) in CA*cv* than FC*cv*. A greater instrumental force (reflecting more hardness and less crunchiness) was required for breaking CA*cv* than FC*cv*.

Table II. Chemical and structural analysis of the two almond cultivars of different origin (Apulia and California)

	Almond	
Parameter	Carmel (CA*cv*)	Filippo Cea (FC*cv*)
Humidity (%)	3.91 ± 0.08	3.92 ± 0.04
Proteins (%)	22.19 ± 0.10**	17.70 ± 0.32
Lipids (%)	49.12 ± 1.10**	56.38 ± 0.12
Fatty acids (%)		
Myristic acid ($C_{14:0}$)	0.04 ± 0.00	0.02 ± 0.00
Myristoleic acid ($C_{14:1}$)	0.04 ± 0.00	0.02 ± 0.00
Pentadecylic acid ($C_{15:0}$)	0.00 ± 0.01	0.00 ± 0.01
Palmitic acid($C_{16:0}$)	6.09 ± 0.02*	5.76 ± 0.08
Palmitoleic acid ($C_{16:1}$)	0.41 ± 0.01	0.42 ± 0.01
Margaric acid ($C_{17:0}$)	0.05 ± 0.00	0.05 ± 0.01
Heptadecenoic acid ($C_{17:1}$)	0.11 ± 0.00	0.08 ± 0.00
Stearic acid ($C_{18:0}$)	1.51 ± 0.01**	3.32 ± 0.01
Oleic acid ($C_{18:1}$)	65.87 ± 0.03**	71.87 ± 0.06
Linoleic acid ($C_{18:2}$)	25.74 ± 0.03**	18.25 ± 0.03
Arachidic acid ($C_{20:0}$)	0.07 ± 0.00	0.11 ± 0.01
Linolenic acid ($C_{18:3}$)	0.07 ± 0.01	0.07 ± 0.00
Behenic acid ($C_{22:0}$)	0.02 ± 0.00	n.d.
Ashes (%)	2.89 ± 0.01	2.72 ± 0.04
Carbohydrates (%)	21.90 ± 1.08	19.28 ± 0.20
K_{232}	2.41 ± 0.03**	1.71 ± 0.03
K_{270}	0.12 ± 0.00	0.17 ± 0.02
Total phenol content (mg/kg)	90.29 ± 9.33**	1432.85 ± 33.35
Force max (N)	41.00 ± 1.65***	32.70 ± 1.37

Data are expressed as mean±SEM of triplicate experiments, except for Force max (n=15 experiments); *P=0.01; **P<0.001; *** P=0.002 vs. FC*cv*.

Sensory evaluation

The study of sensory evaluation (Table III) showed that CA*cv* had significantly (P<0.01) higher VAS scores than FC*cv* for smell, texture, and appearance. The sub-analysis based on the semi-quantitative (0-3) scale showed that CA*cv* had significantly (P<0.01) higher scores than FC*cv* for sweet, hardness, oval shape,

Table III. Scores of sensory evaluation for two almond varieties in the whole group of subjects (N=60).

		Almond	
		Carmel (CA*cv*)	Filippo Cea (FC*cv*)
DESCRIPTORS			
Smell[1]		60.0 (50.0-70.0)*	50.0 (32.5-63.8)
Aroma			
	wood[2]	0 (0-2)	0 (0-1)
	straw[2]	0 (0-0)	0 (0-0)
	tobacco[2]	0 (0-0)	0 (0-0)
	other[2]	0 (0-0)	0 (0-0)
Flavour			
	almond[2]	1.5 (0-3)	1 (0-2)
	straw[2]	0 (0-0)	0 (0-0)
	wood[2]	0 (0-1)	0 (0-1)
	tobacco[2]	0 (0-0)	0 (0-0)
	rancid[2]	0 (0-0)	0 (0-0)
	mould[2]	0 (0-0)	0 (0-0)
Taste[1]		70.0 (52.5-80.0)	60.0 (50.0-83.8)
	sweet[2]	2 (1-2)*	1 (1-2)
	salad[2]	0 (0-0)	0 (0-0)
	bitters[2]	0 (0-1)	0 (0-1)
	acid[2]	0 (0-0)	0 (0-0)
	persistence[2]	2 (2-3)	2 (1-3)
Texture[1]		70.0 (50.0-80.0)*	60.0 (42.5-70.0)
	hardness[2]	2 (1-3)*	1 (1-2)
	crunchiness[2]	2 (1-3)	2 (1-3)
	chewiness[2]	2 (1-2)	2 (1-2)
	stickiness[2]	0 (0-1)	0 (0-1)
	greasiness[2]	1 (0-2)	1 (0-2)
	astringency[2]	0 (0-1)	1 (0-1)
Appearance[1]		72.5 (60.0-90.0)*	50.0 (40.0-70.0)
	oval[2]	2.5 (2-3)*	2 (1-3)
	pleasantness[2]	2 (2-3)*	1 (1-2.75)
	roughness[2]	2 (2-3)*	2 (1-2)
	colour intensity[2]	2 (2-3)	2 (1-3)

Data are expressed as median (IQR) of [1]Visual analogue scales (0-100 mm) and [2] semiquantitative scale (0-3); *P<0.01 between similar descriptors.

Table IV. Scores of sensory evaluation for two almond varieties in subjects according to age and Body Mass Index (BMI)

		Almond	
		Carmel (CA*cv*)	Filippo Cea (FC*cv*)
Age <65 years			
Lean (N=20)[1]			
	Smell	55.0 (42.5-60.0)*	40.0 (22.5-58.8)
	Taste	72.5 (50.0-80.0)*	50.0 (41.3-68.8)
	Texture	75.0 (51.3-88.8)°	60.0 (42.5-71.3)
	Appearance	75.0 (60.0-90.0)°	50.0 (42.5-67.5)
Obese (N=20)[2]			
	Smell	55.0 (50.0-80.0)	50.0 (30.0-68.8)
	Taste	75.0 (60.0-88.8)	70.0 (50.0-90.0)
	Texture	65.0 (50.0-95.0)	62.5 (50.0-77.5)
	Appearance	80.0 (70.0-100)°	50.0 (30.0-80.0)
Age ≥65 years			
Lean (N=20)[1]			
	Smell	70.0 (42.5-77.5)	50.0 (40.0-70.0)
	Taste	70.0 (42.5-80.0)	70.0 (42.5-97.5)
	Texture	65.0 (50.0-80.0)*	50.0 (30.0-67.5)
	Appearance	70.0 (42.5-80.0)	50.0 (42.5-80.0)

[1]BMI <30 Kg/m^2; [2]BMI≥30Kg/m^2. Data are expressed as median (IQR) of Visual analogue scales (0-100 mm); *P<0.05, °P<0.01 CA*cv* vs. FC*cv*.

pleasantness, and roughness. The analysis of VAS scores for sensory evaluation by major descriptors according to age and BMI is reported in Table IV. In subjects aged <65 and lean, scores of smell, taste, texture and appearance were all higher for CA*cv* than FC*cv*. In subjects aged <65 and obese, only appearance scored higher for CA*cv* than FC*cv*. In subjects aged ≥65 and lean, only texture scored higher for CA*cv* than FC*cv*.

Gastrointestinal motility

The results of the functional gastrointestinal motility studies in response to a standard (Nutridrink) and two almond test meals are reported in Table V and Fig. 1.

Fasting gallbladder volumes were comparable across the three days of the test with means ranging from 23 to 26 mL. The ingestion of each test meal induced a mean gallbladder response of 62%, 47%, and 60% to Nutridrink, CA*cv* and FC*cv*, respectively. In particular, the residual gallbladder volume in response to the test meal both as mL and % fasting volume was comparable between Nutridrink and FC*cv* test meals but significantly larger (12.0±0.6 mL) in response to the CA*cv* test meal. The emptying speed was comparable for each test meal, ranging from 19 to 22 min (Table V). The graphic analysis of the emptying curves (Fig. 1A) compares the time-dependent changes of the gallbladder volume (mL and percent of fasting volume), AUC, emptying speed. A less complete emptying is observed in response to CA*cv*.

Basal (fasting) antral areas were small and comparable during the three days of the tests with means ranging from 4.7 to 4.8 cm^2. The ingestion of each test meal induced a sudden antral dilatation (expressed as max postprandial area). However, the effect was similar and significantly greater (mean 36%) with both CA*cv* and FC*cv* than with Nutridrink. Nevertheless, residual antral areas and the estimated emptying speed remained comparable in response to the three test meals (Table V). The graphic analysis of the emptying curves (Fig. 1B) shows the time-dependent changes of the antral areas. A trend was shown towards smaller percentage postprandial areas in response to CA*cv* test meal than FC*cv* test meal.

Orocecal transit time

The ingestion of each test meal induced a consistent increase of H_2 levels in exhaled air, as marker of OCTT (111 min after Nutridrink, 80 and 82 min in response to CA*cv* test meal and FC*cv* test meal, respectively, P=0.00002 for both almonds *vs*. Nutridrink) (Fig. 2). Therefore, with both *cultivars*, OCTT was approximately 27% shorter than OCTT observed with Nutridrink.

Table V. Gastrointestinal motility studies in response to liquid test meal and two almond test meals in 24 healthy subjects.

	Nutridrink Test meal	Carmel (CA*cv*) Test meal	Filippo Cea (FC*cv*) Test meal
Gallbladder emptying			
Fasting gallbladder vol. (mL)	22.8±1.2	26.2±1.6	24.8±1.8
Residual gallbladder vol. (mL)	8.5±0.6	12.0±0.6*	9.9±1.0
Residual gallbladder vol. (%)	37.8±1.8	46.8±2.0°	39.7±2.0
AUC (mL x 120 min)	1590±108	2014±105*	1854±107
AUC (% x 120 min)	7007±240	7946±245*	7556±241
Half-emptying time (min)	19±1	22±2	21±2
Gastric emptying			
Basal antral area (cm^2)	4.7±0.2	4.8±0.1	4.7±0.1
Max. postprandial antral area (cm^2)	10.6±0.3	13.7±0.3*	14.0±0.2*
Residual antral area (cm^2)	4.8±0.1	4.9±0.1	4.7±0.1
AUC (cm^2 x 120 min)	764±20	847±21°	928±21*
AUC (% x 120 min)	3310±190	2941±195	3826±198^
Half-emptying time (min)	29±1	29±4	32±1
Orocecal transit time (min)	111±6	80±4*	82±5*

Each test meal randomly given to the same subject. AUC, area under curve; data are expressed as means±SEM; *P<0.05 *vs*. Nutridrink; °P<0.05 *vs*. Nutridrink and FC*cv*; ^P<0.05 *vs*. CA*cv*.

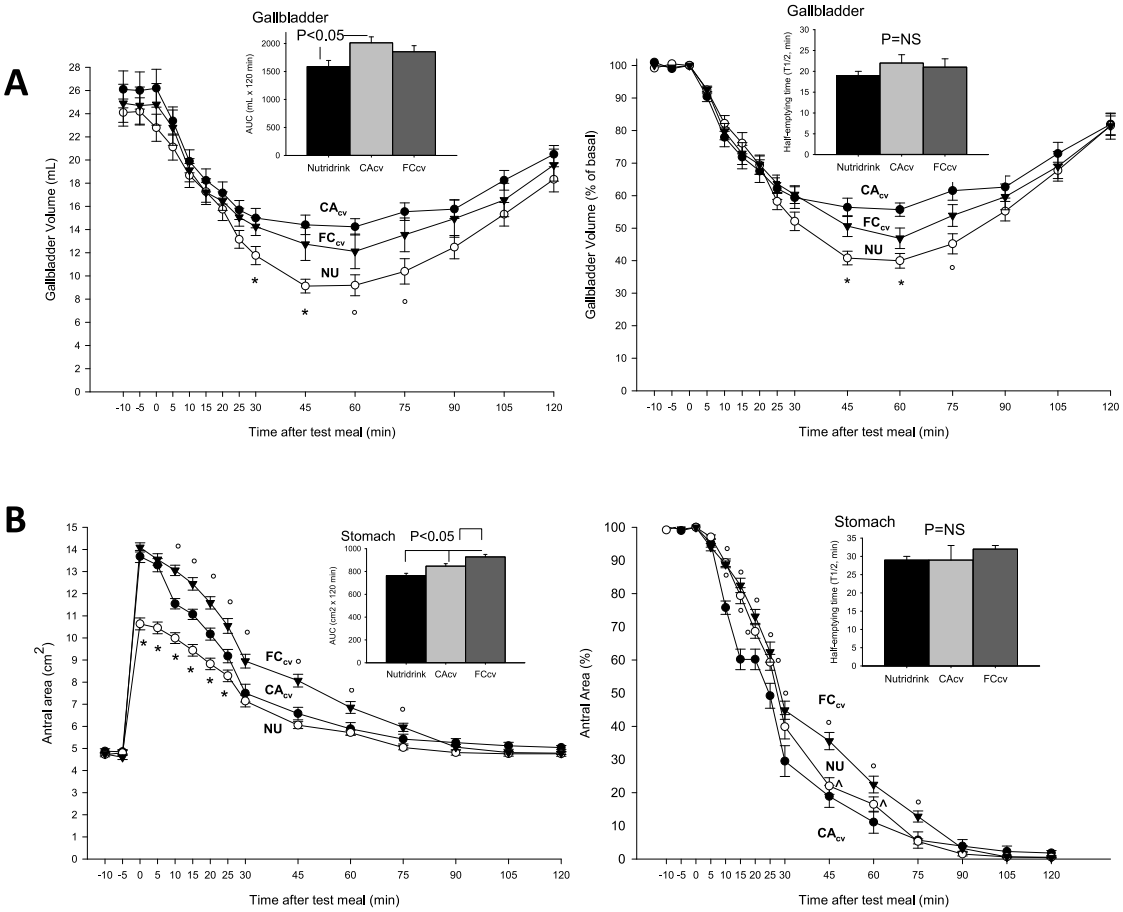

Fig. 1. Gallbladder (A) and gastric (B) emptying curves in response to the ingestion of three test meals: (1) NU: Nutridrink 200 mL + lactulose 15 mL (10 g) to a final volume of 215 mL; (2) FC*cv*: almond test meals with 24 g of Filippo Cea cultivar with 175 mL of water + 15 mL lactulose to a final volume of 215 mL; (3) CA*cv*: almond test meals with 24 g Carmel cultivar with 175 mL of water + 15 mL lactulose to a final volume of 215 mL. A) Time-dependent changes of gallbladder volume are given as means of mL (left) and area under curve (AUC, inlet), and as percentage of fasting volume (right) and half-emptying time (inlet). B) Time-dependent changes of antral areas are given as means of cm^2 (left) and area under curve (AUC, inlet), and as percent of maximal area (right) and half-emptying time (inlet). Statistics: *P<0.05 *vs*. CA*cv* and FC*cv*; °P<0.05 *vs*. CA*cv*; ^P<0.05 *vs*. FC*cv*.

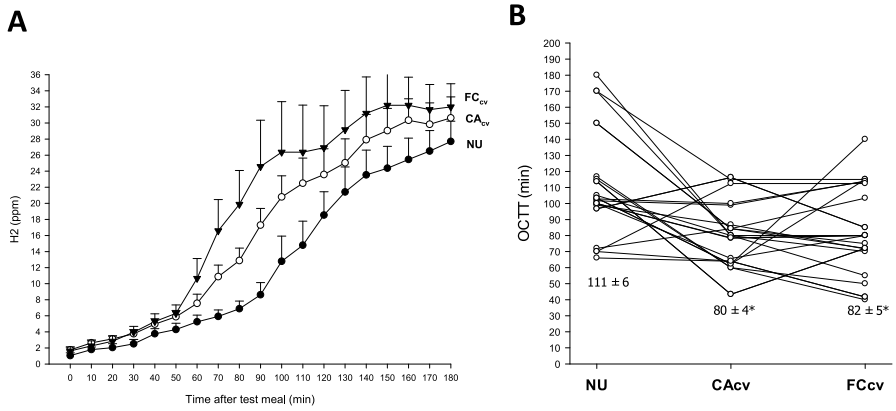

Fig. 2. Study of orocecal transit time (OCTT) in response to the ingestion of three test meals: (1) NU: Nutridrink 200 mL + lactulose 15 mL (10 g) to a final volume of 215 mL; (2) FC*cv*: almond test meal with 24 g of Filippo Cea cultivar with 175 mL of water + 15 mL lactulose to a final volume of 215 mL; (3) CA*cv*: almond test meal with 24 g Carmel cultivar with 175 mL of water + 15 mL lactulose to a final volume of 215 mL. A) Time-dependent curves of H_2 levels (ppm) in exhaled air. (mean±SEM). B) Orocecal transit time (OCTT). Each symbol indicates individual OCTT, while mean±SEM are reported below. *: significant difference from Nutridrink (P<0.05).

DISCUSSION

The present study provides a comprehensive evaluation of the chemical and texture features, and of the influence of almond ingestion on the individual sensory perception and gastrointestinal motility in healthy subjects of different age and body size. Two different *cultivars* i.e., the Apulian Filippo Cea and the Californian Carmel were compared in a blind randomized fashion. The results showed marked differences between the *cultivars*, and open interesting perspectives in terms of nutraceutical properties.

Since antiquity, almonds are an important nutritional resource for humans [41]. Over time, almond has become increasingly important not only as a food but also for its use in cosmetics, pharmaceuticals and the food industry [42]. World almond consumption in 2014 was 1,054,231 metric tons, with 0.15 kg per capita considering the total world population [43]. Italy is one of the major world consumer, with more than 40,000 metric tons of almonds consumed in 2014, 0.68 kg per capita considering the total Italian population, and 1.37 kg per capita considering the percentage of the consuming population [43].

Differences between the two *cultivars* were already evident by chemical analysis, with protein and lipid content comparable to that previously reported [15, 17, 18]. The most striking difference was the total phenol content (10 times higher in the FC*cv* than in CA*cv*). However, we found that the total phenol content in CA*cv* was much lower than that reported in literature [44, 45], possibly due to transport and storage, leading to oxidation phenomena. This hypothesis is supported by the significantly higher value of the K_{232}, a marker of lipid oxidation phenomena in almonds [46] observed in CA*cv*, as compared with FC*cv*. Phenol compounds and their bioaccessibility contribute to healthy nutraceutical effects of almonds; for example phenol compounds in almond skin have been linked with positive health effects as the reduction of oxidative stress and inflammation [47-52]. A number of studies also link the consumption of almonds with lower levels of serum cholesterol and triglycerides, due to their content in polyunsaturated fatty acids [10, 11]. Furthermore, almonds have a low glycemic index and do not adversely impact insulin sensitivity, therefore reducing risk factors linked to diabetes [4]. Almonds are also an excellent source of bioavailable α-tocopherol, which protects against oxidation of low-density lipoprotein (LDL) cholesterol [4], and are indicated in the diet of elderly people because they help to increase bone mineral density [53].

The complete sensory evaluation assessed the degree of desirability of the two almond samples and the existence of possible differences in desirability, according to advanced age and obesity. Apparently, scores related to "appreciation" and appearance-related indices (olfactory, kinaesthetic-tactile and visual perceptions) were higher for CA*cv* than for FC*cv*. Taste, however, was almost comparable between the two *cultivars*, although CA*cv* scored more sweetness than FC*cv*. This study provides additional and novel information because it shows that both obesity and advanced age can significantly influence sensory evaluation. Indeed, lean subjects have more complete perception than obese and elderly subjects. Differences point to distinct anatomical and pathophysiological processes in obesity and aging. Obese subjects display a significant correlation between elevated BMI and the presence of smell and taste dysfunction [54-57]. Visceral fat correlates with obesity and excreted adipokines [58] may alter the perception of odours [57, 59]. Also, studies found a negative correlation between the olfactory functions and age [57, 60], and negative effects of aging on taste [60]. Elderly subjects can lose their sense of smell and the ability to discriminate between smells. Mechanisms include the decrease of number of fibres in the olfactory bulb and olfactory receptors [61], or the neurological and cognitive decline (including Alzheimer's disease) [60, 62]. Finally, chewing problems associated with teeth loss and dentures can also interfere with taste sensations in older people [60], and such aspects deserve further investigations.

The ingestion of the almonds generated significant effects on gastrointestinal motility in healthy subjects. We decided *a priori* to administer a standard amount of CA*cv* or FC*cv* almonds (24 g, ≈50% fat) to promote a reproducible fat-induced gallbladder response. A consistent cholecystokinin-mediated gallbladder emptying occurs with 12 g of ingested fat [26] and in this study the estimated fat content was 11.8 g for CA*cv* and 13.5 g for FC*cv* test meals. The isovolumetric composition of the test meals, moreover, provided accurate analyses of the emptying curve and kinetic parameters of gastric and small intestinal motility. Findings were compared with Nutridrink, the standard test meal adopted by our group [26, 32].

Of note, the ingestion of FC*cv* almonds was associated with a 15% smaller residual gallbladder volume (meaning more emptying) than that observed after the ingestion of the same amount of CA*cv*. This difference can be partly explained by the slightly increased lipid content of FC*cv*. However, the influence of additional factors (i.e. content in polyphenols) cannot be ruled out.

Data on gastric motility showed that the ingestion of both *cultivars* caused a fast antral dilatation, as compared with the standard isovolumetric Nutridrink (i.e. 215 mL). Further studies should assess if this finding is associated with a more pronounced satiety feeling after almond ingestion, as compared with other meals containing a similar amount of nutrients. Previous studies found effects of almonds on fullness and hunger levels [63, 64], an acute satiating effect of almond ingestion, and a dose-dependent enhanced satiety following an almond snack in the midmorning [65]. Furthermore, a recent study showed comparable postprandial hunger, desire to eat, fullness, and neural responses to visual food stimuli after the ingestion of almonds or baked food, linking these results with energy and macronutrient contents [66]. The extent and timing of gastric emptying were comparable after the ingestion of almonds and test meal. Postprandial areas, however, tended to be smaller after CA*cv* than after FC*cv*, likely due to the higher lipid content of the FC*cv*. This emptying pattern, however, did not significantly alter the final antral emptying speed.

The small intestinal transit time in response to the test meal was comparable to that observed in previous studies [28, 32], while it was 27% more rapid after both almonds.

The gastrointestinal response observed with almonds suggests their frequent consumption in distinct metabolic abnormalities. Almonds consumption is inversely associated with the incidence of cardiovascular disease [4, 67-69] and with body weight [66, 70-72]. FC*cv*, with higher lipid content than CA*cv* might act as a "natural" prokinetic agent on a hypomotile gallbladder, a condition at increased risk for biliary sludge or cholesterol cholelithiasis [73]. Also, FC*cv*, highly enriched in phenol content, could better counteract the ongoing oxidative stress in several chronic metabolic disorders. These hypotheses, however, need to be confirmed by specific clinical studies.

CONCLUSION

The present study shows that the FC*cv* and the CA*cv* *cultivars* differ in chemical composition and structure, likely influencing distinct sensory evaluation in lean, obese and elderly subjects. Gastrointestinal motility shows also specific features, i.e. greater gallbladder emptying to FC*cv*, similar gastric emptying to FC*cv*, CA*cv* and test meal and small intestinal transit time to FC*cv*, CA*cv* faster than after test meal. Results point to a potential use of CA*cv* or FC*cv* as valuable nutraceutical tools, to be confirmed by further clinical studies.

Conflicts of interest: None.

Authors contributions: G.D., M.T.M., G.C. and P.P. conceived and designed the experiments; G.D. and M.P.L. performed the clinical experiments; C.S. and F.C. performed the chemical experiments; G.D., A.D.C. and P.P. analyzed the data; M.T.M. and P.P. resulted Promotors; P.P. resulted Primary Investigator; G.D., A.D.C. and P.P. wrote the paper.

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