SUPPLEMENTARY MATERIAL

Preservation of vitamins content in Cuccìa using an innovative method of processing

R. Bognanni[a], G. Gallo[b], V. Di Stefano[c] and M.G. Melilli[a*]
* Corresponding author.

[a] Institute for Agricultural and Forest Systems in the Mediterranean, National Council of Research, Catania, Italy. mariagrazia.melilli@cnr.it; [b] Assessorato regionale dell'agricoltura, dello sviluppo rurale e della pesca mediterranea Regione Sicilia, Palermo, Italy; [c] Department of Biological, Chemical, and Pharmaceutical Science and Technology (STEBICEF), University of Palermo, Palermo, Italy.

Cuccìa is a traditional Sicilian food prepared by boiling whole durum wheat kernels, in water, for many hours. This process destroys the vitamins E and B contents of crude kernels. It was rated a method to prepare the Cuccìa, preserving the vitamin content. Four varieties of durum wheat were processed comparing the traditional cooking method (TR-boiling for 5/6 hours), and an innovative one (IN-grains scarification, germination, and cooking at 50 °C for 2 hours). On soups obtained the content of biotin, niacin and α-amylase activity were determined. ANOVA showed the cooking method influences biotin and niacin content having values from 0.56 and 0.72 ng ml-1 (raw grain) and values close to 0 (TR), while only a 10% decrease (IN) respectively for both vitamins. On the contrary, α-amylase activity was reduced with IN method. The IN method combined with ancient grains, produces the soup with a good vitamin B amount.

Experimental

Plant material, location of trials and crop managements

This study was carried out in 2007-2008 at Caltagirone in the fields of the "Stazione Consorziale Sperimentale di Granicoltura per la Sicilia" (Lat. 37°14′ Long. 14°30′, 350 m.a.s.l., sandy clay soil) (Italy).

The kernels of four varieties of durum wheat (two commercial varieties, *Simeto* and *Arcangelo*, and two traditional varieties *Timilia* and *Russello*), were tested. Simeto, registered in 1988, was obtained by Capeiti x Valnova; Arcangelo, registered in 1983, has Creso/Appulo pedigree. Russello was released during the 1910s and it is a selection from the landraces "Russie"; Timilia is a landrace (Venora & Blangiforti 2017), often sown in the spring in Sicily. Conventional nitrogen/non-irrigated (N90), representing the standard agronomic condition applied by farmers in South Italy, was applied. Plants were harvested after the physiological maturity on June. Kernels was obtained by a thresher.

Cooking methods comparison

Soaking In order to reduce the time/temperature of cooking process and preserve the vitamins an hydration test, simulating homemade traditional Cuccìa, was performed. In particular twenty caryopsides, for each accessions were transferred to a 200 mL beaker, and aqueous solutions of 8% w/ v NaHCO$_3$ was added up to a total volume of 150 mL. Sodium hydrogen carbonate was purchased from Sigma Chemical Co. (ST. Louis, MO). The beakers were left in a growth chamber at a 25 °C constant temperature, and every hour up to the 8th hour, then at 24 h after initial imbibition, the caryopsides were drained, removing the free water by using a blotting paper, then they were weighted and replaced into the flask. After soaking, the traditional cooking, used as control, and the new cooking process of Cuccìa were performed. From the hydration test, it was establish the time for all the accessions for the hydration process, as pre-treatment before cooking kernels to obtain the Cuccìa. For all the accessions, the weight of the caryopsides remain constant after 7 hours of soaking.

Traditional Cooking. After 7 hours of imbibition, the soaked drained caryopsides (corresponding to 100 g of dried seeds) were cooked to the boiling point with 1 L of

distilled water (1:10 dried seed: water ratio, w/v) on a low flame until reaching the cooking point (about 7 hours).

Innovative process. The soaked drained caryopsides, were subjected to the following steps: I) scarification process, by a laboratory stone mill (Partisani Maxi) to produce micro-lesions on the kernels. The two mill stones were set at the maximum distance allowed by the equipment; II) rest of the scarified caryopsides for 20 hours at 18 °C and 95% relative humidity in a Termostabil C5 propagator; III) Cooking under vacuum (Rotavapor Büchi 011). The cooking procedure was divided into 3 stages: a) Cooking under vacuum at 50 °C for 30 minutes; b) Resting under vacuum for 1 hour; c) Cooking under vacuum at 50 °C until optimal cooking point was reached for each accession (determined by panel test at different intervals of cooking). The vacuum was obtained using a BOC Edwards XBS10C pump.

Optimal cooking time. The optimal cooking time (OCT) was evaluated according to the AACC-approved method 66-50, as described by Padalino et al. (2017).

Chemical analyses

On crude kernels and processed kernel (*Cuccìa*) the chemical characterization was performed for the vitamins B7 and B3 and α-amylase activity. Enzyme immunoassay for the quantitative determination of Biotin (Vitamin B7) was obtained from Tecna, while for Niacin (Vitamin B3) was obtained by R-Biopharm.

A Tecan Sunrise plate spectrophotometer (Switzerland), running Magellan 3.2 software has been used for the quantification. Determination of α-amylase activity was performed using the Falling Number 1400 system (ISO Standard n. 3093 – 1974 – ICC Standard n. 107/1 – 1995 e method AACC 56-81 B 1992).

Sensory analysis

Sensory analysis was performed by ten experienced panelists, that evaluated at regular intervals of 3 minutes the optimal cooked point for each varieties. The members of the panel, aged between 28 and 55, were chosen from the staff of the Experimental Station for Wheat Crop. The tasters were required to express their judgement and assess each described characteristic (colour, crunchiness, stickiness, chewiness, odour, taste, aftertaste, global evaluation). Each characteristics was evaluated by means of a 1 to 8

structured scale where 1: dislike very much; - 8: like extremely (Patsias et al. 2006). According to the scale, a score of 5 was taken as the lowest limit of acceptability. Using statistics software (Big Sensory Test), the panel leader elaborated the assessments of the various tasters, rendering objective the sensorial analysis performed on *Cuccìa*.

Data analysis

Data of technological and chemical characterization of the crude and cooked kernels (Cuccia) were subjected to the Barlett's test for homogeneity of variance and then analysed using the analysis of variance (ANOVA). The means were statistically separated on the basis of Student-Newmann-Kewls test when the 'F' test of ANOVA for treatment was significant at least at 0.05 probability level. Significance was accepted at $P< 0.05$ level. The percentage data were arcsin-transformed ($\sqrt{\%}$) before analysis (Snedecor, G.W. and Cochran 1989). The data are reported without transformation.

References

AACC, 2000. American Association of Cereal Chemistry-AACC. Approved methods of analysis of the AACC (10th ed.)St Paul (2000)

Padalino L, Costa C, Conte A, Melilli MG, Sillitti C, Bognanni R, Raccuia SA, Del Nobile MA. 2017. The quality of functional whole-meal durum wheat spaghetti as affected by inulin polymerization degree. Carbohydr Polym. 173.

Patsias A, Chouliara I, Badeka A, Savvaidis IN, Kontominas MG. 2006. Shelf-life of a chilled precooked chicken product stored in air and under modified atmospheres: Microbiological, chemical, sensory attributes. Food Microbiol. 23:423–429.

Snedecor, G.W. and Cochran WG. 1989. Statistical methods. 7th ed. Ames: The Iowa State University Press, Usa.

Venora G, Blangiforti S. 2017. I grani antichi siciliani Manuale tecnico per il riconoscimento delle varietà locali dei frumenti siciliani. Palermo: Le Fate.

Table S1: Analyses of variance for hydration process, Vitamin B7, Vitamin B3 and α-amylase activity and partitioning of the treatment mean squares (MS expressed in absolute value – AV - and percent of total) into main effects and interactions.

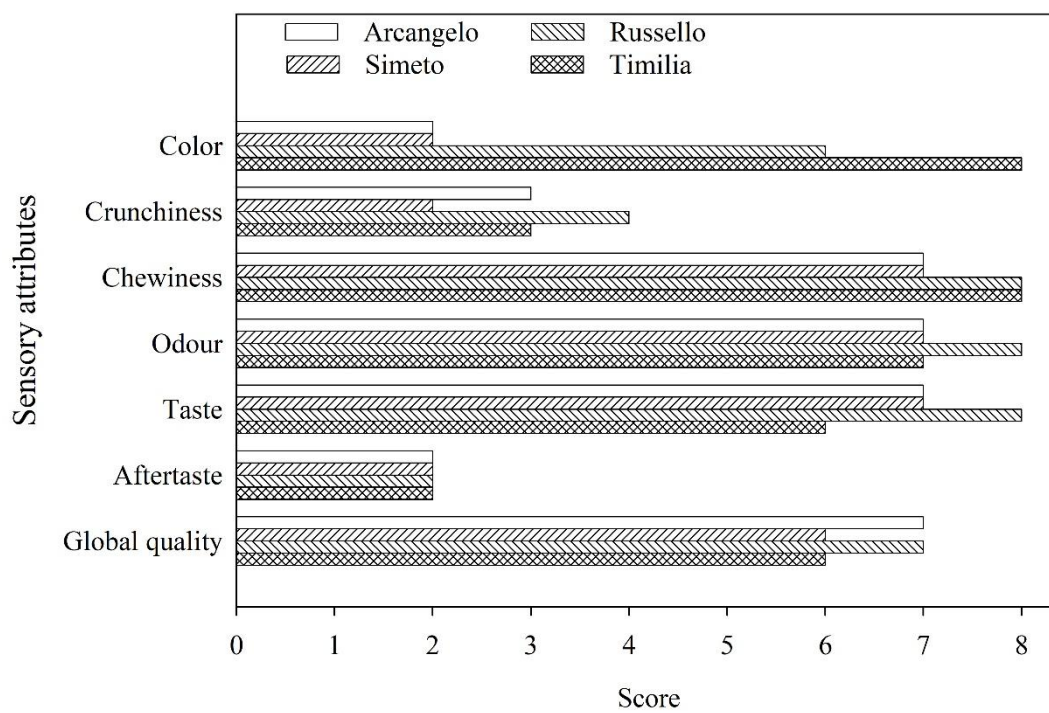| Traits	Accession (A)		Time of soaking (T)		A x T	
	AV	% of total	AV	% of total	AV	% of total
Vitamin B7	0.0073	8.1	0.809	89.4	0.023	2.5
Vitamin B3	0.007	0.5	1.381	99.3	0.003	0.2
α-amylase activity	54240	32.3	100184	59.6	13643	8.1

Figure S1: Descriptors of Cuccìa prepared with the studied accessions, on average of the two cooking processes.