

ISSN 1128-7969

Numero 1

Gennaio - Aprile 2023

Anno 52

pubblicazione quadrimestrale

Sped. in abb. post. 70%

Filiali di Roma

FOSAN 

Ente di Ricerca per lo Studio  
degli Alimenti e della Nutrizione

LA RIVISTA DI  
**SCIENZA** DELL' **ALIMENTAZIONE**  
*Journal of Food Science and Nutrition*



I fiori edibili tra tradizione e innovazione: la valorizzazione di prodotto attraverso lo studio della componente aromatica e del contenuto in flavonoidi

Statistical Evaluation and Validation of a method for the Determination of Fragrance in Italian rice by Panel Test

Panoramica della situazione PFAS nei corpi idrici e organismi acquatici del Mediterraneo



# Statistical Evaluation and Validation of a method for the Determination of Fragrance in Italian rice by Panel Test

Valutazione Statistica e Validazione di una metodologia per la Determinazione dell' Aroma nel Riso Italiano attraverso l'Analisi Sensoriale (Panel Test)

Cinzia Simonelli<sup>1</sup>, Mauro Cormegna<sup>1</sup>

(<sup>1</sup>Ente Nazionale Risi (ENR); Laboratorio di Chimica Merceologia e Biologia Molecolare (LCM) – Rice Research Centre, Strada per Ceretto 4, 27030 Castello D' Agogna (PV), Italy)  
Doi 10.4458/5826-02

## Abstract

Aromatic rice constitute a small but special group of varieties which are considered in some part of the world as best in quality. This rice was long been popular in the orient of Asia, and now becoming more popular in middle est, Europe and United States.

Aromatic rices are characterized by releasing, after cooking, an aroma (fragrance) similar to the popcorn, as a result of the presence of a mixture of molecules including, in particular, 2-acetyl pyrroline. The presence of fragrance is one of the characters to evaluate when new rice varieties are registered in the Italian Register of Varieties (DLgs n.131/2017). The determination is carried out by the Chemical Laboratory of Ente Nazionale Risi with an internal accredited method.

It consists of performing sensorial evaluation through a panel test and defining the absence or presence (weak or strong) of the aroma on the cooked rice samples, prepared using a standardized method. The panel is selected, trained, monitored and maintained according to the procedures defined in the ISO 8586 and ISO 11132 standards. Through the application of this method it is possible to characterize rice varieties of interest to the breeder, for the seed producers' companies, but also for the big organized distribution that carries out the quality control of its products.

In a regulatory framework where there is no standard method for determining the aroma of rice, the work presented here is intended as a starting point for standardization of this specific determination. In this context the use of variance is shown in the evaluation of the selected assessors and their performance.

**Key Words:** Aromatic Rice, Scented Rice, Fragrance, Aroma, Validation, Discrimination Test

For a rice eater, all rice probably has a slight aroma. This is especially true for the Japanese who are perhaps extraordinarily sensitive to agreeable or disagreeable odour and flavor of their rice. Nonetheless it should be realized that this odour is suitable and hardly identifiable by those who are not experts of rice. There is, however, a special class of rice which has a distinct, clearly identifiable, pleasant fragrance. Such rices are known with the name of aromatic rice or scented rice. Its distinct fragrance is discernible to all – rice-eaters or not – both in uncooked and cooked state. And as the scent is very pleasant, it is no wonder that this rice is highly prized throughout the rice countries. It is difficult to know about the origin of this aromatic rice or of its fragrance, but there are references to this exquisitely good-smelling rice being used in the royal kitchens of yore and in other stories. These aromatic rices are not confined to any particular region or country but are dispersed throughout the rice-growing countries (Bhattacharya, 2011).

In aromatic varieties pleasant aroma is not only associated with cooked rice. Quite often these varieties emit aroma in the field at the time of flowering (Singh et al., 2000).

Aromatic rice has a flavor and aroma similar to that of roasted nuts or popcorn. The scent of aromatic rice is a highly heritable trait and reportedly is under the control of from one to four genes, depending on the population studied (Champagne, 2004).

The volatile compounds of cooked scented rice were analyzed and more than 100 components were found. Traditional rice had higher amounts of 4-vinylphenol, 1-hexanol and 1-hexanal but lower amounts of indole as compared to scented rice. Scented rice had an unidentified compound and  $\alpha$ -pyrrolidone, which was not present in traditional rice. However, none of the individual compound identified had the characteristic odour of cooked scented rice (Singh et al., 2000). A total of 14 compounds including in particular 2-acetyl-1-pyrroline (2AP) were detected specifically in scented rice cultivars (Hin-

ge et al., 2016). Many researchers were involving in identifying the compound responsible for the pleasant aroma in aromatic rice in the 20th century. However, due to its unstable nature, 2AP was discovered very late, in 1982. Buttery and co-workers found 2AP to be the principal compound imparting the pleasant aroma to basmati and other scented rice varieties. Since then, 2AP has been identified in all fragrant rice (*Oryza sativa* L.) varieties and a wide range of plants, animals, fungi, bacteria and various food products (Wakte et al., 2016).

### Different type of rice and their classification

Rice can be purchased cooked or uncooked, canned or dehydrated and also in frozen forms. Few foods are packaged so extensively and are offered in so many combinations as rice. To meet the many special requirements of packaged foods, rice undergoes varying degrees of processing, including regular milled, parboiled, precooked, and brown (Kushwaha, 2016).

Brown rice retains the bran layer (containing many vitamins and minerals as well as fiber), as this has not been polished off to produce white rice. In nature exist varieties of rice with naturally pigmented caryopsis (black, red, purple) that actually received increased attention because of their antioxidant properties (Kushwaha, 2016, Petroni et al., 2017).

### Aromatic varieties in the world

Scented or aromatic rice is preferred in some areas of Asia and draws a premium price in certain special market. The Middle East consumers prefer rices with strong aroma. They feel that rice without a distinctive aroma is like food without salt. For consumers in Europe, a trace of aroma is an objectionable trait, because for them any scent signals spoilage and contamination. Most of the high-quality preferred varieties in major rice growing countries are aromatic. Examples are the Basmati rices of India and Pa-

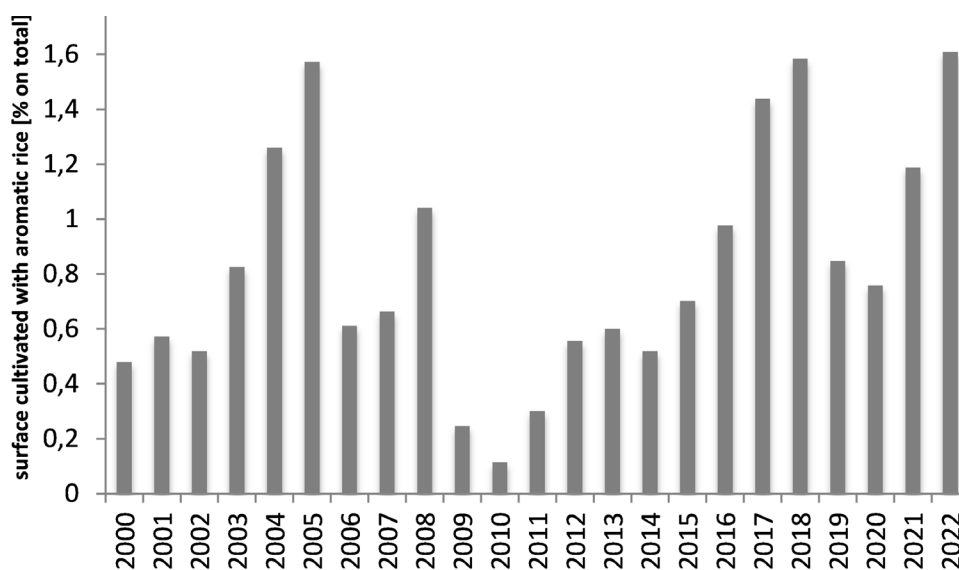


Fig. 1 - Percentage of cultivation of aromatic rice on the total Italian surface cultivated with rice

kistan, Dulhabhog of Bangladesh, Khao Dawn Mali and Leuang Hawn of Thailand, Milfor of the Philippines, Rojolele of Indonesia, Sadri varieties of Iran, Barah of Afghanistan and Della of the United States. These are characterized by long slender grains, intermediate gelatinization temperature, high elongation ratio and strong aroma (Singh et al., 2000).

### The market of aromatic rice in Italy

In Italy there is a market for aromatic rice too. The aromatic rice varieties consumed as milled are: Apollo, Armida CL, Asia, Brezza, Elettra, Febo, Fragrance, Gange, Gelso, Giano, Giglio, Gioia, Iarim, classified as long B; Profumato Gwang, RG300A, classified as long A according to the Italian Legislative Decree n. 131 (D.Lgs. 131, Registro Varietale AA 2022/2023). Then we have pigmented black rice (dehulled rice): Ebanomax, Eclisse, Gioiello, Il Moro, Nero Beppino, Nerone, Nerone 2, Nerone Gold, Penelope, Venere, Violet Nori (characterized by medium ker-

nel); Artemide, Jolly nero, Nairobi one (long B); Alfiero, Jemma, Verelè (long A). As pigmented red rice (dehulled rice) we can classify: Orange Nori (medium), Solitario (long A), Avana Gold, Ermes, Fiamma, Rosso Gwang (long B), Aniride (round kernel).

In Italy, the cultivation of aromatic rice varieties was introduced in 2000 and the first cultivated variety was Gange. To date, the cultivated area with the aromatic rice varieties listed above, amounted to be below 2% of the national total, with the fluctuations shown in Figure 1.

It is interesting to evaluate how the market of aromatic rice varieties has changed over time (Figure 2). Only in 2005 began the cultivation of the first aromatic rice variety with colored pericarp (black), Venere, which increased production over the years, also supported by other varieties that have been selected. Only in 2013 the cultivation of the first red varieties in Italy began, with Ermes. While the surface cultivated with milled aromatic rice is fluctuating and remains, in recent years, below 700 hectares, there

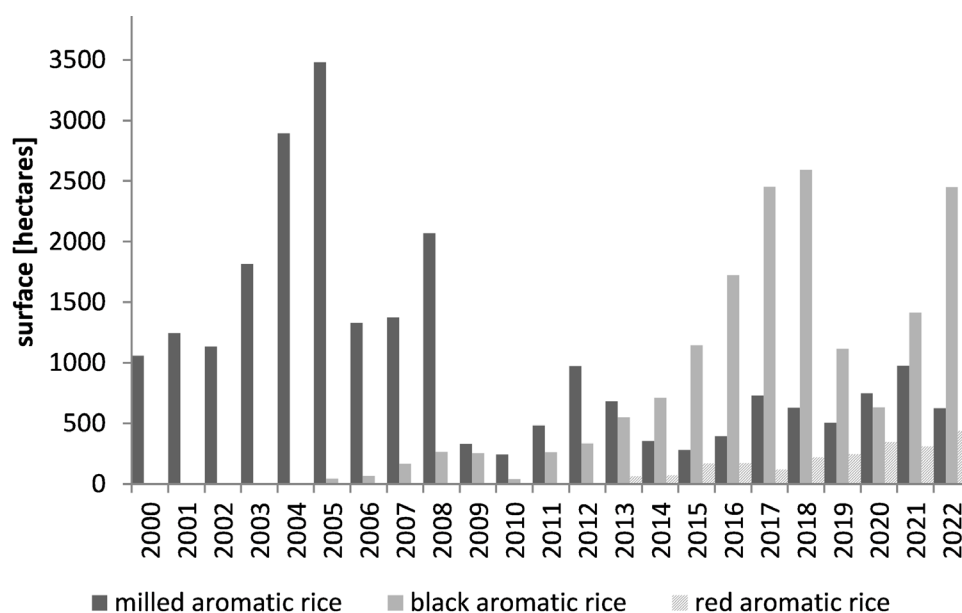


Fig. 2 – Italian surface cultivated with aromatic rice varieties

is a notable increase in that of black rice and a modest, but growing production of red rice (production data from ENR archive). This is due, in addition to the pleasantness of these cultivars, also to the increased attention of consumers to the remarkable beneficial effects they have on health (Petroni et al., 2017).

It is reasonable to think that the aromatic varieties grown in Italy have as destiny the local consumption, given the limited production.

### The determination of the aroma

One of the first simple laboratory technique to evaluate rice for presence of aroma was developed at IRRI (International Rice Research Centre) in 1971. It already consisted of a sensory evaluation of cooked milled or brown rice (Singh et al., 2000), but it never been standardized.

Since 1977, with the first study of Bullard and Holguin, the volatiles in rice are normally determined by collecting the volatiles, separating the compounds by gas chromatography and then

identifying the compounds by gas chromatography technique coupled with a mass spectrometry detector (GC/MS) (Singh et al., 2000).

Sood and Siddiq in 1978 developed an assay for determining the scent from plant material by adding potassium hydroxide (KOH) to the plant sample, which released the aroma (Singh et al., 2000).

In literature there are also a lot of studies in which the presence of the aroma character is determined by molecular markers (Singh et al., 2000).

### The rapid determination of the aroma in rice and its importance

The presence of the aroma is one of the characters to be evaluated when new rice varieties are registered in the National (Italian) Register of Varieties.

The determination is carried out by the Chemical Laboratory (LCM) of Ente Nazionale Risi (ENR) with an internal method described below (MP23).

Usually the LCM applied standard methods for the specific determinations on rice (for exam-

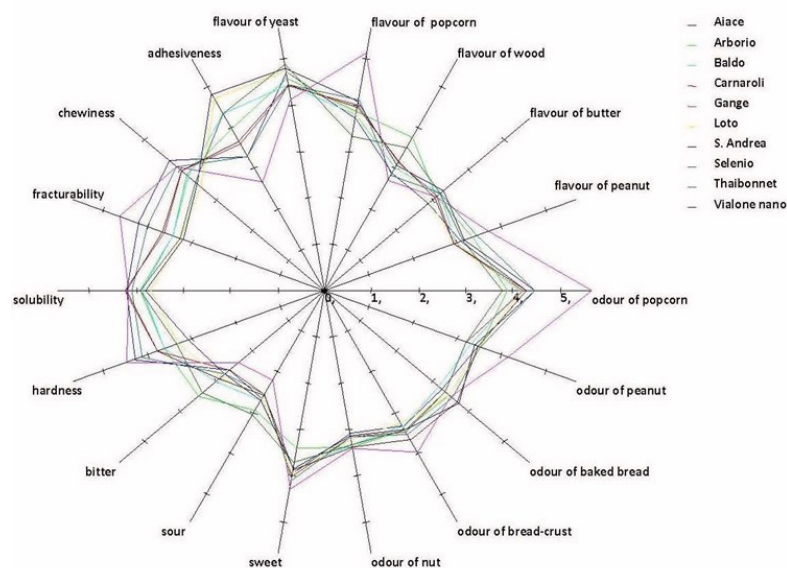


Fig. 3: Spiderplot showing sensory profile of 10 Italian rice varieties

ple for nutritional and chemical characterization, metals contents), but if they are not available, the LCM will develop and validate internal methods. This is what happens with the determination of the presence of the aroma in rice.

Through the application of the MP23 method it is possible to characterize rice varieties of interest to the breeder, for the seed producers' companies, but also for the large distribution that carries out the quality control of its products.

Currently the only standard method for the evaluation of the aroma is the CPVO (Community Plant Variety Office) protocol. However, it presents several problems: it is applicable only to brown rice and requires the addition of a chemical reagent (KOH) to the sample, making the aroma emitted artifact and not similar to that of cooking.

Hence the need to create an evaluation method that considered the sensory analysis on cooked rice.

## Aromatic rices: the current regulatory framework

Several teams developed rice aroma/flavor lexicons with Western US, French, Asian or Italian (Simonelli et al., 2017) consumers. The number of flavour attributes can vary from 7 to 20, but several appear to be shared by consumers from different culture. This is particularly the case of the specific aroma of rice and of scented rice, which appears in several lexicons but with different denominations depending on the culture of the consumers ("pandan like"<sup>1</sup>, "pop-corn", "brioche", "nutty") (Bao, 2019).

To the current state of the art, there is no national or international standards relating to the definitions or methods for determining the aroma in rice.

The definitions available in standard concerns the ISO 5527:2015 which reports: aromatic rice "rice containing a natural aromatic odour" and

<sup>1</sup> It is a common practice in Asia to include Pandan (*Pandanus amaryllifolius*) leaves in cooking non-aromatic rice to give it an aroma (Singh et al., 2000).

the ISO 7301:2021 with the sentence: “*aromatic rice: variety of rice releasing a particular aroma (e.g. roasted nuts, popcorn) that increases during cooking*”. It appears extremely generic because any varieties of rice still has its own aromatic note, as seen in several scientific papers and as emerged also from different studies conducted by ENR on Italian rice varieties (Simonelli et al., 2017).

In the radar chart reported in Figure 3 it is indeed possible to note that only the Gange variety (the only one Italian aromatic variety evaluated) has an odour and flavour of popcorn; all other Italian rice varieties have other types of odour and flavour.

In specific and authoritative texts of sensory analysis (E. Pagliarini, 2002) it is possible to find the following definition: aroma “*fragrance or smell of a product that is perceived through the nose*”. At present there is also no full agreement in the scientific community on the use of the word “aroma” due mainly to translation problems between different languages (SISS, 2012). In fact, for example, as the ISO 5492:2008 standard clarifying, the English term “aroma” and the French term “arôme” do not have the same meaning. Also as regards the term “odour” there are linguistic ambiguities. In fact, the terminology used in the sensory analysis reports the definition: odour “*sensation perceived by means of the olfactory organ in sniffing certain volatile substances*” (ISO 5492). This is the sensorial attribute used in the characterization of the different rice varieties shown in Figure 3, but in the current Italian, instead, the term odour would take on a negative connotation.

Historically, in specific texts (Singh et al., 2000; Kushwaha, 2016), when the aromatic varieties of rice are mentioned, reference is made indistinctly to the presence of “aroma” or “fragrance”. Furthermore, “aromatic” rice varieties are also referred to as “scented” rice varieties.

## The aim of the study

The purpose of this study is to validate the inter-

nal method for the determination of the fragrance (aroma) in milled and brown rice, with the application of specific international standards (ISO 8586 and ISO 11132). In this context, the use of variance is applied in the evaluation of the selected assessors and their performance.

## MATERIALS AND METHODS

### Materials

The internal method consists of performing sensorial evaluation through a panel test and defining the absence or presence (weak or strong) of the aroma on the cooked rice samples, prepared using a standardized internal method (of LCM), as follows.

### Description of the sensory experiment

The preparation of the test rice samples is reported in Figure 4.

Sensory analysis shall be conducted in a dedicated test room. Extraneous activities, including preparation of the samples, should not be allowed during the tests, as these can lead to biases results.

In each routine analytical session there must be an odd number of “expert sensory assessors” (with a minimum of three). Each assessor has a beaker for each cooking rice sample to be analyzed in the quantity of 20 g, prepared as reported in Figure 4.

It is important to analyze a maximum of three samples in each session for each assessor to avoid fatigue. The time of day at which the test is conducted is important; performance is generally considered optimum at mid-morning and mid-afternoon. A maximum of two sessions per day is recommended (ISO 6658).

The assessors have to discriminate only one olfactory stimulus: the fragrance of rice.

Provide the results as “aromatic” or “non-aromatic” rice. When a rice variety is defined as

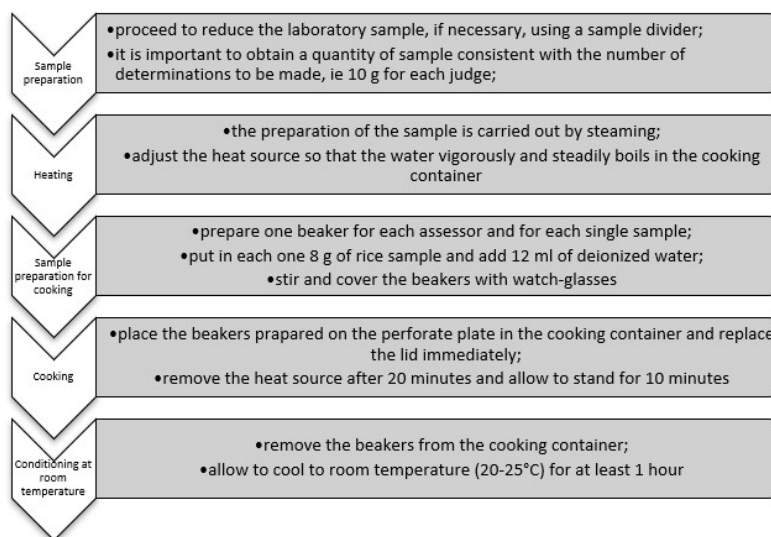


Fig. 4: schematic description for the preparation of rice samples (milled or brown) for the determination of fragrance

aromatic, the assessors evaluate whether the aroma is strong or weak.

## Panel

The panel is selected, trained, monitored and maintained according to the procedures defined in the ISO 8586 and ISO 11132 standards.

For the selection of assessors, according to the ISO 8586, the LCM chooses participants motivated and interested in further developing their sensory skills and willing to participate.

The LCM opted for an internal recruitment. The initial selection procedures involved all the staff of the Rice Research Center and 12 people proved to be compliant with continuing the training of assessors.

Candidates are available to attend both training and subsequent assessments. All the judges declare themselves habitual consumers of rice in its different preparations and they are erudite on the meaning of fragrance in rice. No ability to describe products (in this case, rice) and verbalize sensation is required because this is not a descriptive test.

The type of analysis for which the screening

is carried out concerns only in the olfactory perception of the rice samples. For each evaluator in selection, 10 samples of rice are analyzed, even in different sessions, of which 5 are aromatic and 5 are not aromatic, according to the method described in Figure 4 (two sessions with 3 samples and two sessions with 2 samples). A maximum of 2 errors in the evaluation are allowed (80% of the results must be correct), for example when the aroma of an aromatic rice reference sample is not perceived.

For the training of assessors, a number of judges equal to one and a half times or more than twice as required by the application of the method must be trained; it is optimal that 6 people are trained as expert sensory assessors. The judges will be instructed not to evaluate based on their personal tastes, but to be objective. In order to carry out olfactory training, substitutes or artificial preparations are not used. Only milled or husked rice samples will be considered, as defined in the purpose of the method. Candidates must be trained on two olfactory levels related to the aroma of rice: presence or absence. For the determination of the aroma, it is required that the assessors discriminate on the presence



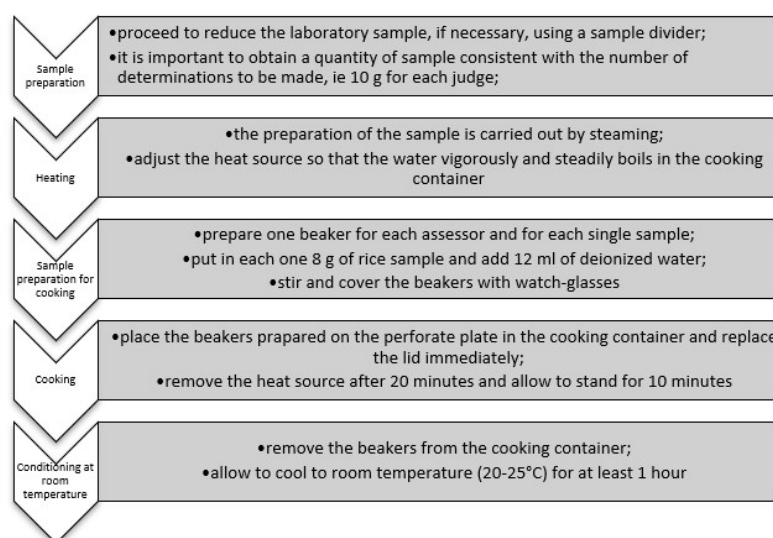


Fig. 5: critical steps in the validation of the method

/ absence of the aroma character. If the aroma is present, the judges must evaluate the weak or strong aromaticity.

## Validation of the method

The validation of the internal method is performed in accordance with the specific (sensory) standards (ISO 8586 and ISO 11132) and ISO/IEC 17025 and involved as reported in Figure 5.

The statistical method used is the analysis of variance with an ordinal dependent variable according to the ISO 8586, recommended for the evaluation of analytical data in sensory determinations. This standard is also used for the evaluation of analysis of variance in the choice of selected assessors for scoring and for monitoring the performance validation.

## RESULTS AND DISCUSSION

### Cooked milled rice

An assessment by each assessor of three samples from six cooked milled rice varieties gave the results shown in Table 1 using a 3-point scoring

system (1: non-aromatic; 2: aromatic – weak; 3: aromatic – strong). A number of 3-5 “expert sensory assessors” are employed during routine analyzes. Table 1 shows the results of four judges’ performance evaluation (validation). Milled and brown rice was tested by the same assessors.

The analysis of variance table is then constructed as shown in Table 2.

The overall analysis of variance is then calculated as shown in Table 3.

It would be concluded that all assessors have low residual standard deviations (Table 2) and statistically significant variation between the samples, in particular for assessors 1 and 3. The standard deviations are comparable and low, so we can say that there are differences between samples (we have to consider that the scale is narrow, from 1 to 3), confirmed by Table 3.

Variation between assessors is not significant because the mean of the scores are comparable.

It is possible to notice that the two samples that do not possess the aroma gene (CRLB1 and Gladio) and therefore do not express fragrance, are univocally evaluated as non-aromatic (mean: 1,00).

For the other varieties, all aromatic, it is instead possible to appreciate a scale of intensity. The rice

variety that results to possess a more intense fragrance is larim which will be used in the routine analytical process as a reference sample.

The statistical significance of interaction between assessors and samples is determined by testing the ratio  $MS_6/MS_7$  against critical values in tables of the F-distribution with  $v_6$  e  $v_7$  degrees of freedom ( $F_{0,05;15;48} = 1,88$ ).

The statistical significance of interaction between assessors is determined by testing the ratio  $MS_5/MS_7$  against critical values in tables of the F-distribution with  $v_5$  e  $v_7$  degrees of freedom ( $F_{0,05;3;48} = 2,80$ ).

The statistical significance of the variation between products is determined by comparing the ratio  $MS_4/MS_6$  with critical values of the F-distribution with  $v_4$  e  $v_6$  degrees of freedom ( $F_{0,05;5;15} = 2,90$ ;  $F_{0,001;5;15} = 7,57$ ).

Table 1: Results of the assessors (cooked milled rice)

Rice varieties (cooked milled rice)	Assessor								Mean
	1		2		3		4		
	score	mean	score	mean	score	mean	score	mean	
larim	3		3		3		3		2,83
	3	3,0	3	2,7	3	2,7	3	3,0	
	3		2		2		3		
Gange	3		3		3		2		2,58
	2	2,7	3	2,7	3	3,0	2	2,0	
	3		2		3		2		
Basmati	3		3		3		2		2,50
	3	3,0	2	2,3	2	2,7	1	2,0	
	3		2		3		3		
CRLB1	1		1		1		1		1,00
	1	1,0	1	1,0	1	1,0	1	1,0	
	1		1		1		1		
Gladio	1		1		1		1		1,00
	1	1,0	1	1,0	1	1,0	1	1,0	
	1		1		1		1		
Basmati 2	2		2		2		3		2,08
	3	2,7	1	1,7	2	1,7	2	2,3	
	3		2		1		2		
Mean	2,22		1,89		2,00		1,89		2,00

Table 2: Analysis of variance – Data not combined (cooked milled rice)

Source of variation	Degrees of freedom, n	Assessors							
		1		2		3		4	
		MS	F	MS	F	MS	F	MS	F
Between samples	$v = 5$	2,76	24,80 <sup>a</sup>	1,82	8,20 <sup>b</sup>	2,40	14,40 <sup>a</sup>	1,82	8,20 <sup>b</sup>
Residual	$v = 12$	0,11		0,22		0,17		0,22	
	Residual standard deviation	0,33		0,47		0,41		0,47	

<sup>a</sup> significant at the level  $\alpha = 0,001$  ( $F_{0,001;5;12} = 8,89$ )

<sup>b</sup> significant at the level  $\alpha = 0,05$  ( $F_{0,05;5;12} = 3,11$ )

Table 3: Analysis of variance – Combined data (cooked milled rice)

Source of variation	Degrees of freedom, $\nu$	SS	MS	F
Between assessors	$\nu = 3$	1,33	$MS_3 = 0,44$	0,06 <sup>b</sup>
Between samples	$\nu = 5$	39,50	$MS_4 = 7,90$	26,33 <sup>a</sup>
Interaction	$\nu = 15$	4,50	$MS_6 = 0,30$	1,66 <sup>b</sup>
Residual	$\nu = 48$	8,67	$MS_7 = 0,18$	
Total	71	54,00		
<sup>a</sup> significant at the level $\alpha = 0.001$				
<sup>b</sup> not significant at the level $\alpha = 0.05$				

## Cooked Brown Rice

At one session, four assessors gave scores for one attribute (fragrance) on three replicates of six cooked brown rice.

Also, in the case of brown rice, all assessors have low residual standard deviations (Table 5) and statistically significant variation between the samples, were suitable.

Variation between assessors is not significant because the mean of the scores are comparable (Table 4). On the other hand, the assessors/samples interaction is not significant (Table 6), and it is not possible to assert that the assessors have disagreement about the ranking of the samples.

Table 4: Results of the assessors (cooked brown rice)

Rice varieties (cooked brown rice)	Assessor								Mean
	1		2		3		4		
	score	mean	score	mean	score	mean	score	mean	
Gioiello	2	2.0	2	2.0	2	2.3	3	2.7	2.25
	2		2		2		2		
	2		2		3		3		
Diamante	2	2.7	2	2.3	3	2.7	2	2.3	2.50
	3		2		2		3		
	3		3		3		2		
CRLB1 (brown)	1	1.0	1	1.0	1	1.0	1	1.0	1.00
	1		1		1		1		
	1		1		1		1		
Il Moro	3	3.0	2	2.0	3	2.3	3	2.7	2.50
	3		2		2		3		
	3		2		2		2		
Il Cardinale	2	1.7	2	1.3	2	1.3	1	1.0	1.33
	2		1		1		1		
	1		1		1		1		
Iarim (brown)	3	3.0	3	2.7	3	2.7	3	3.0	2.83
	3		3		3		3		
	3		2		2		3		
mean	2.44		2.06		2.33		2.28		2.28

Table 5: Analysis of variance – Data not combined (cooked brown rice)

Source of variation	Degrees of freedom, $\nu$	Assessors							
		1		2		3		4	
		MS	F	MS	F	MS	F	MS	F
Between samples	$\nu = 5$	1.96	17.60 <sup>a</sup>	1.16	6.93 <sup>b</sup>	1.52	5.48 <sup>a</sup>	2.36	14.13 <sup>b</sup>
Residual	$\nu = 12$	0.11		0.17		0.28		0.17	
	Residual standard deviation	0.33		0.41		0.53		0.41	
<sup>a</sup> significant at the level $\alpha = 0,001$ ( $F_{0,001;5;12} = 8,89$ ) <sup>b</sup> significant at the level $\alpha = 0,01$ ( $F_{0,01;5;12} = 5,06$ )									

Table 6: Analysis of variance – Combined data (cooked brown rice)

Source of variation	Degrees of freedom, $\nu$	SS	MS	F
Between assessors	$\nu = 3$	1,04	$MS_5 = 0,35$	1,92 <sup>b</sup>
Between samples	$\nu = 5$	32,07	$MS_4 = 6,41$	35,52 <sup>a</sup>
Interaction	$\nu = 15$	2,88	$MS_6 = 0,19$	1,06 <sup>b</sup>
Residual	$\nu = 48$	8,67	$MS_7 = 0,18$	
Total	71	44,65		
<sup>a</sup> significant at the level $\alpha = 0.001$ <sup>b</sup> not significant at the level $\alpha = 0.05$				

It is possible to notice that, again, the Iarim sample which possess the aroma gene, express fragrance even in the brown form and is univocally evaluated as aromatic (mean: 2,83 comparable to the mean value in the milled form). The sample that do not possess the aroma gene (CRLB1 in brown form)

do not express fragrance and is univocally evaluated as non-aromatic (mean: 1,00).

For the other varieties, consisted of pigmented brown rice, it is instead possible to appreciate a scale of intensity with this order: Il Cardinale (non-aromatic), Gioiello, Diamante, Il Moro.

## Evaluation of performance of assessors

As reported previously, at one session, four assessors gave scores for one attribute on three replicates of six samples for milled rice (Table 1) and brown rice (Table 4). It is now possible to evaluate, according to ISO 11132, the performance of assessors.

The bias reported in Table 7 is the difference between the assessor's mean and the overall mean,

both in Table 1 (for milled rice) and in Table 4 (for brown rice).

An individual bias reported in Table 8 is the difference between an assessor's mean for a sample and the panel mean for that sample, both in Table 1 (for milled rice) and in Table 4 (for brown rice).

Table 7: Individual biases and residual SDs

Assessors	Milled rice		Brown rice	
	Bias	Residual SD	Bias	Residual SD
1	0,22	0,33	0,16	0,41
2	-0,11	0,47	-0,22	0,41
3	0,00	0,41	0,05	0,58
4	-0,11	0,47	0,00	0,58

Table 8: Individual sample bias terms

Sample	Milled rice				Brown rice			
	1	2	3	4	1	2	3	4
Iarim	0,17	-0,17	-0,17	0,17	-0,25	-0,25	0,08	0,42
Gange	0,08	0,08	0,42	-0,58	0,17	-0,17	0,17	-0,17
Basmati	0,50	-0,17	0,17	-0,50	0,08	-0,25	0,42	-0,25
CRLB1	0,00	0,00	0,00	0,00	0,50	-0,50	-0,17	0,17
Gladio	0,00	0,00	0,00	0,00	0,33	0,00	0,00	-0,33
Basmati2	0,58	-0,42	-0,42	0,25	0,17	-0,17	-0,17	0,17
SD, s	0,26	0,18	0,28	0,35	0,25	0,16	0,22	0,29

Table 9: Regression and correlation statistics (milled rice)

Parameter	Assessor							
	Milled rice				Brown rice			
	1	2	3	4	1	2	3	4
Correlation, $R^2$	0.94	0.95	0.90	0.82	0.79	0.90	0.82	0.88
Slope, b	1.16	0.95	1.06	0.87	0.93	0.86	0.96	1.31
Intercept, a	-0.08	0.01	-0.11	0.15	0.34	0.10	0.16	-0.69

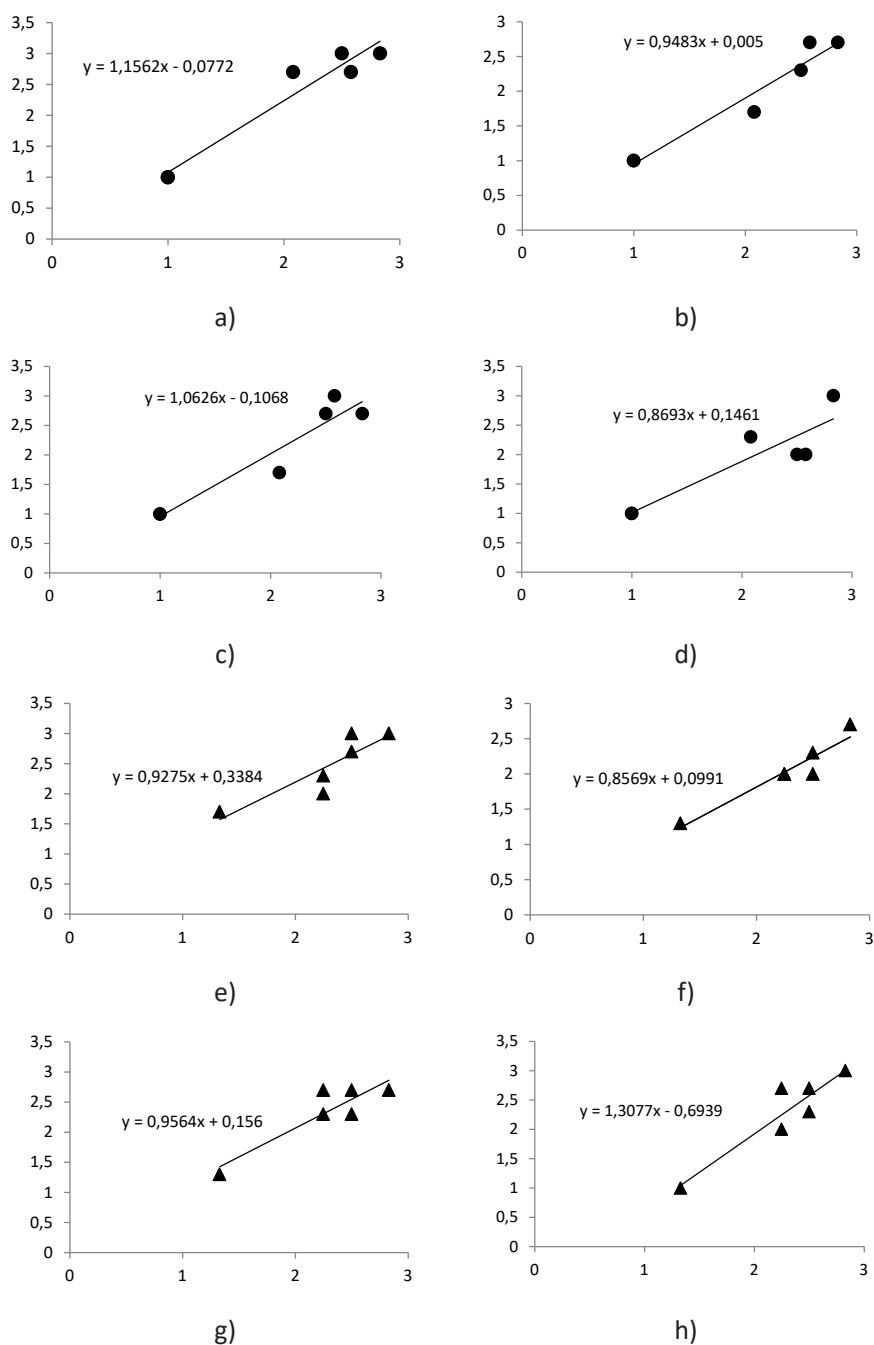


Fig. 6: scores of assessors 1 to 4 [a) to d) for milled rice; e) to h) for brown rice] plotted against the panel means

Figure 6 shows each assessor's scores plotted against the panel means for milled cooked rice and for brown cooked rice.

The ideal plot is one showing complete agreement between an assessor and the panel mean,

with points close to a line of slope,  $b = 1.00$ , and intercept,  $a = 0.00$ . The correlation coefficient should be close to  $+1.00$ .

The regression and correlation statistics for the four assessors are shown in Table 9.

It is possible to notice that the residual standard deviation (SD), reported in Table 8, is for all the assessors low and homogeneous, which indicates good homogeneity of the panel both in milled and in brown cooked rice.

In this validation there were two true values, which all panelists found: the Gladio and CRLB1 varieties are not aromatic, therefore their score should always be 1. This is the proof of trueness, which has been respected.

Evaluating the Figure 6 and the parameters reported in Table 9 for milled cooked rice, it is noticed that the assessor 4 has the lowest correlation coefficient, he has probably used more restrictive parameters than the other judges. Assessor 2 is the one with the highest correlation coefficient, it also has a very close intercept to 0 and a slope close to 1.

In the case of brown cooked rice, assessor 1 has the lowest correlation coefficient, he probably used more stringent parameters than the other assessors for this kind of product. Assessor 2, again, is the one with the highest correlation coefficient.

For the brown rice the correlations are less performing than the milled one.

## Overall panel performance

From Table 3 (milled rice) and Table 6 (brown rice), it can be seen that interaction was not significant at the 0.05 level, indicating that the panel members were consistent in their differences.

The degree of variation in assessors means can be described by the assessors SD:

$$s_a = \sqrt{\frac{MS_5 - MS_7}{n_q \cdot n_r}}$$

For milled cooked rice,  $s_a = 0.12$  and for brown milled rice  $s_a = 0.10$ .

## Repeatability of the panel

The repeatability of the panel can be estimated

from the repeatability of the individual assessors. This is inversely related to the error SD, se:

$$s_e = \sqrt{\frac{MS_6 - MS_7}{n_r}}$$

Where:  $MS_6$  and  $MS_7$  are reported in Table 3 and 6;  $n_r$  is the number of replicates per samples.

For milled cooked rice,  $s_e = 0.20$  and for brown milled rice  $s_e = 0.06$ .

## Monitoring the performance with Shewhart chart

Shewhart charts demonstrated whether a method or process is operating satisfactorily or whether an abnormal event has occurred and action is needed. The repeatability of the panel was monitored over a number of sessions. After each session, the data were analyzed and the repeatability determined. It was plotted on a Shewhart control chart.

Shewhart charts have lines drawn on them, representing the limits of normal performance. Warning limits are set so that they should contain 95% of sessions, if the panel is performing normally. The action limits are set so that only 0.1% of occurrences should be above the upper limit or below the lower limit in those circumstances.

To set the limits for a Shewhart chart, previous data from a range of sessions during which a panel is performing normally are required.

At the moment a small number of sessions have been carried out in the LCM (only 3 for kind of rice), but the control charts have nevertheless been initially set for the parameters: overall panel performance ( $s_a$ ) and repeatability of the panel ( $s_e$ ). The chosen control chart for the evaluation of the performances is the standard deviation (s) chart, according to ISO 7870-2.

Estimated control limits would be set at  $\pm 3s$  (the central line is  $\bar{x}$ ). For a number of observation  $n = 3$ , only the value of  $\bar{x}$  is expected and is equal to 2.568 (it will be possible to represent both control limits when  $n = 6$ ); see Figure 7 to 10.

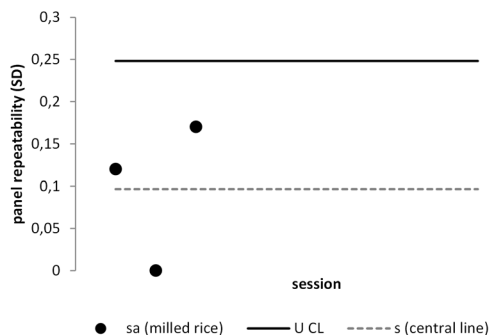


Fig. 7: Shewhart chart for fragrance in rice (cooked milled rice), overall panel performance

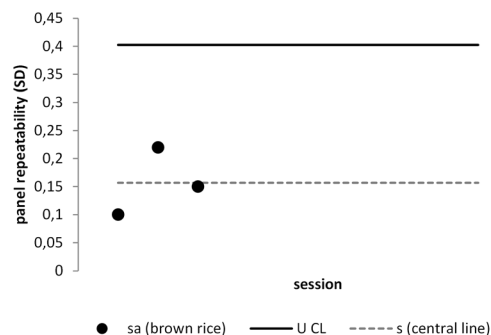


Fig. 8: Shewhart chart for fragrance in rice (cooked brown rice), overall panel performance

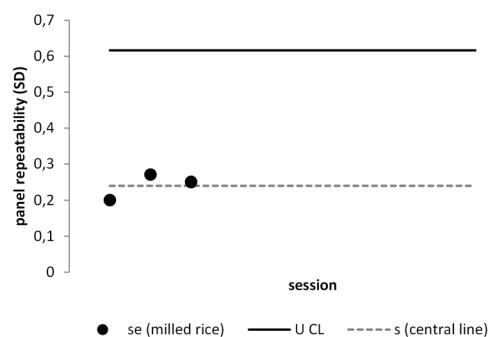


Fig. 9: Shewhart chart for fragrance in rice (cooked milled rice), repeatability of the panel

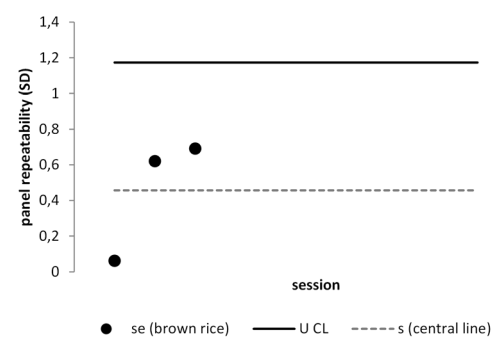


Fig. 10: Shewhart chart for fragrance in rice (cooked brown rice), repeatability of the panel

## CONCLUSIONS AND FUTURE RESEARCH THRUSTS

The aromatic rice varieties, thanks to the work of the Italian breeder, are cultivated in Italy and in order to be introduced in the market, they must follow a precise registration process in the Italian National Register. Among the different properties to be evaluated there is also the presence of aromaticity which, to date, cannot be evaluated with a standardized, robust and reliable method as it is not available.

The Chemical Laboratory of Ente Nazionale Risi has developed the internal method here illustrated here; this internal method is to be considered validated for cooked milled rice and cooked brown rice.

The use of the statistical method proposed and recommended in ISO 8586 demonstrates its applicability in this method where only one sensory parameter is considered, in a limited range of values (from 1 – absence of aromaticity - to 3 – strong aromaticity).



As shown in the statistical tables, variation between assessors is not significant, so the panel of assessors of LCM is homogeneous. There are differences between samples so it is possible to discriminate from aromatic and non-aromatic rice (and between strong or weak fragrance).

The analytical determination is performing on cooked milled and brown rice, also pigmented (black and red). The performance will be evaluated in the future also on parboiled rice.

With the effective application of the statistical evaluation of the data, it is possible to assess whether a sample is suitable to constitute the reference standard. In this specific case, it was possible to state that the Gange variety is less suitable to constitute the reference material with respect to larim. The latter is in fact used as a reference material in the analytical routine, but also in the training of assessors (also according to "A" - "not A" test, ISO 8588). Moreover, with the change of variety as reference material, there were less analytical ambiguities in the routine.

The evaluation of aromaticity on raw rice proved to be much less effective than cooked rice, for which the proposed method works very well.

Currently all the performances evaluated by the Shewhart control charts, are under control both for cooked milled and brown rice.

## Acknowledgements

Special thanks to all assessors: R. Audisio, C. Bocca, L. Campanini, P. Trabella, A. Zone, M. Vigino; WG Rice and other Cereals (UNI); Dr. E. Losi (Market Area - Ente Nazionale Risi).

## References

Bhattacharya K R. 2011. Rice Quality - A guide to rice properties and analysis. Woodhead Publishing.

- Bao J. 2019. Rice - Chemistry and Technology, 4th Edition. American Association of Cereal Chemists.
- Champagne E T. 2004. Rice - Chemistry and Technology, 3rd Edition. American Association of Cereal Chemists.
- CPVO-TP/016/3 del 01/08/2015. Protocol for tests on distinctness, uniformity and stability. *Oryza sativa* L. RICE. Adopted on 01/10/2015 (Ad. 38: Decorticated grain: aroma, p.19).
- Italian Legislative Decree 4th august 2017, n. 131. Disposizioni concernenti il mercato interno del riso, in attuazione dell'articolo 31 della legge 28 luglio 2016, n.154. Gazzetta Ufficiale della Repubblica Italiana, n. 209, 7-9-2017.
- Kushwaha, U.K.S. 2016. Black Rice. Springer.
- Hinge V R, Patil H B, Nadaf A B. 2016. Aroma volatile analyses and 2AP characterization at various developmental stages in Basmati and Non-Basmati scented rice (*Oryza sativa* L.) cultivars. *Rice*. 9:38.
- ISO 5492:20108. Sensory analysis - Vocabulary.
- ISO 5527:2015. Cereals: Vocabulary.
- ISO 6658:2017. Sensory analysis - Methodology - General guidance.
- ISO 7301:2021. Rice - Specification.
- ISO 8588:2017. Sensory analysis - Methodology - "A" - "not A" test.
- ISO 7870-2:2013. Control charts - Part 2: Shewhart control charts.
- ISO 8586:2012. Sensory analysis - General guidelines for the selection, training and monitoring of selected and expert assessors.
- ISO 11132:2012. Sensory analysis - Methodology - Guidelines for monitoring the performance of a quantitative sensory panel.
- ISO/IEC 17025:2017. General requirements for the competence of testing and calibration laboratories.
- Petroni K, Landoni M, Tomay F, Calvenzani V, Simonelli C, Cormegna M. 2017. Proximate Composition, Polyphenol Content and Anti-Inflammatory Properties of White and Pigmented Italian Rice Varieties. *Universal Jour-*

- nal of Agricultural Research*. **5**(5): 312-321.
- Registro Varietale per l'annata agraria 2022/2023 aggiornato al 31 agosto 2022.
- Simonelli C, Cormegna M, Tonello M. 2016. Valutare i risi aromatici. *L'Assaggio*. n°55, autunno 2016 (in Italian).
- Simonelli C, Galassi L, Cormegna M, Bianchi P. 2017. Chemical, Physical, Textural and Sensory Evaluation on Italian Rice Varieties. *Universal Journal of Agricultural Research*. **5**(2): 104-112.
- Simonelli C, Cormegna M. 2018. Determination of Fragrance in Rice by panel test – Project of a validated method and development of a standard. International meeting: Eurosense 2018 (Verona, 2-5 September), Scientific Poster.
- Singh R K, Singh U S, Khush G S. 2000. Aromatic Rices. Science Publishers, Inc.
- SISS, Società Italiana di Scienze Sensoriali, 2012. Atlante sensoriale dei prodotti alimentari. Tecniche nuove.
- Wakte K, Zanan R, Hinge V, Khandagale K, Nadaf A, Henry R. 2016. Thirty-three years of 2-acetyl-1-pyrroline, a principal basmati aroma compound in scented rice (*Oryza sativa L.*): a status review. *J Sci Food Agric*. Published online in Wiley Online Library.