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Instrumental measurement of the texture of hard-boiled egg yolks enriched with different levels of conjugated linoleic acid

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Abstract

There is increased interest in enhancing the conjugated linoleic acid (CLA) content of food products because of its potential health benefits. Inclusion of CLA in the diets of laying hens has led to the incorporation of CLA into eggs, changes in yolk fatty acid (FA) composition and non-acceptable greater firmness of hard-boiled egg yolks. This study was designed to develop instrumental tests to determine the texture characteristics of hard-boiled egg yolks obtained from hens fed diets supplemented with different levels of CLA and other fat sources. Two compression tests have established relationship between some FA levels added to diets and the firmness of boiled egg yolks. There were significant differences in the compression parameters among egg yolks from laying hens fed diets supplemented with 3 and 5 g kg^{-1} of CLA (without other fats in diet) and commercial egg yolks. Supplementations with 30 and 35 g kg^{-1} of high oleic sunflower oil (HOSO) in diets which included 3 g kg^{-1} of CLA, decreased compression parameters to levels similar to commercial eggs.

Additional key words: compression test, egg yolk, fatty acid composition, firmness, quality.

Resumen

Medida instrumental de la textura de la yema de huevos cocidos enriquecidos con ácido linoleico conjugado

Existe un creciente interés en aumentar el contenido de ácido linoleico conjugado (ALC) en los alimentos debido a sus potenciales efectos beneficiosos sobre la salud. La formulación de dietas suplementadas con ALC para gallinas ponedoras ha conducido a la obtención de huevos enriquecidos con este ácido graso. Sin embargo, la incorporación de ALC en el huevo produce cambios en la composición de ácidos grasos del mismo y, consecuentemente, un aumento de firmeza en la yema de los huevos cocidos. Este trabajo se ha diseñado con el fin de desarrollar ensayos instrumentales para determinar las características texturales de las yemas de los huevos cocidos obtenidos de gallinas ponedoras alimentadas con dietas suplementadas con diferentes niveles de ALC y otras fuentes de grasa. Dos ensayos de compresión con una máquina universal de ensayos han permitido establecer relaciones entre los niveles de determinados ácidos grasos añadidos a las dietas de ponedoras y la firmeza de la yema de huevos cocidos. Se encontraron diferencias significativas en los parámetros del ensayo de compresión entre las yemas de huevos procedentes de gallinas ponedoras alimentadas con dietas suplementadas con 3 y 5 g kg^{-1} de ALC (sin ninguna otra fuente de ácidos grasos) y las yemas de los huevos comerciales. Las suplementaciones con 30 ó 35 g kg^{-1} de aceite de girasol rico en oleico a dietas con 3 g kg^{-1} de ALC, disminuyeron los valores de los parámetros del ensayo de compresión a niveles asimilables a los de las yemas de huevos comerciales.

Palabras clave adicionales: calidad, composición de ácidos grasos, ensayo de compresión, firmeza, yema del huevo.

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Introduction[1]

Conjugated linoleic acid (CLA) is a mixture of positional and geometric isomers of linoleic acid with two conjugated double bonds at various carbon positions in the fatty acid (FA) chain. It is considered to have health benefits such as anticarcinogenic, antiatherogenic, antidiabetic and antiadipogenic effects (Dhiman, 2000; Pariza *et al.*, 2001). As a result, there has been considerable interest in increasing the CLA concentration in food products for human consumption.

Ruminant foods are the richest source of CLA for humans because of the ability of ruminants to biohydrogenate dietary unsaturated FA with the help of rumen bacteria. Only small amounts of CLA have been found in milk, meat, or eggs from non-ruminants. In egg yolk lipids, CLA was not detected when laying hens were fed a normal concentrate diet (Raes *et al.*, 2002; Yang *et al.*, 2002). However, several workers have shown considerable enhancement of the CLA content of egg yolk, broiler or pork meat through dietary supplementation with CLA (Watkins *et al.*, 2003).

In enriched eggs, the relationship between CLA concentration in the egg yolk and the feed was almost linear up to a CLA concentration in the diet of 5%, but varied with the CLA-isomer used (Du *et al.*, 1999; Jones *et al.*, 2000; Szymczyk and Pisulewski, 2003). Some of these studies indicate that CLA induces changes in the lipid and FA metabolism of laying hens and affects egg quality characteristics. The concentrations of monounsaturated and non-CLA polyunsaturated FA were reduced, whereas those of saturated FA were considerably increased. Further, some work has shown that dietary CLA supplementation (from 0.5% up) altered the yolk texture of hard-boiled eggs (Ahn *et al.*, 1999; Álvarez *et al.*, 2004). These effects have been related to the incorporation of CLA into yolk membrane phospholipids. This increases yolk membrane permeability and facilitates movement of ions through the yolk membrane during storage. Sensory evaluation tests showed that these eggs were not acceptable for consumption, because of their rubbery, elastic texture.

Other FAs can be added to diet to avoid the negative effect of CLA and/or to obtain functional eggs enriched in essential nutrients. High oleic sunflower oil (HOSO) could be used because of its high content of monounsaturated FA, mainly oleic acid (C18:1, 757 g kg^{-1}), which might control the texture alteration caused by CLA supplementation, by restoring the yolk oleic acid concentration to normal levels (Aydin *et al.*, 2001). Other fat sources, such as fish oil (FO) or algae oil (AO), with a high level of Ω-3 polyunsaturated FA, could reduce the saturated/unsaturated FA ratio of egg yolks enriched with CLA. Further, several papers have shown the benefits to human health associated with consumption of eggs which are enriched in Ω-3 FA (Bovet *et al.*, 2007).

Cachaldora *et al.* (2005) previously showed that retention of total Ω-3 FA in egg yolk fat appeared to be highly and positively dependent on the level of dietary docosahexanoic acid (C22:6 Ω-3, DHA) supplementation. Thus, to obtain a similar Ω-3 FA content in commercial enriched Ω-3 eggs, lower dietary levels of oils, rich in DHA, could be added to layer feeds supplemented with CLA to avoid the negative effects of high doses of marine oil on the sensory quality of eggs.

The British Standards Institution defined the texture of an edible material as *the attribute of a substance resulting from a combination of physical properties perceived by the senses of touch (including mouth feel), sight and hearing* (Anonymous, 1975). Texture is defined as a sensory attribute, and can be measured directly by sensory means. Classifications of food texture, based on rheological principles, have been proposed, using both instruments and sensory methods of characterization to monitor product rheological behaviour. Mechanical properties such as hardness, viscosity and elasticity among others, are included in the textural characteristics of food. Food texture is described by sensory terms; beside, the mechanical properties of food tissues are defined in terms of force, pressure and energy. Different compression tests are used as instrumental techniques to determine the mechanical attributes of texture, resistance to mechanical injury and force-deformation behaviour of numerous foods. Compression tests, which measure the variables of the force-deformation curve, have been adapted to different applications to obtain the best instrumental procedure in each research: based compression test using cylindrical probes (Barreiro *et al.*, 1998), compression with a spherical indenter on specimens in their natural form and size (Diezma-Iglesias *et al.*, 2006), compression between parallel plates of samples or probes of defined

[1] Abbreviations used: AO: algae oil; CLA: conjugated linoleic acid; DHA: docosahexanoic acid; EPA: eicosapentaenoic acid; FA: fatty acid; FO: fish oil; HOSO: high oleic sunflower oil.

shape and size. The basic principle underlying the measurement of force-deformation lies in Hertz's theory: the compressive stress between two bodies in contact is proportional to their elastic modulus and is inversely proportional to their radius. Elastic modulus is directly related to firmness for fixed geometry testing elements and samples, thus firmness can be measured by force deformation parameters (ASAE, 2000).

In this work, instrumental compression measurements were designed and applied to hard-boiled egg yolks to determine: (1) which is the more reliable to discriminate among yolks from hens fed different diets, and (2) how instrumental techniques compare with consumer acceptability.

The aim of this study was to develop instrumental tests that determined the textural characteristics of hard-boiled egg yolks laid by hens fed with diets supplemented with different levels of CLA and other FA sources: FO, HOSO and AO.

Material and Methods

Samples eggs were obtained from layers fed diets supplemented with CLA and other fat sources; the level of fat, added in each experiment, was selected based on the results of previous tests. The CLA used in all studies was obtained from BASF Española, S.A. (Tarragona, Spain) and contained 560 g kg^{-1} CLA. High oleic sunflower oil used contained 757 g kg^{-1} of oleic acid. Fish (FO-EPA and FO) and marine algae oils (AO) contained variable amounts of eicosapentanoic acid (EPA; 171, 67 and 12 g kg^{-1} of total FA, respectively) and DHA (80, 173 and 366 g kg^{-1} of total FA, respectively).

Trials lasted 28 days and started after a pre-experimental period of 21 days. Forty-week-old Warren laying hens were individually housed in cages and served as the experimental unit. Five hens were randomly assigned to each diet.

Two compression tests were developed for firmness measurement of hard-boiled egg yolks, to identify the effect of supplementing the diet of laying hens with different levels of CLA and other FA sources. The first test was carried out on full egg yolks, while the second was carried out after cutting shelled eggs in half lengthwise.

Five experiments were carried out, based on different dietary treatments or formulations for the laying hens, and using the first compression test on the hard-boiled egg yolks (flat-plate compression test). In the fifth experiment the second compression test (cylindrical rod compression test), was used with the objective of decreasing the variation in the mechanical measurements.

Experiment 1

Because CLA decreases the unsaturated FA content and alters the texture of egg yolks (Hur *et al.,* 2007), the objectives of this experiment were to determine whether CLA-induced changes could be prevented by feeding FO and if this level of FO altered egg aroma or flavour.

Hens were randomly assigned to each of the 9 dietary treatments formulated by factorially combining three CLA concentrations (1, 3 and 5 g kg^{-1}) and FO (0, 14 and 20 g kg^{-1}) into a basal diet (Table 1). A commercial diet, without additional FA, was also formulated as a control for sensory evaluation and compression tests of the experimental eggs.

Experiment 2

The experimental design was as in Experiment 1 but the CLA level was decreased to 2 g kg^{-1} and HOSO was added in place of FO, to the diets. The nine dietary treatments were applied factorially with three CLA levels (0, 1 and 2 g kg^{-1}) and three HOSO levels (10, 20 and 30 g kg^{-1}) (Table 1).

Experiment 3

In an attempt to produce eggs which were enriched in both CLA and Ω-3 FA, all sources of FA used in previous experiments were combined in Experiment 3: two high levels of HOSO (30 and 35 g kg^{-1}) and two levels of FO (0 and 17 g kg^{-1}) were added to a basal diet containing a CLA at 3 g kg^{-1}.

Experiment 4

In this experiment hens were randomly assigned to each of nine diets formulated by factorially combining three levels of inclusion of total Ω-3 FA (2.9, 3.7 or 4.5 g kg^{-1}) from three marine sources (FO, FO-EPA and

Table 1. Levels of conjugated linoleic acid (CLA), fish oil (FO), high oleic sunflower oil (HOSO) and algae oil (AO) added to the basal diet of Warren laying hens in Experiments 1-5

	Dietary treatment	CLA (g kg^{-1})	FO or AO* (g/g kg^{-1})	HOSO (g kg^{-1})
Experiment 1	Control	0	0	0
	T1.1	1	0	0
	T1.2	1	14[1]	0
	T1.3	1	20[1]	0
	T1.4	3	0	0
	T1.5	3	14[1]	0
	T1.6	3	20[1]	0
	T1.7	5	0	0
	T1.8	5	14[1]	0
	T1.9	5	20[1]	0
Experiment 2	T2.1	0	0	10
	T2.2	0	0	20
	T2.3	0	0	30
	T2.4	1	0	10
	T2.5	1	0	20
	T2.6	1	0	30
	T2.7	2	0	10
	T2.8	2	0	20
	T2.9	2	0	30
Experiment 3	T3.1	0	0	30
	T3.2	3	0	30
	T3.3	3	17[1]	30
	T3.4	3	0	35
	T3.5	3	17[1]	35
Experiment 4	Control	0	0	0
	T4.1	2.5	11[2]	30
	T4.2	2.5	14[2]	30
	T4.3	2.5	17[2]	30
	T4.4	2.5	11[1]	30
	T4.5	2.5	14[1]	30
	T4.6	2.5	17[1]	30
	T4.7	2.5	17[3]	30
	T4.8	2.5	22[3]	30
	T4.9	2.5	27[3]	30
	Commercial-A	0	17[1]	0
Experiment 5	Control	0	0	0
	T5.1	2.5	14[1]	30
	T5.2	3	0	30
	Commercial-B	0	14[1]	0

[1] Fish oil high in DHA: FO. [2] Fish oil low in DHA: FO-EPA. [3] Algae oil. N = 284 eggs.

AO) differing in EPA and DHA proportions, at a fixed level of CLA (2.5 g kg^{-1}) and HOSO (30 g kg^{-1}) supplementation of the basal diet. The actual amount of marine oils added were 11, 14 and 17 g kg^{-1} of FO and FO-EPA, and 17, 22 and 27 g kg^{-1} of AO (Table 1). A commercial layer diet without additional FA (control) and another containing 4.5 g kg^{-1} of Ω-3 FA, (17 g kg^{-1} of FO) (Commercial-A), but no CLA or HOSO, were formulated as controls for sensory evaluation of the experimental diets.

Experiment 5

In Experiment 5 the objective was to try to improve the repeatability of the instrumental tests. Two diets were evaluated. In both diets the HOSO was fixed at 30 g kg^{-1}, CLA was added at 2.5 and 3 g kg^{-1}, and FO at 14 g kg^{-1} was only supplemented in the diet which contained 2.5 g kg^{-1} of CLA (Table 1). These diets were compared to the control diet and a commercial laying hen diet which contained Ω-3 FA from FO (14 g kg^{-1} of FO) (Commercial-B).

Sensory evaluation test

Twelve eggs per treatment, produced at the end of the experimental periods, were randomly selected for sensory evaluation tests. All eggs were kept at 5ºC for 28 d. Twelve trained panellist from the COREN Quality Department were asked to evaluate warm, hard boiled eggs after shelling them and cutting them in half. Panellist score, on a 10-point scale, described their dislike or like of aroma, taste, aftertaste, flavour, intensity of off-flavours and overall acceptability.

Compression tests

Egg samples from each dietary treatment, were kept at 5ºC for 28 d. Eggs were boiled for 15 minutes and cooled to room temperature. Flat plate compression tests (Experiments 1 to 5) and cylindrical rod compression tests (Experiment 5) were performed on 6 and 20 eggs per treatment, respectively. A total of 284 eggs were evaluated.

The machine used for the mechanical tests was a texture analyser TA-XT2 (Stable Micro Systems Ltd., Godalming, UK), a universal machine with a texture analyser micro processor, connected to a PC, and controlled by specific software.

Flat-plate compression test: a first compression test, using single parallel plate-loading configuration was used in Experiments 1 to 5. Shell and albumen were

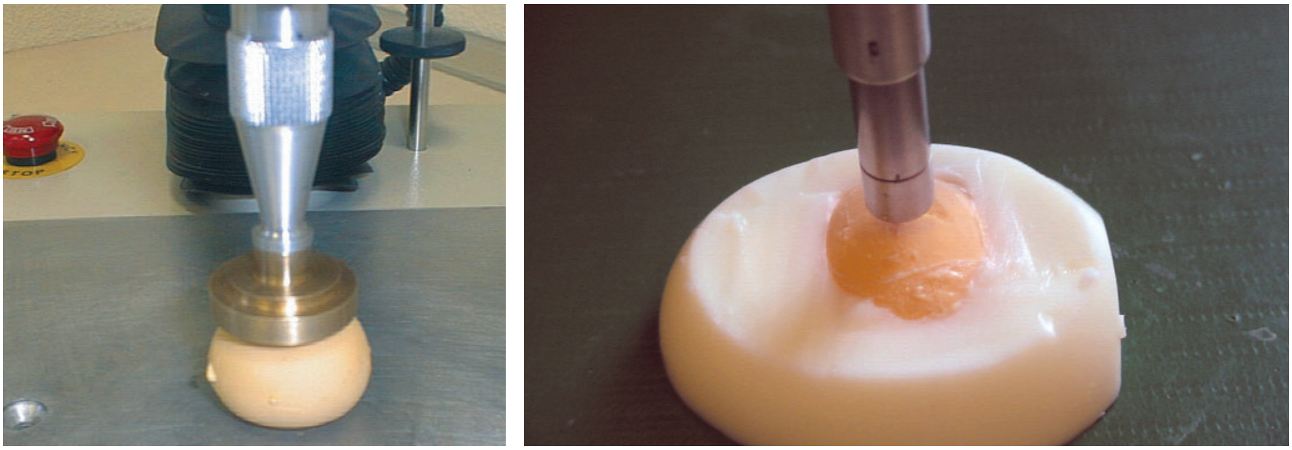

Figure 1. Left: the configuration of the flat-plate compression test on full egg yolks as attached to a texture analyser. Right: the configuration of the cylindrical rod compression test on half egg yolks as attached to a texture analyser. Only 3 mm of deformation was applied, without attaining rupture.

removed by hand and using the texture analyser a maximum deformation of 6 mm was applied at 20 mm min^{-1} on egg yolks, deformation was immediately removed at the same rate. There was one repetition per egg. The compression tool used was a flat-plate of 18 mm of diameter. Full egg yolks were supported on a flat surface with their major axis parallel to the surface (Fig. 1, left). From the force-deformation curve of this test the following parameters were recorded:

— Maximum force: F, in N.

— Not permanent (i.e. elastic) deformation: NPERM, in mm, as a measure of degree of elasticity.

— Absorbed energy during compression, area below the force-deformation loading curve: AREA, in N.mm.

— Force-deformation slope for F and corresponding deformation: F/D, in N mm^{-1}.

Cylindrical rod compression test: a second compression test was carried out with the same texture machine on samples from Experiment 5. A deformation of 3 mm was applied at 20 mm min^{-1} with an 8 mm diameter rod with a spherical tip. No rupture was observed in this test. When single plate-loading or spherical compression tools are used, it is essential that specimens are supported so deformation at the support is negligible. Deformation at the bottom plate will be negligible because the area of contact between the sample and the supporting plate is many times more than the area of contact between the compression tool and the sample (ASAE, 2000). In this compression test, to increase the area of contact between the egg sample and the supporting plate, the hardboiled shelled eggs were cut in half lengthwise (Fig. 1, right). For each half egg the albumen was removed from the top (1×1 cm^2 removed) and two compressions were applied on each half yolk. The same parameters described above were extracted from the force-deformation curves.

Data analysis

Statistica® (version 6, StatSoft, Inc., Tulsa, Oklahoma, USA) software was used for data analysis. The effect of inclusion of CLA and other FA sources in diets was analysed by ANOVA analysis and by t-test for independent samples.

Variability due to the configuration of the compression tests was determined. Therefore, metrological studies of the parameters involved in mechanical measurements were conducted. In the flat-plate compression test applied to full egg yolks, with one measurement per yolk, reproducibility was computed by averaging the standard deviations of the intra-treatments data. These values can be assigned to instrumental error plus the variability due to individual eggs in the sample (excluding treatment effect). The cylindrical rod compression test was applied to half egg yolks with two measurements per half yolk. Means, standard deviations (SDs) and coefficients of variation (CVs) were computed per half egg. Repeatability was calculated by averaging the SDs of the intra-eggs data. In this test repeatability can be assigned to instrumental error. Other levels of variation in the cylindrical rod compression test were computed calculating the SD of means and the CV among eggs, within each treatment. Reproducibility was computed as explained above. Metrological studies were calculated on maximum force values.

Stepwise multilinear regression was used to show the relationship between egg yolk firmness and diet FA content.

Results

Flat-plate compression test

In Experiment 1, panellists rejected eggs from hens fed diets supplemented with 3 or 5 g kg^{-1} of CLA and 14 g kg^{-1} of FO due to excessive hardness and rubbery texture of the boiled yolks. Thus these eggs were not «acceptable» for consumers and were not studied in mechanical compression tests. Therefore, only the treatments with 1 g kg^{-1} of CLA without FO and the three levels of CLA with 20 g kg^{-1} of FO were retained for instrumental firmness studies (treatments T1.1, T1.3, T1.6 and T1.9 in Table 1).

One-way ANOVA was carried out to determine whether 'dietary treatment' had a significant influence on the compression values of egg yolks. There were significant differences ($p < 0.05$) in all the mechanical parameters studied (Fig. 2). The addition of 3 and 5 g kg^{-1} of CLA in diets (T1.6 and T1.9) significantly increased values of the compression parameters. This effect was not reduced by adding 20 g kg^{-1} of FO. There were no differences between control eggs, and eggs from hens fed diets supplemented with 1 g kg^{-1} of CLA (T1.1 and T1.3). There was high variability in treatment T1.3 due to early breakage of one sample egg during the compression test.

With regard to sensory evaluation (Table 2), scores for acceptability, aroma, taste, aftertaste and flavour decreased with increased level of CLA. The intensity of off-flavours increased in eggs with FO and there were significant differences among treatments. High values for all mechanical parameters were obtained from yolks when increased levels of dietary CLA were added to the diet. Inclusion of FO (treatments T1.6 and T1.9) did not significantly decrease yolk hardness (Fig. 2).

In Experiment 2 ANOVA showed there were small, significant differences among diets even though lower levels of CLA (≤ 2 g kg^{-1}) were used in Experiment 2 compared to Experiment 1 (≤ 5 g kg^{-1}).

Univariate factorial ANOVAs were carried out to evaluate the interaction effects of supplementing diets with CLA and HOSO. Maximum force and absorbed energy were the most sensitive mechanical parameters to variation in CLA and/or HOSO level (Table 3).

There was no significant difference in yolk firmness of eggs from diets supplemented with 1 g kg^{-1} of CLA compared to those from diets without CLA. Dietary

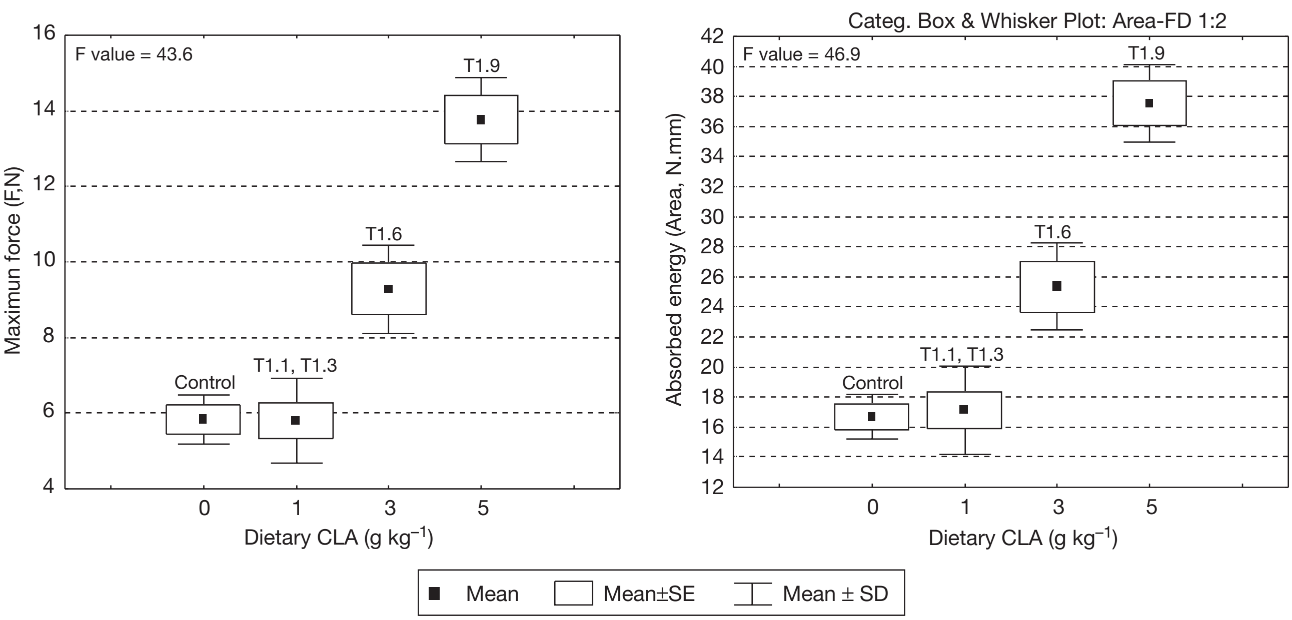

Figure 2. Mean, standard error and standard deviation of maximum force (left) and absorbed energy (right) in the flat-plate compression test, for different levels of dietary CLA in Experiment 1 (N = 6).

Table 2. Sensory evaluation results of samples in Experiment 1. T-test values for independent samples

Treatment	Control	T1.1	T1.3	T1.6	T1.9	SEM[1]
Aroma	9.25[a]	5.83[b]	6.50[b]	5.50[b]	3.52[b]	0.67
Taste	8.75[a]	3.08[b]	2.75[b]	1.33[c]	0.48[c]	0.48
Aftertaste	8.33[a]	3.33[b]	3.17[b]	1.42[c]	0.60[c]	0.58
Flavour	8.67[a]	3.58[b]	3.42[b]	1.58[c]	0.72[c]	0.53
Off-flavour	1.08[a]	4.92[b]	6.00[bc]	7.25[cd]	8.83[d]	0.74
Acceptability scores	9.17[a]	3.67[c]	3.08[c]	0.92[d]	0.04[d]	0.48

[a,b,c,d] Means with different superscripts within the same row are significantly different ($p < 0.05$). [1] Standard error of means ($n = 12$).

supplementation of the basal diet with 2 g kg^{-1} of CLA and 10 or 20 g kg^{-1} of HOSO gave the hardest egg yolks. However, yolks from hens fed diets supplemented with 2 g kg^{-1} of CLA and 30 g kg^{-1} of HOSO reached maximum forces and absorbed energies similar to the other treatments, *i.e.* with 0 or 1 g kg^{-1} of CLA (Table 4). Therefore, HOSO levels were set at 30 and 35 g kg^{-1} in Experiments 3 and 4.

In this experiment all eggs irrespective of treatment were acceptable to panellists. Nevertheless, the sensory evaluation tests showed that the organoleptic quality of the egg yolks were impaired when the CLA level was increased in the diets. Yolks from hens fed diets supplemented with 2 g kg^{-1} of CLA (T2.7, T2.8, T2.9) had the lowest acceptability scores: from 8.25 for diets without CLA and 10 g kg^{-1} of HOSO (T2.1) to 5.25 for diets with 2 g kg^{-1} and 20 g kg^{-1} of HOSO (T2.8). The addition of 30 g kg^{-1} of HOSO to diets supplemented with 2 g kg^{-1} of CLA (T2.9) improved their sensory acceptance to 6.75. Pooled standard error of means ($n = 12$) for this trait was 0.44.

The combined addition of HOSO (30 g kg^{-1}) and CLA (up to 2 g kg^{-1}) gave eggs with an appreciable level of CLA and acceptable sensory quality.

In Experiment 3, there was no significant difference among diets in compression parameters. When the control values from Experiment 1 were compared to diets in Experiment 3 the only significant difference was for treatment T3.3 (3 g kg^{-1} CLA, 17 g kg^{-1} FO, 30 g kg^{-1} HOSO). However the sensory evaluation test showed that diets supplemented with 17 g kg^{-1} of FO were not acceptable, regardless of the level of HOSO.

Table 3. Factorial ANOVA for selected mechanical parameters in flat-plate compression test in Experiment 2. Factors are dietary CLA (0 and 2 g kg^{-1}) and HOSO (10, 20 and 30 g kg^{-1}) levels

	Maximum force (N)			Absorbed energy (N · mm)		
	CLA	HOSO	CLA * HOSO	CLA	HOSO	CLA * HOSO
Sum of squares	15.6	12.2	15.3	134.9	127.7	144.9
Degrees of freedom	2	2	4	2	2	4
Mean square	7.8	6.1	3.8	67.5	63.9	36.2
F-value	5.8	4.6	2.8	9.6	9.1	5.2
p	**	**	*	*	*	*

Marked effects are significant with: * $p < 0.05$ or ** $p < 0.01$.

Table 4. T-test for independent samples. Effect of dietary treatment on flat-plate compression test. Experiment 2

Treatment	T2.1	T2.2	T2.3	T2.4	T2.5	T2.6	T2.7	T2.8	T2.9
Maximum force (F, N)	3.9[ab]	3.31[a]	9[ab]	3.76[a]	3.93[ab]	2.99[a]	5.17[bc]	5.89[c]	3.21[a]
No permanent deformation (NPERM, mm)	2.9[ade]	3.04[abde]	3.15[abde]	3.10[abcde]	3.26[abcde]	3.27[bcd]	3.42[c]	3.04[de]	2.84[e]
Absorbed energy (AREA, N · mm)	12.9[ad]	12.78[ad]	13.56[abd]	13.14[ad]	14.13[ab]	11.04[ad]	19.9[bc]	20.4[c]	11.51[d]
Slope (F/D, N mm^{-1})	0.78[abcde]	0.75[abe]	0.86[acd]	0.78[abcde]	0.88[acde]	0.67[be]	0.91[cd]	1.09[d]	0.68[e]

[a,b,c,d,e] Means with different superscripts within the same row are significantly different ($p < 0.05$).

In Experiment 4 egg yolks with CLA, HOSO and three different Ω-3 FA sources had compression parameter values similar to those of Control and Commercial-A, eggs. Therefore these eggs, based on their texture, were acceptable for consumption. An increased level of dietary Ω-3 FA reduced all sensory traits evaluated by panellists. However, neither dietary source nor the interaction with Ω-3 FA affected any measured traits. Panellist sensory evaluation of the control diet was better than for the commercial diet with Ω-3 (Commercial-A). However, it was not reduced by the inclusion of 2.5 g kg^{-1} of CLA with 30 g kg^{-1} of HOSO in the FO, FO-EPA and AO diets. No significant differences in sensory evaluation values were found because of Ω-3 FA source.

In Experiment 5, eggs from experimental diets had lower maximum force values than control and commercial eggs enriched with Ω-3 (Commercial-B). There were no significant differences among treatments ($p > 0.05$).

Analysis of all data from the flat-plate compression test for Experiments 1 to 5 showed that the variability and differences in maximum forces between control treatments were similar to the variability and differences in diets supplemented with CLA, HOSO and Ω-3 FA sources. Only yolks from layers fed diets supplemented with 3 and 5 g kg^{-1} of CLA, without HOSO (treatments T1.6 and T1.9), had compression parameter values which were different, and higher, than the other treatments (Figs. 2 and 3). Categorized box and whisker plots for elastic deformation, absorbed energy and slope showed patterns parallel to the maximum force plot and had similar F-values for ANOVA (data no shown).

Figure 4 shows a 3-dimensional surface plot of means per treatment of absorbed energy, dietary CLA and dietary HOSO: the decrease in yolk firmness, in response to diets with the same level of CLA, with increased levels of HOSO can be seen.

Predictive models were developed to estimate mechanical parameters using stepwise linear regression. Best results were obtained using a forward stepwise multilinear regression, where the maximum force in compression test (means per treatment of data from flat-plate compression test were used; ranged from 3.04 to 13.75 N) was predicted in terms of level of supple-

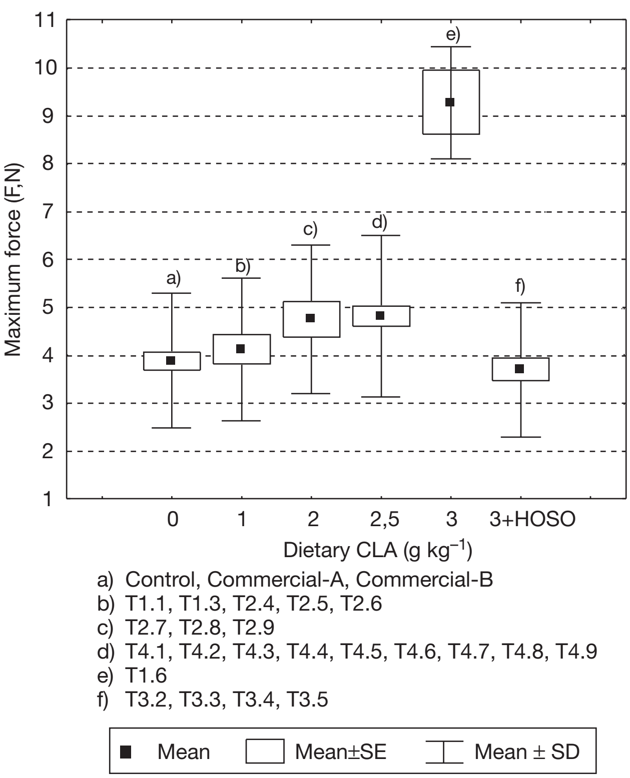

Figure 3. The mean and standard deviation of maximum force in the flat-plate compression test, categorized for dietary CLA level from pooled data of all experiments (Experiments 1-5, except for diet T1.9).

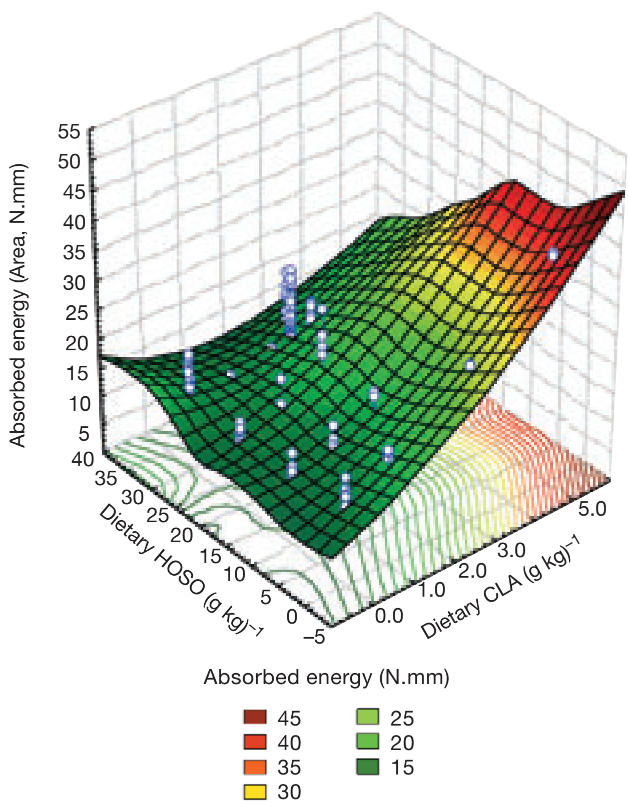

Figure 4. The 3D surface plot of absorbed energy in the flat-plate compression test, dietary HOSO and dietary CLA for Experiments 1 to 5.

Table 5. Means of parameters from the cylindrical rod compression test, for treatments in Experiment 5 (N = 20)

	Control	T5.1	T5.2	Cmmercial-B
Maximum force (F, N)	1.21^{a}	1.33^{bc}	1.30^{b}	1.38^{bc}
No permanent deformation (NPERM, mm)	1.72^{a}	1.67^{b}	1.66^{b}	1.61^{c}
Absorbed energy (AREA, N · mm)	1.79^{a}	1.96^{bc}	1.91^{b}	2.02^{bc}
Slope (F/D, N mm^{-1})	0.40^{a}	0.43^{bc}	0.42^{b}	0.45^{bc}

^{a,b,c} Means with different superscripts within the same row are significantly different ($p < 0.05$).

mentation of CLA (from 0 to 5 g kg^{-1}) together with level of supplementation of HOSO (from 0 to 35 g kg^{-1}) to the basal diet. The FO or AO levels in the diets were not included as they were not significant. The multiple correlation coefficient R of the model was 0.8, and the adjusted coefficient of determination (R^2) was 0.62.

Cylindrical rod compression test

Results from cylindrical rod compression test (Experiment 5) are shown in Table 5. Egg yolks from hens fed the control diet had the lowest mean values in the cylindrical rod compression test parameters compared with the other treatments in Experiment 5. In this experiment control eggs had the highest relative mean values in the flat-plate compression test. Thus, more measurements are required to determine if the two tests are equivalent.

Metrological study

Standard deviations of means and CVs per treatments were computed for the maximum force of both compression tests. Averages of SDs and CVs per treatment are shown in Table 6.

The cylindrical rod compression test had the best reproducibility as the intra treatment CV decreased to 13.63%. The CVs intra egg and intra half egg were 9.81% and 5.44 % respectively due to variation in the contact surface between the sample and rod and microstructure variations in the eggs could explain the variation intra half egg. The increase in CV of intra half eggs means compared with the CV intra eggs could be partially due to the contact between the sample egg and the supporting plate.

Discussion

Previous work, focussed on egg chemical composition has shown that feeding CLA to hens alters the texture of egg yolks in hard-boiled eggs. This is important because when CLA is incorporated into commercial eggs to provide a health benefit, the sensory and quality properties of the food should not be compromised. Following the proposal for further research of Watkins *et al.* (2003), this work proposes an objective procedure to determine the optimal enrichment level with regard to its impact on egg texture quality.

Table 6. Metrology features: standard deviations (SD) averages and coefficients of variation (CV) averages of the data per treatment and per egg for maximum force

		SD (N)	CV (%)
Flat-plate compression (all experiments)	Reproducibility (n = 6)	1.22	26.88
Cylindrical rod compression test (Experiment 5)	Reproducibility (n = 20)	0.18	13.63
	Repeatability:		
	— Intra egg (n = 2)	0.13	9.81
	— Intra half egg (n = 2)	0.069	5.44

	Segregation ability (n = 80)	
	Range (N)	Range/SD
Flat-plate compression (Experiment 5)	4.27	5.3
Cylindrical rod compression test (Experiment 5)	1.5	6.8

In this work the compression test on whole boiled yolks showed that eggs from hens fed diets supplemented with 3 or 5 g kg^{-1} of CLA had increased maximum force values from 4.2 N (average for commercial eggs) to 9.3 and 13.8 N respectively. This effect was reduced by addition of HOSO. Diets with 3 or 2.5 g kg^{-1} of CLA and 30 or 35 g kg^{-1} of HOSO had maximum force values of 3.7 and 6.5 N, which is similar to the firmness of commercial eggs. Aydin *et al.* (2001) reported that addition of olive oil prevented CLA-related changes in the pH of egg yolk and albumen and in yolk colour; colour defects in yolk and albumen were related to a rubbery, pasty, viscous texture, but no experimental data of texture parameters was included in this work.

Dietary Ω-3 FA supplementation did not modify mechanical characteristics of egg yolks, but, according to Cherian *et al.* (2002), it impaired other sensory attributes not related to texture such as aroma, taste and the intensity of off-flavour. As Rose *et al.* (2006) reported an unpleasant fishy taste is responsible for low acceptability of marine oil supplements.

The reproducibility of the instrumental procedure was calculated as the CV of the means of maximum force per treatment (average of compression test on full yolks: 26.88%). This value could be considered as instrumental error plus variation due to the presentation of the sample in the test. To try and minimize this variation, the second compression test was carried out increasing the contact area between the sample and the supporting plate. The CV in this compression test fell to 13.63%. Therefore, instrumental error was significantly reduced. This suggests that the cylindrical rod compression test is more precise than the flat-plate compression test of whole yolks for sensing differences in hard-boiled egg yolks related to supplementing basal layer diets with CLA, HOSO and FO or AO.

Future research should be focus on the development of a sensory protocol, which should include descriptors that adequately characterise texture characteristics of hard boiled egg yolks. Simultaneous instrumental measurements and sensory evaluations focused on texture attributes of yolks could improve understanding of the relationship between mechanical parameters and consumer acceptability.

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References

AHN D.U., SELL J.L., JO C., CHAMRUSPOLLERT M., JEFFREY M., 1999. Effect of dietary conjugated linoleic acid on the quality characteristics of chicken eggs during refrigerated storage. Poultry Sci 78, 922-928.

ÁLVAREZ C., CACHALDORA P., MÉNDEZ J., GARCÍA-REBOLLAR P., 2004. Effects of conjugated linoleic acid addition on its deposition in eggs of laying hens, fed with no other fat source. Span J Agric Res 2(2), 203-209.

ANONYMOUS, 1975. Glossary of terms relating to the sensory analysis of food. British Standard 5098. British Standards Institution, London.

ASAE, 2000. STANDARD ASAE S368.4 DEC00. Compression test of food materials of convex shape. American Society of Agricultural Engineers, St Joseph, Michigan, USA.

AYDIN R., PARIZA M.W., COOK M.E., 2001. Olive oil prevents the adverse effects of dietary conjugated linoleic acid on chick hatchability and egg quality. Am Soc Nutr Sci 131, 800-806.

BARREIRO P., ORTIZ C., RUIZ-ALTISENT M., DE SMEDT V., SCHOTTE S., BHANJI Z., WAKELING I., BEYTS P.K., 1998. Comparison between sensorial and instrumental measurements for mealiness assessment in apples. A collaborative study. J Texture Stud 29(5), 509-525.

BOVET P., FAEH D., MADELEINE G., VISWANATHAN B., PACCAUD F., 2007. Decrease in blood triglycerides associated with the consumption of eggs of hens fed with food supplemented with fish oil. Nutr Metab Cardiovas Dis 17(4), 280-287.

CACHALDORA P., DE BLAS J.C., GARCÍA-REBOLLAR P., ÁLVAREZ C., MÉNDEZ J., 2005. Short communication. Effects of type and level of supplementation with dietary n-3 fatty acids on yolk fat composition and n-3 fatty acid retention in hen eggs. Span J Agric Res 3(2), 209-212.

CHERIAN G., GOEGER M.P., DU A., 2002. Dietary conjugated linoleic acid with fish oil alters yolk n-3 and trans fatty acid content and volatile compounds in raw, cooked, and irradiated eggs. Poul Sci 81, 1751-1577.

DHIMAN T.R., 2000. Conjugated linoleic acid: A food for cancer prevention. Feedstuffs 72, 24-32.

DIEZMA-IGLESIAS B., VALERO C., GARCÍA-RAMOS F.J., RUIZ-ALTISENT M., 2006. Monitoring of firmness evolution of peaches during storage by combining acoustic and impact methods. J Food Eng 77, 926-935.

DU M., AHN D.U., SELL J.L., 1999. Effect of dietary conjugated linoleic acid on the composition of egg yolk lipids. Poultry Sci 78, 1639-1645.

HUR S.J., PARK G.B., JOO S.T., 2007. Biological activities of conjugated linoleic acid (CLA) and effects of CLA on animal products. Lives Sci 110, 221-229.

JONES S., MA D.W.L., ROBINSON F.E., FIELD C.J., CLANDININ M.T., 2000. Isomers of conjugated linoleic acid (CLA) are incorporated into egg yolk lipids by CLA-fed laying hens. J Nutr 130, 2002-2005.

PARIZA M.W., PARK Y., COOK M.E., 2001. The biologically active isomers of conjugated linoleic acid. Prog Lipid Res 40, 283-298.

RAES K., HUYGHEBAERT S., DE SMET S., NOLLET L., ARNOUTS S., DEMEYER D., 2002. The deposition of conjugated linoleic acid in eggs of laying hens fed diets varying in fat level and fatty acid profile. J Nutr 132, 182-189.
ROSE E.L., BRUCE J.H., 2006. Effects of a liquid egg product containing fish oil on selected cardiovascular disease risk factors: a randomized crossover trial. Food Res Int 39(8), 910-916.
SZYMCZYK B., PISULEWSKI P.M., 2003. Effects of dietary conjugated linoleic acid on fatty acid composition and cholesterol content of hen egg yolks. Br J Nutr 90, 93-99.
WATKINS B.A., FENG S., STROM A.K., DEVITT A.A., YU L., LI Y., 2003. Conjugated linoleic acids alter the fatty and compositions and physical properties of egg yolk and albumen. J Agr Food Chem 51, 6870-6876.
YANG L., HUANG Y., JAMES A.E., LAM L.W., CHEN Z.Y., 2002. Differential incorporation of conjugated linoleic acid isomers into egg yolk lipids. J Agr Food Chem 59, 4941-4946.