JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Alkylresorcinols as Markers of Whole Grain Wheat and Rye in Cereal Products

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The alkylresorcinol (AR) content of Swedish wheat grain samples, as well as of cereal ingredients and cereal foods containing wheat and rye, was determined. The average total AR content in Swedish wheat was 412 μg/g (ranging between 227 and 639 μg/g), which is lower than that in Swedish rye analyzed in a previous study. The relative composition of AR homologues was consistent for wheat samples and differed markedly from that of rye. Notably, the ratio of the homologues C17:0/C21:0 was ~0.1 in wheat and ~1.0 in rye, indicating that it can be used to distinguish between those two cereals. The AR content in cereal foods commonly consumed in Sweden varied widely, from nondetectable levels in white wheat flour and products not containing the outer parts of wheat and/or rye to >900 μg/g in some whole grain rye products. AR content in cereal foods was calculated from their recipes using average AR values for the cereal ingredients determined in this study. As there was a good correlation between calculated and analyzed AR levels in cereal foods ($R^2 = 0.91$), it is possible to estimate the proportion of whole grain wheat and/or rye in a given cereal product on the basis of AR content and C17:0/C21:0 ratio. ARs appear to be good markers of whole grain wheat and rye in foods, and their analysis may be an objective way to identify foods rich in whole grain wheat and/or rye or brans thereof.

KEYWORDS: Alkylresorcinols; cereals; cereal foods; marker; whole grain; wheat; rye

INTRODUCTION

Foods containing whole grain cereals have been identified as providing significant health benefits over refined grains and have been linked to a decreased risk of diabetes, obesity, heart disease, and some cancers (*1*). The importance of whole grain cereals in human nutrition has now been recognized by the U.S. Food and Drug Administration (FDA) (*2*), the U.K. Joint Health Claims Initiative (JHCI) (*3*), and the Swedish Nutrition Foundation (SNF) (*4*), all of which authorize health claims linking consumption of whole grain cereals and a reduced risk of heart disease. A food product can be eligible for whole grain health claim if it contains ≥51% whole grain ingredients by weight. Currently, the dietary fiber content of whole grain wheat is used as the standard for determining whether a food product is "whole grain" or not. However, a number of problems have been identified using whole grain wheat as a standard for all cereals (*5*), and more specific markers for whole grain are needed to ensure proper labeling of whole grain cereal products. Alkylresorcinols (ARs) were suggested to serve as possible markers of whole grain wheat and rye products (*6*).

ARs are amphiphilic 1,3-dihydroxybenzene derivatives, with an odd-numbered alkyl chain at position 5 of the benzene ring. There are five main AR homologues in wheat and rye, which differ in the length of the saturated alkyl chain (C17:0−C25:0) (*7*). Wheat and rye also contain small amounts of AR analogues with unsaturated double bonds and keto or hydroxyl groups on their alkyl chains (~5% in wheat and ~15% in rye) (*8*−*10*). ARs are present in high amounts in wheat and rye grains (~0.03−0.15% of dry kernel weight) and in low levels in barley (40−100 μg/g) but not in other human foods (*6*). Although ARs are also present in high amounts in triticale, this cereal is not commonly consumed by humans.

Complete recovery of ARs from baked cereal products after extraction with hot 1-propanol showed that ARs were not lost during the processing as previously suggested (*11*, *12*). It is possible that ARs are trapped in "starch−lipid" complexes and are not completely recovered using the extraction procedure for whole grains (*6*). Because ARs are found in only the bran fractions of wheat and rye, they are linked only with products containing either whole grain wheat, whole grain rye, and/or the brans of these cereals. Therefore, ARs can be useful markers for cereal products containing whole grain wheat and rye.

The aim of this study was to investigate the variation of AR content and homologue compositions in Swedish wheat grain samples, cereal ingredients, and cereal foods. On the basis of these results, a relationship between analyzed and calculated AR content (from the AR content of cereal ingredients) was determined to evaluate whether analyzed AR content can be a

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10.1021/jf049726v CCC: $27.50
Published on Web 12/07/2004

Table 1. Alkylresorcinol[a] Content and Homologue Composition in Spring and Winter Wheat Grain Samples Grown in Sweden

	total ARs (μg/g of DM)		homologue composition (%)										ratio of C17:0 to C21:0	
			C17:0		C19:0		C21:0		C23:0		C25:0			
cultivar	Ö[b]	S[b]	Ö	S	Ö	S	Ö	S	Ö	S	Ö	S	Ö	S
spring														
SW Vinjett	634	639	4	5	38	41	51	49	6	5	1	1	0.1	0.1
IGP Triso	600	519	4	4	33	37	52	51	10	7	2	1	0.1	0.1
LP Fasan	588	468	3	4	31	34	55	53	9	8	2	1	0.1	0.1
SW Vals	534	437	4	4	37	37	52	52	6	6	1	1	0.1	0.1
SW Dragon	557	421	4	5	37	42	52	49	6	4	1	1	0.1	0.1
SW Milljet	541	356	3	4	34	38	53	51	8	6	2	1	0.1	0.1
SW Estrad	432	298	3	4	34	37	54	53	8	6	2	1	0.1	0.1
Zel Tybalt	496	297	4	4	36	39	50	49	8	7	2	1	0.1	0.1
range	432–634	297–639	3–4	4–5	31–38	37–42	50–54	49–53	6–10	4–8	1–2			
mean	548	430	4	4	35	38	52	51	8	6	1	1	0.1	0.1
winter														
SW Zel Marshal	346	427	4	4	37	34	51	52	7	9	1	2	0.1	0.1
SW Hadm Tarso	316	425	6	5	42	39	47	48	5	7	1	1	0.1	0.1
Ceb Ritmo	300	422	4	4	37	33	50	52	7	9	1	2	0.1	0.1
SW Kopral	313	414	5	4	40	37	49	51	6	7	1	1	0.1	0.1
PBIS Kris	258	384	6	5	44	38	45	49	5	7	1	1	0.1	0.1
SW Kosack	353	334	6	6	40	41	44	45	9	7	3	1	0.1	0.1
SW Harnesk	227	311	6	5	40	35	47	51	6	8	1	2	0.1	0.1
HT Olivin	244	283	4	5	37	37	50	51	7	7	2	1	0.1	0.1
range	227–353	283–427	4–6	4–6	37–44	33–41	44–51	45–52	5–9	7–9	1–3	1–2		
mean	295	375	5	5	39	37	48	50	7	8	1	2	0.1	0.1

[a] Means of triplicates (CV < 5%). [b] Ö, Östergötland; S, Skåne.

Table 2. Mean Alkylresorcinol Content and Mean Homologue Composition in Cereal Ingredients

		total AR (μg/g of DM),	homologue composition mean (%)					ratio of
raw material	*n*	mean ± SD	C17:0	C19:0	C21:0	C23:0	C25:0	C17:0 to C21:0
whole grain wheat	32	412 ± 118	4 ± 1	40 ± 3	50 ± 3	7 ± 1	1 ± 1	~0.1
whole grain rye[a]	30	726 ± 117	23 ± 2	32 ± 2	26 ± 2	11 ± 1	8 ± 1	~0.9
whole grain barley	6	8 ± 10	10 ± 4	12 ± 0	23 ± 4	13 ± 1	43 ± 1	~0.4
rye bran	6	2758 ± 728	23 ± 0	32 ± 0	25 ± 0	11 ± 0	9 ± 1	~0.9
sifted rye flour (100%)	6	99 ± 35	24 ± 1	35 ± 1	27± 1	9 ± 0	5 ± 1	~0.9
sifted rye flour (40%)[b]	6	37 ± 15	17 ± 3	35 ± 2	33 ± 3	10 ± 2	5 ± 1	~0.5
wheat bran	6	2211 ± 605	5 ± 0	35 ± 3	50 ± 1	8 ± 2	2 ± 2	~0.1
white wheat flour	6	nd[c]						

[a] Data from Ross et al. (*13*). [b] Containing sifted wheat flour of higher extraction rate. [c] Not detected (detection limit = 5 μg/g).

good marker for the presence of whole grain wheat and/or rye and/or bran in cereal foods.

MATERIALS AND METHODS

Cereals and Cereal Products. Thirty-two samples of Swedish spring and winter wheats (*Triticum aestivum*) grown in Östergötland and Skåne in 2001−2002 were provided by the Unit of Applied Field Research, Swedish University of Agriculture Sciences, Uppsala, Sweden. Cereal foods including 16 commercial crisp breads and 4 cereal ingredients were donated by Wasabröd AB (Filipstad, Sweden), and 6 commercial cereal products and 14 cereal ingredients were donated by Cerealia AB (Järna and Malmö, Sweden). Soft breads (six types) were baked in the experimental bakery of our department. The remaining samples were purchased from local supermarkets in Uppsala and Stockholm, Sweden. If required, samples were freeze-dried and, directly before analysis, milled to flour to pass a 0.5 mm sieve using a Cyclotech 1093 sample mill (Tecator AB, Höganäs, Sweden).

Chemicals and Reagents. Methyl behenate (C22:0, fatty acid methyl ester, Larodan Fine Chemicals AB, Malmö, Sweden) was used as internal standard. All of the solvents and reagents were of analytical grade (Merck, Darmstadt, Germany) and were used without further purification.

Extraction and Analysis of Alkylresorcinols. ARs were extracted from intact cereal grains or milled fractions of raw cereals using ethyl acetate and analyzed by gas chromatography (GC) (*13*). Cereal foods (breads and other processed cereal products) were extracted with hot 1-propanol (*14*) and analyzed as above (*6*).

Comparison of Calculated and Analyzed Alkylresorcinol Content in Cereal Foods. The average AR content of 32 wheat grain samples, 30 rye grain samples, and 6 other cereal ingredients (six replicates), including wheat bran, sifted wheat flour, rye bran, sifted rye flour (100%), sifted rye and wheat flour (40% sifted rye flour and 60% sifted wheat flour), and whole grain barley, were used to calculate the total AR content in wheat and rye products. On the basis of the recipes provided by the manufacturers, the average AR content of the cereal ingredients was used to calculate the AR content, and the calculated AR content was plotted against the analyzed AR content.

Statistics. All values are reported on a dry matter (DM) basis, and each sample was extracted and analyzed in triplicate. Results were analyzed using Student's *t* test (Statistica '99 version 5.5, StatSoft Inc., Tulsa, OK). Differences were considered to be significant at $P < 0.01$.

RESULTS AND DISCUSSION

Content of Alkylresorcinols in Swedish Wheat and Rye Grain Samples. The AR contents in wheat cultivars commonly grown in Sweden were determined (**Table 1**), and the results were compared with those of Swedish rye analyzed in a previous

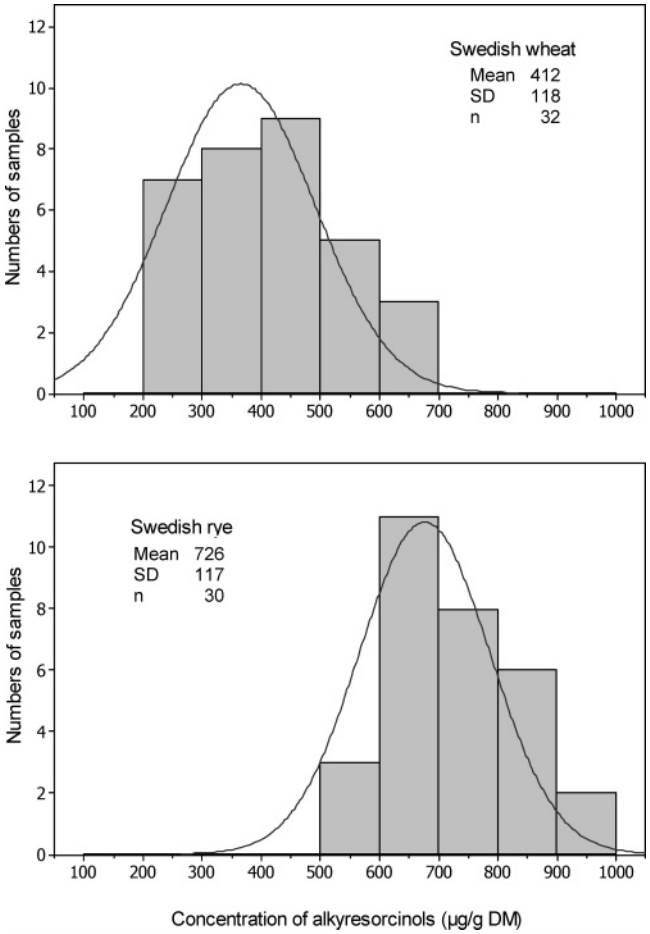

Figure 1. Distribution of total alkylresorcinol content in Swedish wheat and rye grain samples [rye data from Ross et al. (*13*)].

study (*13*). There was a wide variation in total AR content of Swedish wheat, but only a small variation in the relative distribution of AR homologues. The AR content of Swedish wheat grains ranged from 227 to 639 μg/g (mean ± SD, 412 ± 118 μg/g), which is comparable to the content in North American wheat samples (300−700 μg/g) (*15*) but lower than those recently reported for western European wheat samples (595−1429 μg/g) (*6*). The average total AR content in Swedish wheat analyzed in this study is generally lower than that in Swedish rye. Of wheat samples analyzed, 90% contained 200−600 μg of AR/g, whereas 83% of the rye samples contained 600−900 μg of AR/g. The overlap in AR content (500−700 μg/g) (**Figure 1**) makes it difficult to distinguish between whole grain wheat and rye on the basis of only total AR content.

Figure 2 shows that the AR homologue composition of wheat and rye differs markedly as reported previously (*6*). The dominant AR homologues in wheat are C21:0 and C19:0, wheres the dominant AR homologues in rye are C17:0, C19:0, and C21:0. Although it is not possible to distinguish between wheat and rye on the basis of total AR content, it is possible to distinguish between the two on the basis of homologue composition. The ratio of the homologues C17:0 and C21:0 in wheat was ∼0.1, whereas in rye it was ∼1.0. Thus, the ratio of C17:0 to C21:0 may be a tool to distinguish between the two cereals in a cereal product, as will be discussed below.

Spring wheat samples have higher AR contents (mean ± SD, 489 ± 105 μg/g) than winter wheat samples (mean ± SD, 335 ± 64 μg/g). Because winter wheat grain had a higher thousand-kernel weight than spring wheat grain, the apparent decrease in AR content is possibly a dilution effect due to deposition of

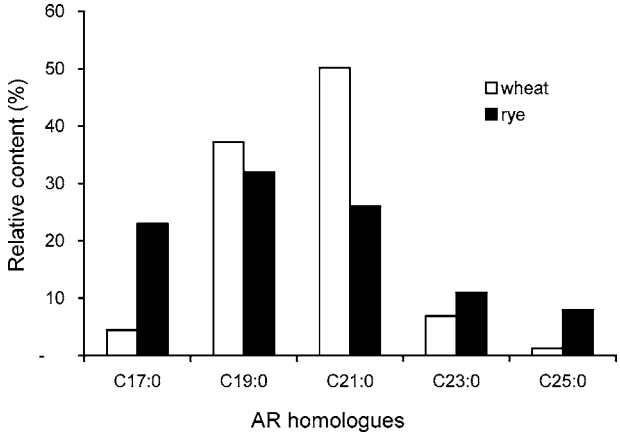

Figure 2. Relative distribution of alkylresorcinol homologues in wheat and rye.

starch and protein during kernel development, whereas the amount of AR remains the same (*15*). Spring wheat grown in Östergötland had significantly higher AR content than that grown in Skåne ($P < 0.01$), whereas winter wheat had a significantly lower AR content in Östergötland than in Skåne ($P < 0.01$). The same cultivars grown in different locations differ in their AR content, and cultivars with a higher amount of ARs in one location tend to have a higher amount of ARs in the other location as well, in agreement with the previous study on rye (*13*). This suggests that both cultivar and environmental factors have an important effect on AR content in cereal grains. However, more extensive field trials are required to test these effects more rigorously.

Alkylresorcinol Levels in Cereal Foods. AR content was determined in 8 different common cereal ingredients used for the production of cereal foods (**Table 2**), as well as in 45 commercial cereal foods commonly consumed in Sweden (**Table 3**). As expected (*6*), ARs were found in high levels in whole grain wheat and rye and in only very low levels in naked barley. ARs were not detected in white wheat flour, but were detected in low levels in sifted rye flour, due to the difficulty of separating the aleurone layer from the rest of the endosperm in rye (*16*). The C17:0/C21:0 ratio was consistent for all wheat-based ingredients (∼0.1) and rye-based ingredients (∼1.0). Commercial sifted rye flour (40% sifted rye flour and 60% sifted wheat flour) has a C17:0/C21:0 ratio of ∼0.5, which is believed to be due to the presence of ARs in the sifted wheat flour used. Thus, the C17:0/C21:0 ratio appears to be a good indication of whether a flour or cereal product contains wheat or rye or a combination of these two cereals.

A range of cereal products made from whole grains, refined grains, and grains with added wheat or rye bran, including muesli, breakfast cereals, flours, baked products, and extruded cereals products (e.g., pasta), were sampled and analyzed for their AR contents (**Table 3**). Products made from whole grain wheat or rye, for example, crisp bread, had the highest amounts of ARs and were within a range similar to the content in whole grain wheat and rye. Products with added wheat or rye bran also contained high levels of ARs, whereas products made from sifted rye or wheat flour, as well as products made of other cereals, for example, whole grain barley, oat, and rice, had very low or no ARs.

The C17:0/C21:0 ratio in processed whole grain wheat and rye-based foods is similar to that in cereal ingredients (∼0.1 in wheat and ∼1.0 in rye). The C17:0/C21:0 ratio is altered in cereal products containing a mixture of whole grain wheat and rye or their brans, in which case the ratio varies between 0.1

Table 3. Alkylresorcinol Content in Cereal Foods ($n = 45$)

cereal food	total AR[a] (μg/g of DM)	homologue composition (%) C17:0	C19:0	C21:0	C23:0	C25:0	ratio of C17:0 to C21:0
Rye-Based Whole Grain Foods[b]							
rolled grains (100%)	698	26	34	24	10	8	1.1
whole grain flour (100%)	575	23	33	26	11	7	0.9
crisp bread 1	804	23	33	24	11	8	1.0
crisp bread 2	736	23	34	24	10	8	1.0
crisp bread 3	722	24	34	24	11	8	1.0
crisp bread 4	700	24	34	24	11	7	1.0
crisp bread 5	700	23	33	25	11	7	0.9
crisp bread 6	665	24	33	25	11	7	1.0
crisp bread 7	646	24	34	24	11	7	1.0
crisp bread 8	645	24	33	25	11	8	1.0
crisp bread 9	633	23	33	24	11	8	1.0
crisp bread 10	614	26	33	26	10	6	1.0
crisp bread 11	607	24	34	24	11	8	1.0
crisp bread 12	574	22	35	25	10	7	0.9
crisp bread 13	490	23	34	25	11	8	0.9
soft bread 1	526	26	36	25	8	6	1.0
soft bread 2	347	24	37	25	8	6	1.0
soft bread 3	197	24	39	26	7	4	0.9
soft bread 4	251	23	38	27	8	4	0.9
soft bread 5	686	24	36	26	9	6	0.9
soft bread 6	465	25	35	25	9	6	1.0
Wheat-Based Whole Grain Foods[b]							
whole grain flour (100%)	339	4	35	50	8	2	0.1
crisp bread	420	4	34	48	10	3	0.1
soft bread 1	233	5	43	44	6	2	0.1
soft bread 2	353	6	39	46	6	3	0.1
soft bread 3	202	6	40	45	6	3	0.1
soft bread 4	332	5	38	45	9	4	0.1
spaghetti	154	3	51	37	8	1	0.1
Other Cereal Foods[d]							
sifted flour (100%)	nd[c]						
sifted flour (40% rye, 60% wheat)	44	16	30	30	16	8	0.5
crisp bread 1	926	15	33	36	11	5	0.4
crisp bread 2	184	9	35	42	11	3	0.2
soft bread 1	351	21	39	26	8	5	0.8
soft bread 2	519	23	35	26	10	6	0.9
soft bread 3	258	18	38	32	7	4	0.6
pasta (with rye bran)	402	21	30	26	14	4	0.8
muesli 1	143	12	29	40	12	6	0.3
muesli 2	124	11	31	46	9	3	0.2
whole grain barley flour[e]	49	2	18	28	14	38	0.1
breakfast cereal 1	404	3	32	47	13	4	0.1
breakfast cereal 2	678	16	31	33	12	8	0.5
breakfast cereal 3	nd						
breakfast cereal 4	nd						
breakfast cereal 5	nd						
white wheat bread	nd						

[a] Means of triplicates (CV < 5%). [b] Whole grain foods contain ≥51% DM of whole grain wheat or rye. [c] Not detected (detection limit = 5μg/g). [d] Including foods containing <51% of wheat or rye and foods added wheat or rye bran and cereals other than wheat and rye. [e] C25:0 is the dominant homologue in barley.

and 0.9 (**Table 3**). Because barley is not a common food ingredient and because it contains very low levels of ARs, it did not influence the overall amount of ARs or AR homologue profile in the mixed products tested. Moreover, barley differs from wheat and rye by the dominant homologue C25:0 (*17*). Thus, using both the homologue composition and the total AR content, it is possible to estimate whether a given sample contains whole grain wheat or rye or whether it is a mixture of the two.

Correlation between Calculated and Analyzed Alkylresorcinol Content in Cereal Products. The aim of this study was to determine whether a correlation exists between calculated and analyzed AR content in cereal foods. The AR content of 45 cereal foods (**Table 3**) was calculated from the product recipes supplied by the manufacturers and the mean value of AR levels in cereal ingredients (**Table 2**). By plotting analyzed AR content against calculated AR content, a good correlation ($R^2 = 0.91$) was obtained (**Figure 3**) despite the wide variation in AR content in both wheat and rye grains. The good correlation between AR contents in cereal ingredients and products and their similarity in homologue distribution provide further evidence that ARs remain stable during processing.

Use of Alkylresorcinols as Markers of Whole Grain Wheat and Rye in Cereal Foods. Currently, the content of total dietary fiber (TDF) of whole grain wheat has been suggested by the FDA as the benchmark for determining whether a product is "whole grain" (*5*). Other methods used to estimate bran content of cereals, such as ash or ferulic acid, have also been suggested as markers of cereal bran in foods (*18*, *19*). However, these components are not specific for cereal bran, as they are also present in the endosperm, food additives, and many plant foods. Using these essentially Swedish samples, ARs were shown to

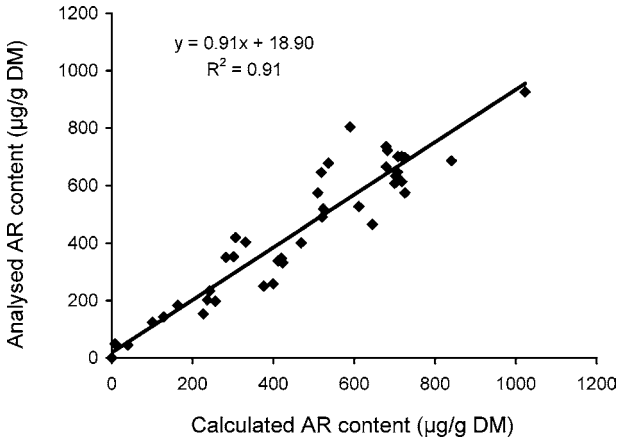

Figure 3. Correlation between calculated alkylresorcinol content based on the average content in cereal ingredients and analyzed alkylresorcinol content in cereal foods ($n = 45$).

be specific markers for the presence of whole grain wheat and/or rye or their bran fractions in foods. However, due to biological variation caused by cultivar or environmental conditions, samples from other countries need to be investigated before ARs can be established as a universal marker. In that case, AR measurements can be an objective way of identifying whole grain wheat or rye in common foods. Moreover, the possibility of rapid analysis of ARs in plasma (*20*) suggests ARs as possible biomarkers of intake of whole grain wheat and rye.

ACKNOWLEDGMENT

We acknowledge Staffan Larsson (SLU, Uppsala, Sweden) for providing wheat grain samples. We thank Dr. Helena Fredriksson (Cerealia AB R&D, Järna, Sweden), Pernilla Ahlbin (Wasabröd AB, Filipstad, Sweden), and Risto Viskari (Fazer AB, Helsinki, Finland) for providing cereal products and information about cereal ingredients in the products.

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Received for review February 19, 2004. Revised manuscript received September 14, 2004. Accepted October 16, 2004. The foundation for Strategic Research (LiFT-program) is acknowledged for their financial support.

JF049726V