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Allium Species, Ancient Health Food for the Future?

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1. Introduction

Allium is the largest and the most important representative genus of the *Alliaceae* family that comprises 700 species, widely distributed in the northern hemisphere, North America, North Africa, Europe and Asia (Tsiaganis et al., 2006). Besides the well known garlic and onion, several other species are widely grown for culinary use and for folk medicine including leek (*Allium porrum* L.), scallion (*Allium fistulosum* L.), shallot (*Allium ascalonicum* Hort.), wild garlic (*Allium ursinum* L.), garlic (*Allium sativum*) and onion (*Allium cepa*) (Lanzotti, 2006; Tsiaganis et al., 2006). Its consumption is attributed to several factors, mainly heavy promotion that links flavour and health. The powerful and unusual flavors of many of these plants and their possible nutritional impact and medical applications have attracted the attention of plant physiologists, chemists, nutritionists, and medical researchers (Graham and Graham, 1987).

Allium roseum is a very polymorphous, widespread species that is represented by 12 different taxa: 4 varieties, 4 subvarieties and 4 forms in North Africa (Cuénod, 1954; Le Floc'h, 1983). In Tunisia, the same authors mentioned the presence of only three varieties: var. *grandiflorum*, var. *perrotii* and var. *odoratissimum*. The *odoratissimum* variety is an endemic taxon in North Africa and a perennial spontaneous weed (Cuénod, 1954). Its flowering stem is about 30-60 cm, leaves are fleshy and very small, flowers are wide, rosy or white coloured and its odour is eyelet (Jendoubi et al., 2001).

In Southern Tunisia, local people on the extension area where *A. roseum* or rosy garlic occurs have extensively developed uses for this species both as a cooking ingredient and a sauce (Najjaa et al., 2011a). *A. roseum* leaves are the main edible part, with a distinctive pungent odour and strong flavour. Besides its culinary use, rosy garlic is also used in folk medicine. Le Floc'h (1983) reported its use for the treatment of headaches and rheumatism. It is also used for the treatment of bronchitis, colds as an inhalation, fever diminution and as an appetizer.

While several studies have provided information about *A. roseum*, detailed studies documenting compositional, nutritional and functional properties are very limited, if not

lacking. The objective of the present study was to characterize chemical composition, nutritional properties, bioactive components, and antioxidant and antimicrobial activities of *A. roseum* grown in Tunisia and to infer their role in human nutrition.

All samples were collected on the same day. To preserve freshness, *A. roseum* samples were transported to the laboratory on ice. Upon arrival, leaves of *A. roseum* samples were cleaned to remove all foreign matter and washed with distilled water. For the analysis of moisture content, pH, carotenoids, vitamin C, and anthocyanidins, samples were frozen and stored at –80 °C until analysis. A second portion of samples was kept fresh, and the leaves were extracted with methanol, then evaporated at 40 °C using a rotary evaporator under high vacuum. The resulting crude extract was used for total phenolic content and flavonoids composition analysis. Fresh leaves were also used to prepare an aqueous extract for allicin determination. The third portion of *A. roseum* samples was air dried in the shade for 24 h at 45 °C. From these air-dried samples, plants were chopped into pieces $\leq$ to 5 cm and ground in a Sorvall Omnimixer into a fine powdery consistency and used for other chemical analyses. The powder sample was packed in a hermetic glass vessel and stored at +4 °C for subsequent analyses. In this study, all methodologies used and data presented are in accordance with FAO standards (Greenfield and Southgate, 2003).

The *A. roseum* methanolic extracts were tested against a panel of spoilage and pathogenic bacteria strains, including the Gram positive *Staphylococcus aureus* ATCC 25923, and *Enterococcus fecalis* ATCC 29212, and the Gram negative *Escherichia coli* ATCC 25922, as well as the yeast *Candida albicans* ATCC 90028, *Candida glabrata* ATCC 90030, *Candida kreusei* ATCC 6258, *Candida parapsilosis* ATCC 22019. The cultures were incubated at 37°C (27°C for the yeast) for 18 h and then diluted in Nutrient Broth to obtain 10^6 CFU/mL.

The recommended methods of the Association of Official Analytical Chemists (AOAC, 1995) were adopted to determine the level of crude protein, water content, ash, carbohydrates, fibres and lipids. Nitrogen content was determined using the Kjeldahl method (AOAC, 1995) and multiplied by a factor of 6.25 to determine the total protein content. Water content was estimated by drying the sample to a constant weight at 70±2 °C (AOAC, 2002). Ash was determined by the incineration of 1.0 g sample in a muffle furnace, at 550 °C for 6 h (Alfawz, 2006). Fibres content was quantified using 2 g sample previously boiled with diluted H_2SO_4 (0.3) using Wende method (AOAC, 1995). Soluble carbohydrates were determined by the phenol-sulphuric acid colorimetric method (AOAC, 1995). Total carbohydrates were calculated by difference as follows: Carbohydrates (%) = 100 - [Proteins (%) + Lipids (%) + Ash (%) + Fibres (%)], according to Alfawz (2006). The ash obtained underwent an acidic hydrolysis and the minerals (Ca, Na, K, Mg, Fe, Zn, Cu, Ni, Mn, Pb, Cd and Cr) were determined separatively, using an atomic absorption spectrophotometer (Hitachi Z6100). Phosphorus content was determined using a spectrophotometer method, based on phosphoric molybdovanadate absorption at 730 nm according to Falade, Otemuyiwa, Oladipo, Oyedapo, Akinpelu and Adewusi (2005).

Fatty acids composition was analysed by gas chromatography. The fatty acids were previously methylated to esters using a born trifluoride methanol complex (14% w/v). The mixture was held one hour at 100 °C.

For the bioactive compound, the total phenolic content of *A. roseum* methanolic extract was determined using Folin-Ciocalteu reagent (Fattouch et al., 2007). Total flavonoids were measured by a colorimetric assay according to Galvez, Martin-Cordero, Houghton

and Ayuso (2005). The vitamin C content was analyzed with 2, 6-dichloroindophenol titrimetric method. Total anthocyanidins were determined using the Reay, Fletcher and Thomas (1998) method. Colorimetric quantification of total carotenoids was determined, as described by Mackinney (1941). Allicin determination was based on Miron et al. (2002) method.

Various concentrations (1 µg/ml up to 10 mg/ml) of *A. roseum* extracts were used to determine the antimicrobial and antifungal activity. Minimum inhibitory concentration (MIC) values were determined by a micro-titre plate dilution method.

The assessment of radical scavenging activity was determined using ABTS (2, 2'-Azino-(bis-3-ethylbenzthiazoline-6-sulfonic acid) di-ammonium salt) radical scavenging activity of the methanolic extracts was determined according to Re, Pellegrini, Proreggente, Pannala, Yang and Rice-Evans (1999). The results were expressed in terms of Trolox equivalent antioxidant capacity (TEAC). The antioxidant activity of the extracts was also evaluated using the DPPH (2, 2-diphenyl-2-picrylhydrazyl) free radical specrophotometrically according to Fattouch et al. (2007).

All analysis mentioned were effected with quality control, than a proper sampling plan was followed with representative samples from the geographic area studied and sufficient replications of the sample were used to ensure statistically reliable and valid data. The analyses of the nutrient contents samples were made in our laboratory where ISO/CEI 17025 (2005) was respected to assure the quality of results.

2. Results and discussion

2.1 Nutritional composition

The proximate chemical and nutritional composition of *A. roseum* edible part collected from Tunisia is listed in Table 1.

Components	Mean Value*
Soluble carbohydrates (g/100 g DW[a])	32.80 ± 0.21
Protein (g/100 g DW[a])	22.70 ± 1.51
Fibre (g/100 g DW[a])	12.30 ± 0.05
Ash (g/100 g DW[a])	7.20 ± 1.31
Fat (g/100 g DW[a])	3.60 ± 0.29

*Values are means ± SD, $n = 3$.
[a]: Dry weight

Table 1. Content of soluble carbohydrates, protein, fibre, ash and fat in *Allium roseum* L. expressed as g/100 g of dry weight basis. (Moisture content 81.2 ± 2.6 g/100 g fresh weight).

2.1.1 Water content and pH

Water content is important because it affects the plant's properties. Compared to related vegetables, the *A. roseum* water content is lower than that of *A. porrum* varing from 83 to 89% (Tirilly and Bourgeois, 1999) and the *A. cepa* (89%) (Dini et al., 2008). The *A. roseum* is rather

neutral (pH = 6.80 ± 0.05) compared to that of garlic (pH = 6.05) (Haciseferoğullari et al., 2005).

2.1.2 Sugars, proteins, fibres and lipids

Soluble carbohydrates represent the most abundant *A. roseum* leaves nutrients class (> 30%); as has also been observed in onion bulbs (Moreau et al., 1996) and garlic (Haciseferoğullari et al., 2005). The total carbohydrates content in this species calculated by difference is 54.2 g/100 g DW. Compared to the carbohydrates content of *A. porrum* (5 to 11%) (Tirilly and Bourgeois, 1999) and to aerial parts of other *Alliums* (5 to 12%) (Brewster, 1994), the leaves of *A. roseum* are rich sources of soluble carbohydrates. Dietary fibres are considered as unavailable carbohydrates, but nonetheless they still play a very important role in maintaining good health. Interestedly, A. *roseum* aerial part fibres content was higher than that reported for *A. cepa* bulb (1.7%), the edible part of the vegetable. Rosy garlic leaves proteins rate is relatively high compared to *A. sativum* bulbs (9.3%) and *A. cepa* (1.7%) (Haciseferoğullari et al., 2005; Dini et al., 2008). Fats accounted for 0.68% of the fresh weight of *A. roseum*, making them the least abundant class of nutrients. Yet this was higher than typical values of <0.5% fresh weight basis for most plant tissues, and also compared to most *Allium* plants. Where onion, leek and garlic contain 0.15%, 0.25% and 0.42%, respectively, as reported by Haciseferoğullari et al., (2005) and Tsiaganis et al., (2006).

2.1.3 Minerals

A. roseum is characterized by high ash content (Table 1) including macro and micro elements (Table 2). *Allium* ash content ranges from 0.6 and 1.0% and higher values are associated to high dry matter content (Brewster, 1994). The mineral element composition of *A. roseum* exhibited a higher concentration of potassium than calcium and magnesium (Table 2). Minerals are important as constituents of bones, teeth, soft tissues, haemoglobin, muscle, blood and nerve cells and are vital to overall mental and physical well being (Jouanny, 1988). The high content of potassium in *A. roseum* is nutritionally significant in since it contributes to the control of hypertension which results in excessive excretion of potassium (Dini et al., 2008). Calcium is found at relatively high concentration in *A. roseum* (Table 2). Onion leaf calcium concentration (2540 mg/100 g fresh weight) (Boukari et al., 2001) is much higher than that of *A. roseum* leaves but bulb calcium concentration (45 mg/100 g fresh weight) (Adrian et al., 1995) is much lower. Therefore, calcium concentration in *A. roseum* is between that of onion leaves and bulbs. *A. roseum* can be considered as a source of calcium for human nutrition. This is important since calcium mineral deficiency is a world-wide problem; particularly in developing countries where the daily average intake is very low, ranging between 300 and 500 mg for adults (Boukari et al., 2001). The low sodium content of A. *roseum* and consequently low Na/K ratio (0.03) is another indication that *A. roseum* consumption would reduce the incidence of hypertension (Iqbal et al., 2006). *A. roseum* leaves also contains several oligo-elements including iron, zinc, copper and manganese. These values are similar to, but higher than, those of Haciseferoğullari et al., (2005) in *A. sativum.* The iron content of 'rosy garlic' was somewhat higher than that of *A. cepa* (8.1 mg/ 100 g) (Moreau et al., 1996). The magnesium, iron and phosphorous levels are adequate. Cadmium, lead and chromium were below the detection limit, as observed by Moreau et al., (1996) for onion.

Component	Concentration*
Major elements	
Potassium	1530.500 ± 0.036
Calcium	712.500 ± 0.048
Magnesium	101.900 ± 0.007
Sodium	46.500 ± 0.003
Na:K ratio	0.030
Anions	
Chlorides	724.00 ± 0.01
Sulfates	437.00 ± 0.03
Phosphates	219.00 ± 6.40
Nitrates	< 0.03
Heavy metals	
Iron	10.110 ± 0.002
Manganese	2.000 ± 0.001
Zinc	1.800 ± 0.001
Copper	1.100 ± 0.006
Nickel	< 0.013
Lead	< 0.035
Cadmium	< 0.007
Chromium	< 0.006

*Values are means ± SD, $n = 3$.

Table 2. The mineral content of *Allium roseum* expressed in mg/100 g of dry weight basis

2.1.4 Fatty acid composition

The fatty acid composition of *A. roseum* leaves is given in Table 3. Chromatographic analysis revealed twelve compounds. Unsaturated fatty acids accounted for most of the fatty acids (85 %) and were represented mainly by linolenic, linoleic, oleic and gadoleic. Five saturated acids (palmitic, stearic, myristic, arachidic and margaric), accounted for ~15% of the total fatty acids. Myristoleic, palmitoleic and heptadecanoic acids were found as minor compounds. The overall fatty acid profile of *A. roseum* reveals a good source of the nutritionally essential linolenic and oleic acids (Zia-Ul-Haq et al., 2007). Linoleic and linolenic acids are the most important essential fatty acids required for growth, physiological functions and maintenance (Pugalenthi et al., 2004). While the major fatty acid in *A. roseum* was linolenic acid, linoleic acid was most abundant in onion, garlic and leek where it represents about 50% of the total (Tsiaganis et al., 2006). The same authors demonstrated that garlic oils contain relatively high levels of linoleic acid, and that myristoleic acid ($C_{14:1}$) was absent in onion. As a consequence, the *A. roseum* fatty acid composition quality is comparable to that of *A. sativum* (Tsiaganis et al., 2006). It could be concluded that fatty acid composition varies within the species. We may note that the most abundant fatty acids are similar to those found in the oil of other *Allium* species. The less abundant fatty acids are present in *A. roseum* but at a lower concentration than reported by Tirilly and Bourgeois (1999) in *A. porrum*, Moreau et al. (1996) in *A. cepa* and Tsiaganis et al. (2006) in *A. sativum*. Overall, the *A. roseum* fatty acid composition was not qualitatively different from that of the other species.

Fatty acid	Percentage*
Myristic ($C_{14:0}$)	0.78 ± 0.11
Myristoleic ($C_{14:1}$)	0.12 ± 0.08
Palmitic ($C_{16:0}$)	12.82 ± 0.33
Palmitoleic ($C_{16:1}$)	0.50 ± 0.18
Margaric ($C_{17:0}$)	0.16 ± 0.13
Heptadecanoic ($C_{17:1}$)	0.15 ± 0.12
Stearic ($C_{18:0}$)	0.98 ± 0.23
Oleic ($C_{18:1}$)	2.87 ± 0.70
Linoleic ($C_{18:2}$)	25.68 ± 0.44
Linolenic ($C_{18:3}$)	52.68 ± 0.41
Arachidic ($C_{20:0}$)	0.19 ± 0.17
Gadoleic ($C_{20:1}$)	2.55 ± 0.32
Total	
Saturated	14.93 ± 0.11
Monounsaturated	6.19 ± 0.11
Polyunsaturated	78.37 ± 0.11

*Values are means ± SD, n = 3.

Table 3. Fatty acid composition of *Allium roseum*

2.2 Bioactive compounds

The content of potential health-promoting substances, flavonoid, total phenolic content, vitamin C, tannin, anthocyanidin, carotenoids and allicin in the wild *A. roseum* growing in the arid region of Tunisia is listed in Table 4.

2.2.1 Phenolic compounds, flavonoids, anthocyanidins, carotenoids and vitamin C contents

Total phenolic content of *A. roseum* expressed in equivalent catechol was higher than that reported for garlic (61.8 mg/100 g FW) and onion (31.0 mg/100 g FW) (Kaur and Kapoor, 2002). Although, shallots had the highest total phenolic content (114.7 mg/100 g) among the bulb onion varieties tested by Lanzotti (2006). However, this content is lower than that of rosy garlic leaves. Significant correlations were observed between the total phenolic content of *A. roseum*, and antioxidant activity, suggesting that phenolic compounds would be the major contributors to the antioxidant capacity of *A. roseum* (Najjaa et al., 2011a). Moreover, tannins and flavonoids were detected by several other authors and are usually less abundant in several other species of *Allium (A. cepa*, *A. ascalonicum*, *A. sativum) (*Bozin et al., 2008; Zielinskaa et al., 2008). Flavonoids are important secondary plant metabolites. The flavonoids content of rosy garlic is seven time that of garlic (0.5 mg/100 g) (Miron et al., 2002). *Allium* species are among the richest sources of dietary flavonoids and contribute significantly to the overall intake of flavonoids (Slimestad et al., 2007). *In vitro* and *in vivo* pharmacological tests have shown that flavonoids exhibit the following variety of actions: (i) antioxidative (Boyle et al., 2000); (ii) reduction of cardiovascular disease (Janssen et al., 1998) and (iii) reduction of carcinogenic activity (Steiner, 1997).

The high *A. roseum* vitamin C content (1523.35 mg/100 g DW) may be an important reason that it has been reputedly used as a traditional Tunisian medicine for treating rheumatism and cold. Furthermore, its high vitamin C content confers considerable nutritional value. *A. roseum* leaves had high anthocyanidin content (1239.62 µg/100 g DW). Much is known about the anthocyanins of *A. cepa* bulbs, and leaves of *A. victorialis* and *A. schoenoprasum* (Terahara et al., 1994; Fossena et al., 2000; Slimestad et al., 2007). Moreover, *A. roseum* had a typical carotenoids content (Table 4) of leafy vegetables, which is higher than those of legumes and fruits (Combris et al., 2007).

Substances	Mean value*
Phenolic compounds (mg CA/100g DW [ab])	736.65 ± 1.51
Flavonoids (mg CE/g DW [ac])	3.37 ± 0.32
Anthocyanidin (µg CE/100 g DW [ac])	1239.62 ± 6.79
Vitamin C (mg/100 g DW [a])	1523.35 ± 74.72
Total Carotenoids (µg/100 g DW[a])	242.25 ± 48.84
Allicin (mg / 100g DW [a])	657.00 ± 0.49

*Values are means ± standard deviations of triplicate determination (Mean ± SD (n = 3)).
[a] DW = dry weight
[b] Total phenolic contents expressed as as mg catechol (CA) equivalents per gram of dry weight
[c] Total flavonoid and anthocyanidin content were expressed as mg catechin (CE) /100 g dry weight

Table 4. *Allium roseum* L. var. *odoratissimum* bioactive substances content.

2.2.2 Allicin content

Garlic antibacterial bioactive principal was identified as diallylthiosulphinate and was given allicin as trivial name since 1944. This bioactive substance is also detected in *A. roseum* with a concentration equivalent to 0.0328 µg/mL. This result is similar to that mentioned by Miron et al. (2002) in garlic (0.0308 µg/mL). Allicin (diallylthiosulfinate) is the most abundant organosulfurous compound, representing about 70% of the overall thiosulfinates formed upon garlic cloves crushing (Miron et al., 2002).

2.3 Antioxidant activity

The antioxidant activities of leaf extracts were assessed and confirmed using two functional analytical methods based on the radicals (ABTS and DPPH) scavenging potential, as recommended by Sànchez-Alonso et al., (2007). A good correlation was found between DPPH and ABTS methods (R^2=0.827), indicating that these two methods gave consistent results. The extracts obtained were all able to inhibit the DPPH, as well as ABTS radicals (Table 5). The antioxidant potential was 378.89 mg Trolox/100g DW with the DPPH method, and 399.99 mg Trolox/100g DW with the ABTS. In comparison to previous data based on the ABTS scavenging capacity, *A. roseum* leaf extracts were comparable or higher than other investigated species known to be rich in antioxidants including strawberry (25.9), raspberry (18.5), red cabbage (13.8), broccoli (6.5), and spinach (7.6) (Proteggente et al., 2002). Significant correlations were observed between the TPC of *A. roseum*, and antioxidant activity (R^2=0.828 for TPC vs. DPPH and R^2=0.925 for TPC vs. ABTS), suggesting that polyphenolic compounds are the major contributors to the antioxidant capacity of *A. roseum*.

Regarding the favourable redox potentials and relative stability of their phenoxyl radical, these biomolecules are considered to be human health promoting antioxidants (Acuna et al., 2002).

Extracts	DPPH (mg Trolox /100g DW)	ABTS (mg/100g DW)
Methanol (75%)	378.80±5.55	399.90± 4.59

Table 5. Free radical scavenging activity of *A. roseum*

2.4 Antibacterial activity

The *in vitro* antibacterial effects of the *A. roseum* extracts obtained with the methanolic extract values are presented in Table 6. The results showed that *A. roseum* extracts have great potential as antimicrobial agent against the tested bacteria. *C. albicans* and *C. glabrata*, were the most sensitive tested organisms to the extract with the MIC values were 0.63 and 2.5 mg/ml, respectively.

The strong antifungal activity was observed against *C. albicans* and *C. glabrata* may be related to the high level of polyphenols content. Cai et al. (2000) showed that several classes of polyphenols such as phenolic acids, flavonoids and tannins serve as plant defence mechanism against pathogenic microorganisms. In fact, the site and the number of hydroxyl groups on the phenol components increased the toxicity against the microorganisms.

Strains	MIC (mg/ml)
Escherichia Coli ATCC 25922	10±1.20
Enterococcus Faecalis ATCC29212	10±0.57
Staphylococcus aureus ATCC 25923	10±0.60
Candida albicans ATCC 90028	0,63±1.85
Candida glabrata ATCC 90030	2,5±1.20
Candida kreusei ATCC 6258	10±2.13
Candida parapsilosis ATCC 22019	10±1.41

MIC, Minimum Inhibitory Concentrations as (mg ml^{-1}).

Table 6. Minimal inhibitory concentrations of extracts of A. roseum on bacterial growth

3. Conclusion

This study revealed that *A. roseum* var. *odoratissimum* growing in Tunisia had a high soluble carbohydrates, crude protein and dietary fibre contents, compared to other *Alliums*. Its mineral content was high in potassium, and calcium. The mineral composition of 'rosy garlic' is sufficient in Ca, P, K, Cu, Fe, Zn and Mg so that it can meet many macronutrient and micronutrient requirements of the human diets. As a consequence, a diet based on *A. roseum* would help in preventing deficiencies in potassium, calcium, iron and magnesium. Furthermore, edible part oil included 15% saturated and 85% unsaturated fatty acids. Linolenic acid and palmitic acid were the most abundant unsaturated and saturated fatty acids, respectively. This fatty composition confers to the *A. roseum* oil considerable nutritional value, acting on physiological functions and reducing cardiovascular, cancer and arthroscleroses diseases occurrence risk. The most abundant phytonutrients found in *A.*

roseum (polyphenolic compounds, flavonoids, anthyacinidins, vitamin C and allicin) exhibit a positive effect on human health as antioxidants and antibacterial compounds. Since the chemical composition of *A. roseum* has not been reported before, this report provides a starting point for comparison to the other *Allium* genus vegetables and it confirms the potentially important positive nutritional value that *A. roseum* can have on human health. Since *A. roseum* is a rich source of many important nutrients and bioactive compounds responsible for many promising health beneficial physiological effects, it may be considered a nutraceutical that serves as a natural source of necessary components to help fulfil our daily nutritional needs and as a functional food as well as in ethnomedecine .

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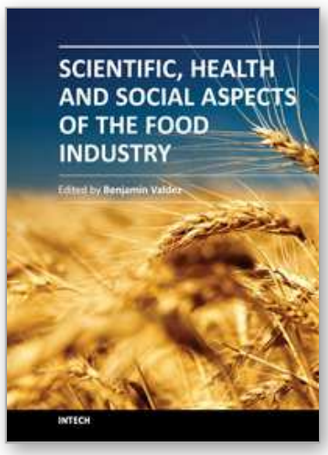

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This book presents the wisdom, knowledge and expertise of the food industry that ensures the supply of food to maintain the health, comfort, and wellbeing of humankind. The global food industry has the largest market: the world population of seven billion people. The book pioneers life-saving innovations and assists in the fight against world hunger and food shortages that threaten human essentials such as water and energy supply. Floods, droughts, fires, storms, climate change, global warming and greenhouse gas emissions can be devastating, altering the environment and, ultimately, the production of foods. Experts from industry and academia, as well as food producers, designers of food processing equipment, and corrosion practitioners have written special chapters for this rich compendium based on their encyclopedic knowledge and practical experience. This is a multi-authored book. The writers, who come from diverse areas of food science and technology, enrich this volume by presenting different approaches and orientations.

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