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Article

Establishment of Alkylresorcinols Cut-off Point to Screen the 100% Whole Wheat Flour

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Abstract: Alkylresorcinols (ARs) are recognized biomarkers of whole grains, exhibiting biological functions such as antioxidant activity and inhibition of cancer cell proliferation, and correlated with nutrients, including fat, dietary fiber, and carbohydrates. ARs can be regarded as a valuable nutritional indicator for evaluating the nutritional quality of whole wheat flour. **Objectives:** This study aimed to assess the ARs content in Chinese commercial whole wheat flour, establish a nutritional quality cut-off point based on ARs, and screen the 100% whole wheat flour. **Methods:** In this study, high-performance liquid chromatography (HPLC) was used to determine the ARs content in 17 Chinese wheat grains and 22 commercial whole wheat flours. Meanwhile, the ARs content in wheat grains reported in other relevant studies was also included. Then, the ARs cut-off point of whole wheat flour was calculated, and this value was applied to distinguish the nutritional quality of the commercial whole wheat flour and screen the 100% whole wheat flour. **Results:** The average total ARs content in the 22 commercial whole wheat flours was 19.8 mg/100 g, ranging from 2.9 to 77.9 mg/100 g, presenting a bimodal distribution, with 40.9% exceeding the cut-off point. Consequently, approximately 40.9% of commercial whole wheat flour can be classified as 100% whole wheat flour. **Conclusions:** This study provides a nutritional quality cut-off point for Chinese commercial whole wheat flours, indicating a notable variation in ARs content and identifying a substantial proportion that falls short of the established nutritional cut-off point.

Keywords: alkylresorcinols; 100% whole-wheat flour; nutritional quality; cut-off point

1. Introduction

Whole grains have risen to prominence recently, celebrated for their rich dietary fiber, vitamins, minerals, and diverse bioactive compounds [1,2]. Studies have shown that inadequate consumption is associated with a higher risk of chronic non-communicable diseases [3–5]. Refining dietary patterns and bolstering whole grain intake have become key strategies for boosting public health [6,7]. Echoing this sentiment, the Dietary Guidelines for Chinese Residents (2022) also advocate for increased whole grain consumption. Wheat, being the planet's most extensively cultivated food crop [8], and whole wheat flour, a cornerstone of whole grain foods, are particularly significant in this context. With the market for whole wheat flour products in China on the rise, the challenge lies in identifying reliable indicators to accurately and objectively assess the nutritional quality of these commercial offerings.

Alkylresorcinols (ARs) stand out as biomarkers for whole grains, occurring predominantly in the bran of wheat and rye [9]. Their concentration in the germ and endosperm is notably low [10,11]. ARs have been found to correlate positively with a range of macronutrients and micronutrients, including protein, fat, ash, total dietary fiber, vitamin E, B1, B2, total mineral elements, and various fatty acids [14]. In contrast, a negative correlation exists between ARs content and available carbohydrates, positioning ARs as a valuable nutritional indicator for evaluating the nutritional quality of whole wheat flour. This study sets out to establish a cut-off point for nutritional quality assessment of commercial whole wheat flours using ARs as a marker, with an evaluation of 22 such products.

2. Materials and Methods

2.1. Calculation of the ARs Cut-Off Point for Whole Wheat Flour

2.1.1. ARs Levels in Wheat Grains Samples

For the purpose of calculating the ARs cut-off point of whole wheat flour, seventeen samples of wheat grains were collected in this study. Seventeen samples of wheat grains (*Triticum aestivum*), grown in Shaanxi Province, Shandong Province, and Heilongjiang Province of China, including "Zhoumai 26," "Zhoumai 27," "Xinong 822," "Xinong 3517," "Xinong 979," "Jinmai 47," "Jinmai 54," "Xiaoyan 22 (Xi'an)," "Xiaoyan 22 (Xianyang)," "Xiaoyan 22 (Baoji)," "Aikang 58," "Zhengmai 366," "Yumai 49," "Bainong 207," "Longmai 33," "Longmai 35," and "Shannong 17." According to the industry standard "Whole Wheat Flour" (LS/T 3244-2015), these wheat grain samples were processed into whole wheat flour.

2.1.2. ARs Content in Wheat Grains Reported in Other Studies

For more accurately calculating the cut-off point of ARs, it is also necessary to incorporate and analyze the ARs content in wheat grains reported in other studies. This additional analysis will provide a more comprehensive understanding of the ARs distribution, enabling a more refined determination of the ARs cut-off point. A literature search was conducted in PubMed, China National Knowledge Infrastructure (CNKI), and Wan Fang Data using the keywords "alkylresorcinols" and "wheat." Studies with unclear specific ARs content in wheat varieties were excluded.

2.1.3. Calculation of the ARs Cut-Off Point

Aggregate the ARs content in wheat grains and describe their distribution. Referring to "Establishment of Reference Intervals for Quantitative Inspection Items in Clinical Laboratories" (WS/T 402 - 2024), calculate the cut - off point with 95% probability, using $\geq 5\%$ value as the ARs cut-off point.

2.2. ARs Levels in Commercial Whole Wheat Flour and Application of ARs Cut-Off Point

2.2.1. ARs Levels in Commercial Whole Wheat Flour Samples

Twenty-two commercial whole wheat flour samples were collected according to sales rankings in 2023. These included 21 brands such as "Hetao," "Jinshahe," "Golden Statue," "Beidahuang," and "Arawana." All these products are claimed to be "whole wheat flour."

2.2.2. Application of ARs Cut-Off Point

The nutritional quality of 22 commercial whole wheat flours was distinguished using the ARs cut-off point. The parts of the flours meeting the cut-off point were identified to determine the proportion of high nutritional quality ones among them. Whole grain and 100% replenished whole wheat flour were provided by China Oil and Foodstuffs Corporation (COFCO). These two samples of whole wheat flour were utilized for the verification of the cut-off point.

2.3. Chemicals and Reagents

Synthetic alkylresorcinols (C17:0, C19:0, C21:0, C23:0, and C25:0, Shanghai Yuanye Bio-Technology Co., Ltd, Shanghai, China) were used for confirmation of peak identity by HPLC. Acetonitrile and ethanol from Fisher Chemical were also used.

2.4. Extraction and Analysis of Alkylresorcinols

ARs extracted from whole wheat flour were analyzed using the HPLC method of Yufei Wang et al. [16]. Two grams of sample was ultrasonic for 30 min in 50mL ethanol and then centrifuged for 5 min at 4200 rpm. The supernatants were filtered and analyzed by HPLC as per Yufei Wang et al. [16]. All quantifications were based on the peak area, and the sample was analyzed in triplicate.

2.5. Statistical Analysis

All values are reported on a dry matter (DM) basis and each sample was extracted and analyzed in duplicate. The total ARs content of 17 wheat grains in China was expressed as mean and standard deviation, the total ARs content of wheat in all retrievable and applicable studies was expressed as median and quartile, and the total ARs content of 22 commercial whole wheat flours in China was expressed as median and quartile. In order to clarify the distribution of total ARs in the 22 commercial whole wheat flours, probability density plots were drawn using Origin 2022 as the plotting software. Statistical analyses were performed using IBM SPSS v.26.0. All plots were generated using Origin2022.

3. Results

3.1. ARs Levels in 17 Wheat Grains of China

The total ARs content of 17 different varieties or origins of wheat grains ranged from 46.7 to 70.5 mg/100g. The average total ARs content was 60.3 mg/100g, with a standard deviation of 7.3 mg/100g. The 95% confidence interval (CI) was 56.6 - 64.1 mg/100g. Among the 17 wheat grains, AR C21:0, as the primary homologues of ARs, accounted for a proportion ranging from 36.7% to 56.5%. AR C19:0 was the second most abundant homologue, with a proportion ranging from 26.1% to 39.1%. AR C23:0 had a proportion ranging from 6.6% to 13.7%. AR C17:0 had a proportion ranging from 5.0% to 8.6%. Moreover, AR C25:0 had a proportion ranging from 4.4% to 8.2% (Figure 1).

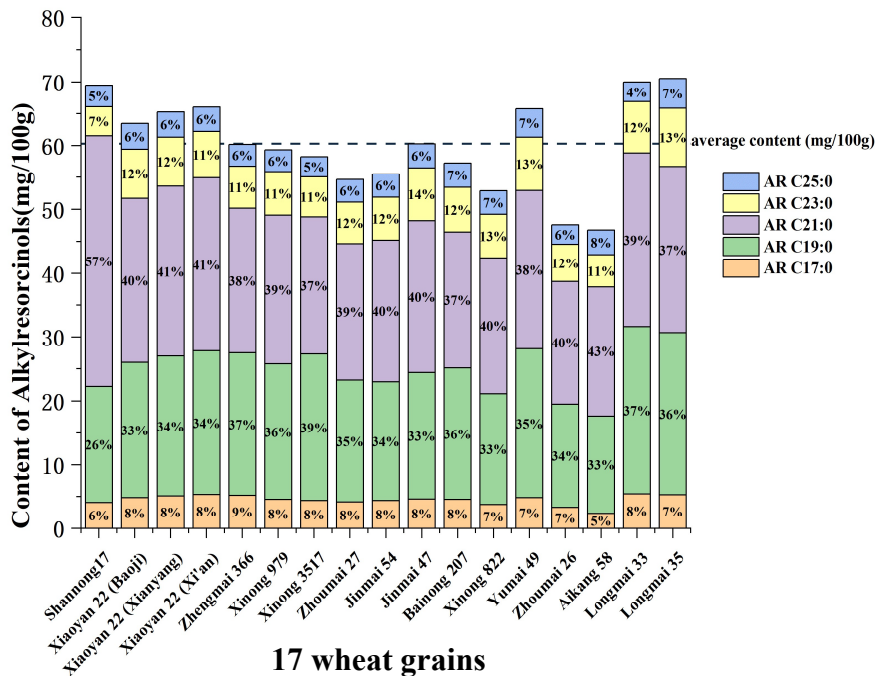


Figure 1. Distribution of alkylresorcinols content in wheat grains (n=17) grown at different locations in China.

This section may be divided by subheadings. It should provide a concise and precise description of the experimental results, their interpretation, as well as the experimental conclusions that can be drawn.

3.2. Calculation of the Cut-Off Point of ARs Content in Whole Wheat Flour

For a more accurate calculation of the cut-off point of ARs content in whole wheat flour, the total ARs content of wheat grains from other research reports needed to be included for a unified analysis. After various literature searches followed the 2.2 method, nine other research papers were ultimately included. The origins of wheat-covered countries include the United States, Canada, France, Poland, Sweden, and Hungary. The detection methods included gas chromatography (GC), spectrophotometry, and high-performance liquid chromatography (HPLC) (Table 1).

Table 1. Contents of Alkylresorcinols in other studies.

country	n	range of total ARs content (mg/100g of DM)	references
China	2	103.6、 107.3	Feiyang Xiong [13]
Sweden	32	22.7~63.9	Chen Y [8]
Sweden	3	48.9~64.2	Ross A B [7]
Austria, France, Kazakhstan, Russia, Spain, Sweden	21	25.1~61.8	Landberg R [14]
United States 、 Canada	59	30.8~65.5	Hengtrakul P [15]
Hungary	176	41.0~60.5	Andersson A A M [16]
Poland	10	56.5~87.9	Kulawinek M [17]
Italian	15	73.7~133.3	Pedrazzani C [18]

The total ARs content values of 17 Chinese wheat grains determined in this study were combined with those of wheat grains from other studies. The distribution range of total ARs content values in wheat grains was obtained as 22.7 -132.1 mg/100g. It was found to show a non-normal distribution and was expressed as 41.0 (41.0, 49.4) mg/100g using the median and quartile.

Referring to the principle of establishing reference intervals for clinical laboratory indicators in the industry standard "Establishment of Reference Intervals for Quantitative Inspection Items in Clinical Laboratories" (WS/T 402 - 2024), to determine the total ARs content limit value in whole wheat flour with a probability of more than 90%, the P5 (35.0 mg/100g) of this set of data was calculated. Then, it was found that ≥ 35.0 mg/100g could ensure that 95% of the ARs content values in wheat were included. A histogram was plotted for the above data to show its distribution level (Figure 2). When the ARs content in whole wheat flour meets the cut-off point, the flour can be considered 100% whole wheat flour.

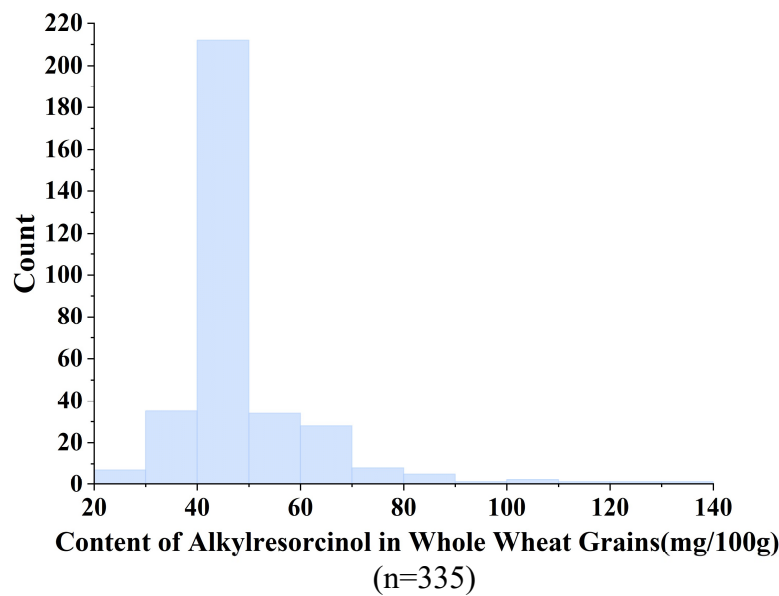


Figure 2. Distribution of alkylresorcinols content in wheat grains.

3.3. ARs Levels in Commercial Whole Wheat Flour and Application of ARs Cut-Off Point

In this study, the total ARs content of 22 commercial whole wheat flours was measured. The results indicated that the total ARs content values of these flours fluctuated within the range of 2.9 to 77.9 mg/100g, with a median of 19.8 mg/100g and quartiles of (7.2, 54.0) mg/100g (Table 2). The total ARs content values in commercial whole wheat flours displayed a bimodal distribution, mainly concentrating in two ranges: 0.0-20.0 mg/100g and 50.0-80.0 mg/100g (Figure 3). These data reveal a significant inconsistency in the distribution of total ARs content in commercial whole wheat flour. AR C21:0 remained the most abundant homologue in commercial whole wheat flours, ranging from 50.8% to 68.5%, followed by AR C21:0, ranging from 25.3% to 32.8%.

Table 2. Distribution of alkylresorcinols content in commercial whole wheat flours (n=22) in China(mg/100g).

ID	ARs					Total ARs
	C17:0	C19:0	C21:0	C23:0	C25:0	
XM01	0.0	0.9	2.0	0.0	0.0	2.9
XM02	3.9	15.9	31.2	4.5	3.3	58.8
XM03	0.4	1.6	3.0	0.0	0.0	5.0
XM04	0.7	2.5	5.2	0.8	0.0	9.2
XM05	3.3	13.6	27.3	3.4	2.1	49.8
XM06	4.6	19.0	39.4	5.0	3.1	71.1
XM07	4.3	17.2	31.2	4.2	2.9	59.8
XM08	0.8	2.9	4.7	0.7	0.0	9.1
XM09	4.7	20.7	43.7	5.5	3.3	77.9
XM10	0.4	1.5	2.7	0.0	0.0	4.5
XM11	0.5	1.9	4.0	0.7	0.0	7.2
XM12	0.6	2.5	5.1	0.8	0.0	9.0
XM13	3.7	12.9	23.7	3.6	2.8	46.7
XM14	2.0	8.2	15.3	2.1	1.6	29.3
XM15	4.3	18.7	39.0	5.0	3.0	69.9
XM16	2.0	7.7	14.6	2.4	1.9	28.6
XM17	3.5	14.2	29.6	4.0	2.7	54.0
XM18	0.4	1.2	2.1	0.0	0.0	3.7

XM19	0.8	3.3	6.0	0.8	0.0	10.9
XM20	0.3	1.1	1.9	0.0	0.0	3.3
XM21	2.9	12.5	26.4	3.4	2.1	47.4
XM22	0.7	1.8	3.7	0.6	0.4	7.2
median	1.4	5.5	10.3	1.5	1.0	19.8
quartile	(0.5,3.7)	(1.8,14.2)	(3.7,29.6)	(0.6,4.0)	(0.0,2.8)	(7.2,54.0)

Note: XM1-22 refers to 22 commercial whole wheat flours.

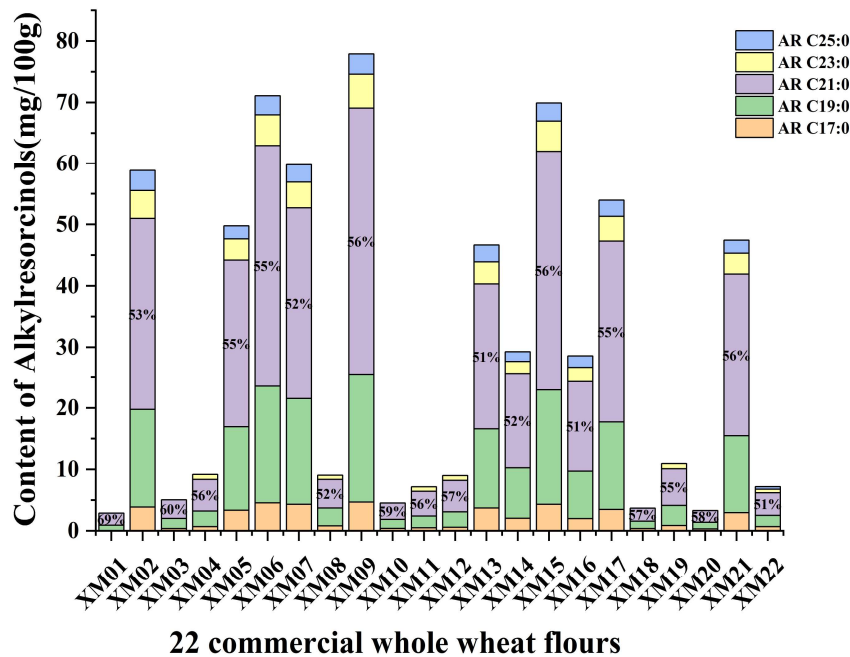
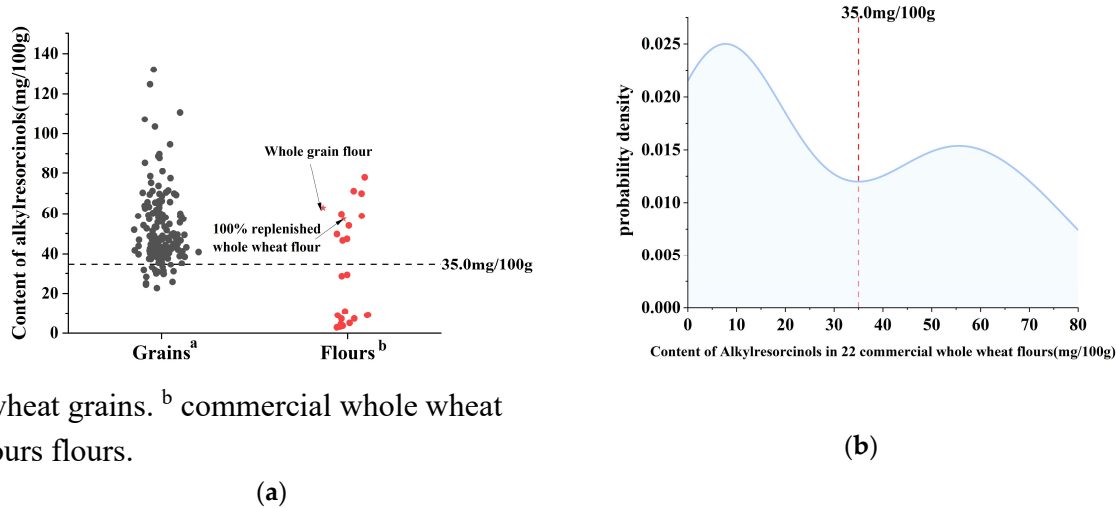


Figure 3. Distribution of alkylresorcinols content in commercial whole wheat flours (n=22) in China.

Based on the ARs content cut-off point of $\geq 35.0\text{mg}/100\text{g}$ in whole wheat flour, this cut-off point can differentiate the portion with higher total ARs content ($50.0\text{--}80.0\text{mg}/100\text{g}$) in 22 commercial whole wheat flours, as well as whole-grain whole wheat flour and 100% replenished whole wheat flour (Figure 4(a)). From the probability density graph (Figure 4(b)), it can be observed that the cut-off point lies precisely in the trough between the two peaks, enabling the screening of 100% whole-wheat flours. Using this point to evaluate the nutritional quality of 22 commercial whole wheat flours reveals that 40.9% meet the requirement.



^a wheat grains. ^b commercial whole wheat flours.

Figure 4. Cut-off point to differentiate commercial whole wheat flours. (a) Distribution of ARs content in wheat grains and commercial whole wheat flours; (b) Distribution of the probability density of ARs content distribution in 22 commercial whole wheat flours and use the cut-off point to screen those with high nutritional quality.

4. Discussion

Whole grains are currently defined chiefly in terms of grain structure. For instance, the American Association of Cereal Chemists (AACC) proposed that whole grains are defined as intact, milled, broken, or flaked glumes. The elemental composition consists of starchy endosperm, germ, and bran, and the relative proportions of the components are the same as those of intact glumes. The definition of whole grains proposed by the American Whole Grains Council is the same as that of the AACC. The difference in expression is that it requires the balance of nutrients in whole grains to be similar to that of natural grains rather than the same [23]. The European Health Grain Association defines whole grains as grains after removing inedible parts (such as husks and valueless substances) that are intact, ground, broken, or flaked. The proportion of the main structural components (starchy endosperm, germ, and bran) should be the same as that of the whole grain [24]. The expression of the concept of whole grains in the group standard "Whole Grains and Whole Grain Food Determination and Labeling General Rules" issued by the Chinese Nutrition Society is that after cleaning but without further processing, the grain kernels with intact caryopsis structure are retained. Although they have undergone grinding, crushing, extrusion, and other processing methods, the relative proportions of the cortex, endosperm, and germ remain consistent with the intact caryopsis. The definitions of whole grains by domestic and foreign scientific research organizations mainly divided whole grains into endosperm, germ, and bran based on the grain kernel structure, and the relative proportions of these three parts were required to be consistent with the intact grain.

The processing and production of whole wheat flour are mainly divided into the whole grain grinding and backfilling methods [25]. The whole grain grinding method directly crushes the whole wheat kernel, and the processing process does not involve the removal of bran [26]. The backfilling method separately stabilizes and crushes the by-products, such as bran and germ, after the production of refined wheat flour. It then backfills them into refined wheat flour according to a certain proportion to make whole wheat flour [27]. The whole wheat flour produced by the whole grain grinding method was most in line with the definitions of whole grains by various organizations mentioned above [28]. However, the high-intensity grinding of the whole grain method quickly led to a high content of damaged starch, gelatinization, protein denaturation, and high enzyme activity. As a result, the whole wheat flour produced by this method took much work to store [29].

On the other hand, whole wheat flour produced by the backfilling method was more accessible to store [29] and had higher product quality. Therefore, the backfilling method of whole wheat flour is the current mainstream production method [30]. In actual processing, the nutritional quality of commercially available whole wheat flour varied due to the different backfilling proportions of bran and germ.

Alkylresorcinols, as phenolic compounds mainly present in bran [31], have various biological functions such as antioxidation [32–34], improvement of insulin resistance [35], inhibition of the proliferation of various cancer cells such as colon cancer, liver cancer, breast cancer, and lung cancer [36–41], protection of nerve cells [42,43], and antifungal properties [44,45]. At the same time, they are biomarkers in whole wheat flour [12] and have a significant correlation with other nutrients [14]. The rapid detection of alkylresorcinols in whole wheat flour can be achieved using liquid chromatography with fluorescence detection. Thus, alkylresorcinols can be used as a practical nutritional quality control indicator for whole wheat flour to determine the nutritional quality of whole wheat flour.

Based on the determination of ARs content in wheat grains, the calculated ARs content cut-off point was $\geq 35.0 \text{ mg}/100 \text{ g}$. This cut-off point was verified by using 22 commercial whole wheat flours. The cut-off point is exactly located in the trough between the two peaks and is capable of screening out 100% whole-wheat flour. At the same time, in this study, the ARs content of the whole grain flour

produced by the whole grain grinding method and the 100% replenished whole wheat flour provided by COFCO was determined. The test results showed that the ARs content of the whole grain whole wheat flour was 62.9mg/100g, and the ARs content of the 100% backfilled whole wheat flour was 57.3mg/100g. Both of them were above this cut-off point, further verifying the feasibility of the ARs cut-off point. Therefore, the cut-off point established in this study can be used to screen out 100% whole - wheat flour. When evaluated by this point, 22 commercial whole wheat flours showed that 40.9% met the cut-off point. Nine brands of whole wheat flour meeting this standard can be identified as 100% whole-wheat flour. In the current market, there are whole wheat flours with high nutrition quality, and there is also a need to improve the nutrition quality of some whole wheat flours.

5. Conclusions

In this study, the cut-off value for alkylresorcinols (ARs) in whole wheat flour was calculated as ≥ 35.0 mg/100g through statistical analysis of total ARs in wheat grains. This point was verified by using commercial whole wheat flour and whole wheat flour processed by two different methods. It was demonstrated that this point could be applied to screen 100% whole wheat flour. The content of alkylresorcinols in 22 commercial whole wheat flours exhibited a bimodal distribution. When evaluating the nutrition quality of 22 commercial whole wheat flours using this point, 40.9% of them met this cut-off point.

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Conflicts of Interest: The authors declare no conflicts of interest.

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